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جامعـــة باجـــي مختـــار-عنابـــة
BADJI MOKHTAR UNIVERSITY-ANNABA

FACULTY OF SCIENCES
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THESIS

Submitted to obtain the doctorate diploma

Specialty: Applied and Fundamental Biochemistry

Study the effect of sesame and pumpkin oils on diabetes in albino Wistar rats fed low zinc diet

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N° DBCH…../2024

Academic year : 2023/2024

Acknowledgements

First of all, praise be to Allah, good and generous, who has never left us and thanks to whom we have been able to go all the way to present this thesis.

I am extremely thankful to my supervisor Mr. **Zine KECHRID**, Professor, Badji Mokhtar Annaba University, who gave me the opportunity to carry out this thesis. I really have the honor to complete this work under his supervision. I also thank him for his valuable scientific advice and his unfailing availability.

My respect and my recognition go to Professor **Mahfoud MESSARAH**, Badji Mokhtar-Annaba University, for accepting the presidency of the jury.

I would also like to thank Professor **Salah ATTALAH**, Brothers Mentouri-Constantine University, and Professor **Mahieddine BOUMENDJEL**, Badji Mokhtar-Annaba University, for the great honor of agreeing to be the examiners of my thesis.

Last but not least, I deeply appreciate my beloved family, who has always supported me through difficult times. Thanks to you all for pushing me forward in every step of my life's journey.

Dedication

My thanks also go to:

My lovers parents who showed constant and intense support during all these years of study, who always encouraged me, and taught me to give my best…

My dear husband Kader…

My wonderful son Mohamed Amir…

My brother Fahd…

My brother Takiy-eddine, his wife Zaineb…

and their daughters Hidaya Belkiss and Farah Halima…

All my family...

My dear friend and sister, Nour Al-Hoda…

My friends and colleagues…

Anyone who participated directly or indirectly in the completion of this work...

I thank you and I dedicate this work to you…

الحمد لله الذي بنعمته تتم الصالحات

Abstract

Zinc is known to play a crucial role in insulin biosynthesis. Therefore, its deficiency may have a deleterious impact on the progression of diabetes and its complications. Given that natural antioxidants are the focus of current researches. So, the aim of this study is to modulate the deleterious impacts of zinc deficiency in the diabetic state using sesame and pumpkin oils by evaluating their effects on carbohydrate metabolism, zinc status, and oxidative stress biomarkers in streptozotocin (STZ)-induced diabetic rats fed zinc-deficient diet.

After induction of diabetes by a single intraperitoneal dose of 60 mg/kg of streptozotocin, male Wistar rats were divided into seven groups (6 each): four groups received an adequate zinc diet; the first group was non-diabetic rats; the second group was diabetic rats; and the other two groups were diabetic groups treated with either sesame oil (6%) or pumpkin oil (5%) as dietary supplements. While the remaining groups were diabetic groups fed zinc-deficient diet, one was untreated, and the others were treated with sesame oil (6%) or pumpkin oil (5%) supplemented in their diet for 27 days.

The results obtained clearly showed that diabetes caused histological changes in pancreatic and kidney tissues, metabolic disorders, zinc status variation, a decrease in the antioxidant defense system and an increase in oxidative stress markers. In addition, insufficient dietary zinc intake led to body weight loss and a decrease in tissues zinc levels (femur, pancreas). Zinc deficiency provoked also an increase in cholesterol and triglycerides, a decrease in lactic dehydrogenase (LDH) and amylase activities, and increase in transaminase activities (GPT). Besides that, low-zinc diet resulted increase markers of oxidative stress, characterized by rise in malondialdehyde (MDA), a reduction in reduced glutathione concentration (GSH), glutathione-S transferase (GST) and catalase (CAT) activities. As well as a disturbed further pancreas and kidney histology. However, supplementation with sesame and pumpkin oils led a significant improvement in the previous parameters.

In conclusion, these findings suggest dietary supplementation of these oils is a powerful factor to attenuate the oxidative severity of zinc deficiency in diabetes through their effective antioxidant capacity.

Keywords: diabetes, zinc deficiency, oxidative stress, sesame, pumpkin.

الملخص

من المعروف أن الزنك يلعب دورًا مهما في التخليق الحيوي للأنسولين. ولذلك، قد يكون لنقصه تأثير ضار على تطور مرض السكري ومضاعفاته. نظرًا لأن مضادات الأكسدة الطبيعية هي محور الأبحاث الحالية، لذا فان الهدف من هذه الدراسة هو تعديل التأثيرات الضارة لنقص الزنك في حالة مرض السكري باستخدام زيوت السمسم واليقطين من خلال تقييم آثارها على ميتابوليزم الكربوهيدرات، ومستوى الزنك، والمؤشرات الحيوية للإجهاد التأكسدي عند الفئران المصابة بالداء السكري المستحدث بالستربتوزوتوسين والتي تتغذى على نظام غذائي منقوص من الزنك.

بعد إحداث مرض السكري بجرعة واحدة داخل الصفاق 60 ملغم/كغم من الستروبتوزوتوسين، تم تقسيم ذكور الجرذان ويستار إلى سبع مجموعات (6 لكل منها): تلقت أربع مجموعات نظامًا غذائيًا كافيًا من الزنك؛ الأولى كانت فئران غير مصابة بالسكري. الثانية كانت فئران مصابة بالسكري. وكانت المجموعتان الأخريان عبارة عن فئران مصابة بالداء السكري تم علاجهاإما بزيت السمسم (6٪) أو زيت اليقطين (5٪) كمكملات غذائية. بينما كانت المجموعات المتبقية عبارة عن مجموعات مصابة بمرض السكري وتلقت نظامًا غذائيًا منقوص من نقص الزنك، لم يتم علاج إحداها، وتم علاج المجموعات الأخرى بزيت السمسم (6٪) أو زيت اليقطين (5٪) المكمل في نظامهم الغذائي لمدة 27 يومًا.

أظهرت النتائج التي تم الحصول عليها بوضوح أن مرض السكر يسبب تغيرات نسيجية في أنسجة البنكرياس والكلى، واضطرابات التمثيل الغذائي، وتغير حالة الزنك، وانخفاض في نظام الدفاع المضاد للأكسدة، وزيادة في مؤشرات الإجهاد التأكسدي، بالإضافة إلى ذلك، أدى عدم تناول كمية كافية من الزنك في الغذاء إلىفقدان وزن الجسم وانخفاض مستويات الزنك في الأنسجة (عظم الفخذ والبنكرياس). أدى نقص الزنك أيضًا إلى زيادة في نسبة الكوليسترول والدهون الثلاثية، وانخفاض في أنشطة اللاكتات ديهيدروجيناز (LDH) والأميلاز، وزيادة في أنشطة إنزيمات ناقلات اللآمين (GPT) بالإضافة إلى ذلك، أدى اتباع نظام غذائي منخفض من الزنك إلى زيادة معايير الإجهاد التأكسدي، والتي تتميز بارتفاع في مستوى الأكسدة الليبيدية (MDA)، وانخفاض في تركيز الجلوتاثيون المختزل(GSH) ، و أنشطة الجلوتاثيون -S- ترانسفيراز(GST) والكاتلاز (CAT) فضلا عن تغير في أنسجة البنكرياس والكلى ومع ذلك، أدت إضافة زيوت السمسم واليقطين إلى تحسن معنوي في المعايير السابقة الذكر.

خلاصة، تشير هذه النتائج إلى أن إضافة هذه الزيوت إلى النظام الغذائي تعد عامل قوي في تخفيف شدة الأكسدة الناتجة عن نقص الزنك في مرض السكري من خلال قدرتها الفعالة المضادة للأكسدة.

الكلمات المفتاحية: مرض السكري، نقص الزنك، الإجهاد التأكسدي، السمسم، اليقطين.

Résumé

Le zinc est reconnu pour jouer un rôle crucial dans la biosynthèse d'insuline. De ce fait, sa carence pourrait avoir un impact délétère sur l'évolution du diabète et ses complications. Étant donné que les antioxydants naturels font l'objet des recherches actuelles. Ainsi, le but de cette étude est de moduler les impacts délétères de la carence en zinc dans l'état de diabète à l'aide d'huiles de sésame et de citrouille en évaluant leurs effets sur le métabolisme du carbohydrates (glucides, lipides et protéines), le statut en zinc et les biomarqueurs du stress oxydatif chez des rats diabétiques induits par la streptozotocine (STZ) et nourris avec un régime déficient en zinc.

Après induction du diabète par une dose intrapéritonéale unique de 60 mg/kg de streptozotocine, les rats Wistar mâles ont été divisés en sept groupes (6 chacun) : quatre groupes ont reçu un régime alimentaire suffisant en zinc; le premier groupe était constitué de rats non diabétiques ; le deuxième groupe était constitué de rats diabétiques ; et les deux autres groupes étaient des rats diabétiques traités avec de l'huile de sésame (6 %) et de l'huile de citrouille (5 %) comme compléments alimentaires. Alors que les groupes restants étaient des groupes diabétiques nourris avec un régime déficient en zinc, l'un d'entre eux n'a pas été traité et les autres ont été traités avec de l'huile de sésame (6 %) ou de l'huile de citrouille (5 %) supplémentée dans leur régime alimentaire pendant 27 jours.

Les résultats obtenus ont clairement montré que le diabète entrainait des modifications histologiques des tissus pancréatiques et rénaux, des troubles métaboliques, une variation du statut en zinc, une diminution du système de défense antioxydant et une augmentation des marqueurs de stress oxydatif. De plus, un apport alimentaire insuffisant en zinc a entraîné une perte de poids corporel et une diminution des taux de zinc dans les tissus (fémur, pancréas). La carence en zinc a également provoqué une augmentation du cholestérol et des triglycérides, une diminution des activités de la lactique déshydrogénase (LDH) et de l'amylase, et une augmentation des activités transaminases (GPT). En outre, un régime pauvre en zinc a entraîné une augmentation des marqueurs du stress oxydatif, caractérisés par une augmentation du malondialdéhyde (MDA), une réduction de la concentration de glutathion réduit (GSH), des activités de glutathion-S transférase (GST) et de catalase (CAT). Ainsi qu'une histologie pancréatique et rénale changé. Cependant, la supplémentation en huiles de sésame et de citrouille a entrainé une amélioration significative des paramètres précédents.

En conclusion, ces résultats suggèrent que la supplémentation alimentaire de ces huiles est un facteur puissant pour atténuer la gravité oxydative de la carence en zinc dans le diabète grâce à leur capacité antioxydante efficace.

Mots clés : diabète, carence en zinc, stress oxydatif, sésame, citrouille.

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Abbreviation's list

4-HNE: 4-hydroxyl nonenal

8-OH-dG: 8-hydroxy-2'-deoxyguanosine

AGEs: Advanced glycation end products

BHT: Buthylated hydroxytoluene

CDNB: 1-chloro, 2.4-dinitrobenzene

DAG: Diacylglycerol

GDM: Gestational diabetes mellitus

DTNB: 5.5′ dithiodis-2-nitrobenzoic acid

IC_{50}: Inhibitory concentration of 50%

GFA: Glucosamine-fructose amidotransferase

GLP-1: Glucagon-like peptide 1

Glu-6-P: Glucosamine-6-phosphate

Glut2: Glucose transporter 2

HbA1c: Glycosylated hemoglobin

HLA: Human leukocyte antigen

HMG-CoA: Hydroxyl-methyl-glutaryl-CoA

$HO^{\bullet}$: Hydroxyl radical

HOCl: Hypochlorous acid

LPO: Lipid peroxidation

MODY: Maturity-Onset Diabetes of the Young

MUFA: Monounsaturated fatty acids

NADPH: Nicotinamide adenine dinucleotide phosphate reduced

NFk-B: Nuclear factor kappa-B

NO^{-}: Nitric oxide

O_2: Singlet oxygen

$O_2^{\bullet-}$: Superoxide anion

$ONOO^{-}$: Peroxynitrite

OS: Oxidative stress

PKC: Protein kinase C

PPAR-γ: Peroxisome proliferator-activated receptor-gamma

R-AGE: Receptor-advanced glycation end products

RER: Rough endoplasmic reticulum

ROS: Reactive oxygen species

RNS: Reactive nitrogen species

Introduction

Introduction

The human body is constantly exposed to various types of agents that lead to the production of reactive species, which are known as free radicals; these radicals cause oxidation of cellular machinery by transferring their unpaired electrons. To counteract the detrimental impacts of these species, the body possesses internal antioxidant systems or acquires exogenous antioxidants from diets that neutralize these species and maintain the body's homeostasis. Therefore, any imbalance between pro-oxidants and antioxidants causes oxidative stress, which in turn causes the development of several pathological disorders, such as diabetes **(Asmat et al., 2016)**.

Diabetes, a prevalent endocrine disease worldwide, which is defined as a heterogeneous set of metabolic disorders. Diabetes is known by chronically elevated blood glucose levels, which are accompanied by disruptions in the metabolism of carbohydrates, lipids and proteins due to a malfunction in the action and/or secretion of insulin. This progressive pathology predisposes the diabetic patient to disabling and/or fatal macro- and microvascular complications, which the latter affect numerous organs **(Reddy and Tan, 2020)**.

Diabetes patients with persistent hyperglycemia generally increase reactive oxygen species overproduction through a variety of pathways. Ultimately, these processes lead to lipid peroxidation, antioxidant dysfunction, and cell membrane damage **(Lotfy et al., 2017)**.

There is no doubt that zinc is ubiquitous and it is one of the most abundant trace minerals in biological systems. Zinc is proven to be important for both human and animal health **(Yusuf et al., 2021)**. As a multipurpose trace element, it is essential for many physiological processes, including the metabolism of carbohydrates and insulin. It also serves as a cellular antioxidant, a regulator in the biosynthesis of insulin, and an element in the structure of many enzymes such as alkaline phosphatase, DNA polymerase, and superoxide dismutase **(Livingstone, 2015; Zhao et al., 2019)**.

Henceforth, there are several motives to suspect that abnormal zinc metabolism, including a zinc deficiency, may contribute to the pathogenesis of diabetes and its sequelae, which is accompanied by an increased susceptibility to oxidative stress and enhanced ROS generation **(Li et al., 2017; Zhang et al., 2017)**.

Recently, plants have gained attention as a source of natural bioactive molecules with powerful antioxidant properties that can be used for their beneficial effects on human health and in the prevention of the development of metabolic diseases such as diabetes **(Al-Ishaq et al., 2019)**. Interestingly, the seeds of plants have received growing attention due to their high nutritional and therapeutic value, which are less toxic and they have fewer side effects and higher efficacy than synthetic drugs **(Dotto and Chacha, 2020)**.

Sesame and pumpkin, as traditional foods or herbal medicines, have a great nutritional value due to their richness in many bioactive components such as fatty acids and phenolic compounds **(Shi et al., 2017; Broznić et al., 2016)**. Indeed, several studies have revealed the antioxidant properties of sesame and pumpkin that give them powerful preventive anti-inflammatory, anti-carcinogenic, antihypertensive activities, anti-diabetic properties, as well as serum lipid-lowering effects **(Sedigheh et al., 2011; Haidari et al., 2016; Aslam et al., 2019; Abd-elnoor, 2019)**. Thus, these plants may be able to prevent a variety of illnesses, including diabetes mellitus.

Based on the previous information's, the current study was undertaken to evaluate the modulatory effects of sesame and pumpkin oils supplementation for the prevention of the development of diabetic pathology by evaluating zinc status, carbohydrate metabolism, and the antioxidant system in streptozotocin-diabetic rats fed a low zinc diet.

This thesis was divided into two essential parts: the first section, "theoretical part". It consists of five chapters including diabetes, zinc, oxidative stress, oxidative stress and diabetes and the plants phytotherapy.

The second part, "experimental part", it was focused on the antioxidant activity and the prophylactic effect of sesame and pumpkin oils in diabetic rats fed dietary zinc deficiency. So, the experimental protocol was presented as follows dosage technics, results presentation, discussion and conclusion.

Chapter I. Diabetes Mellitus

1. Generality

The world is exposed to many chronic diseases. These illnesses are spread and pose a major threat to public health and impose huge costs on medical care. So, this threat required the urgent introduction of new health strategies, measures and policies to help patients from all sides and find radical ways to combat these diseases. Among these diseases, we mention diabetes, a rapidly growing problem that threatens the life of people around the world and whose seriousness lies in its high incidence and mortality. It is the third largest chronic disease after cardiovascular and cerebrovascular diseases **(Chen and Li, 2021)**. It is estimated to affect 578 million people by 2030 and 700 million people by 2045, which prompted scientists to study this health problem **(Tripathy et al., 2021)**.

Diabetes mellitus is a disease of the endocrine system that describes a series of metabolic disorders which are characterized by the presence of chronic hyperglycemia as a result of absolute or relative insulin deficiency and/or insulin resistance, accompanied by impairment of glucides, fat and protein metabolism **(IDF, 2018; Lotfy et al., 2017)**. Chronic hyperglycemia has been shown to be a major factor in the development of diabetic complications as it has deleterious effects on various organs such as nerves, kidneys, eyes and the peripheral vascular system **(Baig and Panchal, 2020)**.

The diagnosis of diabetes is made when a patient presents with the classic symptoms of hyperglycemia: polydipsia (increased thirst), polyuria (frequent urination) and polyphagia (increased hunger) with a random blood concentration above 2 g/L (11mmol/L) or the fasting blood glucose level is above 1.26 g/L (7 mmol/L) **(DiMeglio et al., 2018)**.

As an endocrine hormone, insulin is a small polypeptide of 51 amino acids (8 kDa), which consists of two chains: the α chain contains 21 amino acids and the β chain consists of 30 amino acids. Two interchain disulfide bridges (CysA7 to CysB7 and CysA20 to CysB19) covalently bind chains A and B **(figure 1)**. An intracatenary disulfide bridge connects amino acids 6 and 11 of the α chain **(Aghaei et al., 2020)**.

Insulin is the only hypoglycemic hormone produced and secreted by the β-cells of the pancreatic islets. It is widely known for its key role in controlling glucose homeostasis through stimulating glucose uptake by insulin dependent tissues including liver, muscle and adipose tissue **(Van Niekerk et al., 2020; Vasiljević et al., 2020)**.

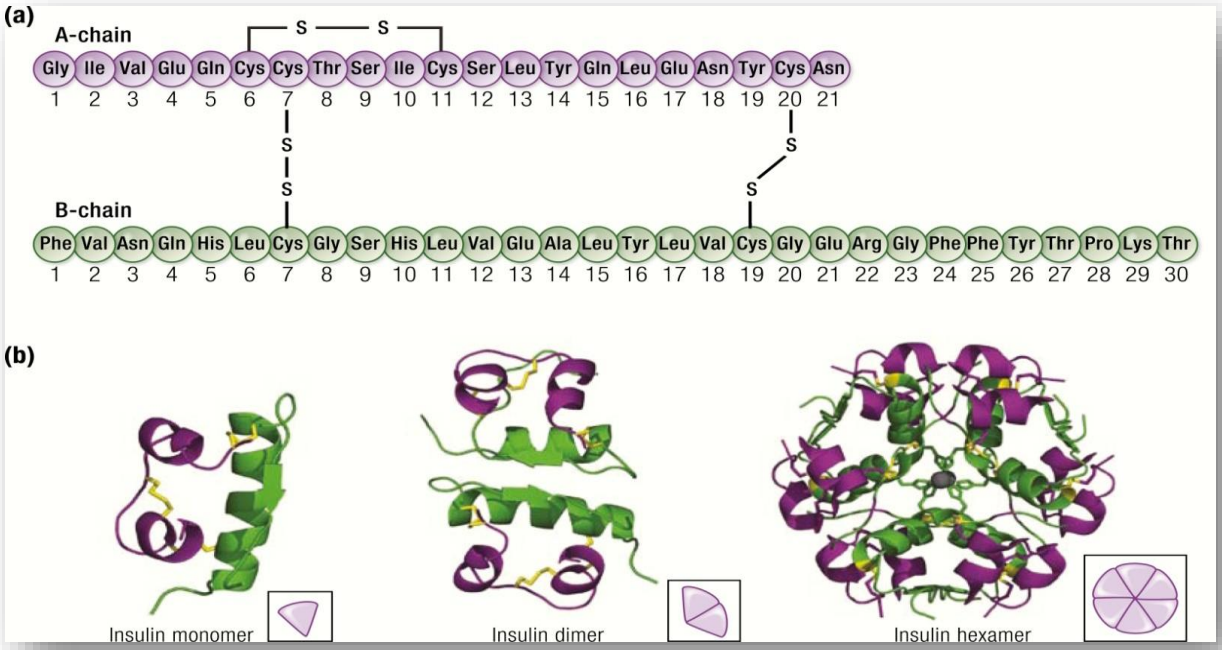


Figure 1. Insulin human structure. a: Amino acid sequence of human insulin. b: Three-dimensional structure of insulin monomer (A-chain in purple; B-chain in green) **(Hirsch et al., 2020)**.

2. Diabetes mellitus types

The diabetes type is defined depending on its natural history, clinical phenotype, genotype, and environmental changes. According to this approach, diabetes can be divided into the following categories: type 1 diabetes, type 2 diabetes, in addition to other forms such as monogenic diabetes, and gestational diabetes **(Leslie et al., 2016)**. It is crucial to understand that hyperglycemia is a common feature of all types of diabetes, but the etiology, pathophysiology, and treatment are differents **(WHO, 2019)**.

2.1. Type 1 diabetes

Formerly known as insulin-dependent or juvenile diabetes, it is primarily an autoimmune process that leads to the destruction of beta cells, resulting in absolute insulin deficiency through the activation of T *cells* and macrophages that infiltrate the pancreatic islets. It accounts for approximately 10 to 20% of all diabetes cases; however, its incidence continues to increase worldwide and it has serious short-term and long-term implications; it usually manifests itself in childhood and adolescence **(Efrat, 2008)**.

This type of diabetes is divided into autoimmune (immune-mediated) diabetes (type 1A) and type 1B or idiopathic diabetes **(Imagawa et al., 2000)**. The absence of autoimmune markers at diagnosis and

the presence of clinical symptoms of diabetic ketoacidosis mainly characterize the latter subcategory **(Aguilera et al., 2004; Piñero-Piloña et al., 2001)**.

Type 1A (5–10% of cases) is an autoimmune disease that results in the selective and progressive destruction of beta cells, which may ultimately lead to the production of autoantibodies against β-cell antigens. It is strongly associated with human leukocyte antigen (HLA) and it leads to severe insulin deficiency **(ADA, 2015)**. Patients with type 1 diabetes should receive lifelong insulin therapy to maintain normoglycemia **(IDF, 2017)**.

2.2. Type 2 diabetes

It is known as non-insulin-dependent diabetes, it is the most common form of diabetes and accounts for approximately 90% of patients with diabetes **(Zheng et al., 2018; Al Hamarneh et al., 2019)**. Persistent hyperglycemia and insulin resistance in peripheral tissues characterize this type **(Olokoba et al., 2012**). Until recently, type 2 diabetes manifested only itself in adults, but children and adolescents are now increasingly affected **(WHO, 2019)**.

Most patients with type 2 diabetes present obesity, which its closely linked to the presence of insulin resistance and represents the main risk factor of this disease **(Eizirik et al., 2020)**. The etiology of T2DM is still unclear, but it is related closely to a family history of diabetes (genetic predisposition) as well as certain factors, particularly obesity, age, diet and ethnicity **(WHO, 2019)**. The remarkable changes in lifestyle, diet and behaviour support the prevalence and treatment of type 2 diabetes **(Zheng et al., 2018)**.

2.3. Gestational diabetes mellitus (GDM)

The gestational diabetes is recognized by "Any degree of glucose intolerance with the onset or first recognition during pregnancy" **(Al Hamarneh et al., 2019)**.

Gestational diabetes is defined as a pancreatic function deficiency to overcome the insulin resistance **(Korkmazer et al., 2015)**, which usually onset in pregnant women during the second and the third trimesters of gestation. It affects around 7 % of all pregnancies.

It is known also that, as long-term consequences, women with GDM have a predisposition to developing type 2 diabetes **(ADA, 2018; Ahmad, 2013)**.

2.4. Monogenic diabetes

Monogenic diabetes is a rare diabetes disease that accounts only for about 1 to 5% of all cases. This form results from a single gene mutation in genes responsible for controlling the production or release of insulin. Although type 1 and type 2 diabetes are caused by multiple genetic defects (polygenic), most patients with monogenic diabetes are misdiagnosed as type 1 or type 2 diabetes **(Poretsky, 2017)**. This

disease includes several subtypes, namely neonatal diabetes mellitus or monogenic diabetes of infancy, dominant hereditary familial forms of early-onset diabetes called Maturity-Onset Diabetes of the Young (MODY), and rarer syndromes associated with diabetes **(Vaxillaire et al., 2012)**.

2.5. Experimental diabetes

Since diabetes poses a serious threat to individual health and causes a societal economic problem, research on its management and treatment has now become one of the most sought-after areas of interest.

Thus, the study of animal models is essential for the development of new and effective ways for treating diseases such as diabetes. A number of animals are exploited in experimental studies related to diabetes, as the animals are physiologically similar to humans **(Kottaisamy et al., 2021)**.

There are several methods for experimental diabetes induction in laboratory animals, either surgical (pancreatectomy), genetic manipulations (transgenic models), a high-calorie diet, and chemical substances. Despite the many ways to induce diabetes, chemical approaches to alloxan- and streptozotocin-induced diabetes represent the most important and preferred experimental models **(Radenković et al., 2016)**.

2.5.1. Streptozotocin-induced diabetes

Streptozotocin (STZ, 2-deoxy-2(([methyl (nitroso) amino] carbonyl) amino)-(α and β)-D-glucopyranose),is a natural antibiotic produced by Streptomyces achromogenes **(Ghasemi and Jeddi, 2023)**. STZ molecule consists of two parts **(figure 2)**: the glucopyranosyl group, which facilitates its uptake by pancreatic β cells through glucose transporter 2 (GLUT2), and a highly reactive nitrosourea group, which destroys pancreatic β cells **(Wu and Yan, 2015)**.

STZ, as a cytotoxic glucose analogue, is an alkylating agent that can add alkyl groups to DNA's double helix structure. In brief, the mode of action of STZ toxicity depends on the DNA alkylation activity of its methyl nitrosourea moiety. The transfer of a methyl group from STZ to the DNA molecule causes DNA structural damage and eventually leads to cell death **(Capdevila et al., 2022)**.

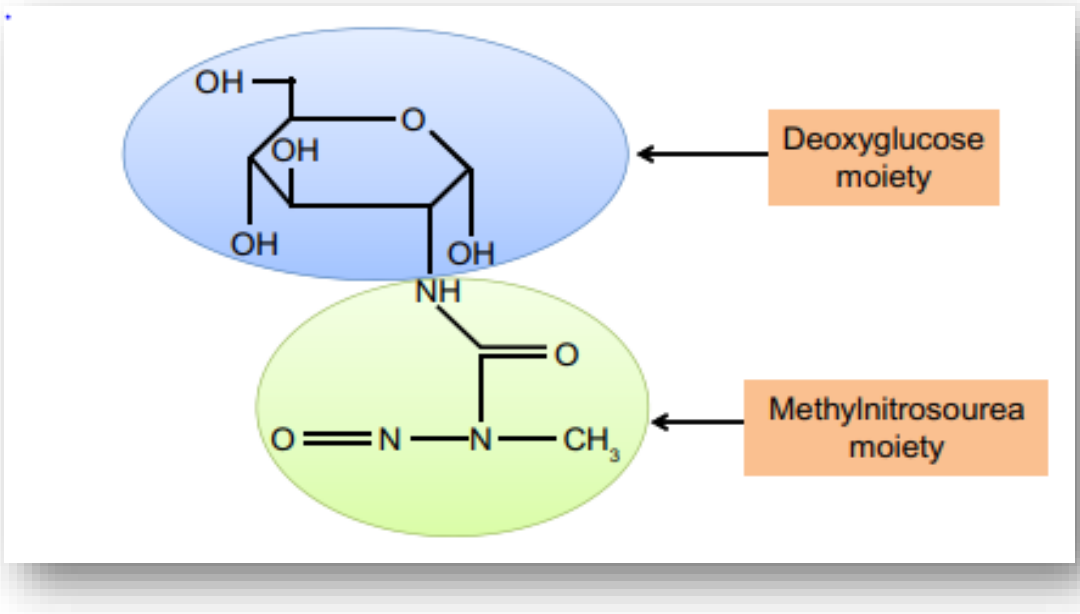


Figure 2. Chemical structure of streptozotocin **(Wu and Yan, 2015).**

3. Complications of diabetes

Complications due to diabetes are a major cause of morbidity, changes in quality of life and mortality in diabetic patients **(Ahmad, 2013)**. These complications are associated with long-term damage and failure of various organs such as kidneys, nerves, eyes and heart. Many factors contribute to the development of diabetes and its complications, but especially to persistent hyperglycemia **(Chawla, 2016)**. The pathological results factors of chronic hyperglycemia affects the vascular system, resulting in both microvasculopathy (retinopathy, nephropathy and neuropathy) and macrovasculopathy (coronary artery disease, peripheral arterial disease and stroke) as shown in **figure 3 (Orasanu and Plutzky, 2009)**.

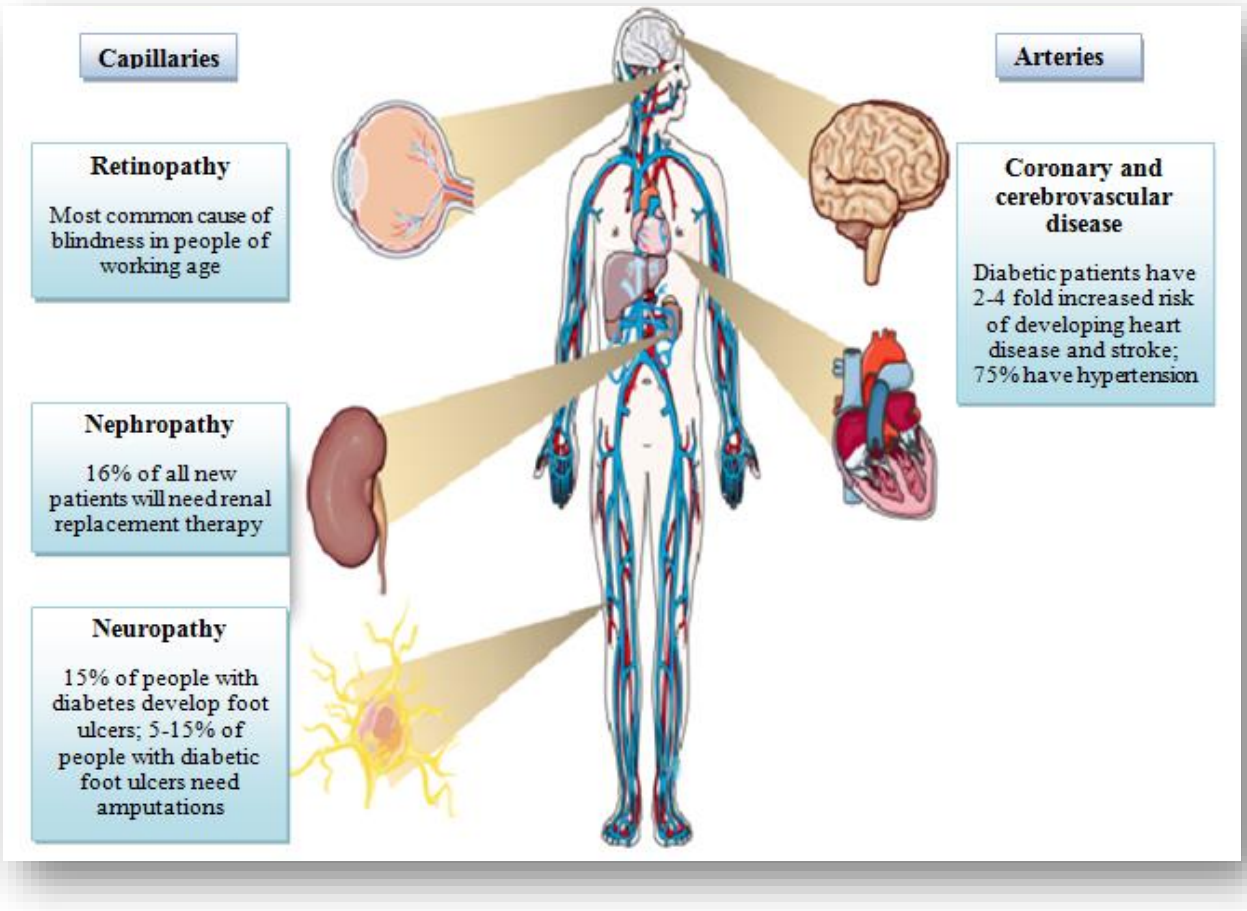


Figure 3. The macrovascular and microvascular complications of diabetes mellitus. Diabetes is a chronic disease mainly associated with the development of macrovascular complications (coronary and cerebrovascular disease), in the arteries; microvascular complications (retinopathy, neuropath and nephropathy), in the capillaries. Vasculopathies significantly affect the rate of mortality and patients' quality of life **(Kaul et al., 2013)**.

4. Diabetes treatments

The primary goal of treatment of this chronic progressive disease is to maintain near-normoglycemic disease to prevent the onset or delay the progression of macro- and microvascular complications.

Lifestyle changes, including diet and physical activity, have been shown to be insufficient to reduce the incidence of diabetes. Continuous insulin therapy is essential for the survival of patients with type 1 diabetes, while overdose of insulin treatment can lead to hypoglycemic episodes. In patients with type 2 diabetes, the appropriate drug treatment is necessary, even the administration of insulin are required to achieve blood glucose control **(Zarkogianni et al., 2015)**. Oral antidiabetic drugs can be divided into seven categories depending on their mechanism of action **(figure 4)**:

4.1. Biguanides (metformin)

This class of drug acts by improving skeletal muscle insulin sensitivity, decreasing hepatic glucose output (suppressing gluconeogenesis and glycogenolysis) **(Cheng and Fantus, 2005)**.

4.2. Sulfonylureas (glibenclamide, glipizide, and glimepiride)

The target of these agents is the ATP-sensitive K^+ channels found in β-cells. Sulfonylureas action is dependent on the inhibition of KATP channels result in membrane depolarization and activation of Ca^{2+} channels, thus stimulating insulin secretion **(Bryan et al., 2005)**.

4.3. Thiazolidinediones (Glitazones)

They are potent and selective agonists of the peroxisome proliferator-activated receptor (PPARγ receptors). Thiazolidinediones act as insulin sensitizers by binding to PPAR-γ in adipose tissue, muscle and liver, thereby reducing insulin resistance. This leads to a reduction in free fatty acid levels and the promotion of lipogenesis **(Mayer et al., 2011; Papoushek, 2003)**.

4.4. Glinides (Meglitinides)

Are another type of insulin secreting drugs, their mode of action is similar to that of sulphonylureas, stimulating insulin release from the pancreatic β cells. Glinides bind to SUR-receptors at the β-cell membrane, which differs from the sulphonylureas binding site **(Lv et al., 2020; Chen et al., 2015)**.

4.5. α-Glucosidase inhibitors (acarbose, miglitol, voglibose)

α-Glucosidase inhibitors works by competitively inhibiting intestinal α-glucosidases, a series of enzymes involved in reducing the digestion of complex carbohydrates and the subsequent delayed absorption of glucose, thereby reducing postprandial blood glucose levels **(Scheen, 2003)**.

4.6. Glucagon-like peptide 1 (GLP-1) analogues

GLP-1 is an intestinal-derived incretin hormone, which regulates blood glucose level through its ability to stimulate insulin secretion, suppresses the secretion of glucagon, and increases pancreatic β cell growth and decrease β cell apoptosis. Additionally, this factor has an action in delaying gastric emptying and inducing satiety **(Cefalu et al., 2014; Renner et al., 2016)**.

4.7. Dipeptidyl peptidase-4 inhibitors

Dipeptidyl peptidase-4 inhibitors are a new class of oral antidiabetic drugs that stimulates insulin release in a glucose-dependent way; it inhibits also glucagon secretion **(Ahren, 2007; Thornberry and Gallwitz, 2009)**.

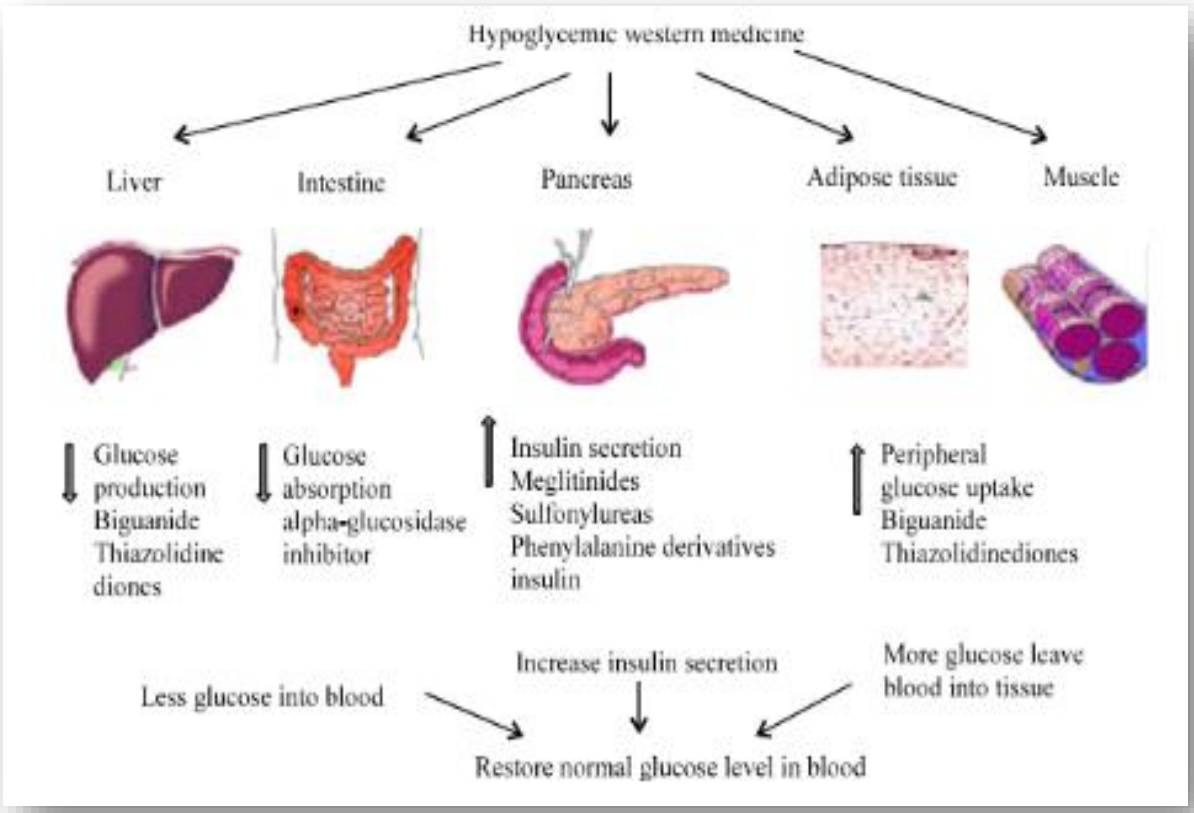


Figure 4. Hypoglycemic medicine action sites in diabetes treatment. Oral hypoglycemic medicines restore hyperglycemia via several mechanisms: insulin secretagogues (sulfonylureas, meglitinides), insulin sensitizers (biguanides, metformin, and thiazolidinediones), alpha-glucosidase inhibitors (miglitol, acarbose) **(Hui et al., 2009)**.

Chapter II. Zinc

1. Zinc

The importance of micronutrients in nutrition and health is undisputable. Among them, zinc is an essential element whose importance for health is increasingly appreciated, and its deficiency may play an interesting role in the emergence of diseases **(Chasapis et al., 2012)**. The relevance of zinc was first reported for the growth of *Aspergillus niger*. Subsequently, it took over65 years to achieve that zinc is an essential element for mice and rats, and an additional 30 years, it was recognized that this is also applies for humans **(Hübner and Haase, 2021)**.

Zinc (Zn) is one of the most important nutritional elements, being essential for the growth and development of all living organisms. The human body contains about 2–3 grams of zinc, so zinc is the second most abundant trace element after iron **(Chasapis et al., 2020)**.

Zinc is a transition metal, has an atomic number 30 and atomic weight 65.37, it appears in group 12 of the periodic table. In biological system, zinc is stable as a divalent cation (Zn^{2+}) and it is neither an oxidizing agent nor or reducing agent owing to its filled d-shell. The chemical properties of this element give zinc an important role in a wide range of biological processes. It has a strong affinity and typically binds to amino acids, proteins, nucleotides and peptides, permitting both structural and catalytic functions **(Kaur et al., 2014; Lee, 2018)**.

2. Food sources

Obviously, the most important dietary source of readily bioavailable zinc is meat **(Kloubert and Rink, 2015)**. Oysters contain more zinc than any other foods. Beef liver, fish, poultry, milk, dairy products, cereals, and legumes contain some of the highest concentrations of zinc **(figure 5)**. Other food sources with a very little zinc including vegetables, fruits. Generally, animal-origin diets are a more preferable source of zinc than plant-origin diets. Since vegetarian foods contain a lot of phytate and casein, which form insoluble complexes with zinc, reducing its absorption **(Imoberdorfa et al., 2010)**.

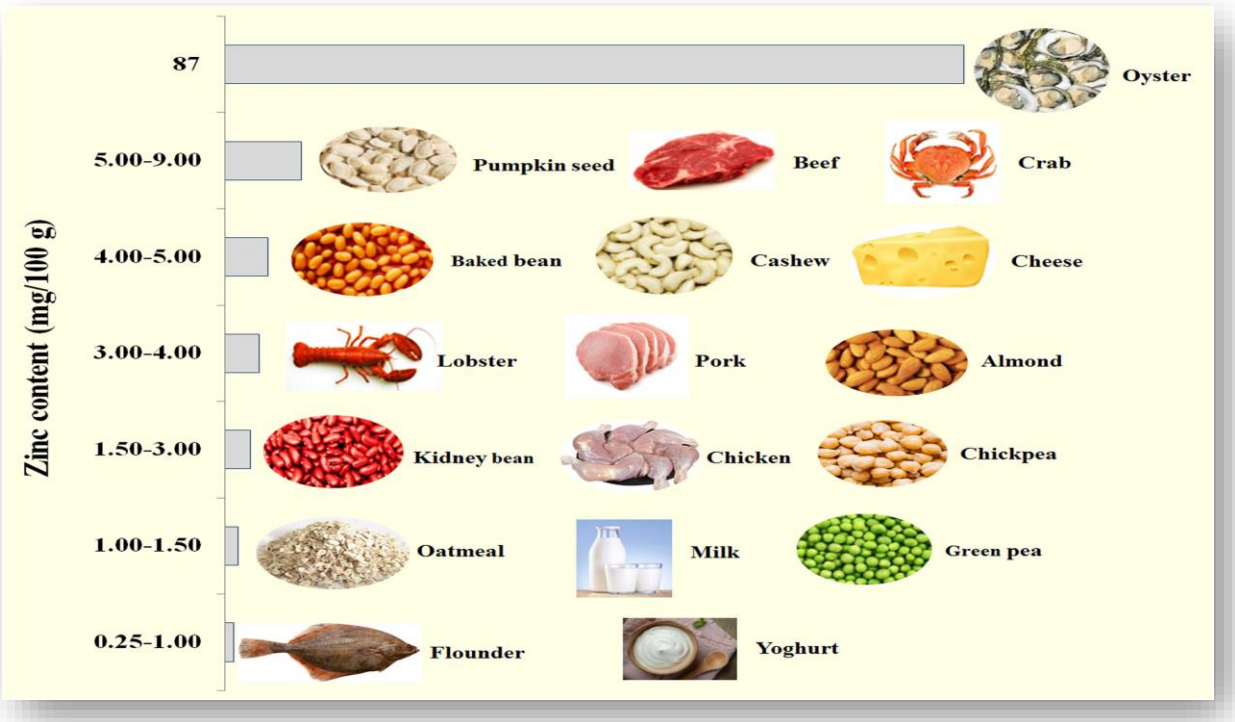


Figure 5. Foods examples, rich in zinc **(Sangeetha et al., 2022)**.

3. Distribution of zinc in human body

Zinc is distributed in all body organs, tissues and secretions. Most of the zinc is present in skeletal muscles 60%, bones 30%, and the remaining all the other tissues **(figure 6)**, with the highest concentrations in the prostate, pancreas, kidneys, liver, spleen and heart. In the plasma is found less than 1% of the total zinc. 80% of the serum zinc is strongly bound to albumin, 18% is tightly bound to α2-macroglobulin, and 2% is bound to transferrin or the amino acids, and a small portion is present as free zinc. However, serum represents a rapidly exchangeable zinc pool of high importance for zinc distribution within the body **(Rashmi et al., 2021; Hara et al., 2017)**.

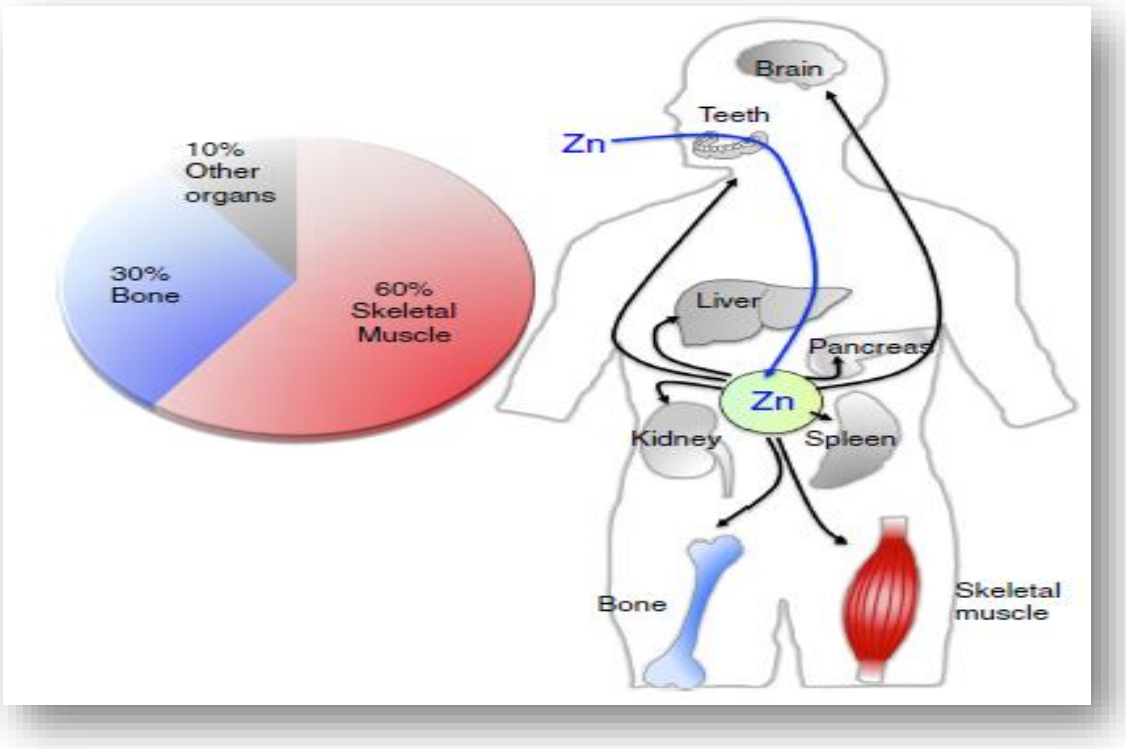


Figure 6. Zinc distribution in the human body. Dietary Zn is uptake by the small intestine and distributed to the organs. Skeletal muscles and bones act as main Zn reservoir tissues **(Hara et al., 2017)**.

4. Zinc metabolism

4.1. Absorption

Prior to the absorption, zinc ions are released from the complex food by the digestive process either directly through dissociation. Hydrochloric acid (HCl) promotes the release of zinc from the feed components or zinc compounds such as $ZnSO_4$, ZnO, and $ZnCl_2$ or indirectly through proteins digestion **(Mir et al., 2020)**. Zinc uptake as divalent cation (Zn^{2+}) takes place at the intestinal brush border membrane, where it is transported from the lumen into absorptive cells of the epithelium **(Maares and Haase, 2020)**. Zinc absorption kinetics are described by two processes, the first is at low zinc concentrations uptake is saturable and occurs by a carrier-mediated, and the second is at higher zinc concentrations, uptake is linear, which indicate passive diffusion process **(Maares and Haase, 2020. Mills, 2013)**.

4.2. Zinc homeostasis

In animal and humans, zinc absorption and endogenous intestinal excretion are the primary mechanisms for maintaining zinc homeostasis **(King et al., 2000)**. Cellular zinc homeostasis is mediated by two protein families of Zn transporters, which have opposite roles as shown in **figure 7**.The Zn-importer (Zip; Zrt-, Irt-like proteins/solute carrier family 39) family comprises 14 proteins members that transport

zinc from the extracellular space or intracellular organelles into the cytosol. Regarding efflux, it is the family of Zn transporter (ZnT)/SLC30A contains 10 proteins members that expel Zn out of the cytosol outside the cell or towards the lumen of intracellular organelles, resulting in a decrease intracellular zinc concentration **(Hara et al., 2017; Kambe et al., 2015)**.

Another way of regulation of zinc homeostasis at the cytoplasmic level is through metallothioneins (MT), which are a cysteine-rich, metal-binding proteins, these proteins buffer the zinc concentration. This process allows a rapid response to an imbalance **(Bonaventura et al., 2015; Kimura and Kambe, 2016)**.

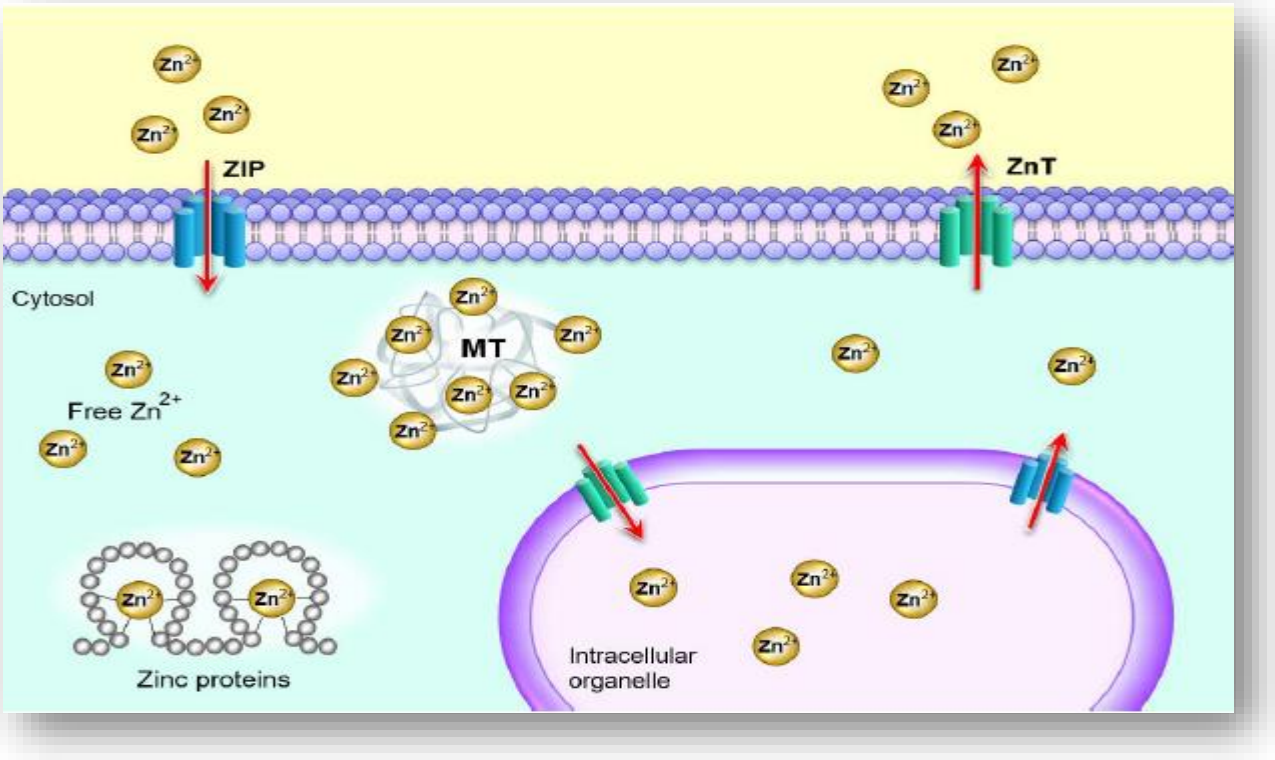


Figure 7. Cellular zinc homeostasis is controlled by the cooperative interactions among metallothioneins (MT), Zrt- and Irt-like proteins (ZIP), and Zn transporters (ZnT). The two families of zinc transporters, ZIP and ZnT, delicately control the movement of zinc into or out the cytosol; MTs bind to zinc to reserve, buffer, and chelate **(Uwitonze et al., 2020).**

4.3. Transport

Circulating zinc transport is provided not by a specific protein, but by several transporters capable of capturing more or less zinc depending on their concentrations and affinities, including albumin, alpha-2-macroglobulin, transferrin, fibrinogen, haptoglobin, C-reactive protein and immunoglobulins like IgGs. Albumin is the main carrier protein of zinc, binding approximately 75–80% zinc in the plasma. Zinc link also to amino acids, cysteine and histidine are the major zinc binding amino acids. The principal function

of these transport proteins is to distribute zinc to the cells of various organs including liver, pancreas, and kidneys.., and at the same time reduce free zinc ion concentration in the plasma **(Mir et al., 2020)**.

4.4. Excretion

Zinc is excreted mainly from the body through faeces. Faecal zinc (about 10 mg/day) corresponds to unabsorbed zinc (67%) from food and endogenous zinc (33%). Renal elimination represents only about 5% of normal daily intake. The zinc filtered at the glomerular. Then, it is reabsorbed at the distal tubule and re-excreted at the proximal. Renal metallothioneins plays an important role as a defense mechanism against zinc leaking out in the urine **(Lestienne, 2004)**. Other body fluids such as sweat, desquamation, semen, milk are also important ways for zinc losses **(Faa et al., 2008)**.

5. Zinc deficiency

Zinc is an important trace element in human health that even a small deficiency is a disaster **(Chasapis et al., 2012)**. Deficiency of Zn can occur generally from inadequate dietary intake and poor absorption or because of increased loss or increased demand.The most common cause worldwide is inadequate intake as a result of a low zinc diet or food contains high phytate**(Livingstone, 2015)**. The clinical manifestations of zinc deficiency are very varied. Indeed, zinc deficiency can lead to a number of functional abnormalities including: growth retardation, dermatitis, diarrhea, dysgeusia, immune dysfunction, recurrent pneumonias, impaired wound healing, night blindness, agerelated macular degeneration, anorexia, hypogonadism, reduced muscle strength, osteoporosis, and impaired cognition **(Gunturu and Dharmarajan 2020)**.

6. Zinc functions

6.1. Physiology role of zinc

Zinc is not only an important nutrient, but it is an essential trace element has wide roles of vital physiological processes. Mostly, it is required for the function of many proteins, it is estimated that 10% of the proteins encoded by the human genome need zinc for their functions **(Eide, 2011)**.

Zinc participates in numerous metabolic processes, as a structural, catalytic, and a regulatory component **(King et al., 2015)**. So, more than 300 catalytically active Zn metalloenzymes (hydrolases, transferases, oxyreductases, ligases, isomerases, and lyases class) and more than 2000 zinc transcription factors have been recognized. Therefore, Zn is an integral component of many proteins and enzymes, and participates in most metabolisms including saccharides, lipid, protein and nucleic acid synthesis **(Miao et al., 2013)**.

6.2. Antioxidant role of zinc

An antioxidant may be defined as any agent that hinders a free radical reaction. Zinc is not an antioxidant in the same sense as vitamin E. It does not interact directly with oxidant species, but prefers to exert its effects in an indirect way **(figure 8) (DiSilvestro, 2000; Stefanidou et al., 2006)**.

- ✓ Zinc is an inhibitor of NADPH oxidase (nicotinamide adenine dinucleotide phosphate oxidase), which is plasma-membrane- associated enzyme that catalyzes the production of oxygen superoxide ($O_2^{\bullet}$) from oxygen O_2, resulting in decreased ROS formation **(Prasad, 2014)**.

- ✓ Zinc plays an important role in the stabilization of membranes.

- ✓ Zinc is a cofactor with copper in SOD (superoxide dismutase), a key enzyme that catalyzed the dismutation of $O_2^{\bullet}$ to H_2O_2 **(Marreiro et al., 2017)**.

- ✓ Zinc influences the expression of glutamate-cysteine ligase, which is involved in glutathione synthesis. This has a twofold effect of zinc acts on the neutralization of free radicals directly by glutathione or indirectly as a cofactor of glutathione peroxidase (GSH-Px) **(Eide, 2011)**.

- ✓ Zinc induces the production of metallothionein, rich in cysteine, which is an excellent scavenger of hydroxyl radicals (OH) **(Prasad et al., 2004)**.

- ✓ Zinc effectively competes with iron and copper membrane binding sites. Membrane-bound iron and copper can stimulate the generation of radicals from lipid peroxides. Thereby, the replacement of these metals by zinc would prevent the formation of highly reactive oxidants **(Oteiza, 2012)**.

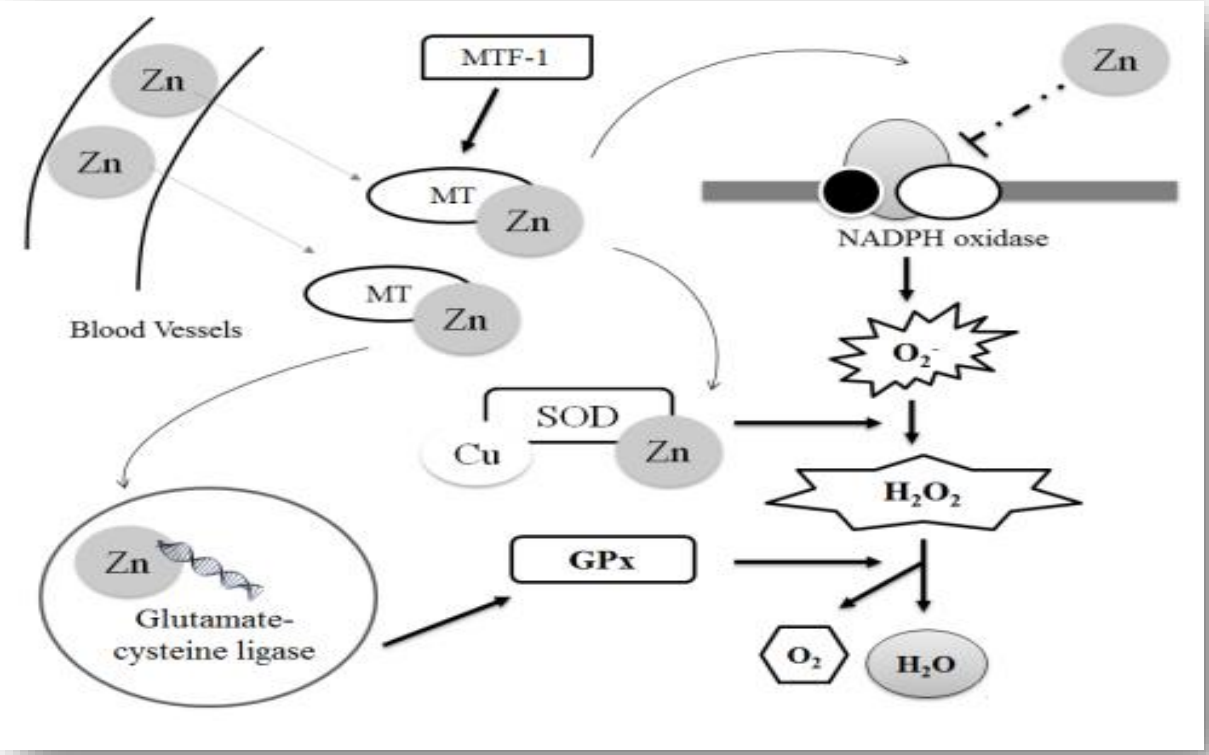


Figure 8. Participation of zinc in antioxidant mechanisms. GSH-Px: Glutathione peroxidase; MT: Metallothionein; NADPH: nicotinamide adenine dinucleotide phosphate; SOD: superoxide dismutase enzyme; Zn: Zinc **(Marreiro et al., 2017)**.

7. Zinc and insulin

It has been known for a decades that a physical-chemical association exists between insulin and zinc. A healthy pancreas contains high zinc,but it is greatly reduced in diabetic patients. In fact, most of the zinc in the pancreas is found in beta cells, and is concentrated within the dense core insulin-secreting granules. Importantly, Zn^{2+} ions are an essential factor for insulin processing and storage. After synthesis at the level of the lumen of the rough endoplasmic reticulum (RER), Then, pro-insulin is transported into the Golgi apparatus and stored in the form of hexamers in the presence of zinc ions. After the condensation of pro-insulin hexamers into secretory granules, they are latter transformed into active insulin (after the cleavage of the C-peptide). So, zinc is an important for the storage of insulin in the secretory vesicles. In other words, it is necessary for the crystallization **(Chabosseau and Rutter, 2016)**.

Regarding the role of zinc in insulin action in peripheral tissues, this element has insulin-like properties, particularly by stimulates glucose uptake in insulin-dependent tissues such as adipose tissue and muscle.

Zinc inhibits glucagon secretion through the activation of ATP-dependent potassium channels by Zn^{2+} ions and inhibiting the activation of voltage gated calcium channels **(Ruz et al., 2019)**.

8. Zinc and diabetes

Zinc has a major role in insulin synthesis, storage, and secretion. So, its abnormalities such as deficiency, undoubtely involved in the pathogenesis of diabetes and its complications. Zn deficiency affects the ability of β cell to produce insulin, and a reduction in the sensitivity of peripheral tissue cells to insulin **(Jurowski et al., 2014; Sun et al., 2018)**. The element is also a necessary cofactor in antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase.Therefore, it is obvious that any alterations in Zn metabolism, like the non-availability of adequate Zn, it leads to contribute to the tissue damage in diabetes**(Barman and Srinivasan, 2022)**. Diabetics are the most susceptible to zinc deficiency, as a result of the excessive loss of zinc in the urine and impaired intestinal absorption **(Kazi et al., 2008)**.

Chapter III. Oxidative stress

1. Definition

Oxygen, a vital molecule, can have harmful effects on the body through the formation of free radicals (reactive oxygen species). Gerschman and Hartman had already used the toxicity of oxygen and the "free radical theory" to explain the aging process in the mid-1950s **(Haleng et al., 2007; Bourgoin, 2012)**. Sies first defined oxidative stress (OS) as "an imbalance between the pro-oxidants and antioxidants in favor of the oxidant species, leading to potential damage" **(figure 9)**. When ROS combats and affects the cellular antioxidant defense system, oxidative stress results from either increased free radical formation or a reduced physiological activity of antioxidant defense against free radicals. In biological systems, free radicals play roles as beneficial and toxic compounds. They have beneficial effects in moderate or small amounts and they are involved in various physiological functions such as immune defense, cell signaling, genetic expression and redox regulation. On the other hand, their overproduction can damage the integrity of several biomolecules including lipids, proteins and DNA, leading to increased oxidative stress in various human diseases such as diabetes mellitus, cardiovascular diseases and neurodegenerative diseases **(Phaniendra et al., 2015)**.

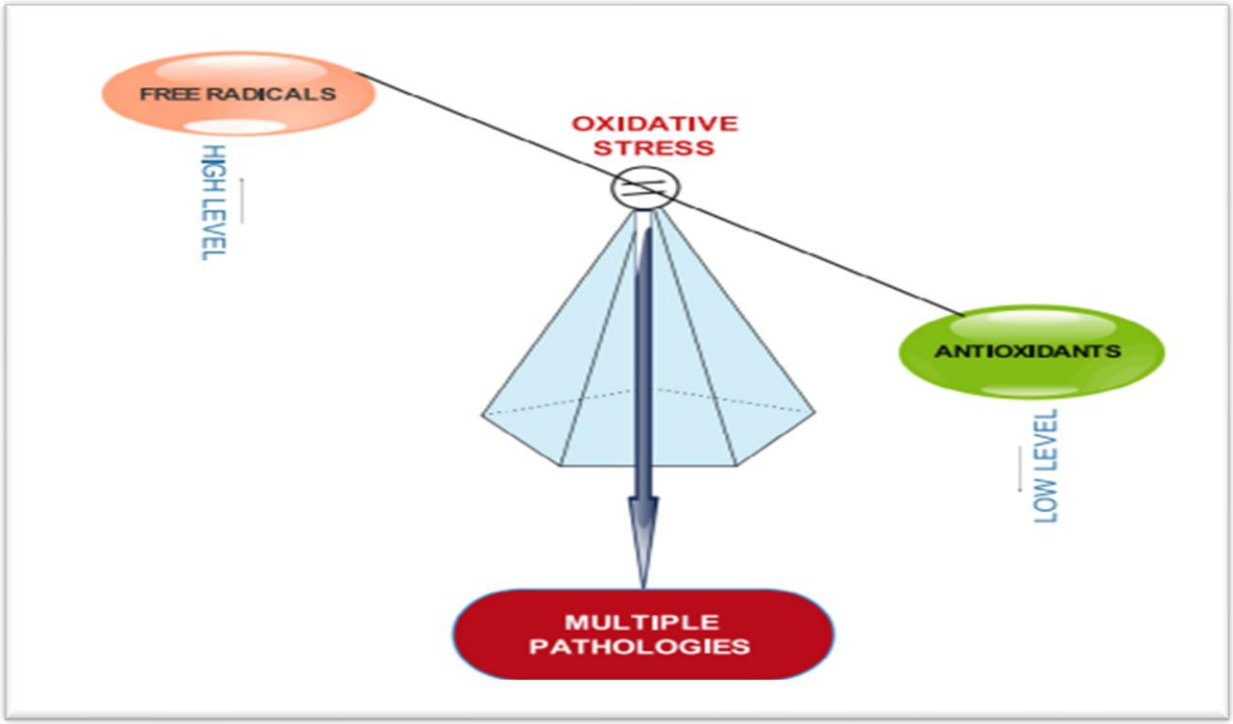


Figure 9. The imbalance between free radicals and antioxidants **(Ighodaro and Akinloye, 2018)**.

2. Free radicals

A free radical can be defined as a molecule or atom that contains one or more unpaired electrons in the valence shell or outer orbit. This specificity makes it unstable, short-lived and highly reactive towards other molecules. The free radicals are high reactivity; they can withdraw electrons from other compounds to achieve stability, thus generating new free radicals **(Phaniendra et al., 2015)**. In various fields of biology and medicine, free radicals are commonly referred to as reactive oxygen species (ROS) or reactive nitrogen species (RNS) **(Poprac et al., 2017)**. Reactive oxygen species (ROS) are oxygen-derived molecules that react with and oxidize most biomolecules. They represent an important class due to their diverse effects on biological processes. ROS include free radicals such as hydroxyl radicals (OH), superoxide anion radicals ($O_2^{\cdot -}$) and nitrogen oxide ($NO^{\cdot}$), as well as non-radical molecules like hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and peroxynitrite ($ONOO^-$) **(Firuzi et al., 2011)**.

3. Sources of ROS

3.1. Exogenous sources

Among the many exogenous factors associated with an increased production and/or a decrease in the elimination of free radicals **(Rioux, 2009)**, the exogenous sources as follows **(figure 10)**:

- Diet (antibiotics, alcohol, coffee, foods rich in protein and/or fat and/or with a high glycemic index, low consumption of antioxidants).
- Atmospheric CO_2.
- Pollutants (cigarette smoke, air pollution and occupational metals (transition metals such as mercury, iron, cadmium and nickel).
- Medicines (cancer treatments).
- Radiation (ionizing, ultraviolet, microwave).
- Dermal absorption (insecticides, drugs).

3.2. Endogenous sources

3.2.1. Mitochondria

In cells, about 90% of ROS are produced by mitochondria, which are represents the main source of production. This production is mainly due to the loss of electrons at the level of complexes I and III of the mitochondrial respiratory chain that leads to the formation of superanions **(Tebboub, 2019)**.

3.2.2. NADPH oxidase

NADPH oxidase is a membrane complex enzyme expressed in many tissues and blood cells. It creates superoxide anions by transferring electrons from NADPH to an oxygen molecule (O_2), creating $NADP^+$, H^+ and $O_2^{\bullet-}$. These last two compounds react with each other to form hydrogen peroxide H_2O_2 **(Lavie, 2015)**.

$$NADPH + 2O_2 \xrightarrow{\text{NADPH oxidase}} NADP^+ + O_2^{\bullet-} + H^+$$

3.2.3. Xanthine oxidase

Xanthine oxidase is a cytosolic enzyme that generates ROS by reducing hypoxanthine to xanthine and xanthine to uric acid. This enzyme is found in the blood, capillary endothelial cells and particularly in the liver and intestines **(Berry and Hare, 2004)**.

3.2.4. Peroxisomes

In mammals, peroxisomes play an indispensable role in various biochemical processes, including the synthesis of phospholipids, the oxidation of fatty acids, bile acids, and the degradation of amino acids. Peroxisomes contain several oxidoreductases that produce H_2O_2 as part of their catalytic activity.

Mammalian peroxisomes may contain also xanthine oxidoreductase and inducible nitric oxide synthase (NOS_2), which are two potential sources of superoxide anions **(Van Veldhoven, 2010)**.

3.2.5. Endoplasmic Reticulum

The enzymes of the endoplasmic reticulum help catalyze reactions to detoxify fat-soluble drugs and other toxic metabolites. The best known of these enzymes is cytochrome P450, which oxidizes unsaturated fatty acids and xenobiotics, producing ROS **(Servais, 2004)**.

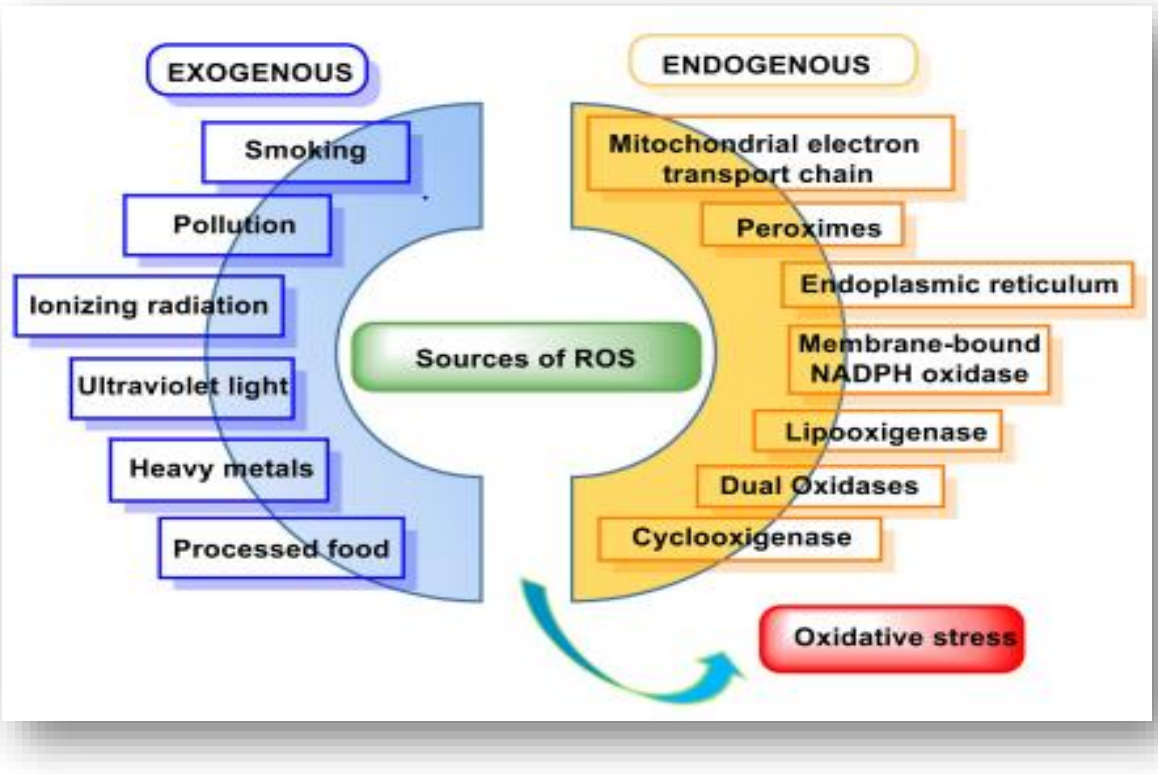


Figure 10. A schematic representation of exogenous and endogenous sources involved in the ROS production that cause oxidative stress in the human body **(Curieses Andrés et al., 2023)**.

4. The molecular targets of free radicals

When there is an imbalance between free radicals formation and antioxidant defenses, the former are produced in higher concentrations, leading to oxidative stress. Because these free radicals are highly reactive, they can cause deep changes to various molecules targets, including lipids, proteins, and nucleic acids **(Phaniendra et al., 2015)**.

4.1. Lipids

Membrane lipids, in particular the polyunsaturated fatty acid residues of phospholipids, are the preferred target of oxidation by free radicals **(Phaniendra et al., 2015)**. Lipid peroxidation represents one of the direct biochemical consequences of the production of ROS. This is a chain reaction triggered by a radical that leads to the formation of unstable hydroperoxides (ROOH), which are broken down to aldehydes, altering the cell membrane, loss integrity of the cell and its organelles **(Valko et al., 2006; Rao et al., 2011; Gentile et al., 2017)**. Lipid peroxidation leads also to the formation of many toxic end products, including malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). Both react with proteins and DNA, then they have mutagenic and carcinogenic potential **(Guéraud et al., 2010)**.

4.2. Protein oxidation

Protein oxidation can be induced by free radicals **(Phaniendra et al., 2015)**. In other words, ROS oxidize various amino acids that are present in proteins. These amino acids are very sensitive to oxidative stress, such as: sulfur amino acids (cysteine, methionine), basic amino acids (arginine, lysine) and aromatic amino acids (phenylalanine, tyrosine and tryptophan). Proteins modified by oxidation lose their biological properties and become more susceptible to enzyme proteolysis **(St-Louis, 2011)**.

Depending on the intensity of oxidative stress, proteins undergo various modifications in the protein chains. These modifications are generally modeled by the presence of transition metals (Fe^{+2}, Cu^{+2}). Since they can be divided into two categories: on the one hand, those that break the peptide bonds and modify the peptide chain, and on the other hand, the modifications of the peptides by the addition of the products resulting from lipid peroxidation such as 4-HNE. Ultimately, these modifications can lead to loss of structural or enzymatic activity of the protein **(Phaniendra et al., 2015; Levine, 2002)**.

4.3. DNA oxidation

DNA is also a target of radical attacks. Indeed, the most common oxidative damage caused by hydroxyl radicals ($HO^{\bullet}$) involves oxidative bases. ROS can induce oxidation, particularly of guanine, as it is the most easily oxidized of the four DNA bases. The base can react with $HO^{\bullet}$ to form 8-hydroxy-2\'-deoxyguanosine (8-OH-dG) **(Kino et al., 2017)**. So, the 8-OH-dG level detected in several biological systems, which is used as a biomarker of the degree of oxidative stress **(Altieri et al., 2008)**. Free radical attacks DNA molecule by becoming carcinogenic or causing mutations on the DNA strands **(Loft et al., 2008)**.

5. Antioxidant system

An antioxidant is a molecule that prevents, reduces, delays or completely intercepts the effects of free radicals, thereby protecting the body from oxidation. **Halliwell and Gutteridge (1990)** defined an antioxidant as “any substance which, at a low concentration compared to oxidizable substrates, inhibits or significantly retards the oxidation of that substrate.” In brief, the antioxidant protects biological systems from the toxic effects of free radicals, there are two types of antioxidants systems, including enzymatic and non-enzymatic **(figure 11)**.

5.1. Enzymatic antioxidants

The human system has a number of enzymes that neutralize the formation of reactive species in cells. The most important include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) **(Ighodaro and Akinloye, 2018)**.

5.1.1. Superoxide dismutase

Superoxide dismutase (SOD) are metalloenzymes that catalyze the dismutation of superoxide ions ($O_2^{\bullet}$) into a hydrogen peroxide molecule (H_2O_2) and oxygen (O_2). In mammals, three isoenzymes are described: cytoplasmic Cu/Zn-SOD (SOD1), mitochondrial Mn-SOD (SOD2) and extracellular Cu/Zn-SOD (SOD3), which differ depending on the chromosomal location of the gene, their metal content, their concentration, quaternary structure and its cellular location **(Fukai and Ushio-Fukai, 2011)**.

$$2O_2^{\bullet} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

5.1.2. Catalase

Catalase (CAT) is a tetrameric ferriheme oxidoreductase, which catalyzes the decomposition of H_2O_2 to H_2O and O_2 **(Grigoras, 2017)**.

$$2\,H_2O_2 \xrightarrow{Catalase} 2\,H_2O + O_2$$

Although CAT is ubiquitous, it is found mainly in peroxisomes. Depending on the H_2O_2 concentration, CAT has two enzymatic activities. At high H_2O_2 concentrations, CAT acts catalytically, as described in the previous reaction. However, at low H_2O_2 concentration and in the presence of a suitable hydrogen donor such as ethanol, methanol, phenol, etc., it has a peroxidic effect and thus removes H_2O_2 **(Kodydková et al., 2014; Moldogazieva et al., 2018)**.

5.1.3. Glutathione peroxidase

Glutathione peroxidase (GSH-Px) is a selenocysteine peroxidase that breaks down hydrogen peroxides (H_2O_2) into water; and lipid peroxides to their corresponding alcohols using GSH as a cofactor, it is located mainly in the mitochondria and sometimes in the cytosol. This enzyme plays a crucial role in inhibiting the lipid peroxidation process, thereby protecting cells from oxidative stress **(Ighodaro and Akinloye, 2018; Cardoso et al., 2017)**.

$$H_2O_2 + 2\,GSH \xrightarrow{GSH-Px} 2\,H_2O + GSSG$$

$$ROOH + 2\,GSH \xrightarrow{GSH-Px} ROH + H_2O + GSSG$$

5.2. Non-enzymatic Antioxidants

5.2.1. Endogenous

a. Glutathione GSH

Glutathione (GSH) is a tripeptide of (γ-glutamyl-cysteinyl-glycine) that is distributed primarily in the cytosol but is found also in other intracellular organelles including mitochondria, peroxisomes, endoplasmic reticulum (ER), and the nucleus. Liver is the principal location for its synthesis and distribution. It represents the largest thiol group in the cell (-SH) and is present in either reduced (GSH) or oxidized form (GSSG). The GSH/GSSG ratio is considered an excellent marker of lipid peroxidation and it is used to measure redox status. GSH acts directly as a strong ROS scavenger and detoxifies oxidants indirectly via enzymatic reactions. In addition, as a cofactor of several antioxidant enzymes (GSH-Px, GST); and regeneration of α-tocopherol and ascorbic acid to their active forms **(Marí et al., 2009; Aslani and Ghobadi, 2016)**.

b. Uric acid

Uric acid is able to scavenge radical species and acts as a chelator of metal ions, which are involved in prooxidant generation **(Strazzullo and Puig, 2007)**.

5.2.2. Exogenous

a. Vitamins

- **Vitamin E**

Vitamin E is a fat-soluble vitamin. Eight forms have been reported (α-, β-, γ-, and δ-tocopherol, and α-, β-, γ-, and δ-tocotrienol), but the most active form is α-tocopherol. The hydrophobic nature of vitamin E allows it to insert itself into the fatty acids of the cell membrane and lipoproteins where it plays a protective role in preventing the spread of lipid peroxidation induced by oxidative stress **(Traber and Atkinson, 2007)**.

- **Vitamin C**

Vitamin C is commonly known ascorbic acid. It is a powerful water-soluble antioxidant. An excellent ROS scavenger can protect various biological substrates (proteins, lipids and DNA) from oxidation. In addition, vitamin C is able to reignites vitamin E. On the other hand, vitamin C allows maintaining the plasma concentration of glutathione, thus reducing lipid peroxidation **(Ali et al., 2020)**.

b. Trace elements

Trace elements such as copper (Cu), zinc (Zn), manganese (Mn), selenium (Se) and iron (Fe) are essential elements in defending against oxidative stress. All antioxidant enzymes require a cofactor to maintain their catalytic activities. Therefore, glutathione peroxidase (GSH-Px) requires selenium. Manganese, copper and zinc are components of superoxide dismutase (SOD); iron is an integral component of catalase (CAT) **(Wołonciej et al., 2016)**.

- **Selenium**

Selenium plays a key role in protecting cells and their constituents against free radical attack. This function is due to its presence in the active site of glutathione peroxidase and the antioxidant activities of selenoproteins **(Ramoutar and Brumaghim, 2010)**.

- **Copper**

At physiological concentration copper is the cofactor of SOD, however, it can play a role in the production of ROS (Fenton reaction) when its concentration is high **(Haleng et al., 2007; Kazi et al., 2008)**.

- **Zinc**

Zinc is an essential cofactor of SOD. It protects also the thiol groups of proteins. It can inhibit ROS formation reactions induced by transition metals such as iron or copper **(Haleng et al., 2007)**.

- **Manganese**

Manganese is one of the essential trace elements; it enters into the structure of many enzymes, in particular SOD. Excess manganese can produce reactive oxygen species **(Bocca et al., 2017)**.

c. Polyphenols

Polyphenols are secondary metabolites of plants. The polyphenol family includes more than 8,000 compounds. On the basis of the number of phenol rings that they contain and structural elements that link these rings together, polyphenols may be classified into different categories including phenolic acids, flavonoids, stilbenes and lignans **(Pandey and Rizvi, 2009)**. The chemical structure of polyphenols gives them certain capacities, in particular antioxidants, which allows them to scavenger free radicals and chelate metals (due to the hydroxyl group). They can also inhibit lipid peroxidation and in particular the formation of oxidized LDL (Low Density Lipoprotein) **(Jaldappagari et al., 2013)**.

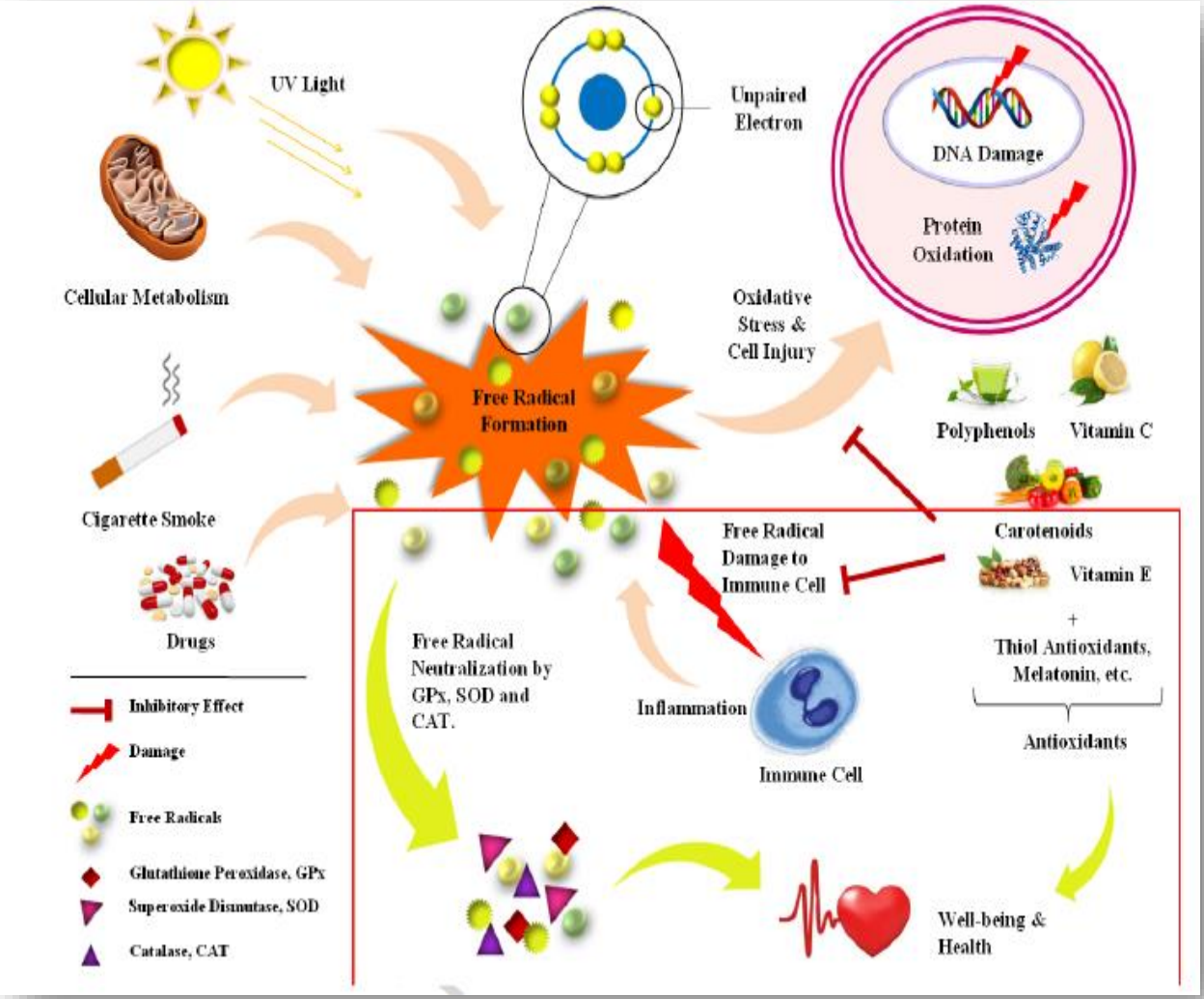


Figure 11. A schematic diagram of the sources of ROS, enzymatic and non-enzymatic molecules involved in antioxidant defense and biological targets **(Aslani and Ghobadi, 2016)**.

Chapter IV. Oxidative stress and diabetes

1. Molecular pathways associated with oxidative stress in diabetes mellitus

Chronic hyperglycemia is known to be the main cause of increased oxidative stress in diabetes. Otherwise, several pathways may be involved in the development of oxidative stress in persistent hyperglycemia **(figure 12)**, such as: glucose autoxidation, increased activity of the polyol signaling pathway, activation of protein kinase C (PKC), increased formation of advanced glycation end products (AGEs) and increased flux of the hexosamine pathway or the overproduction of superoxide anion by the mitochondria" **(Rochette et al., 2014)**.

1.1. Glucose autoxidation

In the presence of transition metals, glucose oxidized in its enediol form, resulting in the formation of an anionic enediol radical. This radical releases superoxide anions ($O2^{\bullet -}$) through the reduction of molecular oxygen. This reaction simultaneously results in the formation of a carbonyl compound α-acetolaldehyde, which, in the presence of transition metals, triggers the Fenton reaction with the formation of hydroxyl radicals ($HO^{\bullet}$) **(Hunt et al., 1988; Bonnefont-Rousselot et al., 2000)**.

1.2. Polyol pathway

Under hyperglycemic condition, glucose metabolism via glycolysis and the pentose phosphate pathway is interrupted upon saturation of hexokinase, this enzyme responsible for the phosphorylation of glucose to glucose-6-phosphate. In this case, the polyol pathway involves aldose reductase, which converts glucose into sorbitol by oxidizing NADPH to $NADP^+$ and reducing the amount of NADPH, and then converts sorbitol into fructose under the action of sorbitol dehydrogenase activity, which needs NADPH as a cofactor. During this reaction, a reduction in NADPH levels can reduce the regeneration of glutathione, a powerful radical scavenger, and thus an increase in free radical production, leading to an induction of oxidative stress **(Yaribeygi et al., 2019)**.

1.3. Hexosamine pathway

In a hyperglycemia state, the normal glycolysis pathway induces the hexosamine pathway. In this pathway, the fructose 6-phosphate (F-6-P) molecule is metabolized by glucosamine fructose amidotransferase (GFA) into glucosamine 6-phosphate (Glu-6-P), which is later converted into Uridine diphosphate NAcetylglucosamine (UDP-GlcNAc) under UDPNAcetylglucosamine synthase activity. UDP-GlcNAc is the end product of the hexosamine pathway and serves as a substrate for the addition of an N-acetylglucosamine to serine or threonine residues of proteins by modifying their functional properties. Increased glucose influx through this pathway has been shown to play a role in the generation of reactive oxygen species (ROS) **(Yan, 2014; Ighodaro, 2018)**.

1.4. Protein kinase-C activation

In hyperglycemia, increased conversion of glucose to glyceraldehyde-3-phosphate, a product of glycolysis, and its conversion to diacylglycerol (DAG) leads to activation of multiple protein kinase C (PKC) isoforms **(Tarr et al., 2013)**. Nevertheless, PKC activation leads to ROS production through stimulation of NADPH oxidase. Furthermore, stimulation of PKC can induce insulin resistance by inhibiting Akt-dependent nitric oxide synthase function and induce inflammation through the synthesis of the pro-inflammatory factors NFk-B (nuclear factor kappa-B) **(Yan, 2014)**.

1.5. Glycation of proteins

Diabetes increases the risk of AGEs formation due to the high plasma glucose level, which play an important role in glycation of proteins.

Advanced glycation end products (AGEs) form a heterogeneous group of molecules that are formed by a noenzymatic reaction of carbonyl groups of reducing sugars with free amino groups of proteins. The process of their creation takes place in three steps. The first step is the "Maillard reaction," in which glucose reacts with a free amino group on proteins to form an unstable compound, the Schiff base. In the second step, the Schiff base is rearranged molecularly to form a more stable product, the Amadori products. Finally, the slow accumulation of these products after various rearrangements as well as possible oxidation reactions leads to the irreversible formation of glycation end products (AGEs) **(Fakhruddin et al., 2017; Yaribeygi et al., 2019; Papachristoforou et al., 2020)**.

In chronic hyperglycemia, AGEs are actively produced and accumulate in the circulatory system and various tissues, which contributes to vascular complications in diabetes mellitus. The involvement of AGEs in the development of oxidative stress is due to their ability to bind to specific receptors (R-AGEs) present on endothelial and glomerular cells or even macrophages. Activation of these receptors triggers the production of ROS via the activation of NADPH oxidase as well as the activation of the transcription factor NF-kB, which causes the transcription of inflammatory cytokines, thus promoting the development of oxidative stress and an inflammatory response **(Bandeira et al., 2013)**.

1.6. Mitochondrial production of superoxide anions

The mitochondrial respiratory chain is the primary site of superoxide anion production. This production is increased in the presence of high glucose concentrations. During hyperglycemia, the reduced coenzyme NADH (nicotinamide adenine dinucleotide H) produced by glycolysis accumulates, resulting in an excess of electron donors, leading to electronic overload at the level of the mitochondrial respiratory chain and subsequently to overproduction of superoxide anions **(Bonnefont-Rousselot et al., 2004)**. Hyperglycemia is also thought to increase proton (H^+) flux and transmembrane potential gradients across

the inner membrane transporter chain, which stimulates ATP synthase activity, leading to the generation of mitochondrial free radicals **(Yaribeygi et al., 2019)**.

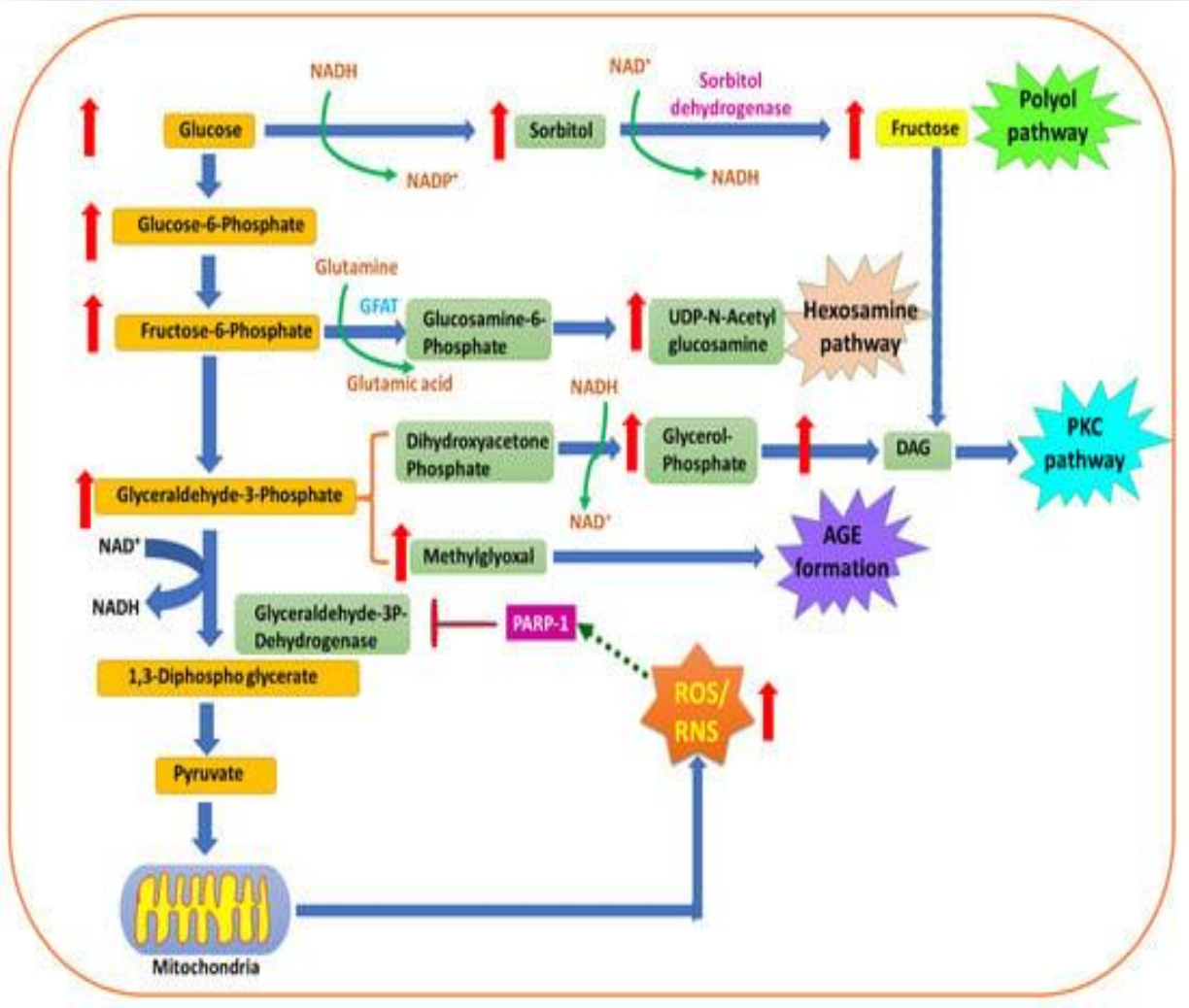


Figure 12. Metabolic pathways activated by hyperglycemia. Four pathways are activated by hyperglycemia: the polyol pathway, hexosamine pathway, protein kinase C (PKC) pathway and the advanced glycation end product (AGE) formation pathway **(Singh et al., 2022)**.

2. Oxidative stress and diabetes mellitus

Oxidative stress has received considerable attention in recent years due to its profound effects on human health, particularly its association with diabetes **(Caturano et al., 2023)**. Increased OS is believed to be a major cause of diabetes complications. On the other hand, oxidative stress (OS) is involved in the pathogenesis of diabetes, it lead to insulin resistance, beta cell dysfunction, and impaired glucose tolerance.

In diabetes, oxidative stress appears to be largely triggered by both higher free radical production and an acute decline in antioxidant defenses. The possible causes of oxidative stress may be autooxidation of glucose; Fluctuations in redox balance, reduced tissue concentrations of low molecular weight antioxidants (GSH, vitamin E and impaired of antioxidant defense enzymes activities such as SOD and CAT) **(figure 13)**.

So, this increase in the production of ROS and the reduced activity of antioxidant defense systems due to hyperglycemia are undoubtedly responsible for the occurrence of diabetes complications **(Kalio and Davis, 2018; Sardu et al., 2019)**.

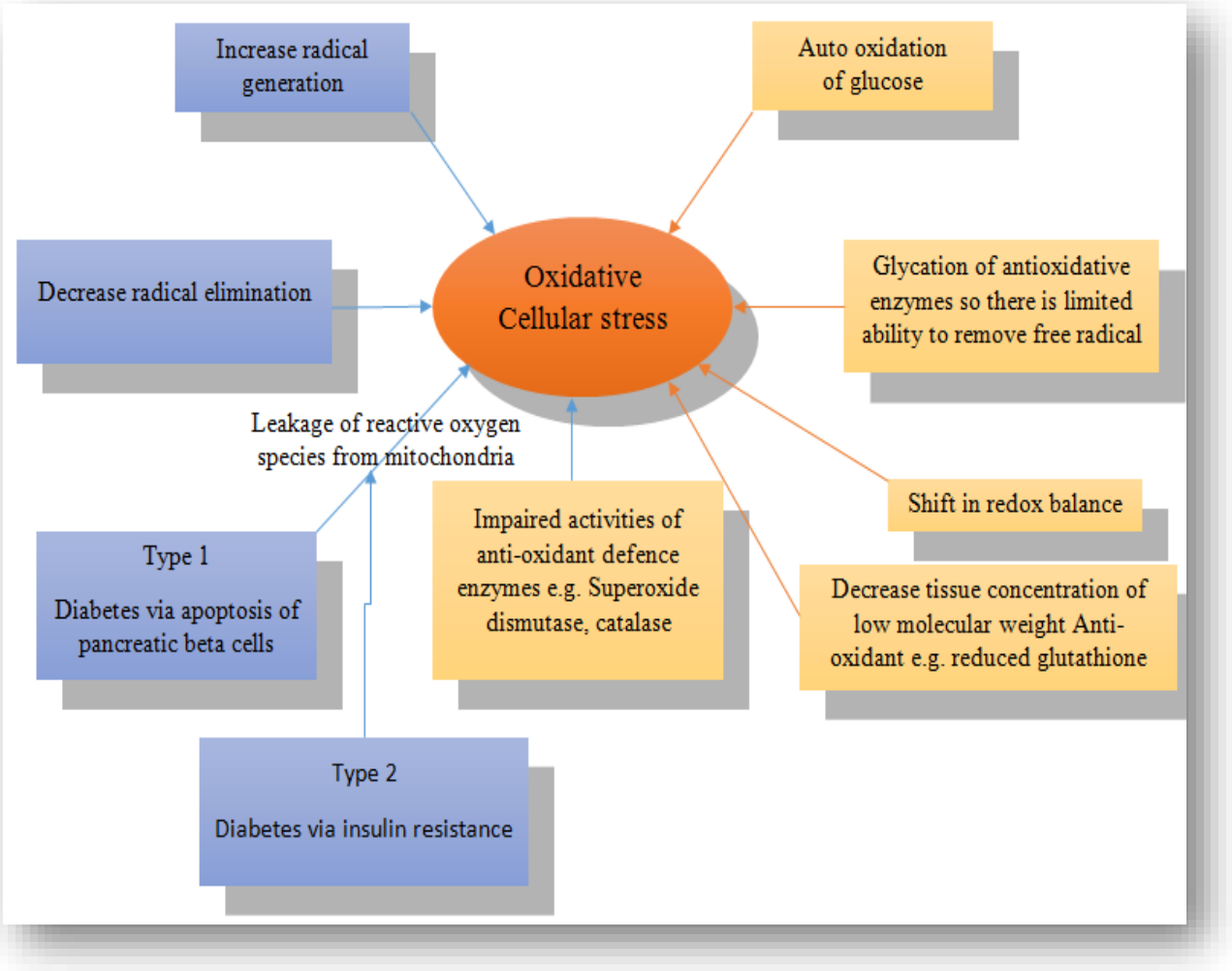


Figure 13. The impacts of oxidative stress on diabetes **(Kalio and Davis, 2018)**.

3. Oxidative stress and beta-cell dysfunction

Oxidative stress induced by ROS generation due to exposure to high glucose concentrations during the development of diabetes is critically involved in the impairment of beta cell function. Since β-cells have a low capacity of antioxidant enzymes such as SOD, GSH-Px and catalase as compared to the liver, this makes it extremely vulnerable to oxidative stress **(Drews et al., 2010; Wang J and Wang H, 2017; Newsholme et al., 2019)**.

Chronic hyperglycemia has negative effects on pancreatic β-cells; it is capable to cause disorders of insulin secretion, a reduction in its proliferation and differentiation, and an increase in apoptosis through various mechanisms **(Kaneto and Matsuoka, 2012; Gerber and Rutter, 2017)**.

4. Effect of zinc deficiency on diabetes through oxidative stress

Oxidative stress plays an important role in the pathology of diabetes and its complications **(Pitocco et al., 2013; Lotfy et al., 2017)**. Zinc occupies an important place among the trace elements; it plays a role in most biological functions. Zinc is necessary for the normal functioning of oxidative defense. This role can be explained by its involvement in the defense against free radicals and their synthesis. In addition, zinc as a cofactor is essential for antioxidant enzymes activities such as Cu-Zn-SOD and GSH-Px activities **(Chasapis et al., 2012)**. Several complications of diabetes may be associated with the overproduction of free radicals, which are accompanied by the decrease in the intracellular concentration of zinc and zinc-dependent antioxidant enzymes **(Powell, 2000)**. Thus, zinc deficiency and oxidative stress are potential etiological factors of diabetes **(Haase and Maret, 2005)**. In brief, zinc deficiency plays a role in the pathogenesis of diabetes mellitus, which is associated with oxidative stress that accelerates cellular and vascular damage **(Kechrid et al., 2007)**.

Theoretical part

Chapter V. Sesame and pumpkin

1. Phytotherapy

1.1. Definition

Etymologically, the term “phytotherapy” is divided into two different terms: “phuton” and “therapeia”, which, from their Greek origin, mean “plant” and “treatment” respectively. Therefore, phytotherapy can be defined as an allopathic discipline whose aim is to prevent and treat certain dysfunctions and/or certain pathological conditions using plants, plant parts or herbal preparations, whether consumed or applied externally **(Chabrier, 2010; Anne Sophie., 2018; Salah Eddine, 2018)**. Only plants that have proven their healing power are interested in phytotherapy. The active ingredients with the highest concentration are selected so that the entire plant, leaves, stems, branches, flower tips, bark, roots, fruits or flowers can be used fresh or dry **(Cazau-Beyret, 2013)**.

1.2. Phytotherapy types

Two types of phytotherapy can be distinguished:

- ✓ A sometimes very ancient traditional practice based on the use of plants depending on empirically discovered virtues. According to the WHO, this herbal medicine is considered a traditional medicine and it is still widely used in some countries, especially developing countries.

- ✓ A practice based on advances and scientific knowledge that searches for active extracts in plants. Thus, the identified active ingredient extracts are standardized. This practice leads, depending on the case, to the production of medicinal products or phytomedicines, the distribution of which is subject to a marketing authorization in accordance with the regulations in the country **(Mohamed and Mohamed, 2012)**.

1.3. Diabetes phytotherapy

Despite significant progress in the treatment of diabetes with synthetic drugs, the search for effective and safe drugs continues because they are easily accessible, affordable and have fewer side effects as compared to synthetic drugs **(Bansode and Salalkar, 2017)**. More than 200 plant species have antidiabetic properties, most of which have been studied through screening tests without revealing their exact mode of action.

Herbal products may contain multiple active compounds, that work in different ways and they can impact multiple biological pathways, e.g.: inhibition of glucose absorption, increase of insulin secretion, improvement of glucose absorption, improvement of pancreatic β-cell function and many antioxidant properties, which thus have an alleviating effect on diabetic symptoms **(Kuc et al., 2021; Jugran et al., 2021)**.

2. Selected medicinal plant species

Due to the richness of medicinal plants in bioactive components especially antioxidants properties; they represent an interesting therapeutic option to reduce the severity of diabetes and its complications. This chapter explores the different therapeutic actions of commonly used plants including sesame and pumpkin (**Dotto and Chacha, 2020; Tanwar and Goyal, 2021)**.

2.1. Sesame (*Sesamum indicum* L.)

2.1.1. Generality

Sesame is an annual, herbaceous plant that is one of humanity's oldest crops **(Honjoya et al., 2021)**. It is grown mainly in tropical and subtropical regions of Asia, Africa and South America. This plant is cool; does not resist frost and hot summers allows it to reach the end of its cycle, i.e. the formation of seeds whose cycle varies between 80 and 180 days **(figure 14) (Lim, 2012; Rebbas et al., 2020)**.

Figure 14. The ***Sesamum indicum* L**. plant. Pictures show leaves, flowers, capsules, and sesame seeds **(Mushtaq et al., 2020)**.

Sesame belongs to the family – Pedaliaceae, which includes dicotyledonous plants. This family contains 16 genera and more than 30 species, the best known of which is ***Sesamum indicum*** that remains the most cultivated **(Honjoya et al., 2021)**.

The sesame seed has been called the “queen of oilseeds” due to its high oil level (50–60%) **(Gadade et al., 2017)**, it ranks 9th among the top13 main oilseed crops, which account for 90% of the world production of edible oil **(Bamigboye et al., 2010)**.

2.1.2. Sesame seed oil

Sesame oil called also gingelly oil or til oil, is used in various food preparations either fresh or fried for flavor. As a traditional healthy food, sesame oil has high nutritional value due to its high content of fatty acids (oleic acid, linoleic acid, palmitic acid, stearic acid) and bioactive compounds including phytosterols and lignans (sesamin, sesamol, episesamin, sesamolin, sesaminol) and vitamin E, which are considered natural antioxidants. Published literatures indicated also that sesame oils contain various polyphenols.

The presence of these functional compounds can have positive effects on human health, for example by preventing the formation of free radicals and reducing oxidative stress. Furthermore, they can protect the oil from oxidative degradation during storage, marketing and use, thereby extending the shelf life of sesame oils **(Ramesh et al., 2005; Bopitiya and Madhujith, 2013; Shi et al., 2017)**. This oil has numerous biological properties such as antioxidant **(Afroz et al., 2019)**, anti-inflammatory **(Deme et al., 2019)**, antihypertensive **(Mushtaq et al., 2020)** and hypocholesterolemic **(Taha et al., 2014)**. In addition, sesame oil has a protective effect in the treatment of diseases associated with oxidative stress, such as diabetes mellitus **(Haidari et al., 2016)**, obesity **(Qin et al., 2019)**, chronic kidney failure **(Liu et al., 2015)**, and neurodegenerative diseases **(Mohamed et al., 2021)**.

2.1.2.1. Hypoglycemic activity

Sesame contains less saccharides, high protein, and high dietary fiber, which can be efficient in the prevention of diabetes complications **(Bigoniya et al., 2012; Ley et al., 2014)**.

Many reports suggest that saturated fatty acids intake are considered a possible factor in diabetes risk. In that case, cooking oils rich in monounsaturated and polyunsaturated fatty acids are effective in reducing diabetes risk **(Sankar et al., 2006; Riserus et al., 2009; Patel et al., 2010; Violi et al., 2015)**. Because sesame oil is a good source of monounsaturated and polyunsaturated fatty acids, it may be helpful in diabetes prevention. In a study on streptozotocin (STZ)-diabetic rats, daily consumption of sesame oil for 42 days showed a hypoglycemic effect, a reduction in glycosylated hemoglobin (HbA1c) levels, as well as an improvement in antioxidant systems **(Ramesh et al., 2005)**.

Several studies indicated that sesamin, a major sesame lignan, is related to lipid metabolism via a series of biochemical actions in both animals and humans, this mention that sesamin could be used as a therapeutic approach in diabetes administration **(Thuy et al., 2017; Mohammad Shahi et al., 2017)**.

Aslam et al **(2019)** investigated the influence of sesame seed oil consumption on blood glucose level, insulin secretion, glycosylated haemoglobin production, and liver antioxidant enzymes activities in patients with type 2 diabetes.

2.1.2.2. Antioxidant activity

Sesame oil contains sesamolin, sesamin and sesaminol lignan compounds, which are known to play an important role in antioxidant activity. Sesame oil could be useful in treating disease-related oxidative stress such as diabetes mellitus, atherosclerosis, chronic kidney failure, and neurodegenerative diseases, such as Alzheimer's disease **(Taha et al., 2014)**.

Sesame oil has long been also considered a daily dietary supplement to increase cellular resistance to lipid peroxidation (LPO) **(Kaur and Saini, 2000)** by lowering LPO and inhibiting the formation of reactive free oxygen radicals. Several studies reported that a single dose of this oil alleviated oxidative stress and liver damage in rats **(Gauthaman and Saleem, 2009)**.

Sesame oil increases the concentration of alpha-tocopherol in the blood and tissues. In addition, sesame oil showed a significant ability to scavenger's free radical in the methanolic fraction due to the presence of phenolic compounds **(Yamashita et al., 1995; Espin et al., 2000)**. Besides to reduce lipid peroxidation and reactive oxidative species production, sesame oil increases antioxidant enzymes activities like glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase under various conditions of oxidative stress **(Hemalatha and Raghunath, 2004; Hsu[a] et al., 2005; Hsu[b] et al., 2006; Aslam et al., 2019)**.

In a study performed on hypertensive patients, sesame oil consumption remarkably decreased oxidative stress and simultaneously maintaining GSH-Px, SOD, and catalase activities **(Sankar et al., 2005)**.

2.2. Pumpkin (*Cucurbita pepo* L.)

2.2.1. Generality

Pumpkin is an annual plant; it is one of the most cucurbits crops cultivated in the world, along with melon, cucumber and watermelon **(Özbek and Ergönül, 2020)**. It is believed to have originated in the ancient civilizations of North and Central America. Then it spread to Europe and other parts of the world during the early 16th century **(Ramadan, 2019)**. This plant is broadly grown in warm weather, after cultivation, full-grown pumpkin fruit can be obtained in an average of 80 to 90 days **(figure 15) (Wang et al., 2020)**.

Figure 15. The ***Cucurbita pepo*** **L**. plant. Pictures show flower, leaves, pepo fruit and slice of pumpkin with its seeds. The fruit can be very variable in size, shape, and color **(Ayyildiz et al., 2019)**.

Pumpkin as a pepo type fruit belongs to the Cucurbitaceae – family, which is one of the largest family in the plant kingdom, containing of greatest number of edible species, and it has five main genera **(Achilonu et al., 2018)**. Usually, ***Cucurbita pepo*** species are recognized as true pumpkin and are cultivate for human consumption and traditional medicine uses **(Caili et al., 2006)**.

Pumpkin seeds contain large amounts of minerals such as magnesium, selenium, zinc, copper and bioactive compounds like tocopherols, and carotenoids, besides their high fat and protein contents. For a long time, pumpkin seeds were consumed as a salty snack after roasting. Nevertheless, the seeds are also referred to as oilseeds due to their very high oil content (50%) **(Meru et al., 2018; Özbek and Ergönül, 2020)**.

2.2.2. Pumpkin seed oil

Pumpkin oil, a dark greenish red oil belongs to the group of expensive and good quality edible oils **(Kulaitienė et al., 2018)**. It is used in some salad's preparation, which gives them a very pleasant taste. As an extremely rich source of various bioactive compounds, that it have functional properties used as potential nutraceutical. In recent years, several studies have shed light on the medicinal properties of pumpkin seed oil, which is known as the dichromatic viscous oil due to its strong antioxidant activity **(Rouag et al., 2020)**.

Pumpkin oil is rich in highly unsaturated and saturated fatty acids, including oleic, linoleic, palmitic, palmitoleic, and gadolic fatty acids. A high content of tocopherols, squalene, phytosterols (Δ7-sterols), and carotenoids (lutein and zeaxanthin) have been also reported in pumpkin seed oils. In addition, Phenolic acids like p-coumaric, vanillic, and ferulic were detected in pumpkin oil. The high valuables contents of

pumpkin oil may make it a suitable alternative medicine or complementary medicine for human health management **(Ramak and Mahboubi, 2019)**.

Several studies have found that pumpkin have many pharmacological properties including anti-inflammatory **(Al-Okbi et al., 2017)**, antihypertensive **(El-Mosallamy et al., 2012; Chahal et al., 2022)**, hepatoprotective **(Abou Seif, 2014)**, antioxidant **(Benalia et al., 2015; Amin et al., 2020)**, and anti-diabetic **(Abd-elnoor, 2019; Adams et al., 2011)**. Besides, the potential effect to prevent prostate disease, arthritis and kidney stones **(Andjelkovic et al., 2010; Procida et al., 2013; Potočnik et al., 2016)**.

2.2.2.1. Hypoglycemic activity

Pumpkin is one of herbal that have anti-diabetic capacity, and its fruits are used for human consumption in diabetic conditions **(Xia and Wang, 2007; Kwon et al., 2007)**. In several reports, pumpkin has shown acute hypoglycemic activity in experimental hyperglycemic alloxan diabetic rabbits, and in type 2 diabetics **(Acosta-Patino et al., 2001; Alarcon-Aguilar et al., 2002)**. In a study conducted on alloxan-induced diabetic rats, consumption of pumpkin seeds powder and oil showed an effect hypoglycaemic, a decrease in glycosylated hemoglobin (HbA1c) levels as well as an increase in insulin secretion **(Abd-elnoor, 2019)**. A study indicated also that pumpkin posse's hypoglycemic properties in diabetic mice, this effect may be explained by either a decrease in intestinal glucose absorption or an increase in peripheral glucose utilization. In addition, pumpkin is rich in pectin, a type of dietary fiber, which when consumed is considered a good factor to control blood sugar level and reduce the need for insulin **(Adams et al., 2011)**.

2.2.2.2. Antioxidant activity

The antioxidant effects of pumpkin seed oil are due to its selenium and vitamin E richness that protect the body from free radicals attack (Krist, 2020). In diabetic rats, pumpkin seeds reduce the effects of oxidative stress by decreasing MDA level and increases CAT and SOD activities; improve the concentration of GSH **(Makni et al., 2011)**. Similarly, polyunsaturated fatty acids (linoleic acid and α-linoleic acid) have been indicated to exhibit a protective role against lipid peroxidation, through increase the level of several cellular antioxidants components, like ascorbic acid, α-tocopherol and GSH **(Abou Seif, 2014)**. Moreover, the essential trace element zinc in pumpkin seeds acts as an antioxidant due to its ability to neutralize the generation of free radicals or directly occupy the iron or copper binding sites **(Shaban and Sahu, 2017)**.

Additionally, Paul et al **(2020)** indicated that the antioxidant capacity of pumpkin seeds oil is attributed to the phenolic compounds, which are able to scavenge free radicals.

Experimental part

Materials and methods

1. Investigation aim

The present study was undertaken to evaluate the effect of dietary zinc deficiency on diabetic state in an experimental model of Wistar rats administered streptozotocin. Meanwhile, evaluating the preventive and curative effects of sesame and pumpkin oils against the impact of diabetes under nutritional zinc deficiency. To this end, we first examined the phytochemical composition of the two oils, evaluated the antioxidant activity through DPPH radical scavenging and β-carotene bleaching inhibition, investigated antihyperglycemic effect, estimated the carbohydrate metabolism, zinc status, and oxidative stress biomarkers.

2. Plant material

Sesame seeds and pumpkin oil "El-Captin mark" were purchased from the herbal market in Annaba, Algeria. The sesame seeds were purified of all impurities in order to extract the oil.

3. Sesame oil extraction

After all impurities were removed, whole sesame seeds were steamed at a temperature not exceeding 90 °C and then pressed with a hydraulic press. The squeezing process was repeated several times until a sufficient amount of oil was obtained. Then the oil was left to stabilize for a few days. So, that it became pure. Finally, the oil was stored in an opaque bottle at 25 °C to protect it from exposure to air, light, and contaminations, which can lead to oxidation and spoilage. This helps maintain its quality and stability until until it is used in the experiments (the oil was extracted in the nature touch center, for oil extraction, Constantine, Algeria).

4. Phytochemical study

4.1. Total Polyphenol Content

Principle

Polyphenols assay was carried out with Folin Ciocalteu's reagent according to the colorimetric method cited by Li et al **(2007)**. The reagent was a yellow acid, consists of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). It was reduced during the oxidation of phenols into a mixture of blue oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}) **(Ribéreau, 1968)**. The resulting color is proportional to the amount of polyphenols present in oils, with a maximum absorption at 760 nm.

Operating method

- To 0.2 ml of oil extract (1mg/ml), add1ml of diluted Folin-Ciocalteu's reagent (10%).
- The mixture was incubated for 4 min at room temperature.
- Add a volume of 0.8 ml of sodium carbonate solution (75 mg/ml).
- Then, the sample was incubated (the reaction mixture) in a dark at room temperature for 2 hours.
- A calibration curve was produced in parallel under the same conditions of samples, using gallic acid as a standard at different concentrations.
- The absorbance was read against the blank at 760 nm.

4.2. Total flavonoids content

Principle

The total flavonoid content of each sample was estimated by a colorimetric method using the aluminium chloride reagent ($AlCl_3$). This reagent forms a yellow flavonoid-aluminium complex at an absorption of 415 nm **(Turkoglu et al., 2007)**.

Operating method

- 250 µL of diluted sample prepared in methanol (1mg/ml) was mixed with 2550 µL of methanol and 100 µL of 1- M aqueous potassium acetate.
- A 100 µL of 2% aluminium chloride was added to the previous mixture, and shaken vigorously.
- The sample was incubated for 40 minutes at room temperature.
- The measurement was made at 415 nm by a spectrophotometer against a blank.
- A calibration curve was produced in parallel under the same operating conditions using quercetin as a standard at different concentrations.

4.3. Condensed tannins

Principle

The condensed tannin was evaluated by the vanillin assay as described by Hagerman **(2002)**.

Operating method

- 1ml of the diluted sample was mixed with 5 ml of assay reagent (1% vanillin-8% HCL), then the mixture was vigorously stirred.
- 5 ml of concentrated HCL (4%) was added after 1 min.
- The sample was incubated 20 min in a water bath at 30°c.
- The absorbance was performed at 500 nm against the blank.
- A calibration curve was produced in parallel under the same operating conditions using tannic acid as a standard at different concentrations.

4.4. Total saponin content

Principle

The total saponin was estimated according to Shiau et al **(2009)** method.

Operating method

- 50 µL of the diluted sample was mixed with 250 µL of 8% vanillin.
- Placed the test tubes in an ice-water bath, followed by the slow addition of 2500 µL of 72% (v/v) sulfuric acid on the inner side of the tube.
- The sample was incubated for 3 min, and brought to a water bath at 60°c for 10 min, and then cooled.
- The absorbance was measured at 544 nm against a reagent blank.
- A calibration curve was produced in parallel under the same operating conditions using diosgenin (a steroidal saponin) as a standard at different concentrations.

5. Antioxidant activity measurement

The evaluation of the antioxidant activity of sesame and pumpkin oils was carried out by two methods namely; DPPH radical scavenging and inhibition of β-carotene bleaching.

5.1. DPPH free radical scavenging activity

Principle

The DPPH (1, 1-diphenyl-2-picrylhydrazil1-diphenyl-2-picrylhydrazil) is a stable free radical, purple in color. In the presence of free radical scavengers, it is reduced to 2.2 diphenyl 1 picrylhydrazine

by taking a hydrogen atom, this leads to the disappearance of the purple color and the appearance of yellow, by a follow the decolorization kinetics at 517 nm **(Gulcin and Alwasel, 2023)**.

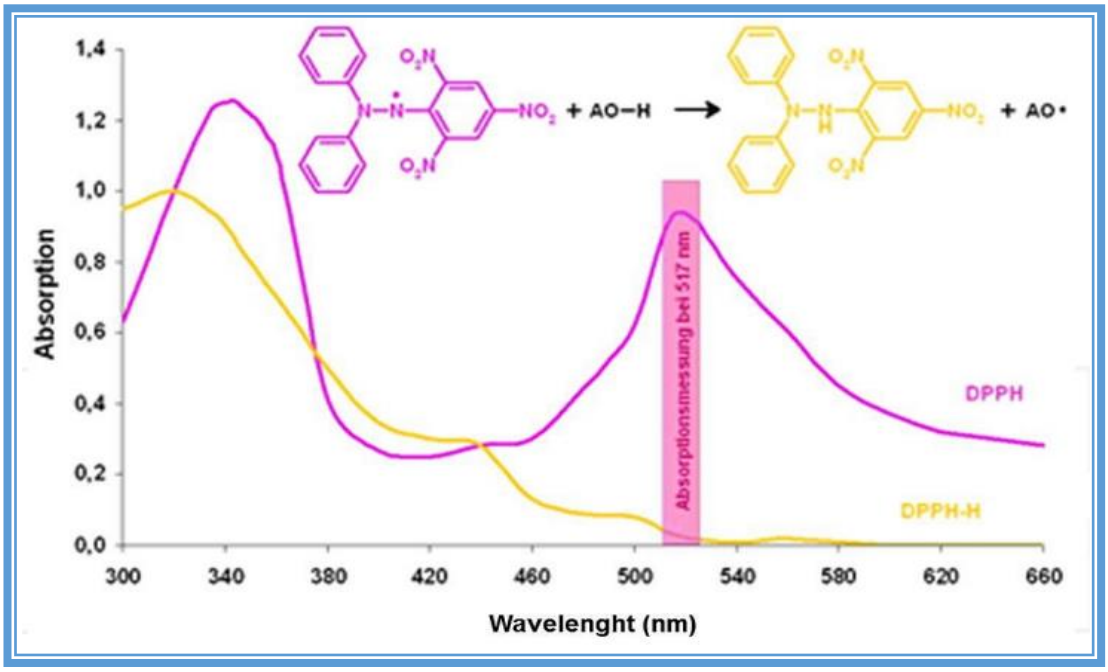


Figure 16 A schematic showing the color change of DPPH from purple to yellow when exposed to an antioxidant substrate **(Mendoza Pérez and Fregoso Aguilar, 2013)**.

Operating method

- Dissolved 2.4 mg of DPPH in 100 ml of methanol to obtain a solution (6×10^{-5}M).
- Introduced in tubes, a volume of 25 µl of different concentrations of the tested oil (1 mg/ml).
- Incubated for 30 minutes with 975 µl of a methanolic solution of DPPH.
- Leave the tubes of the reaction mixture in dark and at room temperature.
- The absorbance was read at 517 nm.
- The negative control was composed of 975 µl of methanolic solution of DPPH and 25 µl of methanol.
- The positive control was represented by a solution of a standard antioxidant ascorbic acid.

The results can be expressed as anti-radical activity or free radical inhibition in percentages (I %) using the following formula:

$$\% \text{ scavenging activity} = [(A0 - A1)/A0] \times 100.$$

Where:

A0: Absorbance of negative control.

A1: Absorbance of test sample or standard.

- ✓ **Calculation of IC_{50}**

IC_{50} or the 50% inhibitory concentration also called EC_{50} (Efficient Concentration 50), was the concentration of the tested sample necessary to reduce 50% of the DPPH radical.

The IC_{50}s were calculated graphically by linear regressions of the drawn graphs; percentage of inhibition as a function of different concentrations of the fractions tested.

5.2. Bleaching activity of β-carotene

Principle

The evaluation of antioxidant capacity by β-carotene bleaching was based on measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides resulting from the oxidation of linoleic acid **(Tepe et al., 2006)**.

The lipid peroxidation inhibition activity of sesame and pumpkin oil was determined using the β-carotene bleaching assay, according to the method described by Kulisic et al **(2004)**.

Operating method

- In the flask, a 20 mg linoleic acid was added plus100 mg of Tween 40, and 1 ml of β-carotene solution (0.1mg/ml chloroform).
- Evaporated the mixture at 50°C for 10 min by a rotary evaporator.
- Slowly 50 ml of oxygenated distilled water was added to the flask under a vigorous agitation in order to obtain an emulsion system of β-carotene/linoleic acid (emulsion A).
- A 0.2 ml of methanolic solution (oils/BHT) was added to 5 ml aliquots of the emulsion in test tubes.

- After gently shaken, the tubes were incubated at 50°C in a water bath for 2 h.
- The absorbance reading of samples was taken immediately (t=0) and at 15 min intervals for 120 min at 470 nm against a blank.

The negative control: without oils, consisting of 0.2 ml methanol and 5 ml emulsion (A).

The blank: A solution consisting of 0.2 ml methanol and 5 ml of the emulsion (B). (Emulsion (B) consisting of 20 mg of linoleic acid, 100 mg of Tween 40 and 50 ml of hydrogen peroxide).

The positive control: BHT was used as a positive control, under the same conditions as the oils.

The antioxidant activity was expressed as a percentage inhibition AA (%) and determined according to the following formula.

$$AA\ (\%) = [1-(A0 - At) / (A^o0 - A^ot)] \times 100$$

Where A0 and A^o0 are the absorbance values measured at initial time of incubation for sample or standard and control respectively, while At and A^ot are the absorbance values measured in the sample or standard and control at t= 120 min.

6. Oral glucose tolerance test (OGTT)

To evaluate the antidiabetic effect of the oils administered during the experimental period, an oral glucose tolerance test was performed by administering glucose solution to the mice in an amount of 2 g/kg body weight orally. Mice were given standard diet. Blood glucose level before and after feed intake were measured at t0 (basal), t1 (30 min), t2 (60 min), t3 (90 min), t4 (120 min) in all groups using a glucometer (Accu-check glucose-meter).

OGTT was conducted after 14 days of treatment, according to the procedure previously described by Gupta et al **(2012)** with slight modification. Briefly, normal mice divided into seven groups (6 each) were treated as follows:

Group 1: control mice received standard diet + 2 g/kg glucose after 90 min.

Group 2: mice received 4% sesame oil in diet + 2 g/kg glucose after 90 min.

Group 3: mice received 6% sesame oil in diet + 2 g/kg glucose after 90 min.

Group 4: mice received 8% sesame oil in diet + 2 g/kg glucose after 90 min.

Group 5: mice received 4% pumpkin oil in diet + 2 g/kg glucose after 90 min.

Group 6: mice received 5% pumpkin oil in diet + 2 g/kg glucose after 90 min.

Group 7: mice received 8% pumpkin oil in diet + 2 g/kg glucose after 90 min.

7. Treatment of rats

7.1. Animals and breeding conditions

Male Albinos Wistar rats (weighing around 150–170 g, male, 10 weeks of age) were acquired from Pasteur institute (Algiers, Algeria). Animals were allocated in polypropylene cages with stainless steel griddle tops and bottoms and stainless-steel food hoppers. Trays were placed under each food hopper to collect spilled food. These rats were subjected to a period of adaptation approximately two weeks, to the conditions of the animal house; at an ambient temperature under a photoperiod of 12-h light/dark. They were given access to diet and water *ad-libitum* throughout the period of the experiment. Studies were handled in accordance to the protocol approved by the Institutional Animal Ethical Committee of Badji Mokhtar University, Annaba.

7.2. Induction of Experimental Diabetes

Experimental animals were administered with freshly prepared streptozotocin (STZ) (Sigma Chemicals Company), dissolved in cold citrate buffer (0.1 M, pH 4.5) at the dose of 60 mg/kg body weight after overnight fasting **(Ghanbari et al., 2016)**. A 10% glucose solution was given overnight to the streptozotocin treated animals to prevent STZ-induced hypoglycemia. One week later of streptozotocin (STZ) injection, the diabetic state (type 1 diabetes) was confirmed by estimating blood glucose level from the tail vein using an Accu-chek glucose meter (Roche Diagnostics, Paris, France); only rats with glucose level over 14 mmol/L were considered diabetic model.

7.3. Diet preparation

In this study, two diets of the rats were prepared according to the method described by Southon et al **(1988)**, one supplemented with zinc (54 mg/kg of feed) intended for the groups whose food was adequate in zinc and the other was poor in zinc (1.2 mg/kg of feed) for groups whose feed was inadequate in zinc. Preparation of the diets was done, by mixing quantities of raw materials and metals as shown in **table 1** and **2**.

Table 1. Diet composition **(Southon et al. 1988)**.

Raw materials (g/kg diet)	Quantity (g/kg diet)	Percentage (%)
Cornstarch	326	32.6
Sucrose	326	32.6
Protein	168	16.8
Fiber (cellulose)	40	4
Mineral mix	40	4
Vitamins mix	20	2
Lipids (corn oil)	80	8

Table 2. Minerals amount in the diet **(Southon et al. 1988)**.

Metal (mg/kg)	Diet adequate in zinc	Diet inadequate in zinc
Zinc	52.1	-
Copper	6.0	6.0
Iodine	0.6	0.6
Manganese	58.5	58.5
Iron	28.6	28.6
Calcium	7.11	7.11
Phosphate	14.02	14.02
Chlorine	3.68	3.68
Potassium	3.34	3.34
Magnesium	0.70	0.70
Sodium	2.39	2.39

7.4. Protocol design

After stabilization of diabetes, rats were divided into seven groups (6 each), which were as follows:

Group 1 (ND): non-diabetic rats fed a sufficient zinc diet.

Group2 (DSZ): diabetic rats fed a sufficient zinc diet.

Group3 (DDZ): diabetic rats given zinc deficient diet.

Group4 (DSZ + SO): diabetic rats fed zinc sufficient diet and supplemented with sesame oil at a dose of 6% **(Ramesh et al., 2005)**.

Group5 (DDZ + SO): diabetic rats received zinc deficient diet and supplemented with sesame oil at a dose of 6%.

Group6 (DSZ + PO): diabetic rats given zinc sufficient diet and supplemented with pumpkin oil at a dose of 5% **(Hassan et al., 2019)**.

Group7 (DDZ + PO): diabetic rats fed zinc deficient diet and supplemented with pumpkin oil at a dose of 5%.

The experimental protocol continued for 27 days. Food intake was measured daily, while animal weight was recorded twice weekly.

7.5. Sacrifice and removal of organs

7.5.1. Blood samples

At the end of the experiment, animals were sacrificed by cervical decapitation. The collected blood samples were centrifuged at 3000 rpm (10 minutes, 4°C), and serum stored at − 20C° in eppendorf tubes for biochemical analysis.

7.5.2. Tissue collection

After dissection, liver, kidneys, pancreas, and femur were excised, stripped of their fatty tissue, rinsed in a 0.9% sodium chloride (NaCl) solution and weighed. One fragment of pancreas, femur, and a fragment of liver of each animal were put in an oven at 80°C for 16 hours to dry, then kept at normal temperature to determine zinc concentration. A second fragment of liver was stored at - 20° C for assaying oxidative stress parameters. The other fragment of pancreas was fixed in 10% formalin solution for the histological study **(figure 17)**.

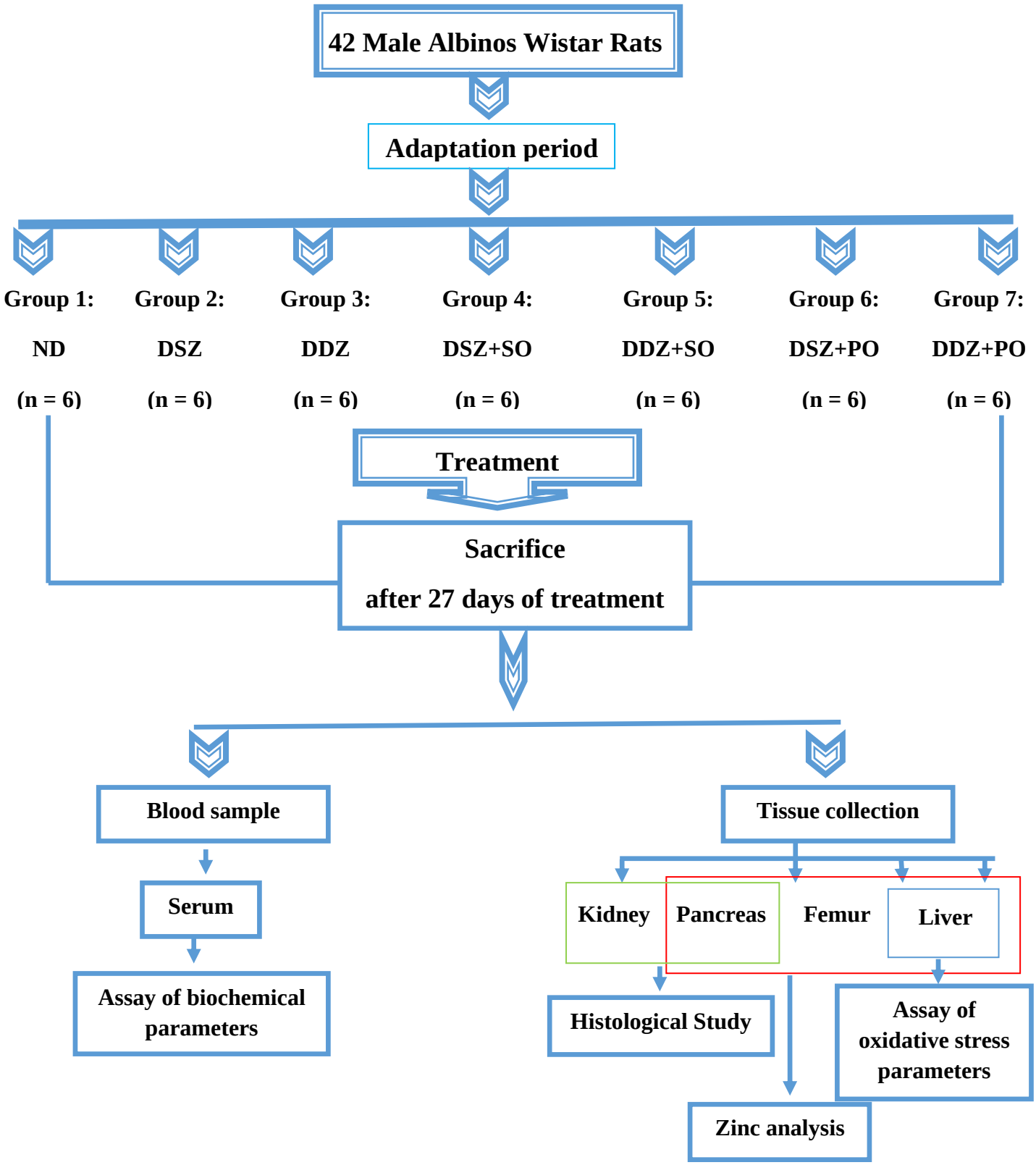


Figure 17. The different steps of the experimental protocol.

8. Zinc Analysis

The measurements of zinc content in liver, pancreas and femurs, which were previously dried, were carried out by heating these organs in silica crucibles at 480 °C for 48 hours using oven. After cooling, the ash was dissolved in concentrated nitric acid, diluted with distilled water, and then filtered with filter paper (Whatman No. 542) for Zn estimation utilizing a flame atomic absorption spectrophotometer (AA-7000 SHIMADUZ France; biochemistry laboratory-Mostaganem university). The zinc concentration was determined by comparison with a standard range of zinc nitrate (1 mg/ml) realised under the same conditions (see annex) **(Southon et al., 1988)**.

9. Dosage of biochemical parameters

The biochemical parameters were measured using commercial test kits from Spinreact, Girona, Spain, using an automatic chemistry analyzer (Mindray BS-130).

9.1. Glucose assay

Blood glucose was measured by an enzymatic reaction according to the method of Trinder **(1996)**. Glucose is converted to gluconic acid and hydrogen peroxide (H_2O_2), under glucose oxidase (GOD) action. In the presence of peroxidase (POD), hydrogen peroxide oxidizes the colorless chromogen (4-aminophenazone) into a red-violet colored compound (quinoneimine) according to the following reactions:

$$\beta\text{-D glucose} + O_2 + H_2O \xrightarrow{GOD} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-aminophenazone} \xrightarrow{POD} \text{Quinone} + H_2O$$

9.2. Aspartate aminotransferase (ASAT) activity assay

Aspartate aminotransferase (ASAT) which is known also as Glutamate-oxaloacetate transaminase (GOT) catalyzes the reversible transfer of an amino group from aspartate to α-ketoglutarate forming glutamate and oxaloacetate under the action of malate dehydrogenase (MDH) and $NADH^+H^+$. Then, oxaloacetate is reduced to malate **(Murray, 1984[a])**, according to the following reactions:

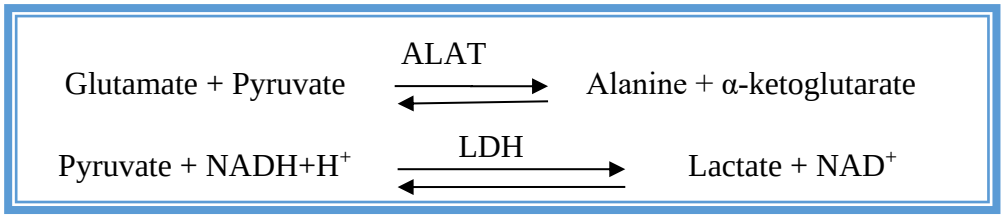
$$\text{Aspartate} + \alpha\text{-Ketoglutarate} \xrightleftharpoons{AST} \text{Glutamate} + \text{Oxaloacetate}$$

$$\text{Oxaloacetate} + NADH + H^+ \xrightleftharpoons{MDH} \text{Malate} + NAD^+$$

The decrease in NADH concentration is directly proportional to the enzymatic activity of aspartate aminotransferase in the sample.

9.3. Alanine aminotransferase (ALAT) activity assay

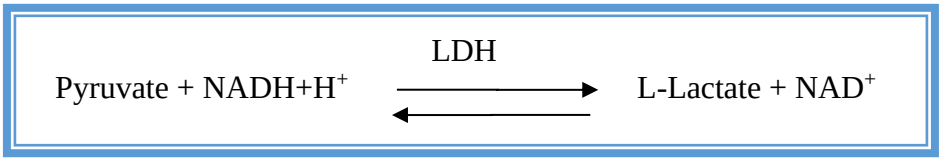
Alanine aminotransferase (ALAT) also called glutamate pyruvate transaminase (GPT) catalyzes the reversible transfer of an amino group from alanine to α-ketoglutarate forming glutamate and pyruvate under the action of lactate dehydrogenase (LDH) and NADH. Then, the pyruvate produced is reduced to lactate, according to the following reactions:



The decrease in NADH concentration is directly proportional to the enzymatic activity of alanine aminotransferase in the sample **(Murray, 1984[b])**.

9.4. Lactate dehydrogenase (LDH) activity assay

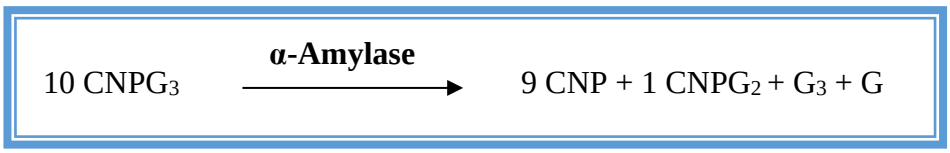
Lactate dehydrogenase (LDH) catalyzes the reduction of pyruvate by NADH according to the following reaction:



The decrease in NADPH concentration is directly proportional to the LDH enzyme activity in the sample **(Pesce, 1984)**.

9.5. α-Amylase activity assay

2-Chloro-4-nitrophenyl α-D-maltotrioside ($CNPG_3$) is enzymatically hydrolyzed by α-amylase to 2-chloronitrophenol (CNP) with the formation of 2-chloro-4-nitrophenyl α-D-maltoside ($CNPG_2$), maltriose (G_3) and glucose (G).



The color intensity of 2-chloronitrophenol (CNP) formed is proportional to the activity of α-amylase in the sample **(Ying Foo et al., 1998)**.

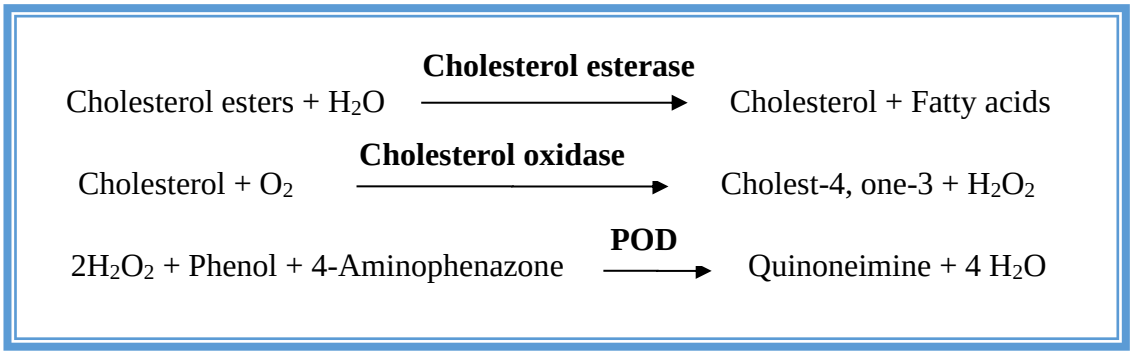
9.6. Direct and total bilirubin assay

Bilirubin is defined as the amount of serum pigment, reacting with diazotized sulphanilic acid to produce azobilirubin at acidic pH, which quantifiable by spectrophotometry.

The intensity of color formed is proportional to the bilirubin concentration in the sample **(Kaplan et al., 1984)**.

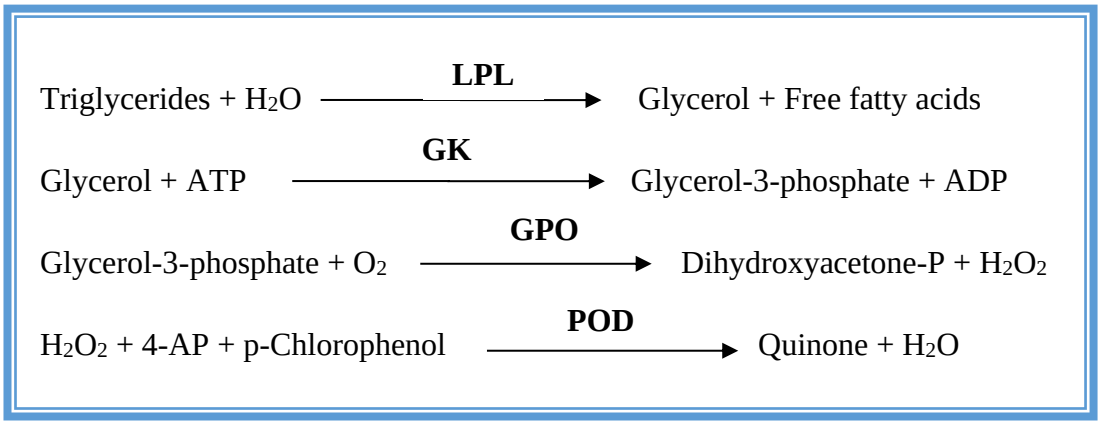
9.7. Cholesterol assay

The cholesterol concentration is determined by an enzymatic reaction according to the Naito method **(1984)**. Under the activity of cholesterol esterase, cholesterol esters hydrolyze to form cholesterol and non-esterified fatty acids according to the following reaction:



9.8. Triglycerides (TGs) assay

Triglycerides are enzymatically hydrolyzed to glycerol and free fatty acids by lipoprotein lipase (LPL). Under the effect of glycerol kinase, glycerol is phosphorylated to form glycerol -3- phosphate (GTP). The latter is oxidized to dihydroxyacetone and H_2O_2 by glycerol-3-phosphate oxidase. In the presence of peroxidase, H_2O_2 combines with 4−aminophenazone and p-chlorophenol to give quinone according to the following reaction **(Kaplan et al., 1984[b])**.



9.9. Total protein assay

The copper sulphates interact with the peptide bonds in an alkaline medium forming a violet (blue-violet) coloration, whose intensity measured is proportional to the quantity of proteins present in the samples, according to the Burtis colorimetric method **(Burtis et al., 1999)**.

9.10. Albumin assay

Albumin reacts with bromcresol green (BCG) in an acid medium to form a colored complex, the color intensity of which is proportional to the amount of albumin in the sample **(Gendler, 1984)**.

9.11. Creatinine assay

The dosage of creatinine is based on the reaction of creatinine with sodium picrate. This is the JAFFE reaction. Creatinine reacts with alkaline picrate forming a colored complex. The intensity of the color formed is proportional to the concentration of creatinine in the sample **(Murray, 1984)**.

9.12. Urea assay

Urea is enzymatically hydrolyzed into ammonia ions (NH_4^+) and carbon dioxide (CO_2). In the presence of the catalyst nitroprusside, the ammonia ions formed react with salicylate and hypochlorite (NaClO) to form green indophenol according to the following reaction **(Kaplan, 1984)**.

$$\text{Urea} + H_2O \xrightarrow{\textbf{Urease}} (NH_4^+)_2 + CO2$$

$$NH^+_4 + \text{Salicylate} + \text{NaClO} \xrightarrow{\textbf{Nitroprusside}} \text{Indophenol}$$

9.13. Uric acid assay

Uric acid is oxidized by uricase to allantoin and hydrogen peroxide, which under the influence of peroxidase, 4 aminophenazone (4-AP), and 2-4 dichlorophenolsulfonate (DCPS), forms a red compound of quinoneimine, whose intensity is proportional to the concentration of uric acid in the sample according to the following reaction **(Schultz, 1984)**.

$$\text{Uric acid} + 2\,H_2O + O_2 \xrightarrow{\textbf{Uricase}} \text{Allantoin} + CO2 + 2\,H_2O_2$$

$$2\,H_2O_2 + 4 - AP + DCPS \xrightarrow{\textbf{POD}} \text{Quinoneimine} + 4\,H_2O$$

10. Determination of oxidative stress parameters

10.1. Preparation of tissue homogenate

The evaluation of oxidative stress was measured in liver tissue. One gram of liver was taken from all the seven studied groups. Then the tissues were crushed and homogenized in 2 mL of Tris-buffered saline (50 mM Tris, 150 mM NaCl, pH 7.4). After, a centrifugation of the cell suspension 10000×g, 4°C, 15 min, the resulting supernatant was aliquoted in eppendorf tubes and stored at (- 20°C) until utilization.

10.2. Tissue Protein Assay

Bradford's reagent develops in the presence of proteins to a quantifiable blue color at 595 nm; the intensity is proportional to the quantity of proteins present in the sample.

Principle

The amine (-NH2) of the proteins react with a reagent based on phosphoric acid, ethanol and Coomassie blue (G250) to form a blue color complex. The appearance of this color reflects the degree of ionization of the acid medium and the intensity corresponds to proteins concentration **(Bradford, 1976)**.

Operating method

- Take 0.1 ml of the homogenate.
- Add 5 ml of Bradford's reagent.
- Shake and let the mixture stand for 5 min for color stabilization.
- Read at 595 nm the optical density, against the blank.
- The optical density obtained was reported on a calibration curve.

Protein concentrations are determined depending on the calibration curve of bovine serum albumin BSA (1 mg/ml) produced under the same conditions (see annex).

10.3. Estimation of malondialdehyde concentration (MDA)

The lipid peroxidation level in liver homogenate was determined as MDA according to Buege and Aust **(1978)**, which is one of the end products formed during the free radical-mediated breakdown of polyunsaturated fatty acids.

Principle

The principle of this assay is based on MDA condensation in acid and hot medium with thiobarbituric acid, to form a pink colored complex between two molecules of thiobarbituric acid. The complex can be measured by absorption spectrophotometry at 530 nm.

Operating mode

- Take 375 µl of the homogenate.
- Add 150 µl of the TBS buffer solution (50 mM Tris, 150 mM NaCl pH 7.4).
- Add 375 µl of the TCA-BHT solution (TCA 20%, BHT 1%).
- Shake and centrifuge at 9000 g for 10 min at 4°C.
- Take 400 µl of the supernatant
- Add 80 µl of HCl (0.6 M).
- Add 320 µl of the solution: Tris-TBA (26 mM Tris, 120 mM TBA).
- Mix and incubate in a water bath at a temperature 80°C for 10 min.

The optical densities were measured at 530 nm against reagent blank.

The concentration of MDA in the sample was expressed in nmol/ mg protein, according to the formula:

$$\textbf{[MDA] (nmol / mg protein)} = \frac{\textbf{OD} \times 10^6}{\boldsymbol{\varepsilon} \times \textbf{L} \times \textbf{X} \times \textbf{df}}$$

• C: Concentration of MDA in nmol/mg of protein.

• OD: Optical density at 530 nm.

• ε: Molar extinction coefficient of MDA = 1.56 x 10^5 M^{-1} cm^{-1}.

• L: Optical path length = 1 cm.

• X : Sample protein concentration (mg/ml).

• df: Dilution factor: df = 0.2083.

10.4. Estimation of reduced glutathione level (GSH)

The glutathione assay was estimated according to the method of Jollow et al **(1974)**.

Principle

This assay is based on the measurement of the optical absorbance of 2-nitro-5-marcapturic acid, which results from the reduction of 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) by the (-SH) groups of glutathione.

Operating mode

- Take 0.8 ml of the homogenate.
- Add 0.2 ml of the salicylic acid solution (0.25%).
- Shake and leave for 15 minutes in an ice bath.
- Centrifugal at 9000g for 5min.
- Take 0.5 ml from the supernatant.
- Add 1 ml of the Tris buffer, pH 9.6.
- Mix and add 0.025 ml of 5.5 dishio-bise-2-nitrobenzoic acid (DTNB) at 0.01M.
- Leave for 5 min at room temperature and read the optical densities at 412 nm against reagent blank.

The concentration of GSH was obtained by the following formula:

$$\textbf{[GSH] (nmol GSH/mg protein)} = \frac{\textbf{OD x 1 x 1.525}}{\textbf{13.1 x 0.8 x 0.5 x mg protein}}$$

- OD: Optical density
- 1: Total volume of the deproteinization solutions used (0.8ml homogenate, 0.2ml salicylic acid).
- 1.525: total volume of the solutions used in the GSH assay in the supernatant (0.5 ml supernatant + 1 ml tris + 0.025 ml DNTB).
- 13.1: Absorbance coefficient (concerning the –SH group at 412 nm).
- 0.8: Volume of the homogenate.

- 0.5: Volume of supernatant found in 1.525 ml.

10.5. Estimation of glutathione peroxidase activity (GSH-Px)

The enzymatic activity of glutathione peroxidase (GSH-Px) was measured by the method of Flohe and Gunzler **(1984)**.

Principle

This method is based on the reduction of hydrogen peroxide (H_2O_2) in the presence of reduced glutathione (GSH), the latter is transformed into (GSSG) under the impact of GSH-Px according to the following reaction:

$$H_2O_2 + 2\,GSH \xrightarrow{\textbf{GSH-Px}} GSSG + 2H_2O$$

Operating mode

- Take 0.2 mL of the homogenate.
- Add 0.4 mL of GSH (0.1 mM).
- Add 0.2 mL of TBS buffer solution (50 mM Tris, 150 mM NaCl, pH 7.4).
- Incubate in a water bath at 25°C for 5 min.
- Add 0.2 mL of H_2O_2 (1.3 mM) to initiate the reaction, leave to react for 10 min.
- Add 1 mL of TCA (1%) to stop the reaction.
- Put the mixture in ice for 30 min.
- Centrifuge for 10 minutes at 3000 rpm.
- Take 0.48 mL of the supernatant.
- Add 2.2 mL of TBS buffer solution.
- Add 0.32 mL of DTNB (1 mM)
- Mix and after 5 minutes read the optical densities at 412 nm against the blank.

The enzymatic activity of GPx was expressed as micro-mole of oxidized GSH per milligram of protein (µmol GSH/mg protein) according to the formula:

$$\textbf{[GPx] (µmol GSH/min/mg protein)} = \frac{\textbf{OD Sample – OD Standard} \times \textbf{0.04}}{\textbf{OD Standard}} \times \frac{\textbf{5}}{\textbf{mg protein}}$$

- OD Sample: Optical density of the sample.
- OD standard: Optical density of the standard.
- 0.04: Substrate concentration (GSH).

10.6. Estimation of Glutathione-S-transferase activity (GST)

The glutathione S-transferase activity was determined using Habig et al **(1974)** method.

Principle

This method is based to provide or supply the enzyme with a 1-chloro, 2.4-dinitrobenzene (CDNB) as a substrate, which is easily conjugates with glutathione under the action of GST. The conjugation reaction of these two products leads to the formation of a new molecule (GSH-CDNB) which absorbs light at a wavelength of 340 nm.

Operating method

- Let the GSTs contained in the homogenate to act on a mixture (GSH + CDNB) at a temperature of 37°C and at a pH of 6.5.
- Measure the variation in optical density (due to the appearance of the GSH-CDNB complex) for 1 minute over 5 minutes at a wavelength of 340 nm.

Reagents	Blank (µL)	Assay (µL)
Phosphate buffer (0.1 M) pH 6.5	850	830
CDNB (0.02 M)	50	50
GSH (0.1 M)	100	100
Supernatant	-	20

The enzymatic activity of GST was obtained by the following formula:

$$\textbf{[GST] (nmol CDNB /min/mg protein)} = \frac{\textbf{OD sample / min – OD blank / min}}{\textbf{9.6 × mg protein}}$$

- OD sample/min: Optical density of the sample per minute.
- OD Blank/min: Optical density of blank per minute.
- 9.6: GSH-CDNB extinction coefficient expressed in $mM^{-1}.Cm^{-1}$.

10.7. Estimation of catalase activity (CAT)

CAT activity was determined using the Aebi method **(1984)**.

Principle

The catalase enzymatic activity assay is based on the decrease in absorbance at 240 nm, which is due to the dismutation of hydrogen peroxide (H_2O_2) into water and oxygen by catalase.

$$2\,H_2O_2 \xrightarrow{CAT} 2\,H_2O + O_2$$

Operating method

- Add a 780 µL of phosphate buffer and 200 µL of H_2O_2 to 20 µL of homogenate to react, at a temperature of 25°C.
- The quantity of the homogenate was determined according to the quantity of proteins, which must be between 1 and 1.5 mg/mL, ie a quantity of 10 to 20 µL of diluted homogenate.
- The reading of the absorption was realised after 15 seconds of delay and during 60 seconds of measurement.

Reagents	Blank (µL)	Assay (µL)
Phosphate buffer (0.1 M) pH 7.4	800	780
H_2O_2 (0.5 M)	200	200
Supernatant	-	20

The activity of CAT was calculated by the following formula:

$$\textbf{[CAT] (µmol } H_2O_2\textbf{/ min/ mg protein)} = \frac{\textbf{ΔOD/ min}}{\textbf{ε × L × X × df}}$$

- ΔOD: Variation of optical density per minute.
- ε: Extinction coefficient of H_2O_2 (0.04 mM^{-1}. Cm^{-1}).
- L: Width of the cuvette (1 cm).
- X: Quantity of proteins in mg/ml.
- df: Dilution factor of the H_2O_2 in the buffer (0.02).

10.8. Estimation of superoxide dismutase activity (SOD)

The activity superoxide dismutase (SOD) was determined according to the method, which was described by Misra and Fridovich **(1977)**.

Principle

The activity of SOD is determined by its ability to inhibit 50% of epinephrine auto-oxidation in an alkaline medium at a wavelength of 480 nm. An increase in absorbance at this wavelength reflects the extent of auto-oxidation, evidenced by the formation of adrenochrome, which results in the development of a pink coloration.

Operating method

- Mix 10 µL of the homogenate with 970 µL of EDTA sodium carbonate buffer.
- Add 20 µL of epinephrine.

- Start the stopwatch and read the sample and blank absorbance's every minute for 4 minutes at 480 nm.

Reagents	Blank (µL)	Assay (µL)
EDTA sodium carbonate buffer (0.05 M) pH 10.2	970	970
Epinephrine (30 mM)	20	20
Supernatant	10 µL TBS	10

The activity of SOD was calculated by the following formula:

$$\textbf{SOD (IU/ mg protein)} = \frac{\Delta OD}{\varepsilon \times L \times X} \times df$$

- SOD (IU/mg protein): quantity of enzyme capable of inhibiting epinephrine auto-oxidation by 50%/min.
- ΔOD: (ΔA sample/min - ΔA blank/min) at 480 nm.
- ε: Molar extinction coefficient of epinephrine (4.2 mM^{-1}. Cm^{-1}).
- L: 1cm.
- X: Quantity of proteins (mg/ml).
- df: 10.

11. Histological study

The histological sections were performed at pathological anatomy laboratory Ibn zohr hospital, Guelma city. The technic for the histological examination was previously described by Hould **(1984)**. It includes the following steps:

- **Fixation**

The pancreases and kidneys were fixed in a 10% formalin solution. Afterwards, the organs were cut transversely and placed in special cassettes with perforated walls to allow the passage of liquids.

- **Dehydration**

It was carried out by immersion in ethanol baths at increasing degrees (70°, 96°, 100°), and then in two xylene baths. Finally, the tissues were ready for embedding in paraffin.

- **Inclusion**

This step was realised by immersing the cassettes in two successive baths of paraffin for 2 h each at a temperature of 58°C.

- **Section cutting**

The paraffin blocks were cut beforehand before undergoing microtome sections of 3 µm to 5 µm. The paraffin ribbons obtained were spread on microscope slides, then smoothed out and fixed with heated gelatinous water.

- **Deparaffinization-Hydration**

The sections must be deparaffinised before staining. In other words, they were rehydrated by immersing them in successive baths of: xylene, ethanol (of decreasing degree), subsequently, they washed in running water.

- **Staining**

The hematoxylin-Eosin (Hematein-Eosin) technique was used by an automaton according to the following steps:

The purplish-blue coloration of the basophils (nuclei) was obtained after incubation of the slides in a Harris hematoxylin bath for 15 minutes. Then, the sections were immersed in acid alcohol (1 to 2 dives) in order to differentiate them. A rinsing with a water bath was carried out and the observation by the microscope allowed the verification of the differentiation of the slides. The bluing of the slides in an ammoniacal water bath and immersion in an eosin bath (15 seconds to 2 minutes) which stains the acidophilic structures (cytoplasm) pink. All these baths were separated by water rinses. Finally, the preparations were then mounted by EUKITT, dried and then observed under an optical microscope (LEICA DM-750).

12. Statistical Analysis

Statistical analysis was carried out using Graph Pad Prism 8. Comparison among groups was assessed by using a One-Way ANOVA test followed by Turkey's post hoc test. The results were expressed as the mean ± SEM and comparing the different groups were as follows:

- a: comparison of DSZ group vs ND group,
- b: comparison of DDZ group vs DSZ group,
- c: comparison of DSZ+SO, DSZ+PO groups vs DSZ group,
- d: comparison of DDZ+SO, DDZ+PO groups vs DDZ group.

The differences were considered to be:

- (a, b, c, d) significant when ($p<0.05$),
- (a1, b1, c1, d1) highly significant when ($p<0.01$),
- (a2, b2, c2, d2) very highly significant when ($p<0.001$).

Experimental part

Results

1. Phytochemical studies and antioxidant activity of sesame oil and pumpkin oil

1.1 Phytochemical results (chemicals contents)

- The total polyphenols contents of the oils, as estimated by the Folin-Ciocalteu reagent method, were determined from a calibration curve, plotted using gallic acid as standard y= 0.002x+0.137 and R^2= 0.994 and expressed in milligrams of gallic acid equivalent per gram of oil (mg GAE/g oil).

- The flavonoids content were calculated from a calibration curve (y= 0.005x+0.137) and R^2= 0.994 and produced by quercetin. Expressed in milligrams quercetin equivalent per gram of oil (mg QE/g oil).

- The tannins content were estimated from a calibration curve (y= 0.140x+0.020) and R^2= 0.995, reported in milligrams of tannic acid equivalent per gram of oil (mg TAE/g oil).

- The saponins content were determined from a calibration curve (y= 0.411x+0.004) and R^2= 0.990 and produced by diosgenin. Expressed in milligrams diosgenin equivalent per gram of oil (mg DGE/g oil) (see annex). The results obtained are reported in **table 3**.

Table 3. Amount of total polyphenols, total flavonoids, condensed tannins, and saponins in the sesame and pumpkin oil.

Oil	Total polyphenols (mg GAE/g oil)	Total flavonoids (mg QE/g oil)	Condensed tannin (mg TAE/g oil)	Total saponin (mg DGE/g oil)
Sesame oil	117.83±0.76	77.4±1.56	4.39±0.03	1.11±0.02
Pumpkin oil	17.32±0.68	7.63±0.82	4.99±0.18	0.63±0.27

The values given represent the mean of three measurements ± SEM.

1.2 Antioxidant activity

In this study, the antioxidants capacities of sesame and pumpkin oils were determined using two different tests: the DPPH free radical scavenging test and the β-carotene bleaching test.

1.2.1. DPPH assay

The values obtained made it possible to draw a curve, which represents the variation in the percentage of anti-radical activity as a function of the concentration of the two oils studied **(figure 18)**.

The antioxidant power of oils was determined from its IC_{50}, a parameter commonly used to measure anti-radical activity, which was defined as the oil concentration necessary to reduce half of the DPPH radical.

Indeed, the low IC_{50} value corresponds to strong antioxidant activity. In this case, the comparison of the IC_{50} compared to the positive control showed that the oils have an interesting antioxidant activity with an IC_{50} for sesame oil = 0.16±0.24 mg/ml, pumpkin oil = 0.30±0.49 mg/ml and ascorbic acid = 0.10 ± 0.02 mg / ml. On the other hand, the results obtained indicated that the two oils have anti-radical activity in a dose-dependent manner.

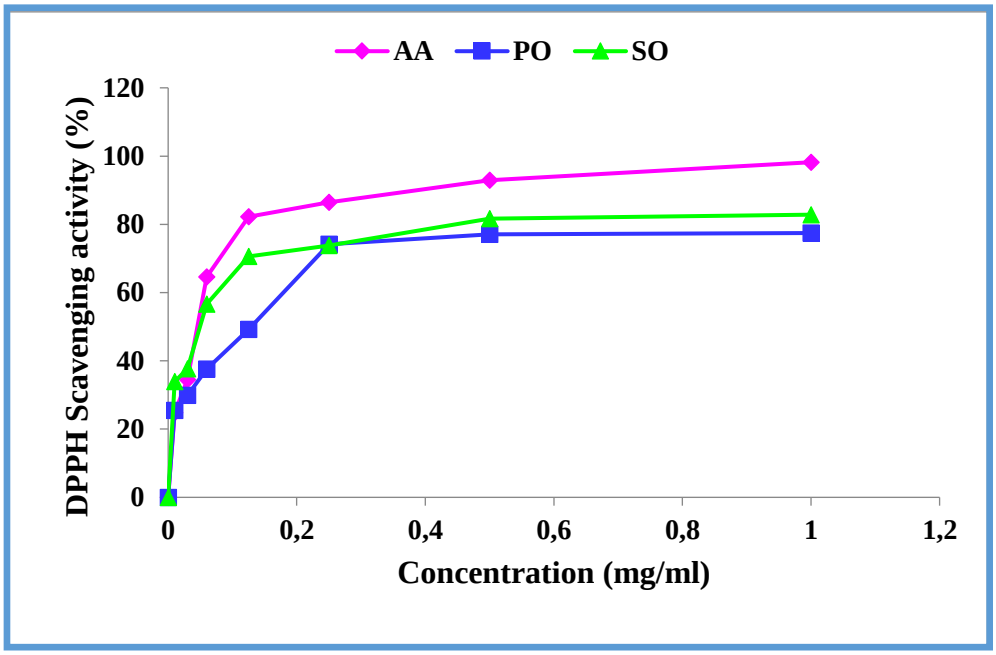


Figure 18. Anti-radical activity of sesame and pumpkin oils. Ascorbic acid was used as a reference antioxidant.

1.2.2. β-carotene Bleaching assay

The kinetics of the oxidation reaction of β-carotene in the absence and presence of sesame and pumpkin oils as well as standard antioxidants are shown in **figure 19**. From the curve, the absorption of the reaction mixture significantly decreased quickly compared to that of the negative control and becomes stable for the oils over a longer period.

According to the inhibition percentage, the standard antioxidant (BHT) showed remarkable β-carotene decolorization inhibition activity with a percentage in the range of 84.55%, while pumpkin oil had recorded a percentage of 45.91% and for sesame oil 36.8%.

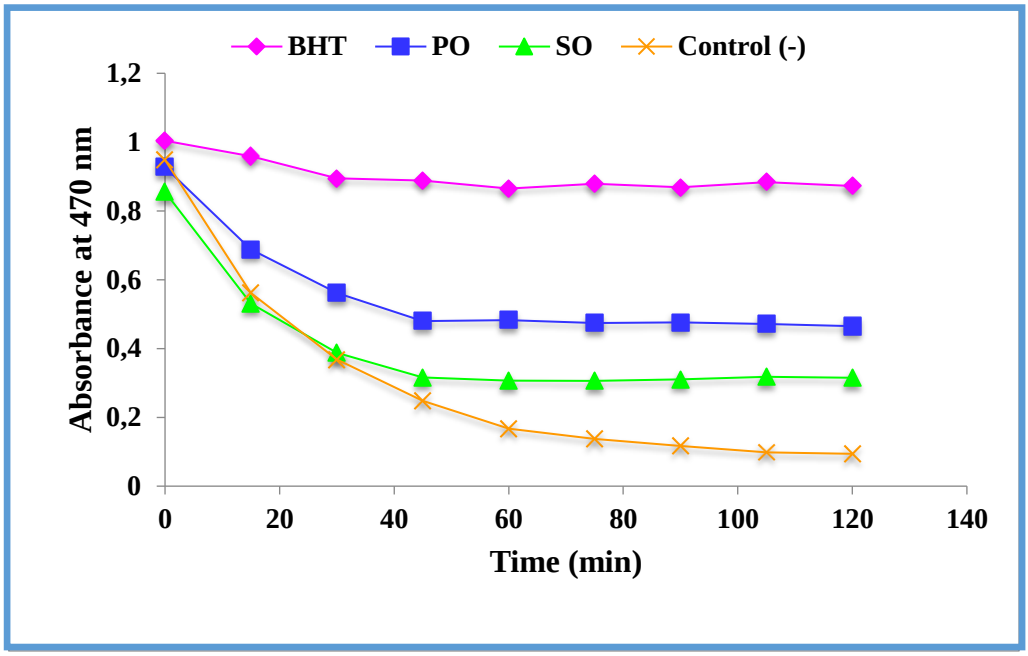


Figure 19. Kinetics of β-carotene bleaching at 470 nm in the absence and presence of sesame oil, pumpkin oil, and BHT.

2. Oral Glucose Tolerance Test (OGTT)

Figure 20 shows the effect of three increasing doses of sesame oil and pumpkin oil in mice rendered hyperglycemic orally of 2 g/kg body weight glucose. Highly significant hyperglycemia ($p<0.001$) was observed in the mouse groups examined 30 minutes after glucose challenge compared to the normal fasting blood glucose of each individual group.

In parallel, a regulation of glycaemia was observed after the peak. It is remarkable improvement in this regulation in mice treated with the two oils in moderate doses (5%, 6%). By comparing blood glucose, point by point, no significant difference was noted after 2 hours. On the other hand, the reduction in the glycaemia of the mice treated from time (1 h) until time (2 h) was very significant ($p<0.001$, $p<0.01$, $p<0.05$) as compared to the normal fasting blood glucose.

According to the results of the tolerance test, the doses of 5 and 6% were chosen to evaluate the antidiabetic effect of sesame oil and pumpkin oil. The other doses were used to compare with the two selected doses in order to select the lower dose with the best effect.

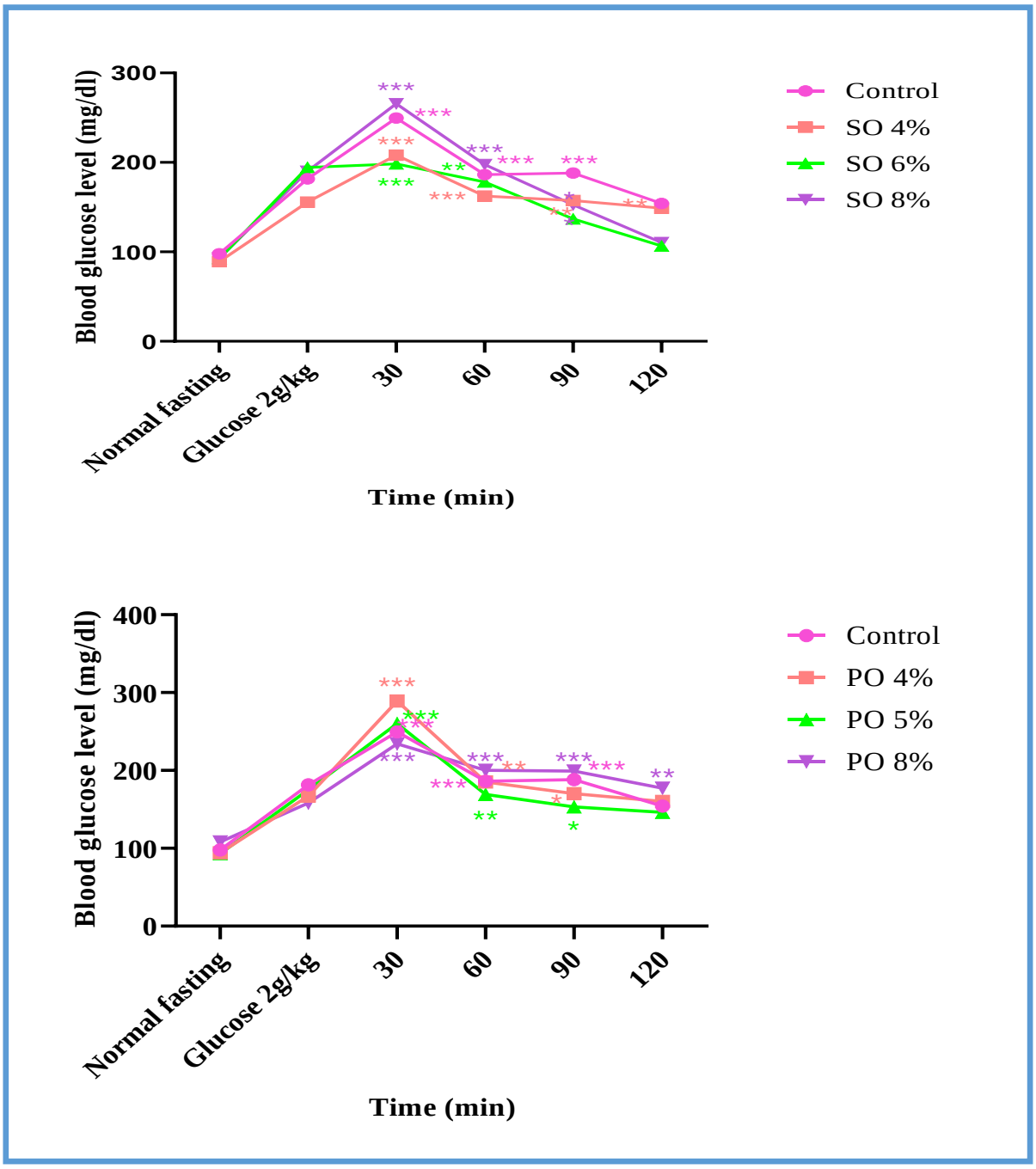


Figure 20. Effect of sesame and pumpkin oils on blood glucose level in hyperglycemic mice following 120 min of glucose administration. *p<0.05; **p<0.01; ***p<0.001 as compared to normal fasting blood glucose of each group.

3. Effect of treatment on body weight and food intake

Table 4 and **figures 21**, **22** indicate the variations in body weight and food consumption of the different groups. The findings obtained showed a very highly significant decrease ($p<0.001$) in body weight and highly food intake ($p<0.01$) of diabetic rats fed a sufficient zinc diet as compared to the non-diabetic animals. Meanwhile, zinc deficiency significantly ($p<0.05$) affected body weight associated with a decrease in food intake ($p<0.05$). Interestingly, sesame and pumpkin oils supplementation increased remarkably body weight ($p<0.05$) in DDZ groups, it was recorded also a highly significant improvement of food intake in the group supplemented with sesame oil ($p<0.001$).

Table 4. Effect of treatment on body weight in the experimental groups.

Parameters	**Experimental groups**						
Body weight (g)	ND	DSZ	DDZ	DSZ+SO	DDZ+SO	DSZ+PO	DDZ+PO
Initial	230.66±11.01	205.83±7.02	201.33±6.91	206.33±6.47	213.66±7.55	208.83±4.32	200.83±9.66
Final	246.2±7.78	168.2±5.73^{a2}	139.7±6.13^{b}	183.66±6.25	167.5±1.74^{d}	181.16±9.41	172.66±7.94^{d}

Values are presented as mean ± SEM and comparisons made between groups (n = 6 rats each group):

DSZ vs. ND: $^{a2}p<0.001$;

DDZ vs. DSZ: $^{b}p<0.05$;

DDZ+SO, DDZ+PO vs. DDZ: $^{d}p<0.05$.

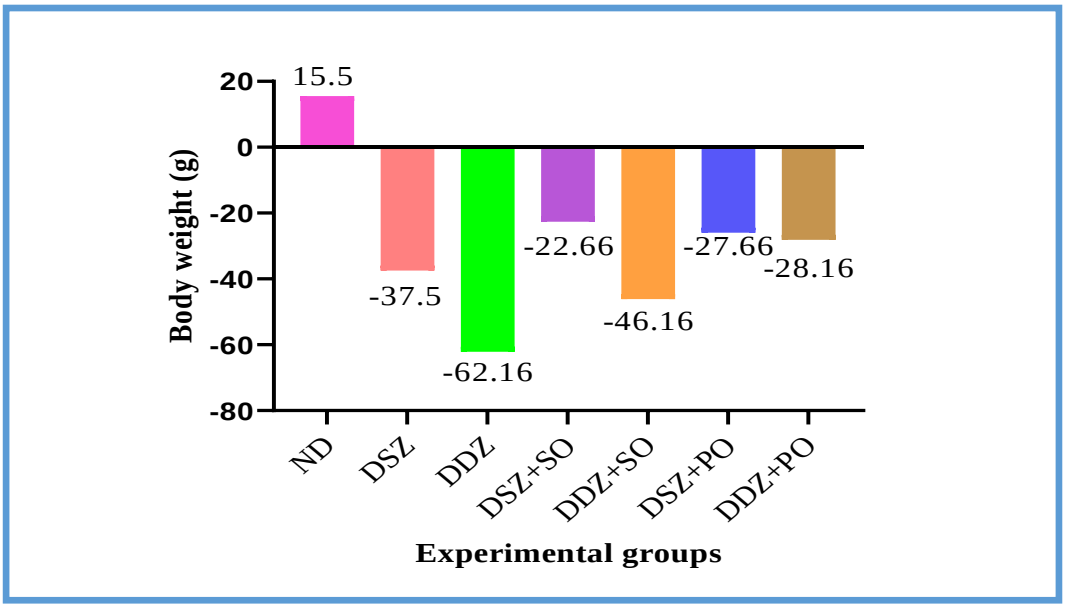


Figure 21. Body weight gain and loss of the groups at the end of treatment.

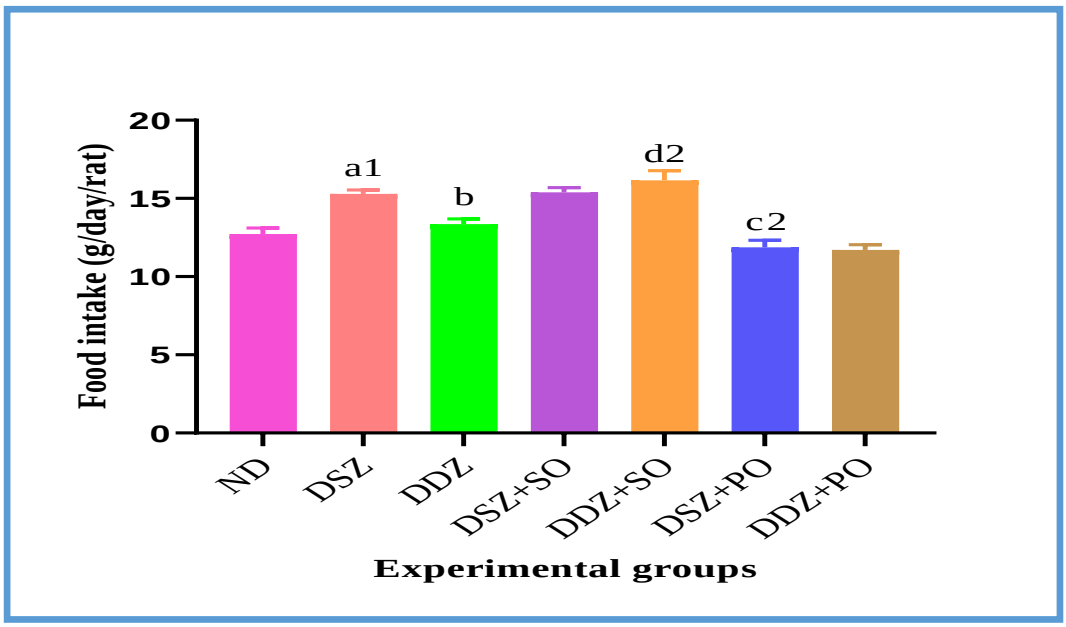


Figure 22. Variation in food intake in studies experimental groups.

Values are presented as mean ± SEM and comparisons made between groups (n = 6 rats each group):

DSZ vs. ND: $^{a1}p<0.01$;

DDZ vs. DSZ: $^{b}p<0.05$;

DSZ+PO vs. DSZ: $^{c2}p<0.001$

DDZ+SO vs. DDZ: $^{d2}p<0.001$.

4. Effect of treatment on carbohydrate metabolism

4.1. On glucides metabolism

4.1.1. On blood glucose

The blood glucose test results are shown in **table 5** and **figure 23**. Diabetic rats fed zinc sufficient diet showed a very significant increase in fasting glucose level as compared to the non-diabetic group ($p<0.001$). It was found also that zinc deficiency caused a slight increase in blood glucose, which is statistically insignificant compared to the DSZ group.

However, the treatment of DSZ and DDZ rats with sesame oil or pumpkin oil, induced improvements in this parameter, which was reflected in a very highly significant decrease ($p<0.001$) in serum glucose level in all treated groups compared to the DSZ and DDZ groups.

Table 5. Effect of treatment on fasting blood glucose in the experimental groups

Parameters	**Experimental groups**						
Blood glucose (mg/dl)	ND	DSZ	DDZ	DSZ+SO	DDZ+SO	DSZ+PO	DDZ+PO
Initial	96.0±4.09	466.66±63.6	482.83±46.1	467.5±68.43	470.0±62.4	536.33±40.71	485.33±48.29
Final	85.50±3.86	441.0±21.6^{a2}	492.3±15.85	250.3±35.9^{c2}	282±18.12^{d2}	181.16±9.4^{c2}	295.8±55.2^{d2}

Values are presented as mean ± SEM and comparisons made between groups (n=6 rats each group):

DSZ vs. ND: $^{a2}p<0.001$;

DSZ+SO, DSZ+PO vs. DSZ: $^{c2}p<0.001$

DDZ+SO, DDZ+PO vs. DDZ: $^{d2}p<0.001$

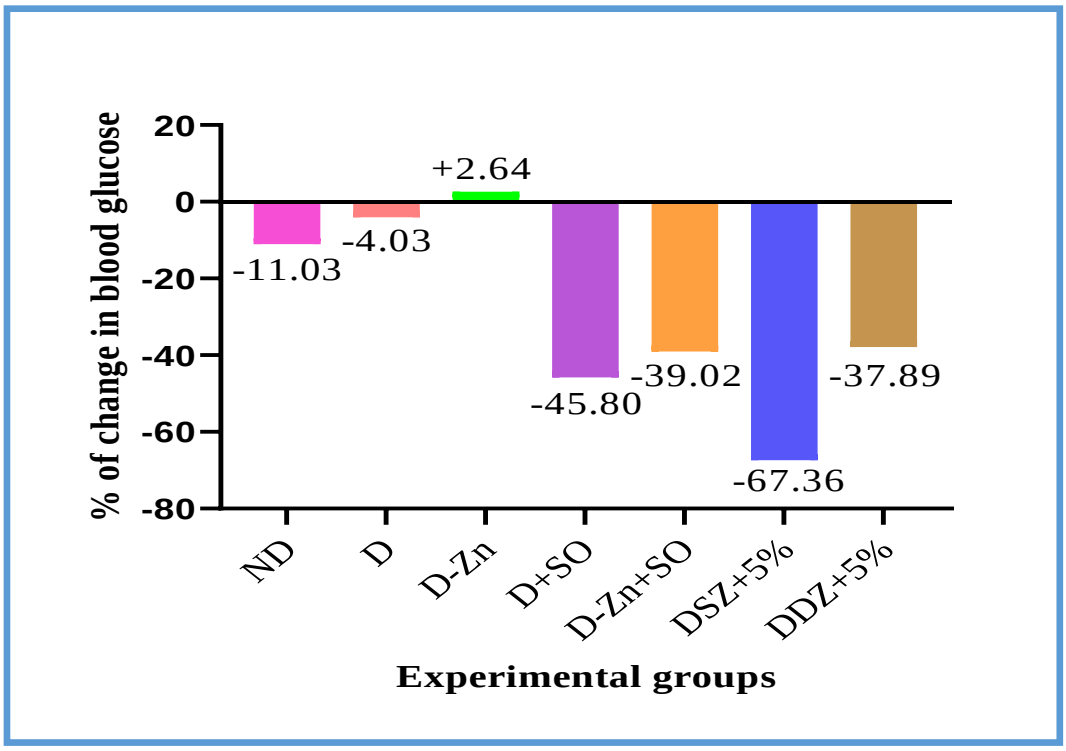


Figure 23. Percentage of change in blood glucose level in studied experimental groups.

4.2. On lipids metabolism

The findings of the lipid profile test indicated a significant metabolic disorder, as evidenced by a significant increase in cholesterol levels ($p<0.05$) in diabetic rats, zinc-deficient diabetic rats had also very high cholesterol and triglyceride levels ($p<0.001$) ($p< 0.01$) as compared to zinc-adequate diabetic rats **(Figure 24)**. On the other hand, administration of sesame oil or pumpkin oil to zinc-deficient diabetic rats significantly restored cholesterol and triglyceride levels ($p<0.001$).

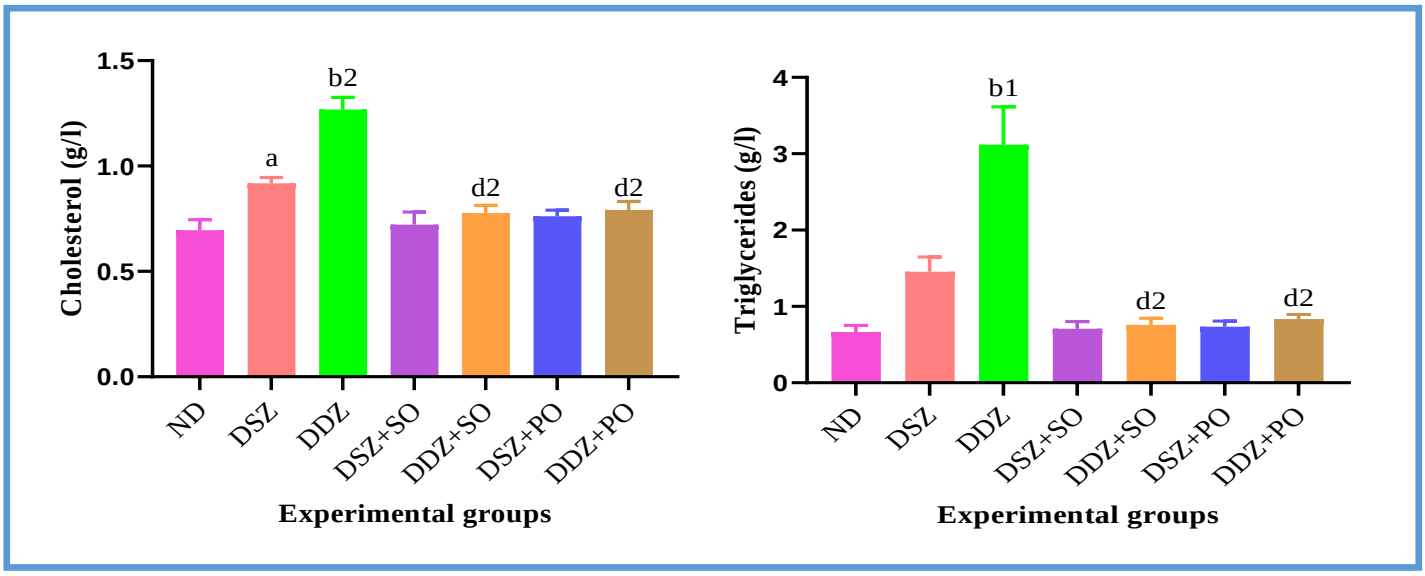


Figure 24. Serum concentration of cholesterol and triglycerides in the studied groups.

Values are presented as mean ± SEM and comparisons made between groups (n = 6 rats each group):

DSZ vs. ND: $^{a}p<0.05$;

DDZ vs. DSZ: $^{b1}p<0.01$; $^{b2}p<0.001$;

DDZ+SO, DDZ+PO vs. DDZ: $^{d2}p<0.001$.

4.3. On transaminases activities (GOT, GPT) and bilirubin level

According to the **figure 25**, diabetes was the cause of hepatic biomarker disturbances. In other words, a significant increase in GOT and GPT was observed in rats fed zinc sufficient diet ($p<0.001$), ($p<0.05$) respectively., a rise in the level of total bilirubin and direct bilirubin was also noticed, but it was not significant. Besides, GPT activity increased more obviously ($p<0.05$) further in zinc-deficient diabetic rats as compared to the DSZ group.

Supplementation with sesame oil or pumpkin oils in the DSZ group diet restored some of these previous parameters (GOT: $p<0.01$, $p<0.05$; total bilirubin: $p<0.05$). While, treatment DDZ group with the two oils showed remarkable efficacy, reflected in the lowering the levels of these parameters (GOT: $p<0.001$, GPT: $p<0.01$, $p<0.001$, total bilirubin: $p< 0.05$, $p<0.01$).

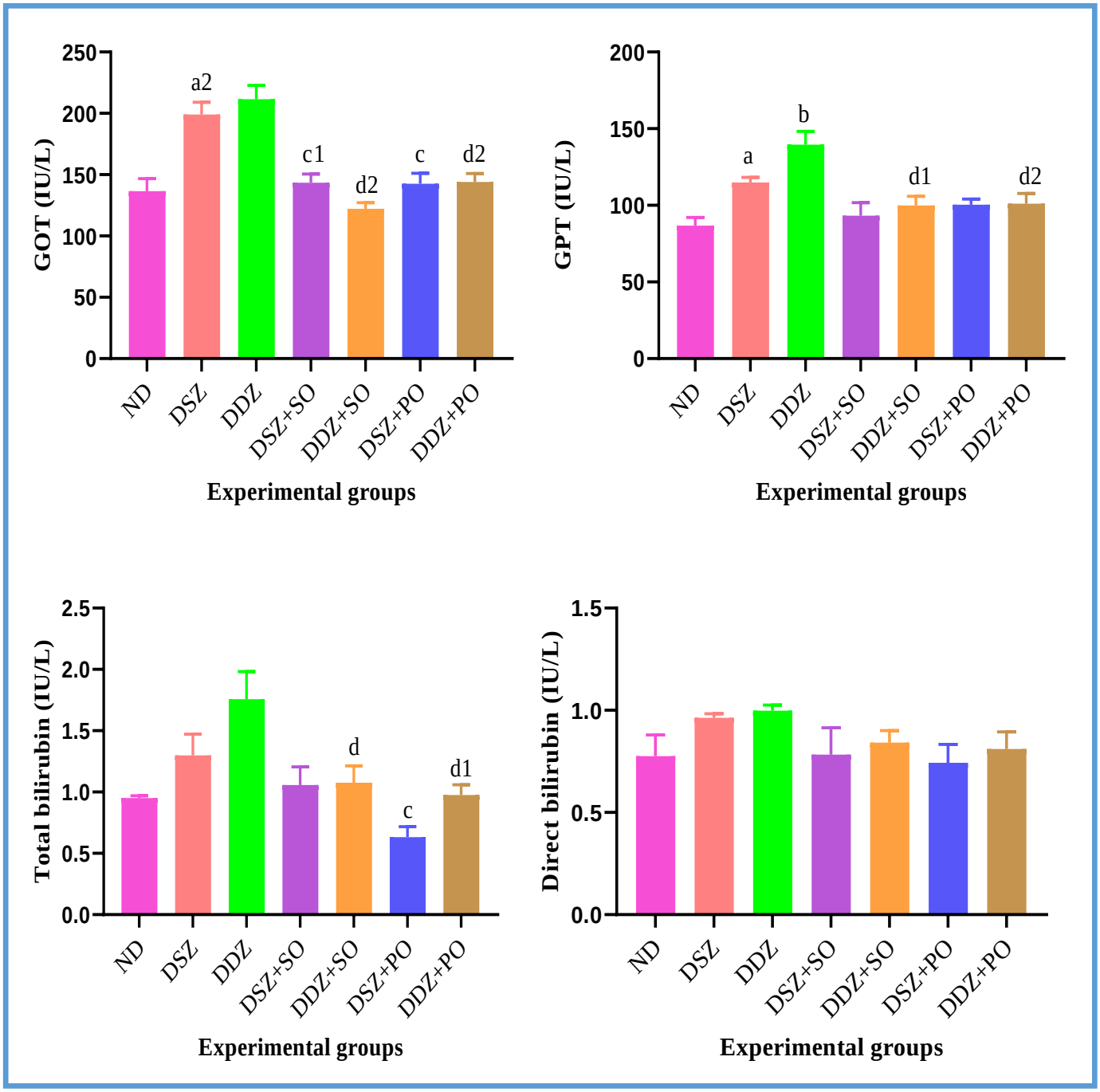


Figure 25. Variation GOT and GPT activities, both total and direct bilirubin concentrations in the studied experimental groups.

Values are presented as mean ± SEM and comparisons made between groups (n = 6 rats each group):

DSZ vs. ND: $^{a}p<0.05$, $^{a2}p<0.001$;

DDZ vs. DSZ: $^{b}p<0.05$;

DSZ+SO, DSZ+PO vs. DSZ: $^{c}p<0.05$, $^{c1}p<0.01$;

DDZ+SO, DDZ+PO vs. DDZ: $^{d}p<0.05$, $^{d1}p<0.01$, $^{d2}p<0.001$.

4.4. On protein metabolism

After four weeks of treatment, the results revealed that diabetes caused a very highly significant decrease in total serum protein and serum albumin concentrations in the diabetic group subjected to a zinc-sufficient diet compared to the non-diabetic group ($p<0.001$), ($p<0.01$) respectively. Furthermore, these protein disorders are associated with a significant increase in serum urea level ($p<0.05$), thereby a similar increase in uric acid and creatinine levels were also observed ($p<0.05$, $p<0.01$) respectively **(Figure 26)**.

In addition, zinc deficiency resulted a greater reduction in serum total protein and serum albumin, with an increase in urea, uric acid, and creatinine levels. However, treatment with sesame or pumpkin oils in the DSZ and DDZ groups showed a significant improvement in the protein profile, illustrated by a significant increase in total protein ($p<0.01$) in all treated groups, as well as a significant reduction in urea ($p<0.05$), creatinine ($p<0.01$) in DSZ group treated with sesame oil. It was noticed also highly decrease of both uric acid ($p<0.01$) and creatinine ($p<0.001$) in DSZ group treated with pumpkin oil.

While, the supplementation of sesame or pumpkin oils to DDZ group showed remarkable efficacy, reflected in the reducing of the levels of urea ($p<0.01$), uric acid ($p<0.01$) and creatinine ($p<0.01$) for sesame and uric acid ($p<0.001$), creatinine ($p<0.001$) for pumpkin.

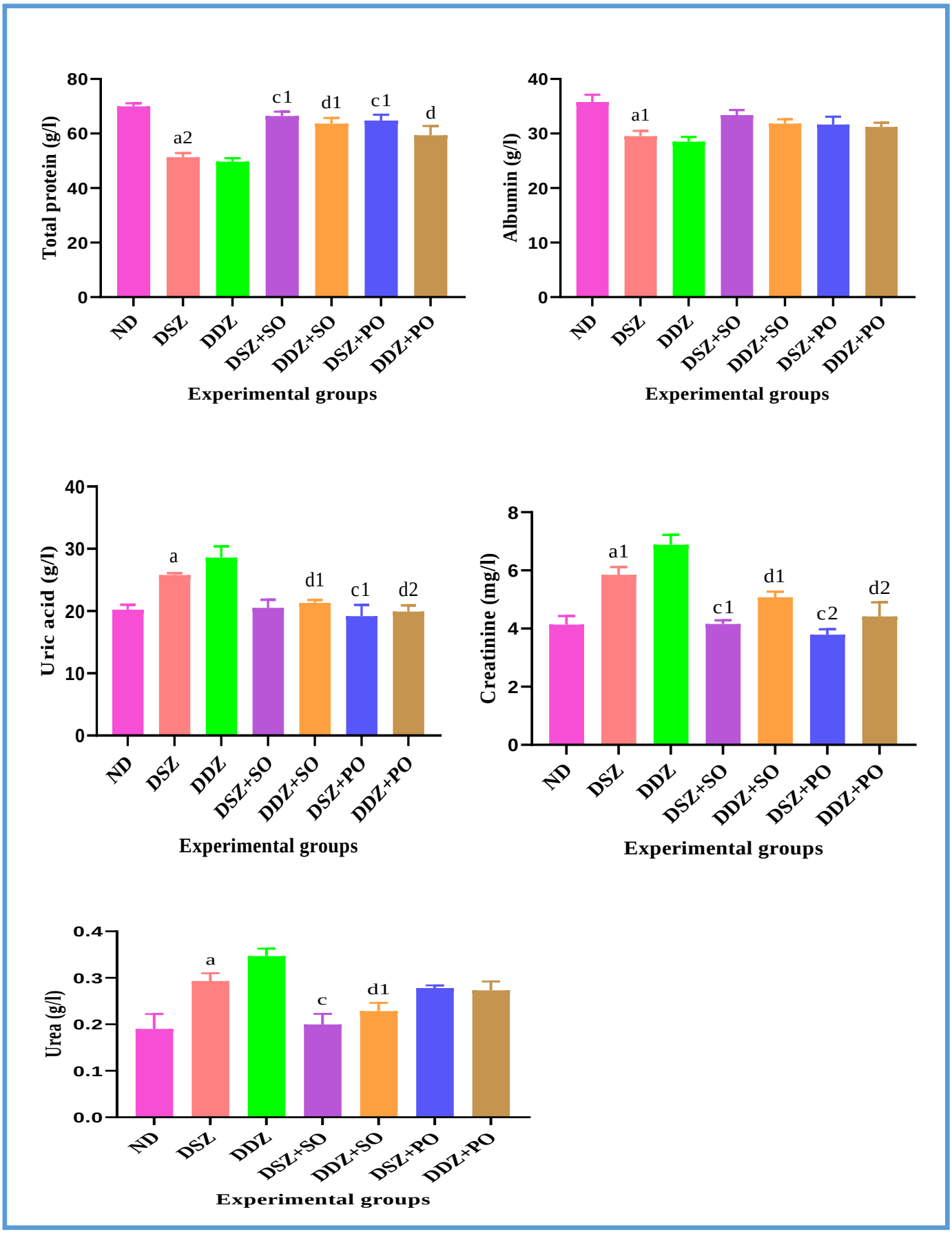


Figure 26. Serum concentration of total proteins, albumin, uric acid, creatinine, and urea in the studied experimental groups.

Values are presented as mean ± SEM and comparisons made between groups (n = 6 rats each group):

DSZ vs. ND: $^{a}p<0.05$, $^{a1}p<0.01$, $^{a2}p<0.001$;

DSZ+SO, DSZ+PO vs. DSZ: $^{c}p<0.05$**,** $^{c1}p<0.01$, $^{c1}p<0.001$;

DDZ+SO, DDZ+PO vs. DDZ: $^{d}p<0.05$, $^{d1}p<0.01$, $^{d2}p<0.001$.

5. Effect of treatment on zinc status and zinc-dependent enzymes

5.1. On the zinc status

As indicated in **figure 27**, the diabetic rats given sufficient zinc diet recorded a significant reduction zinc levels in the liver ($p<0.01$), pancreas ($p<0.01$) and femur ($p<0.001$) as compared to non-diabetic group.

In the same time, zinc-deficiency led to a further fall of zinc concentration in the femur and pancreas ($p<0.05$). Similarly, a similar reduction in hepatic zinc concentration was observed in the same group, but it remains statistically insignificant.

Administration sesame oil to DDZ group increased significantly zinc levels in the femur ($p<0.001$), pancreas ($p<0.001$), and liver ($p<0.01$).

Similarly, treatment with pumpkin oil in the same group led to improvements in zinc concentration in the femur and pancreas ($p<0.01$).

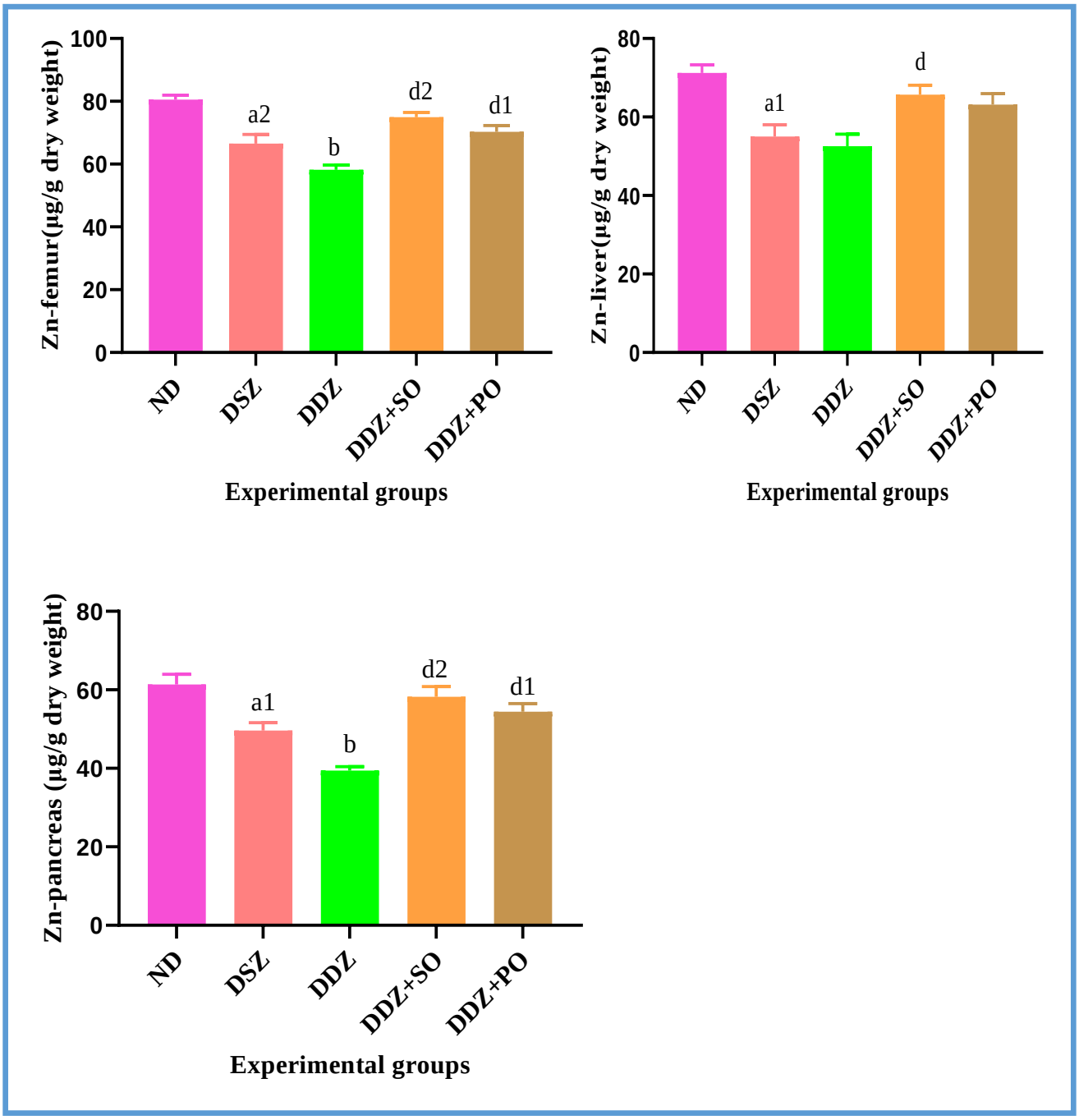


Figure 27. Zinc concentration in the femur, liver, and pancreas in the studied experimental groups.

Values are presented as mean ± SEM and comparisons made between groups (n = 6 rats each group):

DSZ vs. ND: $^{a1}p<0.01$, $^{a2}p<0.001$;

DDZ vs. DSZ: $^{b}p<0.05$;

DDZ+SO, DDZ+PO vs. DDZ: $^{d}p<0.05$, $^{d1}p<0.01$, $^{d2}p<0.001$.

5.2. On zinc-dependent enzymes activities

Lactate dehydrogenase (LDH) and amylase enzymes activities are illustrated in **figure 28**.

The results obtained indicated that diabetes caused a highly significant decrease in amylase activity ($p<0.001$). On the other hand, amylase and LDH activities were significantly reduced in the DDZ group ($p<0.05$, $p<0.001$) respectively as compared to the DSZ group. Whereas, the amylase and LDH activities were significantly increased ($p<0.05$, $p<0.01$) in diabetic rats subjected to a zinc-sufficient diet and treated with sesame oil. Moreover, the supplementation of sesame or pumpkin oils to the diet of the DDZ groups highly significant ameliorated the activity of these enzymes.

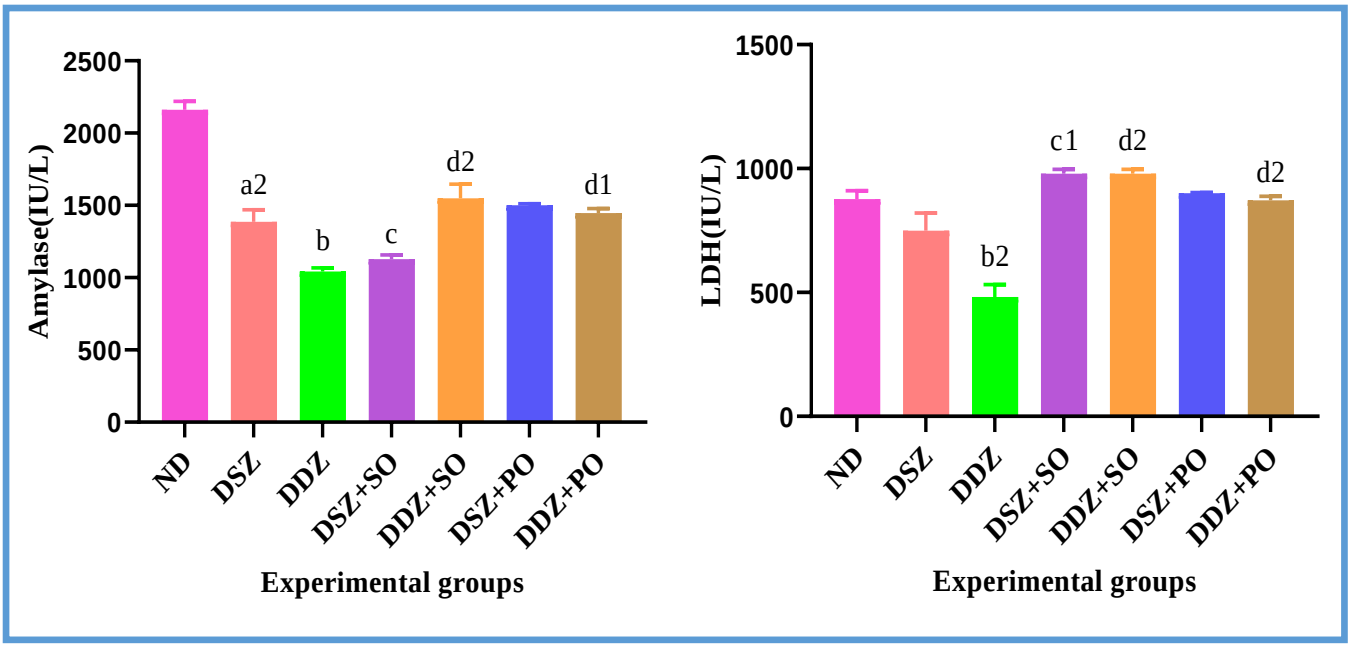


Figure 28. Variation in amylase and LDH activities in the studied experimental groups.

Values are presented as mean ± SEM and comparisons made between groups (n = 6 rats each group):

DSZ vs. ND: $^{a2}p<0.001$;

DDZ vs. DSZ: $^{b}p<0.05$, $^{b2}p<0.01$;

DSZ+SO, DSZ+PO vs. DSZ: $^{c}p<0.05$, $^{c1}p<0.01$;

DDZ+SO, DDZ+PO vs. DDZ: $^{d1}p<0.01$, $^{d2}p<0.001$.

6. Effect of treatment on oxidative stress parameters

6.1. On the concentration of malondialdehyde (MDA) and reduced glutathione (GSH)

Oxidative stress led to a highly significant decrease in GSH content ($p<0.001$) in the diabetic group subjected to a zinc sufficient diet as compared to the non-diabetic group. Meanwhile, depletion of antioxidant defenses in the liver was associated with significant lipid peroxidation, as evidenced by a very significant increase ($p<0.001$) MDA level **(figure 29)**. Moreover, zinc deficiency resulted further highly increase in MDA level accompanied with highly significant decline GSH ($p<0.001$) content.

On the other hand, a significant restoration in GSH and MDA level was observed in the DSZ rats treated with sesame oil or pumpkin oils ($p<0.01$, $p<0.05$). This improvement was greater in DDZ rats supplemented with the two oils ($p<0.001$, $p<0.01$).

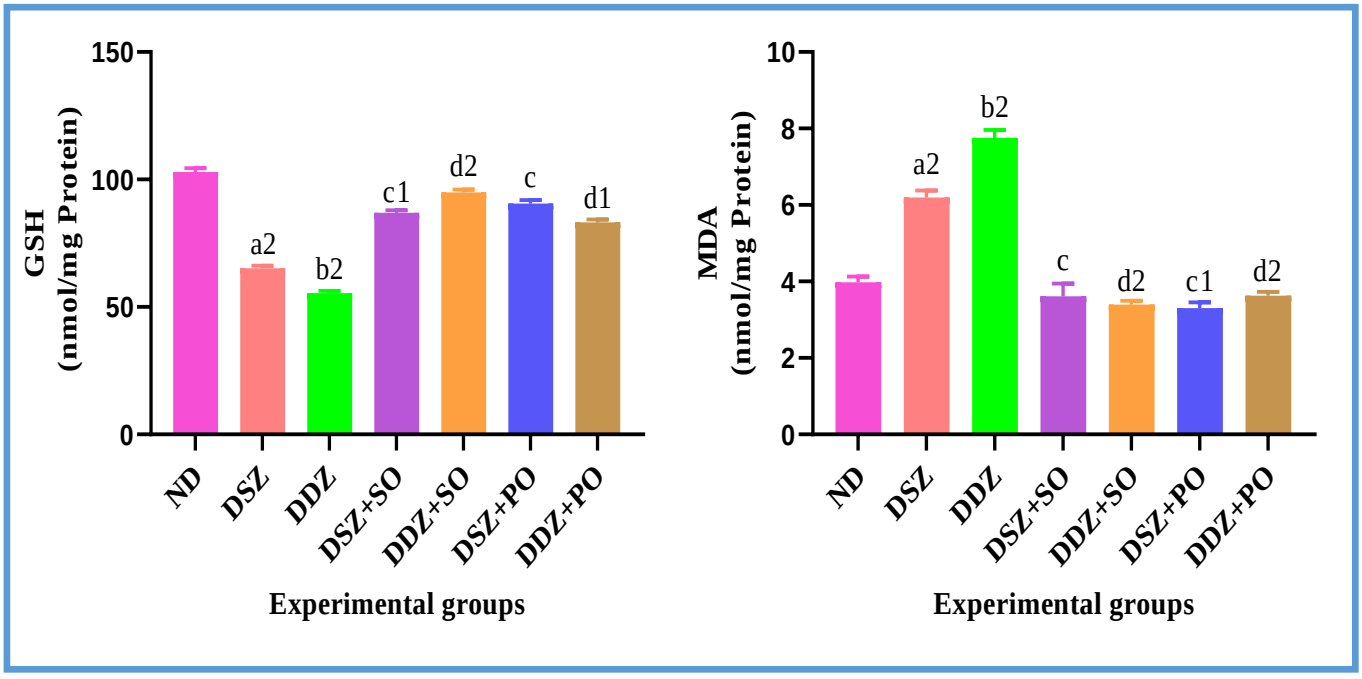


Figure 29. Variation in hepatic MDA and GSH levels in the studied experimental groups.

Values are presented as mean ± SEM and comparisons made between groups (n = 6 rats each group):

DSZ vs. ND: $^{a2}p<0.001$;

DDZ vs. DSZ: $^{b2}p<0.001$;

DSZ+SO, DSZ+PO vs. DSZ: $^{c}p<0.05$, $^{c1}p<0.01$;

DDZ+SO, DDZ+PO vs. DDZ: $^{d1}p<0.01$, $^{d2}p<0.001$.

6.2. On GST, GSH-Px, SOD and catalase activities

The enzymes activities involved in the antioxidant defense system are shown in **figure 30**.

Depending on the findings have been obtained, there is a highly significant decrease in glutathione S-Transferase ($p<0.001$), glutathione peroxidase ($p<0.001$), superoxide dismutase ($p<0.001$) and catalase ($p<0.01$) activities in the diabetic group as compared to the non-diabetic group.

Furthermore, GST and CAT activities were significantly ($p<0.05$, $P<0.001$) reduced due to zinc deficiency diet. However, the treatment of DSZ group with sesame oil significantly increased GSH-Px ($p<0.05$), CAT ($p<0.01$), SOD ($p<0.01$) activities.

The results indicated also that pumpkin oil had a positive effect, which mentioned by a significant increase ($p<0.05$) of GST and GSH-Px activities and highly significant ($p<0.001$) of CAT activity.

On the other hand, administration of sesame oil or pumpkin oils to zinc-deficient diabetic rats highly significant restored the activities of GST ($p<0.001$, $p<0.01$), GSH-Px, ($p<0.05$, $p<0.001$), SOD ($p<0.001$), and CAT ($p<0.001$) as compared to the DDZ group.

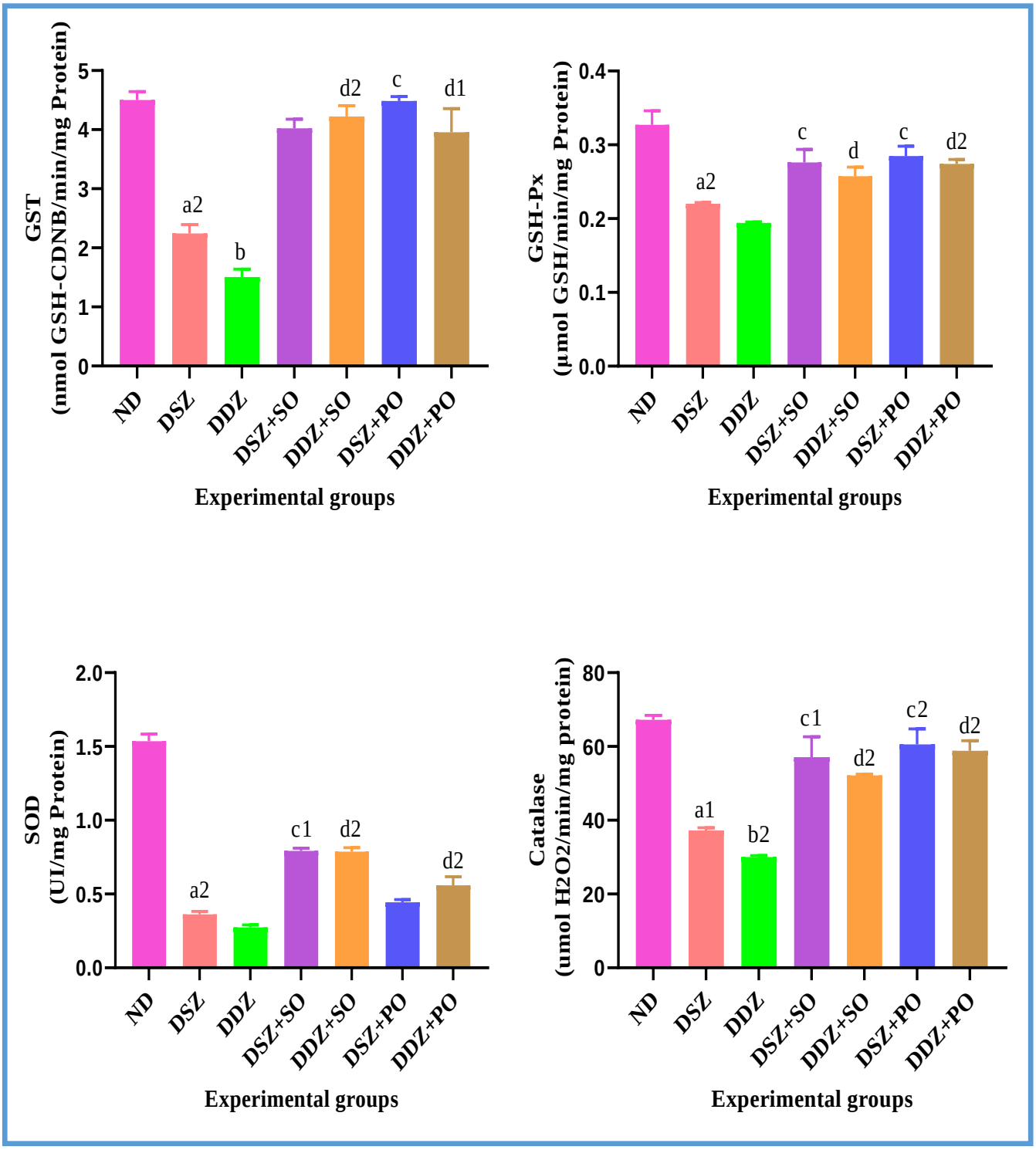


Figure 30. Variation in enzymatic activity of GST, GSH-Px, SOD and Catalase in the liver in the studied experimental groups.

Values are presented as mean ± SEM and comparisons made between groups (n = 6 rats each group):

DSZ vs. ND: $^{a2}p<0.001$;

DDZ vs. DSZ: $^{b}p<0.05$;

DSZ+SO, DSZ+PO vs. DSZ: $^{c}p<0.05$**,** $^{c1}p<0.01$, $^{c2}p<0.001$;

DDZ+SO, DDZ+PO vs. DDZ: $^{d}p<0.05$, $^{d1}p<0.01$, $^{d2}p<0.001$.

7. Histological study

7.1. Effect of treatment on pancreas histology

Figure 31 (A-G) represents the histopathological examination of the pancreas in the different experimental groups.

Microscopic observation of the pancreas of non-diabetic rats revealed normal pancreatic tissue architecture characterized by the presence of normal-sized islets of *Langerhans* (**figure 31** A). However, diabetic rats fed adequate zinc diet had a reduction in the size of the islets of *Langerhans* (**figure 31** B). In addition, greater changes in pancreatic tissue architecture, manifested by the absence of islets of *Langerhans*, damage to acinar cells, and thickening of septa, were noticed in diabetic rats fed zinc-deficient diet (**figure 31** C).

Whereas, the pancreatic section of zinc-deficient or sufficient diabetic rats treated with sesame oil or pumpkin oil showed an improvement in histological abnormalities such as the restored structure of pancreatic islet cells with reducing the intensity of atrophic change in acinar cells (**figures 31** D, E, F, and G).

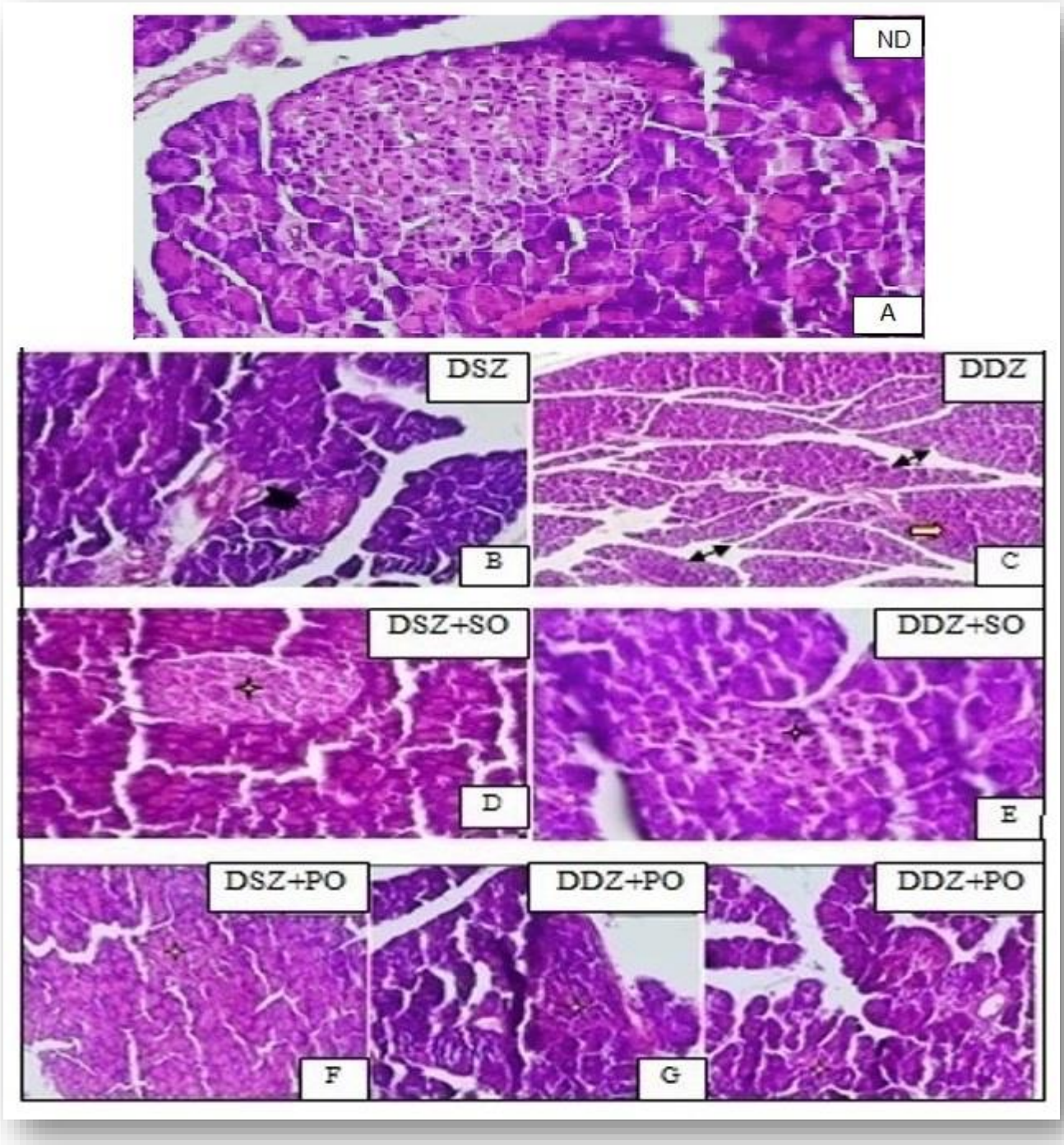


Figure 31. Pancreas histology stained with hematoxylin-eosin from the studied experimental groups.

Section of the pancreas from non-diabetic group (ND) shows the normal architecture of pancreatic islets (**A**). The pancreas from the diabetic group (DSZ) shows reduced *B*-cells size (➡) (**B**).The pancreas from the diabetic group (DDZ) shows entirely lost β-cells and degeneration of acinar cell, thickening of the septa (**C**) (↘↙). Sections of the pancreas from diabetic groups (DSZ/DDZ) treated with sesame or pumpkin oil respectively, indicates a regeneration of pancreatic islets and reduced damage to acinar cells (**D/E/F/G**) (✦). Optic microscopy (400 ×).

7.2. Effect of treatment on kidney histology

Figure 32 (A-G) illustrates the treatment effect on the kidney histology of the studied experimental groups.

In non-diabetic rats, the sections show the normal architecture of the renal tissue with normal size of glomerular and tubular structure (**figure 32** A). In comparison, the section of the diabetic group exhibited degeneration of glomeruli and tubules (**figure 32** B). These morphological alterations are also highlighted in diabetic animals fed zinc deficient diet, which are indicated by cystic dilatation of the renal tubules (**figure 32** C). However, treatment with sesame or pumpkin oils reduced these pathological changes, through the regeneration of glomeruli and tubules (**figures 32** D, E, F, and G).

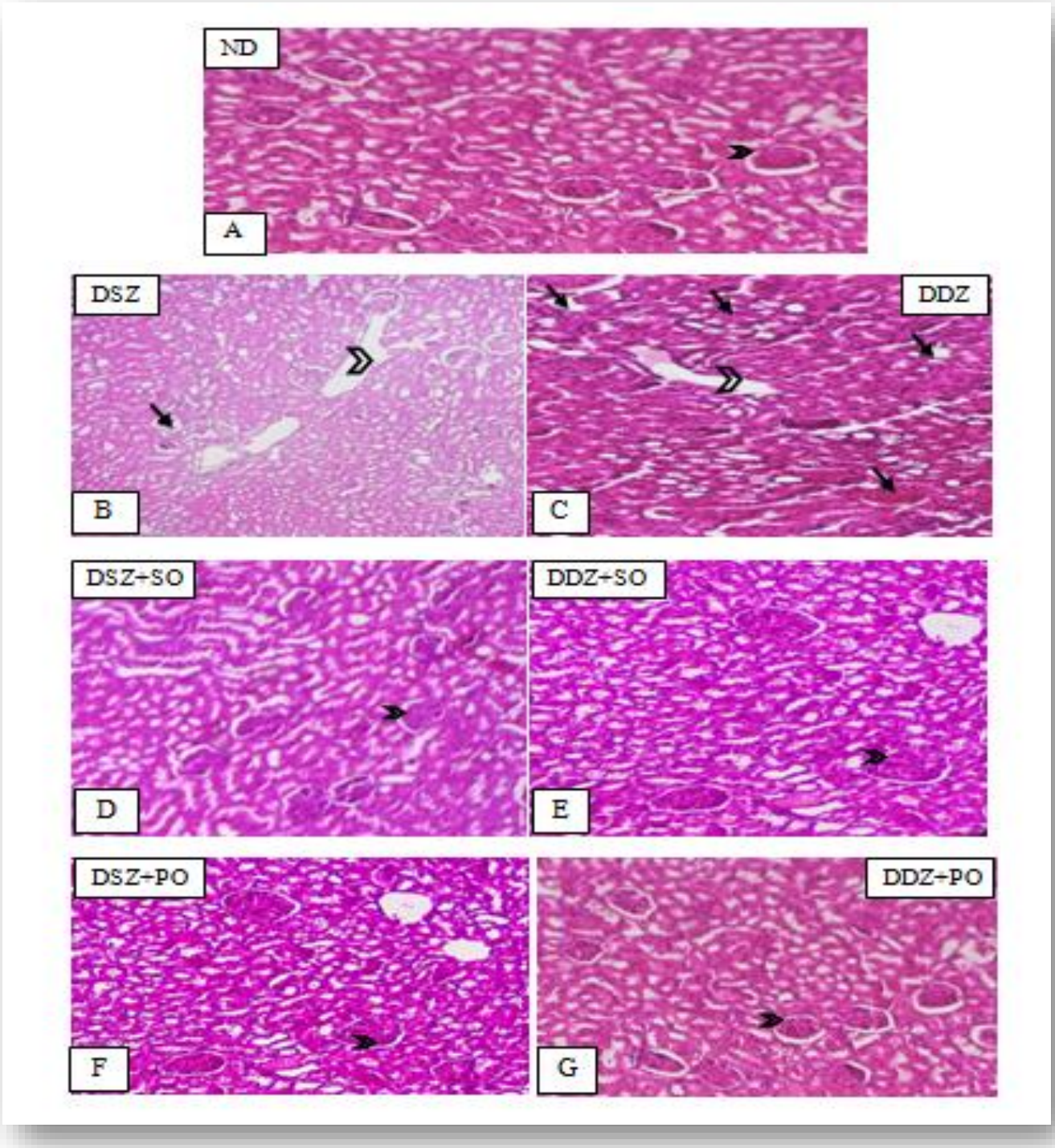


Figure 32. Kidney histology stained with hematoxylin-eosin from the studied experimental groups.

In non-diabetic rats, the section shows normal renal parenchyma with normal glomerular and normal tubular structure (**A**). In diabetic group, there is degeneration of glomeruli and tubules (↘) (**B**). In diabetic zinc deficient diet animals, kidney tissue shows severe degenerative changes of glomeruli (↘), congestion (Σ) with glomerular atrophy associated with dilatation of Bowman's space (↘) (**C**). In DSZ/ DDZ+SO/PO: treated rats. Kidney shows a moderate degree of improvement (➽) (**D**/**E**/**F**/**G**). Optic microscopy (400 ×).

Discussion

Discussion

Diabetes mellitus as a major health problem; is a set of multifactorial metabolic disorders, which contribute together to dysfunctions of the corresponding regulatory systems that affect human health and life quality. The excessive generation of free radicals, as well as a disruption of oxidative stress markers generally accompany this disease. Ultimately, this situation leads to multiple complications that constitute the real problem and diabetes development **(Mohamed et al., 2016)**.

In diabetes, different mechanisms have been proposed to explain the pathogenesis of the complications of this pathology, among which the disturbance in zinc homeostasis stands out. To put simply, the deficiency of this micronutrient increases the risk of diabetes mellitus development and its complications. Literature suggests that zinc plays an important role in β-cell function, insulin action and glucose homeostasis, thereby influencing the pathogenesis of diabetes. In addition, insufficient zinc can be considered one of the possible factors for diabetes complications development due to its antioxidant properties, since zinc is the main component of many antioxidant enzymes **(Hussein et al., 2021; Martins et al., 2022)**.

In that case, zinc is an essential catalytic and structural cofactor for many enzymes and other proteins. It has been known for many years that zinc deficiency causes increased oxidative stress and consequently, increased oxidative damage to DNA, proteins and lipids. Furthermore, many studies have indicated that zinc plays an indirect antioxidant role and that dietary inadequacy may contribute to human diseases such as cancer, diabetes, etc… **(Eide, 2011)**.

Recent years have been characterized by a special interest in the development of medicinal plants as sources of natural bioactive compounds. So, many studies are increasingly focused in the therapeutic effects of antioxidants of natural origin due to their promising biological capacities to protect the human body against the harmful effects of free radicals; delaying the development of many chronic diseases and avoiding rancidity of foods through lipid oxidation **(El-Haci et al., 2012; Hagos et al., 2023)**.

Therefore, the objective of this investigation is to study diabetes mellitus in dietary zinc deficiency condition and explore methods for the treatment based on medicinal plants. To achieve this goal, two plants sesame and pumpkin were evaluated their antioxidant and antidiabetic effects.

The result of the quantitative phytochemical assay of the sesame oil indicated that the total polyphenol content and flavonoids were found in high concentration. However, Saponins and tannins were presented in a little concentration. The levels of these compounds were relatively lower as compared to the findings, which were found by Sani et al **(2013)**.

Pumpkin oil contained also a moderately high concentration of total polyphenols and flavonoid content. However, the saponins and tannins concentrations were few. This richness in polyphenols gives the oils antioxidant capacity, since the antioxidant effect of oils is based on their content of phenolic compounds **(Akin et al., 2018; Kulaitienė et al., 2018; Esmaeilzadeh Kenari and Razavi, 2022)**.

The DPPH antioxidant activity of oils indicated their ability to release hydrogen atoms. Moreover, the comparison of the IC_{50} to the positive standard showed that the oils have considerable or even excellent antioxidant activity. These findings suggest that sesame and pumpkin oils contain free radical scavengers acting as primary antioxidants, which supposed that the action of these antioxidants due to their capacity to give hydrogen atoms **(Mikołajczak et al., 2021; Esmaeilzadeh Kenari and Razavi, 2022)**.

The spectrophotometric evaluation of antioxidant capacity by the β-carotene bleaching assay, which depends on the yellow color losses of beta-carotene due to its interaction with radicals formed by the linoleic acid oxidation in an emulsion. This rate of bleaching of β-carotene may decrease in the presence of antioxidants. Additionally, free radicals play the role of mediator in the β-carotene bleaching phenomenon. Over reaction time, and in the absence of antioxidants, β-carotene undergoes rapid decolonization resulting in reduced absorption of the test solution. According to Liyana-Pathirana et al **(2006)**, an extract that delays or inhibits β-carotene bleaching can be described as a free radical scavenger and a primary antioxidant. Furthermore, the test of inhibition of the oxidation of linoleic acid coupled with that of β-carotene appears very useful as a mimetic model of lipid peroxidation in biological membranes **(Ferreria et al., 2006)**. Thus, from these results, it appeared that the sesame and pumpkin oils presented interesting activity compared to the standard antioxidant (BHT).

In the current study, the body weight of diabetic rats fed an adequate zinc diet was effectively reduced as compared to the non-diabetic group. The reason for this reduction could be due to the body's inability to use carbohydrates as an energy source. On the other hand, this loss might be due to the excessive degradation of fats and proteins **(Sukanya et al., 2020)**.

Meanwhile, diabetic rats fed zinc deficient diet had less body weight gain in comparison with rats fed zinc sufficient diet. Certainly, this weight loss was associated with reduced food intake. This is consistent with previously published reports **(Hamdiken et al., 2018; Tebboub and Kechrid[a], 2021)**. Zinc deficiency has been reported to have a dramatic effect on the body weight. It is well known to cause taste and appetite disturbances, which are often associated with impaired gustine activity, which is a dependent zinc enzyme. So, these disorders in return lead to a decrease in food intake and stimulate early satiety, which may affect growth rate **(Tebboub and Kechrid[b], 2021)**.

However, sesame oil as a dietary supplement attenuated significantly the reduction of weight loss via increased food intake in diabetic rats fed zinc deficient diet than those non-treated one. These effects may be explained by the capacity of sesame oil to reduce hyperglycemia in these animals, and the antioxidative properties of the bioactive compounds present in this oil and their ability to suppress free radicals, which lead to body weight increase **(Haidari, 2016; Ibrahiem, 2016)**. In this context, Bhuvaneswari et al **(2012)** reported that the sesame oil stimulates protein synthesis and inhibits proteolysis, which may be related to the anabolic effect of insulin. Hence, an improving body weight is arguably the side effect of this oil. Supplementation with pumpkin oil revealed also a drastic restoration in body weight of the diabetic group given deficient zinc diet. Despite, the improvement in body weight of rats, there was a further remarkable decrease in food consumption following pumpkin oil treatment. This can be explained by it containing: tannins and gallic acid which responsible of polyphagia diminution **(Abd-elnoor, 2019; Rouag et al., 2020)**.

Zinc concentration in femur, liver, and pancreas tissues showed a noticeable decrease in both diabetic rats fed either zinc-sufficient or zinc-deficient diet. In fact, many studies indicated that diabetes can affect zinc status in many ways. In other words, body zinc content is usually related to altered intestinal absorption and urinary excretion, which is also another factor responsible for this depletion, as a result of renal dysfunction **(Tomat et al., 2011; Sinha and Sen, 2014; Othman et al., 2020)**. These results were correlated with the histological study of kidney, which revealed remarkable histopathological changes in diabetic animals.

On the other hand, the increase in zinc levels in diabetic rats fed a low-zinc diet supplemented with sesame or pumpkin oils was probably due to the hypoglycemic effect of these oils, which can be considered as one of the main factors in maintaining kidney function against the harmful effects of free radicals. Consequently, this might lead to a reduction in excessive zinc loss in urine. These ameliorations were consistent with the results of histological examination of the kidneys, which mentioned that animals benefited from sesame or pumpkin oils supplementation predominantly have a preserved histological appearance.

Diabetes induced a significant increase in blood glucose level in diabetic rats fed a zinc-sufficient diet compared to non-diabetic ones. STZ injection reduced insulin secretion following a destruction in pancreatic islet β cells, which led to this metabolic disorder **(Wu and Yan, 2015; Al-Jaghthmi and Zeid, 2020)**. Additionally, hyperglycemia could result from two fundamental mechanisms, on the first hand by endogenous overproduction of glucose (gluconeogenesis and glycogenolysis), on the other hand, by the disruption in the utilization of glucose by peripheral tissues **(Derouiche and Kechrid, 2016)**.

Furthermore, it was found also a slight increase in blood glucose of the diabetic animals fed a low zinc diet, which is statistically insignificant compared to those under adequate zinc diet. Indeed, it has been suggested that zinc deficiency could be involved in saccharides metabolism disorders; these seem to be correlated with the histological alterations of the pancreas of this group **(Tebboub, 2019)**.

Treatment of zinc-sufficient or zinc deficient rats with sesame or pumpkin oils induced considerable obvious decrease in serum glucose level.

The blood sugar-lowering effect of sesame oil could be explained by its high monounsaturated fatty acid (MUFA) content. MUFA has the ability to protect β-cells from death and improve insulin sensitivity **(Alamri, 2019)**. In addition, the presence of fat-soluble lignans, especially sesamin, sesamolin and sesamol, increase the potential regulation of blood sugar level through numerous mechanisms, including glycogen synthase (UDP-glucose-glycogen glucosyltransferase), a key enzyme involved in the liver synthesis of glycogen. On the other hand, insulin secretion could be mainly induced by improving gene expression involved in glucose uptake and insulin signal transduction pathways **(Atefi et al., 2022)**. Interestingly, these multiple effects were demonstrated by the histopathological improvements of the pancreas.

At the same time, it seems that the anti-diabetic potential of pumpkin oil is attributed to its compounds including tocopherol isomers (α, β, γ and δ), which have the capacity of lowering blood glucose level and amelioration pancreatic characteristics through its antioxidant activities **(Perez Gutierrez, 2016)**. Tocopherols have been reported to be effective in the enhancing peripheral glucose uptake and increasing insulin secretion **(Bharti et al., 2013; Boaduo et al., 2014)**. Additionally, a number of recent studies proved the hypoglycemic effects of pumpkin polysaccharides through affecting glucose metabolism enzymes activities (alpha-amylase and alpha-glucosidase inhibition), promote hepatic glycogen synthesis, inhibit gluconeogenesis and increase *β*-cell islet insulin secretion **(Song and Sun, 2017; Khan et al., 2019;Ji et al., 2023)**.

Transaminases as biomarkers of liver function; are important enzymes in the body's biological processes. These enzymes are involved in the conversion of amino acids into alpha-ketoacids. Diabetes has been reported to cause the breakdown of body proteins into amino acids, which increases the intensity of gluconeogenesis. Thus, under the action of transaminases, these amino acids can be converted into carboxylic acid compounds such as α-ketoglutaric acid and pyruvic acid and finally into glucose, implying strong enzymatic activities of GOT and GPT **(Felig et al., 1970; Nain et al., 2012)**. The increase in transaminases in diabetic rats fed zinc-deficient diet confirms the work of Greeley and Sandstead **(1983)**,

who found evidence of reduced oxidation of the carbon chain of alanine, when zinc was restricted, leading to alanine accumulation in blood.

Interestingly, GOT and GPT activities in diabetic rats fed either sufficient or insufficient zinc diet were restored following administration of sesame or pumpkin oils. This means that both oils can play a mitigating role in liver function via decreasing blood glucose level, subsequently, reduce protein breakdown, the accumulation of amino acids in blood. Therefore, the transaminases activities were suppressed **(Moller and Nair, 2008)**.

In addition, the analysis of liver function parameters did not show a significant increase in serum levels of total and direct bilirubin in diabetic rats given diets either rich or poor in zinc. Nevertheless, supplementation of sesame and pumpkin oil attenuated significantly total bilirubin levels. This amelioration reflects the hepatoprotective properties of these oils. Otherwise, the increased concentrations of total protein and especially serum albumin biosynthesis might be attributed in the recovery of bilirubin levels.

Zn is a central component of several metalloenzymes, including lactate dehydrogenase and amylase **(Jing et al., 2009; Prasad, 2014)**. In the present study, diabetic animals exhibited a significant decrease in serum amylase activity. Meanwhile LDH and amylase activities decreased more in zinc deficient diabetic animals. The decline activities of these enzymes are undoubtedly due to the decrease in zinc concentration **(Derouiche and Kechrid, 2016)**. Similarly, Sun et al **(2006)** and Jing et al **(2009)** reported that amylase and LDH are metalloenzymes that needs zinc as a co-factor for their activities, and that any variation in this metal status could negatively affect the activity of these enzymes.

In contrast, treatment with sesame or pumpkin oils rectified the activity of these zinc-dependent enzymes. However, the mechanism by which sesame or pumpkin oil improved LDH and amylase activities remains unclear, but is likely due to their antioxidative capacities, and restoration of zinc level **(Mohamed and Wakwak, 2014; Dotto and Chacha, 2020)**.

Lipid profile assay has become of crucial importance through its use in the management of several diseases **(Akuyam et al., 2007)**. Diabetes mellitus is related to hyperlipidemia and causes profound abnormalities in lipid concentration and composition, these latter represent an important risk factor for cardiovascular diseases. Many studies have reported that cardiovascular complications associated with diabetes are referred to variations in lipid metabolism **(Akuyam et al., 2007; Qi et al., 2008)**.

In this investigation, an alteration of lipid balance especially cholesterol was demonstrated in diabetic rats receiving a zinc-sufficient diet.

In diabetes case, insulin deficiency could affect the activity of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase a key enzyme for cholesterol biosynthesis. In other words, the inhibition leads hypercholesterolemia. On the other hand, the elevated concentration of cholesterol observed in diabetic animals might be also due to acute insulin deficiency that causes an increase in free fatty acids from adipose tissue, which results in increased production of cholesterol-rich LDL particles **(Tebboub, 2019)**. Moreover, the diabetic rats fed low zinc diet; the levels of cholesterol and triglyceride were higher than that of those receiving a sufficient zinc diet. This was likely as a result of zinc variation that provoked catabolism of lipid stores due to an increase demand of energy **(Kechrid et al., 2012)**.

However, after the supplementation of sesame or pumpkin oils, cholesterol and triglyceride levels in zinc-deficient diabetic rats appeared close to their normal values. This indicated the interesting blood lipid-lowering potential of these oils.

Previous studies have reported that sesamin which is a major lignan of sesame oil, is probably responsible for the decline in these parameters. In brief, sesamin is effective in lipid metabolism by suppressing lipid synthesis and increasing fatty acid oxidation **(Li et al., 2020)**. In this regard, these effects are associated with the down and up-regulation of several enzymes and transcription factors **(Liang et al., 2015; Kim et al., 2017)**. In other words, these effects lead to a decrease in the activity and gene expression of enzymes and transcription factors involved in fatty acid synthesis and increase the activity and gene expression of the fat breakdown transcription factor in the liver. In addition, they found that the hypocholesterolemic effect of sesamin inhibits the intestinal absorption of cholesterol, which increases the secretion of cholesterol into bile **(Ajayi et al., 2012; Devarajan et al., 2016)**. Furthermore, numerous studies reported the hypolipidemic effect of sesame oil is due to its richness in monounsaturated and polyunsaturated fatty acids **(Haidari et al., 2016; Sankar et al., 2011)**.

Pumpkin oil effectively reduces cholesterol synthesis and/or increase cholesterol catabolism in the liver, via the presence of unsaturated fatty acids. Many studies have proven that polyunsaturated fatty acids linoleic acid and oleic acid lowering cholesterol level **(Elsenousy et al., 2019)**. Moreover, the hypolipidemic activity of pumpkin via phytosterols and phenolic compounds could be due to inhibition cholesterol absorption.

Above all the lipid-lowering properties of pumpkin was partly attributed to its fiber composition. Previous data suggested that a high-fiber diet lowers triglyceride levels by inhibiting lipogenesis in liver. **(Sedigheh et al., 2011; Ramadan et al., 2016; Abd- Elnoor, 2019; Elsenousy et al., 2019; Hussain et al., 2022)**.

Under severe oxidative stress conditions, the formation of free radicals leads to a change in the protein. Proteins can be directly damaged by specific interactions of free radicals with particularly sensitive amino acids **(Hassan et al., 2015)**.

The finding of the current study revealed a significant decrease in total protein and serum albumin levels, but an increase in uric acid, urea and creatinine in diabetic rats. This could be due to reduced rate of protein biosynthesis, increased rate of protein catabolism and decreased amino acid uptake **(Al-Jaghthmi and Zeid, 2020)**. Indeed, these results have already consistent with those described by Hamdiken et al **(2017)** under analogous experimental conditions.

Kamal **(2014)** confirmed that increment in urea, uric acid and serum creatinine levels are considered as significant markers of renal dysfunction (glomerular filtration), which directly correlated to hyperglycemia. Diabetes is a crucial factor in this progressive kidney damage. At the same time, the decline in total protein and albumin levels presumably also due to microproteinuria and microalbuminuria, which are important clinical markers of diabetic nephropathy **(Sreekutty and Mini, 2016)**.

The results showed that diabetic animals treated with sesame oil led an increase in level of total protein, with a decrease in urea, uric acid and creatinine. Similarly, diabetic rats treated with pumpkin oil, a significant rise in total protein level, with a decline in the uric acid and creatinine concentrations were noticed. The markedly reduction in the levels of these parameters confirm the ability of sesame and pumpkin oils in controlling blood glucose level and thus reducing protein catabolism. Additionally, this reflects also the protective role of these oils in preservation kidney dysfunction. In fact, these data are supported by the findings of the histological examination of kidneys and the result of Aslam et al **(2019)**.

Diabetes and its complications are closely related to oxidative stress. In that case, the latter plays a crucial role in the development and progression of this pathology **(Zhang et al., 2020)**. The findings obtained clearly indicated the status of oxidative stress in the diabetic group as compared to the non-diabetic group. In brief, the high level of MDA in the liver is a biomarker of lipid peroxidation in diabetes. Several pathways generally produce ROS under hyperglycemia condition. This oxidative stress is not only represented by increased free radical production, but also by the alteration of the redox balance and cellular constituents **(Rains and Jain, 2011)**. ROS react with macromolecules and produce denatured, modified, and non-functional molecules, which induce an abnormal oxidative circumstance. This state of oxidative

stress provokes cellular and tissue damage, with an alteration in the antioxidant defense system leading to the worsening of the disease **(Asmat et al., 2016)**.

GSH, a major endogenous antioxidant that acts as the most abundant cellular thiol resource and provides a suitable system to maintain the cellular redox status, especially in diabetic patients **(Mobasher et al., 2020)**.

In this study, a decrease in glutathione concentration was observed in the diabetic group. The decline in GSH content might be due to the higher consumption of glutathione as a co-substrate by GSH-Px and GR in detoxification reaction **(Krishnan et al., 2019)**. Another explanation, the decreased level of intracellular NADPH via the polyol pathway reduced the GSH content as required for GSH regeneration **(Sekiou et al., 2021)**.

Antioxidant enzymes are the first line of defense against ROS-induced oxidation in the organism including SOD, GSH-Px, and CAT, which are the three main scavenging enzymes that remove toxic free radicals in vivo. Indeed, the induction of diabetes changed obviously the activity of these enzymes.

So, the findings of this investigation could be explained, by the increased production of free radicals following the glucose auto-oxidation and the non-enzymatic glycation of these enzymes due to sustained hyperglycemia **(Qusti et al., 2016; Hamdiken et al., 2018)**. Furthermore, the impaired of hepatic antioxidant enzymes activities could probably be linked to overproduction and accumulation of hydrogen peroxide and superoxide radicals in tissues, which affected the structure and function of these enzymes **(Tebboub and Kechrid[a], 2019)**.

Zinc is known to be an important component of many enzymes and proteins involved in antioxidant defense system **(Özcelik et al., 2012)**. Therefore, its deficiency undoubtedly leads to increased production of free radicals and depletion of antioxidant capacity **(Yousef et al., 2002)**. According to the results obtained, elevated MDA level was detected in rats given zinc deficient diet; this suggests a deleterious effect of zinc deficiency in increasing lipid peroxidation **(Hidalgo et al., 2002)**. In addition, a drastic decrease in GSH, CAT and GST activities were also remarked.

Nevertheless, the inclusion of sesame or pumpkin oils in the diet contributed a high decrease in MDA level and an increase in GSH concentration, CAT, GST, SOD, and GSH-PX activities. The antioxidant activity of sesame oil has been shown to be able to inhibit lipid peroxidation and reduce membrane damage **(Li et al., 2020)**. Otherwise, the effective antioxidant capacity of the sesame oil was owing to its richness in lignans and tocopherols. Likewise, many studies have indicated the antioxidant and radical scavenging property of this oil is attributed to the lignans component **(Wan et al., 2015)**.

Besides that, wide ranges of phenolic compounds such as phenolic acids, flavonoids, and tannins have also been reported to possess antioxidant capacities **(Mahendra Kumar and Singh, 2015)**. It is clear that the bioactive components of sesame oil can prevent harmful of ROS by enhancing cell membrane stability as well as by controlling the radicals generation, by either scavenging them or promoting their decomposition. Alternatively, it may be assumed that the improvement in the endogenous antioxidant enzymes was due to decreased utilization of these enzymes. In addition, the reason for the significant increase in GSH level may be a result of sesame oil being rich in vitamin E **(Moghtaderi et al., 2020)**.

At the same time, pumpkin oil has shown high effectiveness in improving the function of the antioxidant defense system. This protective mechanism could be related to the antioxidant power and antiradical properties of the phenolic group **(Paul et al., 2020)**. These results are consistent with several studies on the antioxidant effects of pumpkin oil, which showed obviously its strong antioxidant potential due to the high phenolic and vitamin E contents **(Kulaitienė et al., 2018)**. Abou-Zeid et al **(2018)** pointed out that the antioxidant capacity of pumpkin oil might be due to the several compounds radical scavenging capacity: including carotenoids, tocopherols, ascorbic acid, and zinc.

Conclusion and perspectives

The development of scientific knowledge regarding the therapeutic properties of bioactive compounds in traditionally used plants has necessitated the development of new antidiabetic treatments. This encouraged us to carry out this investigation, which allowed us to find out whether dietary supplementation with sesame and pumpkin oils, throughout the experiment, could limit the harmful consequences of zinc deficiency in diabetes state. Considering our findings, the main conclusions are as follows:

- The quantitative phytochemical study revealed that the two oils studied are rich in flavonoids and total polyphenols, with a low concentration of condensed tannins and saponins.
- Both oils have antioxidant capacity demonstrated by using DPPH free radical scavenging and β-carotene bleaching.
- Zinc deficiency led to physiological disorders, which were manifested by body weight loss and a reduction in food intake.
- A disorder of carbohydrate metabolism characterized by hyperglycemia throughout treatment.
- A significant disorder in lipid metabolism was also illustrated by an increase in cholesterol and triglyceride levels.
- Dysfunction of liver, which was characterized by an elevation in transaminase activities (GOT, GPT).
- Disturbances of protein metabolism, which was characterized by a significant decrease in total protein and albumin levels and accompanied by an increase of creatinine, uric acid, and urea levels in diabetic rats receiving an adequate zinc-diet.
- Disorders in zinc status were noted by a decrease in zinc concentration in the different tissues including liver, pancreas, and femur in diabetic rats received zinc-deficient diet.

Regarding oxidative stress biomarkers, the results revealed also that zinc deficient diet caused:

- An increase in the level of lipid peroxidation with a considerable reduction in reduced glutathione (GSH) concentration.
- A remarkable decrease in glutathione-S-transferase (GST), catalase (CAT), and superoxide dismutase (SOD) activities.

The supplementation of sesame or pumpkin oils to the diet of zinc-deficient diabetic rats improved the following parameters:

- An improvement in carbohydrate metabolism, which is illustrated by a decrease in blood glucose level.
- Amelioration in the liver biomarkers (GOT, GPT), total bilirubin and the lipid profile (cholesterol, triglycerides).
- Restoration in the protein profile, which is represented by an increase in total protein level with a reduction in uric acid, creatinine, and urea levels.
- Restoration zinc status of the examined organs (pancreas, liver, and femur). Moreover, an improvement the activity of zinc dependent enzymes including amylase and lactate dehydrogenase.
- Regarding the potential of these oils against the harmful impact of dietary zinc deficiency on the oxidative stress biomarkers, In other words, the supplementation of sesame and pumpkin oil revealed significant antioxidant potential, which are reflected in the reduction of the lipid peroxidation (MDA) rate, an increase of reduced glutathione (GSH) level and significant improvement of the antioxidant enzymes including CAT, GSH-Px, GST, and SOD activities

In fact, these findings indicated that sesame and pumpkin oils are two interesting plants with great therapeutic potential. To put it simply, due to their bioactive components, the oil of these plants have powerful antioxidants capacity, which are responsible for the protective effect against oxidative stress in diabetes accompanied with zinc-deficient state. Further studies are needed to assess the role of sesame and pumpkin oil and their compounds as drug candidates for the treatment of diabetes.

Considering the significance of these findings, it would be interesting to continue research by:

- Evaluation the antioxidant and anti-diabetic effects of the combination of sesame and pumpkin oil.
- Separation and evaluation the various compounds in order to determine the mechanism action and molecular mechanisms involved in the observed pharmacological effects.

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Annexes

Annex: Calibration curves

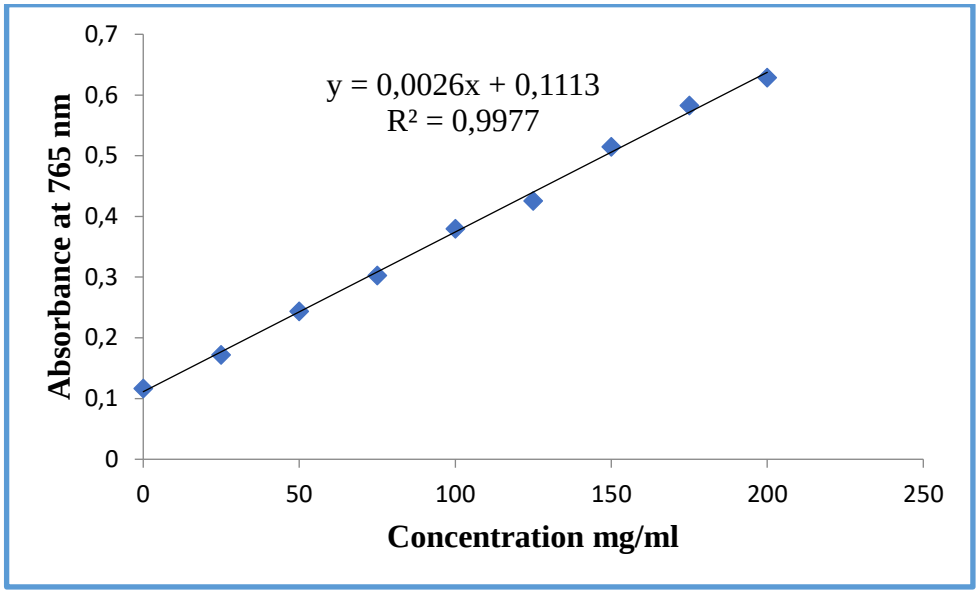

Figure 1. Gallic acid calibration curve for total polyphenol determination.

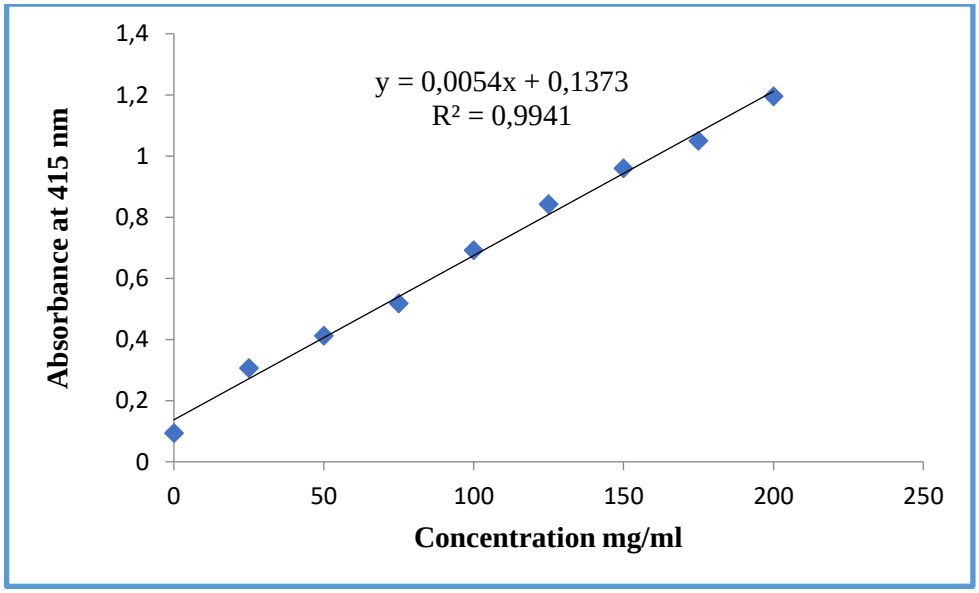

Figure 2. Quercetin calibration curve for flavonoid determination.

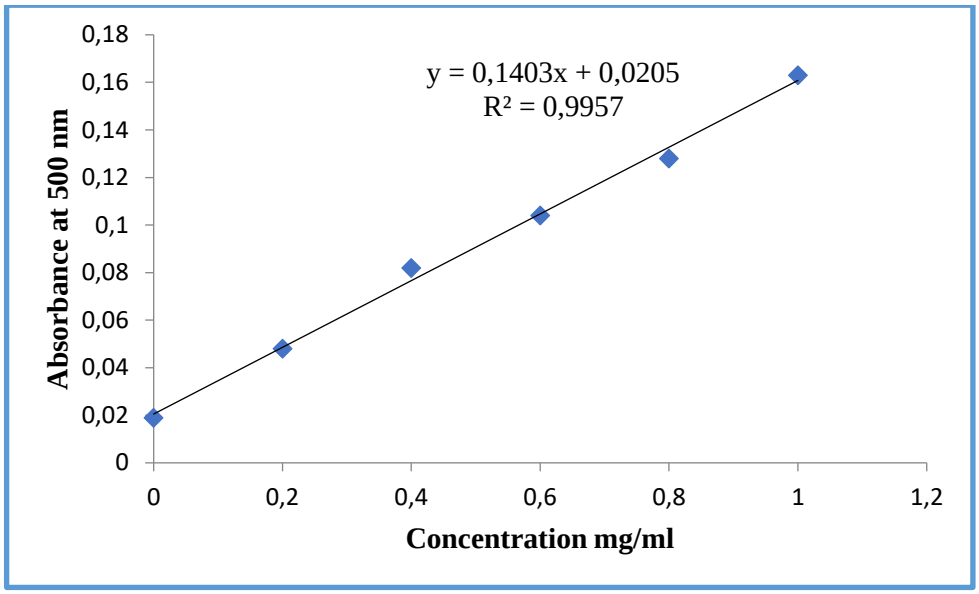

Figure 3. Tannic acid calibration curve for tannin determination.

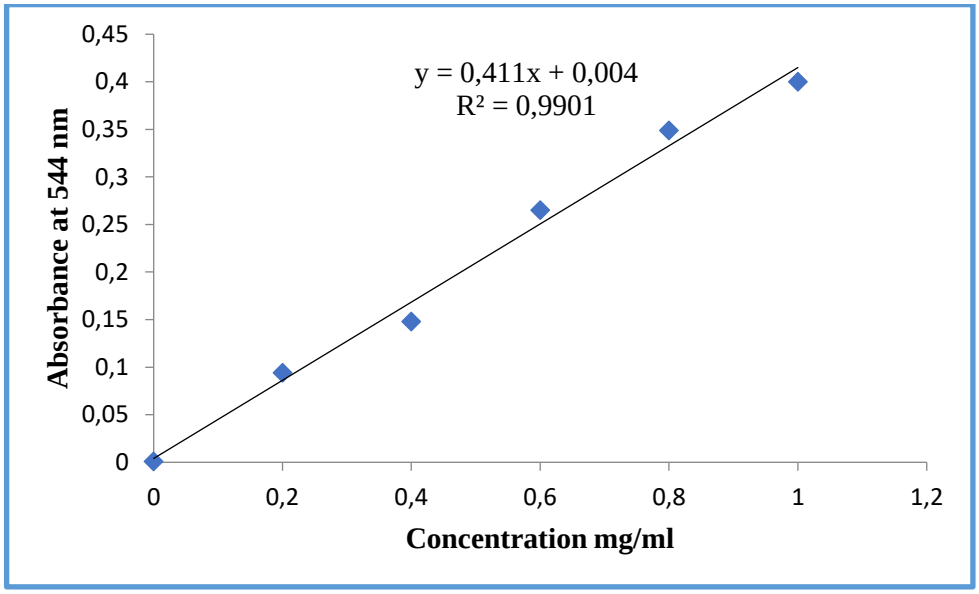

Figure 4. Diosgenin calibration curve for saponin determination.

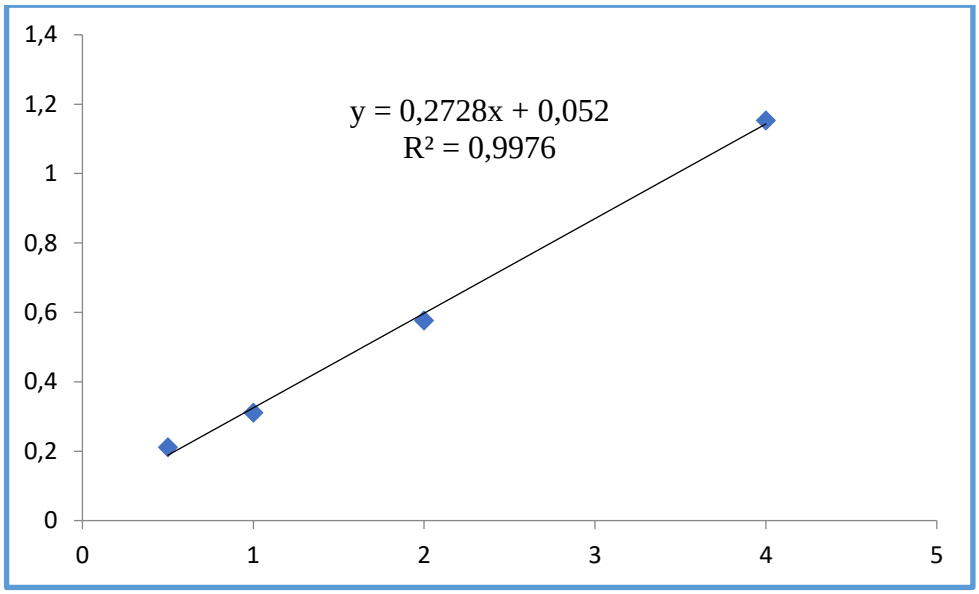

Figure 5. Zinc calibration curve (µg/ml).

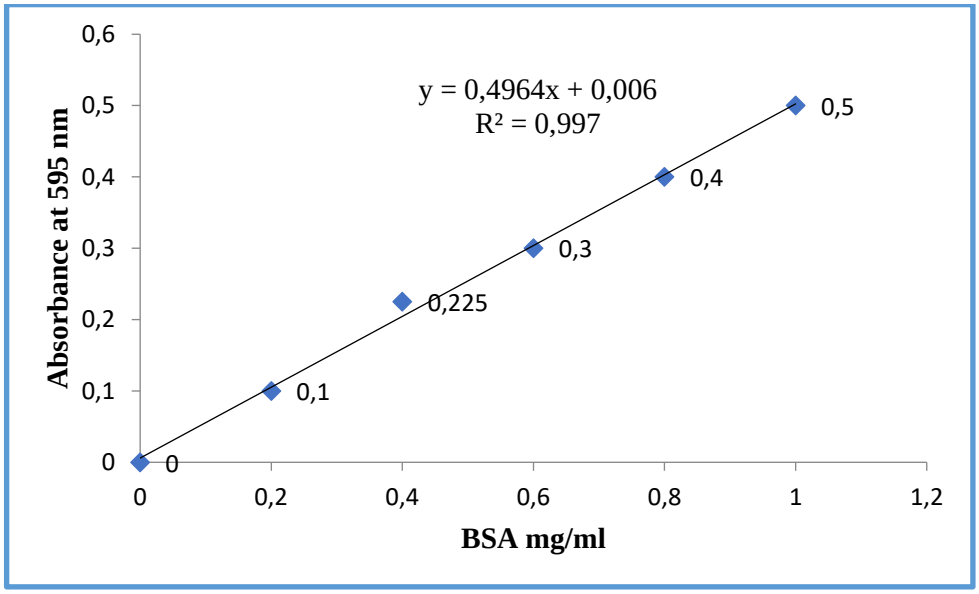

Figure 6. Protein calibration curve.

Scientific production

- **Publication:** Effect of Zinc Deficiency on Blood Glucose, Lipid Profile, and Antioxidant Status in Streptozotocin Diabetic Rats and the Potential Role of Sesame Oil.

Communications

International communications

- Beloucif A, Kechrid Z. Influence of dietary zinc deficiency on diabetic rats. The 3rd Mediterranean congress on bio-analysis from the 13th to 15th December, 2019 in Mahdia, Tunisia.
- Beloucif A, Kechrid Z. The impact of sesame oil on diabetic rats fed zinc deficient diet. The 1st international symposium 'environment & sustainable development', held between 10 and 11 February 2020 in Relizane/ Algeria.
- Beloucif A, Kechrid Z. The effect of sesame oil on blood glucose and lipids profile in diabetic rats. The 30th international congress of the Tunisian society of biological sciences (ATSB) held in Sousse, Tunisia on 25-28 march 2019.
- Beloucif A, Kechrid Z. The beneficial effect of sesame oil on renal parameters in diabetic rats fed zinc-deficient diet. The third international symposium medicinal plants and materials (MPM-2020) organized in university of larbi tebessi-Tebessa- Algeria on February 25 to 27, 2020.

National communications

- Beloucif A, Kechrid Z. Khalfaoui N. The benefic effect of sesame oil on renal biochemical parameters in streptozotocin diabetic rats1er symposium national biomolecules & biotechnologie. Blida, 18-19 décembre 2019.
- Beloucif A, Kechrid Z. Estimation of plasma glucose and effect of sesame oil on antioxidants level in diabetic rats fed zinc-deficient diet. Première journée scientifique sur la biologie des plantes médicinales, tenue à Tébessa le 22 janvier 2020.
- Beloucif A, Kechrid Z. The potential effect of sesame oil on oxidative stress in diabetic albino Wistar rats. Deuxième journée nationale de médicine interne de Guelma (JNMIG) dans le thème est : cœur et diabète.

Biological Trace Element Research
https://doi.org/10.1007/s12011-021-02934-5

Effect of Zinc Deficiency on Blood Glucose, Lipid Profile, and Antioxidant Status in Streptozotocin Diabetic Rats and the Potential Role of Sesame Oil

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Received: 12 April 2021 / Accepted: 20 September 2021

Abstract
Zinc is recognized to have a crucial function in insulin production. As a result, its absence may have a deleterious impact on the progression of diabetes and associated consequences. So, this study was undertaken to evaluate the effect of sesame oil on biochemical parameters, zinc status, and oxidative stress biomarkers in streptozotocin (STZ)-induced diabetic rats fed zinc-deficient diet. Rats were divided into four groups. The first group consisted of non-diabetic rats that were fed in a sufficient zinc diet, whereas the second was a diabetic group which received also sufficient zinc diet, while the third and fourth groups were diabetic rats fed in a deficient zinc diet, one was non-treated and the other was treated with sesame oil 6% diet for 27 days. Zinc deficiency has affected the weight of the diabetic animals. It was also noticed that inadequate dietary zinc intake increased concentrations of glucose, cholesterol, triglycerides, malondialdehyde, and transaminases activities. Furthermore, zinc deficiency feed provoked a decrease in zinc level in tissues (femur, liver, and pancreas); glutathione concentration; and lactic dehydrogenase, amylase, catalase, superoxide dismutase, and glutathione-S-transferase activities. However, sesame oil treatment ameliorated all the previous parameters approximately to their normal values. It was found out that sesame oil supplementation is a potent factor in mitigating the oxidative severity of zinc deficiency in diabetes through its effective antioxidant potential.

Keywords Diabetes · Sesame · Zinc deficiency · Oxidative stress · Antioxidant

Introduction

Human body is exposed consistently to various types of agents that result in the generation of reactive oxygen species (ROS), which are known as free oxygen radicals. In order to counteract the harmful effect of these species, body undertakes many strategies based on endogenous or exogenous antioxidant systems. Any imbalance between pro-oxidants and antioxidants may result in oxidative stress, which eventually leads to many chronic diseases such as diabetes [1], a major endocrine health problem that disturbs the metabolism of carbohydrates, fats, and proteins. Diabetes is principally characterized by hyperglycemia, which is the major factor of diabetic complications, such as neuropathy, retinopathy, nephropathy, and cardiovascular diseases [2]. Prolonged hyperglycemia contributes generally to the process of overproduction of ROS via several mechanisms, particularly glucose auto-oxidation, and the activation of polyol and hexosamine pathways, protein kinase C, and the formation of advanced glycation end products (AGE), which in return contributes to the increase of oxidative stress. Ultimately, these mechanisms cause lipid peroxidation, cell membrane damage, and antioxidant dysfunction [3]. Superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), glutathione-S-transferase as antioxidants enzymes, and non-enzymatic elements such as reduced glutathione (GSH) are parts of different lines in defense mechanisms and strategies in the biological system. Reactive oxygen species are likely to impair the activity of these systems. Nevertheless, antioxidant molecules like vitamins C and E and trace elements as zinc are important for strengthening the body's antioxidant defenses and preventing oxidative stress [4].

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Published online: 06 October 2021

Trace elements as components of the biological structures of many enzymes could be performing a pivotal role in the management and the development of diabetes [5]. Among these trace elements, zinc is an important nutrient with highly relevant functions in humans and animals. It is a co-factor for more than 300 enzymes. Zinc acts as antioxidant factor, which induces the clearance of free radicals and prevents cell membrane damage [6]. Moreover, it is a structural component of the superoxide dismutase enzyme; it also has a major role in the expression of metallothionein and in the regulation of glutathione peroxidase. Another property of zinc is to promote the antioxidant capacity of the cells through competition with copper and iron in the binding sites in the cell membranes. These two metals stimulate the production of lipid peroxides. In this way, the replacement of iron and copper for zinc could prevent lipid peroxidation in diabetic patients [7, 8]. Zinc has an important role in carbohydrate metabolism and insulin action [9]. Regarding the presence of zinc in the regulation of insulin production in pancreatic *β cells*, this element forms an integral part of crystalline insulin. It is reasonable to assume that zinc itself could affect insulin synthesis, storage, and secretion [10]. Henceforth, there are several motives to suspect that abnormal zinc metabolism, such as its deficiency, could play a role in the pathogenesis of diabetes and its complications [11], which is accompanied with an increased vulnerability to oxidative stress, and promotes ROS generation [12].

Sesame is a spice used either fresh or fried for the sake of flavor in various food preparations. Sesame as a traditional healthy food has a great nutritional value due to its high fatty acid content (oleic acid, linoleic acid, palmitic acid, stearic acid) and bioactive compounds including phytosterols, lignans (sesamin, sesamol, episesamin, sesamolin, sesaminol), and vitamin E [13]. It shows numerous biological properties such as antioxidant [14], anti-inflammatory [15], antihypertensive [16], and hypocholesterolemic [17]. Moreover, sesame has a protective effect in managing diseases associated to oxidative stress such as diabetes mellitus [18], obesity [19], chronic renal failure [20], and neurodegenerative diseases [21]. Thus, this study was carried out to evaluate the anti-hyperglycemic activity and examine the modulator effect of sesame oil supplementation for the prevention from the development of diabetic pathology by evaluating body weight gain, zinc status, carbohydrate metabolism, and antioxidant system in streptozotocin diabetic rats fed in a deficient zinc diet.

Materials and Methods

Experimental Animals

Male Albinos Wistar rats (weighing around 150–170 g, male, 10 weeks of age) were acquired from Pasteur Institute (Algiers, Algeria). Animals were allocated individually in polypropylene cages with stainless steel gridded tops and bottoms and stainless steel food hoppers. Trays were placed under each food hopper to collect spilled food. The temperature was maintained at 22° ± 2 °C and humidity was around 40% under a photoperiod of 12-h light/dark. They were given access to diet and water ad libitum throughout the period of the experiment. Studies were handled in accordance to the protocol approved by the Institutional Animal Ethical Committee of Badji Mokhtar University, Annaba (PNR-ANDRS 8/u23/332).

Oil Extraction

After removing of all impurities, whole sesame seeds were steamed at a temperature not exceeds 90 °C, and then pressed using a hydraulic press. The pressing process is repeated several times until the obtention of a sufficient quantity of oil. Finally, the oil was stored in glass bottles at 25 °C until it is used in the experiments.

Qualitative Phytochemical Screening

The sesame oil extract was checked for the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids according to the methods described by Trease and Evans [22]. Chemical components tests were depended on the visual observation of the change in color or the precipitate formation after specific reagent addition.

Firstly, Mayer's test was used to confirm the occurrence of alkaloids. Secondly, the flavonoids were detected by Shinoda's test, whereas Ferric chloride test was used to evaluate the presence of tannins. Thirdly, the existence of terpenoids was assessed by Salkowski test. Finally, froth test was used to reveal saponins.

Quantitative Analysis

Total Polyphenol Content

The total polyphenol content was determined according to the Folin-Ciocalteu colorimetric reagent which was described by Li et al. method [23]. Briefly, 200 µL of oil extract was mixed with 1 mL of diluted Folin-Ciocalteu reagent (10%). Eight hundred microliters of sodium carbonate solution (75 mg/mL) was added after 4 min. Then, the mixture was incubated in a dark at room temperature of 2 h. The absorbance was read against the blank at 760 nm.

The concentration of total polyphenols was calculated via the equation that was obtained using gallic acid, $Y = 0.002x + 0.137$ ($R^2 = 0.994$), and expressed as milligram equivalent of gallic acid (EGA) per gram of extract (mg EGA/g extract).

Total Flavonoid Content

The total flavonoid was estimated according to the method of Turkoglu et al. [24]. Briefly, 250µL of diluted extract was mixed with 2550µL of methanol and 100µL of 1-M aqueous potassium acetate. Then, 100µL of 10% aluminum nitrate was added to the mixture, and shaken vigorously. After 40 min of reaction at room temperature, the absorbance was performed at 415 nm.

The flavonoid concentration was calculated via the equation $Y = 0.005x + 0.137$ ($R^2 = 0.994$), and expressed as milligram of quercetin equivalent (EQ) per gram of extract (mg EQ/g extract).

Condensed Tannin

The condensed tannin content was evaluated by the vanillin assay as described by Hagerman [25]. Briefly, 1 mL of the diluted extract was mixed with 5 mL of assay reagent (1% vanillin–8% HCl), and the mixture was vigorously stirred. It is followed by the addition of 5 mL of concentrated HCL (4%) after 1 min. The absorbance was read at 500 nm after 20-min incubation into a water bath at 30° against the blank. The concentration of tannins was deduced from the calibration ranges, which established with tannic acid following the equation $Y = 0.140x + 0.020$ ($R^2 = 0.995$), and are expressed in milligram of tannic acid equivalent per gram of extract (ETA mg/g of extract).

Total Saponin Content

The total saponin was estimated according to Shiau et al. method [26]. Briefly, 50 µL of the diluted extract was mixed with 250 µL of 8% vanillin. Test tubes were placed in an ice-water bath, followed by the slow addition of 2.5 mL of 72% (v/v) sulfuric acid on the inner side of the wall, incubated for 3 min and brought to a water bath at 60° C for 10 min, and then cooled. Absorbance was measured at 544 nm against a reagent blank. The calculation of the concentration was carried out according to a calibration curve produced by diosgenin (a steroidal saponin), $Y = 0.411x + 0.004$ ($R^2 = 0.990$), and expressed as milligram equivalent of diosgenin (EDG) per gram of extract (mg DGE/g of extract).

Induction of Experimental Diabetes

Experimental animals were administered freshly prepared streptozotocin (STZ) (Sigma Chemicals Company), dissolved in cold citrate buffer (0.1 M, pH 4.5) at the dose of 60 mg/kg body weight after overnight fasting. A 10% glucose solution was given overnight to the streptozotocin-treated animals to prevent STZ-induced hypoglycemia. One week later of streptozotocin (STZ) injection, the diabetic state was confirmed by estimating blood glucose levels from the tail vein using an Accu-chek glucose meter (Roche Diagnostics, Paris, France); only rats with glucose level over 14 mmol/L were considered diabetic model.

Diet Preparation

Diet was prepared according to the method described by Southon et al. [27] and which consists of (in g/kg diet) (Table 1).

Mineral mix was formulated to contain either adequate (54 mg/kg) or inadequate (1.2 mg/kg) quantities of zinc as determined by atomic absorption spectroscopy. The mineral mix was supplied (g/kg diet) by calcium hydrogen orthophosphate, 13; disodium hydrogen orthophosphate, 7.4; calcium carbonate, 8.2; potassium chloride, 7.03; magnesium sulfate, 4; ferrous sulfate, 0.144; copper sulfate, 0.023; potassium iodide, 0.001; manganese sulfate, 0.180; and zinc carbonate, 0.1. The zinc-deficient diet contained no additional zinc carbonate.

Experiment Design

After stabilization of diabetes, rats were divided into four groups (6 each). The first and second groups were non-diabetic (ND) and diabetic (D) controls, and were given sufficient zinc diet containing 54 mg Zn/kg diet. The third and fourth groups were diabetics and received deficient zinc diet containing 1.2 mg Zn/kg diet. One was an untreated group (D-Zn), and the other was treated with sesame oil 6% diet (D-Zn + SO). The treatment lasted for 27 days.

Blood and Tissue Sample Collection

At the end of the experiment, animals were sacrificed by cervical decapitation. The collected blood samples were centrifuged at 3000 rpm and serum stored at − 20C° until biochemical analysis. Pancreas and liver were excised and washed with ice-cold isotonic NaCl saline, and blotted to dry. The right femur was taken and the connective tissues

Table 1 Composition of diet

Diet composition (g/kg diet)	Quantity (g/kg diet)	Percentage (%)
Cornstarch	326	32.6
Sucrose	326	32.6
Protein (egg white solids)	168	16.8
Lipids (corn oil)	80	8
Fiber (cellulose)	40	4
Vitamin mix	20	2
Mineral mix	40	4

and muscle were removed. Liver fragment, pancreas fragment, and femur were weighed and dried at 80 °C for 16 h, and then zinc concentrations were determined. The other fragment of liver was immediately processed for assaying glutathione, malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST). The second fragment of pancreas was utilized for the histological study.

Analytical Methods

Biochemical Assays

The biochemical parameters were measured using commercial kits from Spinreact, Girona, Spain. Kit's references were as follows: cholesterol-1001091, GOT-1001161, GPT-1001171, triglycerides-1001311, lactic dehydrogenase-1001260, and amylase-41201.

Tissue Zinc Analyses

The measurements of zinc in the liver, pancreas, and femurs were realized by heating these organs in silica crucibles at 480 °C for 48 h. Then, the ash was dissolved in hot 12-M hydrochloric acid for Zn assay using a flame atomic absorption spectrophotometer (AA-7000 SHIMADUZ France Atomic absorption spectrophotometer). The accuracy of zinc recovery was tested using standard reference materials, bovine liver and wheat flour, which exceeded 96%. These standards (bovine liver and wheat flour) were prepared and analyzed identically as samples. Zinc standards were prepared from 1.0 mg/mL zinc nitrate standard solution using 5% glycerol to approximate the viscosity characteristics, and to avoid zinc contamination from exogenous sources. All tubes were soaked in HCl (10% v/v) for 16 h and rinsed with double distilled water.

Measurement of Stress Oxidative Parameters

Preparation of Tissue Homogenate

One gram of liver was homogenized in 2 mL of tris-buffered saline (1:2 weight/volume TBS, pH 7.4), and was centrifuged at 10 000 × g for 15 min at 4 °C. The resulting supernatant was used to determine MDA, GSH, SOD, GST, and CAT.

Assay of Lipid Peroxidation

MDA in liver homogenate was determined as the biomarker of lipid peroxidation. The product of the reaction between MDA and thiobarbituric acid (TBA) was determined spectrophotometrically according to Buege and Aust [28]. Absorbance of TBA-MDA complex was determined at 532 nm.

Estimation of Reduced Glutathione

Glutathione (GSH) concentration was estimated using a colorimetric technique as described by Jollow [29]. This technique is based on the development of yellow color when DTNB (5,5-dithiobis-2-nitrobenzoic acid) was added to compounds containing sulfhydryl groups. The absorbance was measured at 412 nm. Total GSH content was expressed as nmol GSH/mg protein.

Assay of Catalase Activity

CAT activity was determined at 25c using the Aebi method [30] and the dismutation of hydrogen peroxide (H_2O_2) was monitored spectrophotometrically at 240 nm for 1 min.

Estimation of Superoxide Dismutase Activity

The activity superoxide dismutase (SOD) was determined according to the method which was described by Misra and Fridovich [31]. Ten microliters of tissue homogenate was added to 970 µL of EDTA sodium carbonate buffer (0.05 M) at pH 10.2. The reaction was started by adding 20 µL of epinephrine (30 mM) and the activity was measured at 480 nm for 4 min.

Assay of Glutathione-S-Transferase Activity

Glutathione-S-transferase (GST, EC.2.5.1.18) acts as a catalyst during the conjugation reaction of glutathione (GSH) to 1-chloro-2,4 dinitrobenzene (CDNB) as a substrate. Its activity was assayed by the method of Habig et al. [32] and absorbance was detected at 532 nm for 5 min.

Effect of Sesame Oil on Oral Glucose Tolerance Test (OGTT)

Oral glucose tolerance tests (OGTT) were carried out as described by Gupta [33] with slight modifications. Briefly, mice were divided into four groups (6 each). Then, they were treated as follows: group one received standard pellet diet. Groups two, three, and four all received sesame oil, respectively, at a dose of 4%, 6%, and 8% diet. OGTT was conducted after 14 days of treatment. On completion of 14 days of treatment, glucose (2 g/kg) was orally administered 90 min. Then, mice were fasted overnight and blood was collected from the tail vein just prior to the glucose loading (normal fasting) and at intervals of 30 min up to 2 h for glucose estimation using a glucometer (Accu-chek glucose meter).

Histological Study

The histopathology examination was performed by light microscopy on the pancreas, which was fixed at 10% neutral formalin solution, after washing with isotonic saline (0.9%). Then, it was dehydrated in graded ethanol and embedded in paraffin. The obtained sections were cut into 5 µm and stained with hematoxylin–eosin.

Statistical Analysis

Statistical analysis was carried out using GraphPad Prism 8 and data was expressed as means ± SEM. Comparison among groups was assessed by using one-way analysis of variance followed by Turkey's post hoc test and differences were considered statistically significant at $p < 0.05$.

Results

Phytochemical Screening

Preliminary phytochemical screening of sesame oil extract indicated the presence of various bioactive compounds such as tannins, flavonoids, saponins, terpenoids, and alkaloids, which possess pharmacological properties. Sesame oil was found to contain higher quantities of total polyphenols (117.83 mg GAE/g extract), total flavonoids (78.4 mg QE/g extract), a small concentration of condensed tannins (4.39 mg TAE/g extract), and saponins (1.11 mg DGE/g extract) (Table 2).

Effect of Treatment on Body Weight and Food Intake

Findings showed a significant decline in body weight with a higher food intake of diabetic rats as compared to non-diabetic rats. Meanwhile, zinc deficiency significantly affected body weight of diabetic rats. However, sesame oil supplementation ameliorated the growth rate and food consumption of D-Zn rats (Table 3).

Tissue Zinc Concentration

As indicated in Table 3, zinc levels in the femur, pancreas, and liver of diabetic rats were significantly lower ($p < 0.001$, $p < 0.01$, $p < 0.01$) compared to the non-diabetic rats. Again, zinc deficiency led to a further fall of zinc in the femur and pancreas ($p < 0.05$). On the other hand, sesame oil supplementation significantly improved femur ($p < 0.001$), liver ($p < 0.05$), and pancreatic ($p < 0.001$) zinc contents.

Blood Biochemical Values

As depicted in Table 4, the results showed a very significant rise in blood glucose ($p < 0.001$), GOT ($p < 0.001$), GPT ($p < 0.05$), and cholesterol ($p < 0.05$) of the diabetic group compared to non-diabetic group. Besides, it was observed that zinc deficiency resulted an increase of GPT ($p < 0.05$),

Table 2 Amount of total polyphenols, total flavonoids, condensed tannins, and saponins in the sesame oil extract

Extract	Total polyphenols (mg GAE/g extract)	Total flavonoids (mg QE/g extract)	Condensed tannin (mg TAE/g extract)	Total saponin (mg DGE/g extract)
Sesame oil	117.83 ± 0.76	77.4 ± 1.56	4.39 ± 0.03	1.11 ± 0.02

Values are means of three replicates ± SEM

GAE gallic acid equivalents; *QE* quercetin equivalents; *TAE* tannic acid equivalents; *DGE* diosgenin equivalent

Table 3 Body weight, food intake, and tissue zinc concentration of non-diabetic rats (ND), Zn-sufficient diabetic rats (D), Zn-deficient diabetic rats (D-Zn), and Zn-deficient diabetic rats given dietary sesame oil (D-Zn + SO)

Parameters	Experimental groups			
	ND	D	D-Zn	D-Zn + SO
Initial body weight (g)	230.66 ± 11.01	205.83 ± 7.02	201.33 ± 6.91	213.66 ± 7.55
Final body weight (g)	246.2 ± 7.78	168.2 ± 5.73^{a2}	139.7 ± 6.13^{b}	167.5 ± 1.74^{c}
Food intake (g/day/rat)	12.71 ± 0.95	15.28 ± 0.64^{a1}	13.36 ± 0.81^{b}	16.15 ± 1.51^{c2}
Femur (µg/g dry weight)	80.56 ± 1.41	66.58 ± 2.90^{a2}	58.22 ± 1.45^{b}	74.92 ± 1.55^{c2}
Liver (µg/g dry weight)	71.20 ± 2.11	55.04 ± 2.97^{a1}	52.57 ± 3.08	65.72 ± 2.36^{c}
Pancreas (µg/g dry weight)	61.36 ± 2.60	49.65 ± 1.98^{a1}	39.42 ± 1.02^{b}	58.25 ± 2.59^{c2}

Values are mean ± SEM; number of animals = 6

$^{a1}p < 0.01$, $^{a2}p < 0.001$ versus ND group

$^{b}p < 0.05$ versus D group

$^{c}p < 0.05$, $^{c2}p < 0.001$ versus D-Zn group

triglyceride ($p < 0.01$), and cholesterol ($p < 0.001$). However, treatment with sesame oil showed a significant restoration of the previous parameters.

Zinc Enzyme Activities

As shown in Table 4, diabetes resulted in highly significant decrease ($p < 0.001$) in amylase activity. Furthermore, zinc deficiency led to an obvious slight reduction in LDH and amylase activities. These activities increased significantly ($p < 0.001$) after sesame oil supplementation.

Hepatic Oxidative Stress Parameters

As far as the hepatic oxidative stress is concerned, the diabetes status caused a rise in MDA level ($p < 0.001$), with a decrease in GSH content ($p < 0.001$) and GST ($p < 0.001$), SOD ($p < 0.001$), and CAT ($p < 0.01$) activities in diabetic rats. In addition, zinc deficiency provoked a significant augmentation of MDA level ($p < 0.0.001$), a significant decline in GSH level ($p < 0.001$) and CAT activity ($p < 0.001$), and a slight decrease in GST activity ($p < 0.0.05$), whereas there was an improvement of these parameters to near normal values after supplementation of sesame oil (Figs. 1, 2, 3, 4 and5).

Oral Glucose Tolerance Test (OGTT)

OGTT was used to determine the anti-hyperglycemic effect of sesame oil. As presented in Fig. 6, glucose administration caused a noticeable increase in blood glucose at 0 min, 30 min, and 60 min after glucose loading in all studied groups compared to normal fasting blood glucose of each one, but at 90 and 120 min, there was a decline in blood glucose concentration in treated groups compared to normal fasting blood glucose.

Pancreatic Histopathology

Microscopic examinations of pancreas sections of non-diabetic and diabetic groups are presented in Fig. 7. The pancreas of non-diabetic rats show normal architecture marked by the presence of islets of Langerhans (Fig. 7A). Conversely, in diabetic group, a reduced B-cell size was observed (Fig. 7B). On the other hand, more histological changes are noted in diabetic rats fed zinc deficiency diet, which revealed absence of B-cells, injury of acinar cells, and thickening of the septa (Fig. 7C), however, an improvement

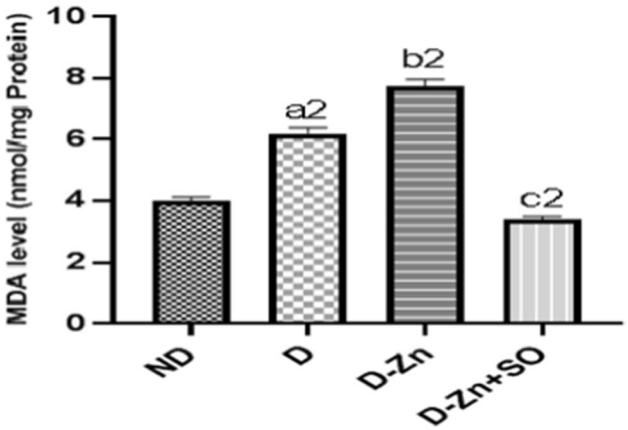

Fig. 1 Liver MDA level of non-diabetic rats (ND), Zn-sufficient diabetic rats (D), Zn-deficient diabetic rats (D-Zn), and Zn-deficient diabetic rats given dietary sesame oil (D-Zn + SO). Values are mean ± SEM of 6 rats. $^{a2}p < 0.001$ versus ND group. $^{b2}p < 0.001$ versus D group. $^{c2}p < 0.001$ versus D-Zn group

Table 4 Mean blood glucose, serum triglycerides, serum cholesterol, serum glutamate oxaloacetate transaminase (GOT), serum glutamate pyruvate transaminase (GPT), serum lactate dehydrogenase (LDH), serum amylase of non-diabetic rats (ND), Zn-sufficient diabetic rats (D), Zn-deficient diabetic rats (D-Zn), and Zn-deficient diabetic rats given dietary sesame oil (D-Zn + SO)

Parameters	Experimental groups			
	ND	D	D-Zn	D-Zn + SO
Blood glucose (mg/dL)	85.50 ± 3.86	441.0 ± 21.65^{a2}	492.3 ± 15.85	282.0 ± 18.12^{c2}
Triglyceride (mg/dL)	66.5 ± 8.64	145.5 ± 19.36	311.7 ± 49.97^{b1}	76 ± 8.40^{c2}
Cholesterol (mg/dL)	69.17 ± 5.89	91.83 ± 2.77^{a}	126.8 ± 5.70^{b2}	77.67 ± 3.66^{c2}
GOT (IU/L)	136.5 ± 10.21	199.2 ± 9.94^{a2}	211.7 ± 11.20	122.2 ± 4.97^{c2}
GPT (IU/L)	86.67 ± 5.37	114.8 ± 3.35^{a}	139.5 ± 8.78^{b}	99.83 ± 6.02^{c1}
LDH (IU/L)	877.0 ± 33.08	749.0 ± 71.70	482.0 ± 50.11^{b2}	979.7 ± 17.39^{c2}
Amylase (IU/L)	2161 ± 59.68	1387 ± 84.19^{a2}	1043 ± 25.57^{b}	1549 ± 97.36^{c2}

Values are mean ± SEM; number of animals = 6

$^{a}p < 0.05$, $^{a2}p < 0.001$ versus ND group

$^{b}p < 0.05$, $^{b1}p < 0.01$, $^{b2}p < 0.001$ versus D group

$^{c1}p < 0.01$, $^{c2}p < 0.001$ versus D-Zn group

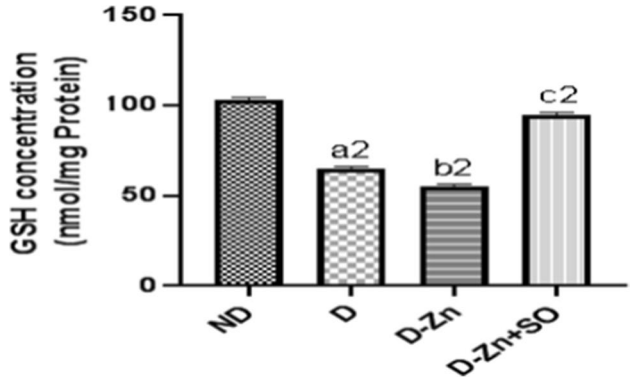

Fig. 2 Liver GSH concentration of non-diabetic rats (ND), Zn-sufficient diabetic rats (D), Zn-deficient diabetic rats (D-Zn), and Zn-deficient diabetic rats given dietary sesame oil (D-Zn + SO); values are mean ± SEM of 6 rats. $^{a2}p < 0.001$ versus ND group. $^{b2}p < 0.001$ versus D group. $^{c2}p < 0.001$ versus D-Zn group

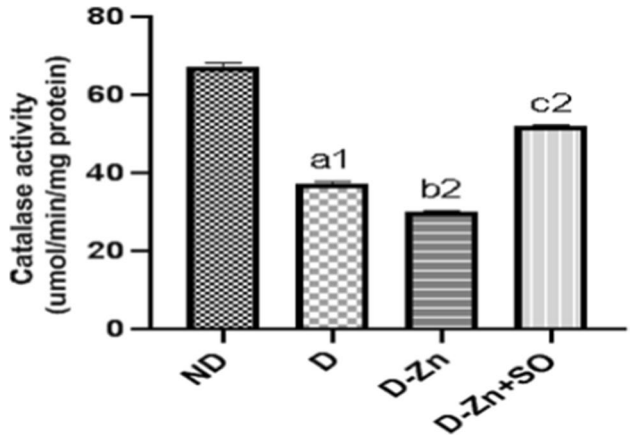

Fig. 3 Liver catalase activity of non-diabetic rats (ND), Zn-sufficient diabetic rats (D), Zn-deficient diabetic rats (D-Zn), and Zn-deficient diabetic rats given dietary sesame oil (D-Zn + SO); values are mean ± SEM of 6 rats. $^{a1}p < 0.01$ versus ND group. $^{b2}p < 0.001$ versus D group. $^{c2}p < 0.001$ versus D-Zn group

of acinar cell structure and regeneration of B-cell in the pancreatic sections of sesame oil-treated rats (Fig. 7D).

Discussion

Nowadays, there is a significant shift towards traditional medicine or phytotherapy due to the cumulative and irreversible bad side effects of modern drugs. However, the use of plants in medicine is primarily based on biologically active compounds, which have many therapeutic properties such as anti-inflammatory, antioxidant, antidiabetic, anticarcinogen, and hypocholesterolemic [34]. Hence, this study focused on evaluating the potential antidiabetic and antioxidant effects of sesame oil on diabetes under nutritional zinc deficiency.

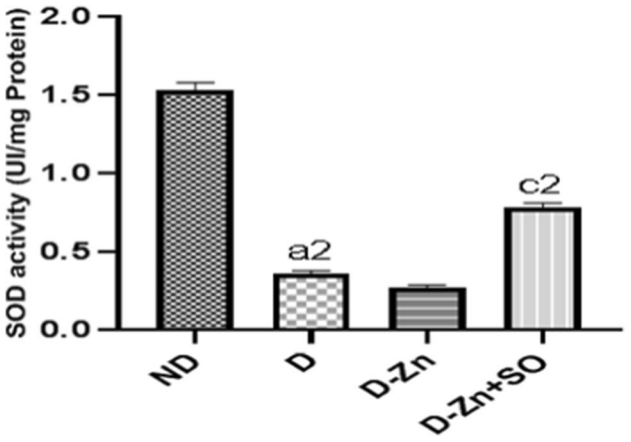

Fig. 4 Liver SOD activity of non-diabetic rats (ND), Zn-sufficient diabetic rats (D), Zn-deficient diabetic rats (D-Zn), and Zn-deficient diabetic rats given dietary sesame oil (D-Zn + SO); values are mean ± SEM of 6 rats. $^{a2}p < 0.001$ versus ND group. $^{c2}p < 0.001$ versus D-Zn group

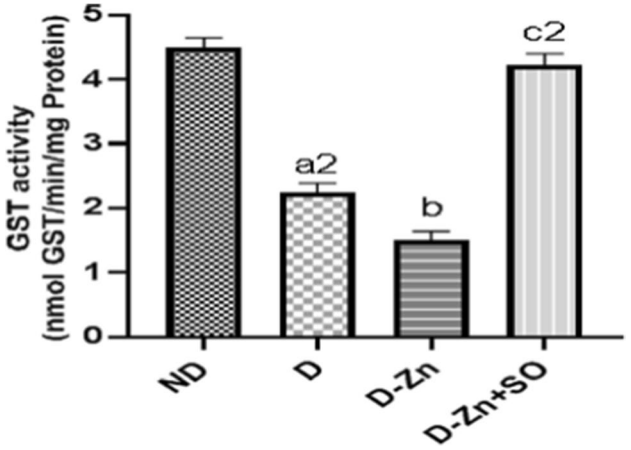

Fig. 5 Liver GST activity of non-diabetic rats (ND), Zn-sufficient diabetic rats (D), Zn-deficient diabetic rats (D-Zn), and Zn-deficient diabetic rats given dietary sesame oil (D-Zn + SO); values are mean ± SEM of 6 rats. $^{a2}p < 0.001$ versus ND group. $^{b}p < 0.05$ versus D group. $^{c2}p < 0.001$ versus D-Zn group

The results of qualitative screening showed the presence of high levels of polyphenols and flavonoid contents in sesame oil, which reflects its strong biological antioxidant properties [35].

The current study showed remarkable body weight losses in a diabetic group in comparison with a normal one. Undoubtedly, muscle wasting, resulting from insufficient use of carbohydrates as a source of energy and the excessive catabolism of fats and proteins, appears to account for weight loss in diabetic rats [36]. Contrarily, diabetic rats fed in a zinc deficiency diet had less body weight gain in comparison with rats fed in a zinc adequate diet. This is consistent with previously published reports [8, 37]. Zinc

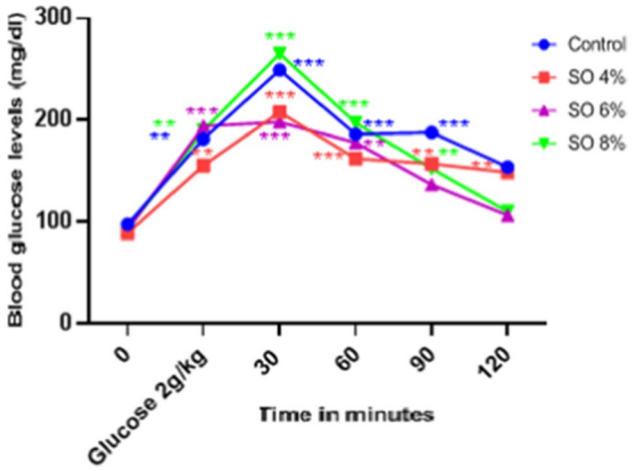

Fig. 6 Effect of sesame oil on blood glucose level in hyperglycemic mice following 120 min of glucose administration. $^{**}p < 0.01$ as compared with normal fasting blood glucose of each group. $^{***}p < 0.001$ as compared with normal fasting blood glucose of each group

deficiency has been reported to have a dramatic effect on the body weight of the rats. It is known to cause taste and appetite disturbances, which are often associated with impaired gustine activity, a dependent zinc enzyme. Thus, these disorders in return lead to a decrease in food intake and stimulate early satiety with eating, which may affect growth rate [38]. However, sesame oil as a dietary supplement attenuated significantly the reduction of body weight and increased food consumption in diabetic rats fed in low-zinc diet than those non-treated group [39]. These effects may be explained by the capacity of sesame oil to reduce hyperglycemia in these animals, and the antioxidative properties of the antioxidants compounds present in sesame oil, and their ability to suppress free radicals, which lead to body weight increase [18]. Another explanation is that the sesame oil stimulates protein synthesis and inhibits proteolysis, which may be related to the anabolic effect of insulin. Hence, an improving body weight is arguably the side effect of this oil [40].

The concentration of zinc in femur, liver, and pancreas tissues showed a noticeable decrease in both diabetic rats fed in either zinc-sufficient or zinc-deficient diet. These results coincide with previously published investigations [41, 42]. Many studies indicated that diabetes can affect zinc metabolism. The decrease in zinc content in diabetic rats is usually related to altered intestinal absorption and the urinary excretion of zinc, which results from a reduction in glomerular filtration rate [43, 44]. Furthermore, the levels of zinc in the diabetic rats fed in low-zinc diet improved after sesame oil treatment. It is likely assumed that supplementation with this oil positively influences oxidative stress damage through ameliorating hyperglycemia, and kidney function resulting in reduction of zinc urinary losses.

The high blood glucose level observed in diabetic rats was certainly the result of insulin deficiency due to depletion of β-cells after STZ injection, or to the increased rate of glycogenolysis and gluconeogenesis [36]. Diabetic rats fed in a zinc-deficient diet and those under adequate zinc diet had no differences in blood glucose level, despite the fact that zinc deficiency exacerbated fasting hyperglycemia associated with reduced circulating insulin [45]. Meanwhile, it was observed that treatment with sesame oil showed a significant decrease in blood glucose. The hypoglycemic effect of sesame oil may refer to its bioactive

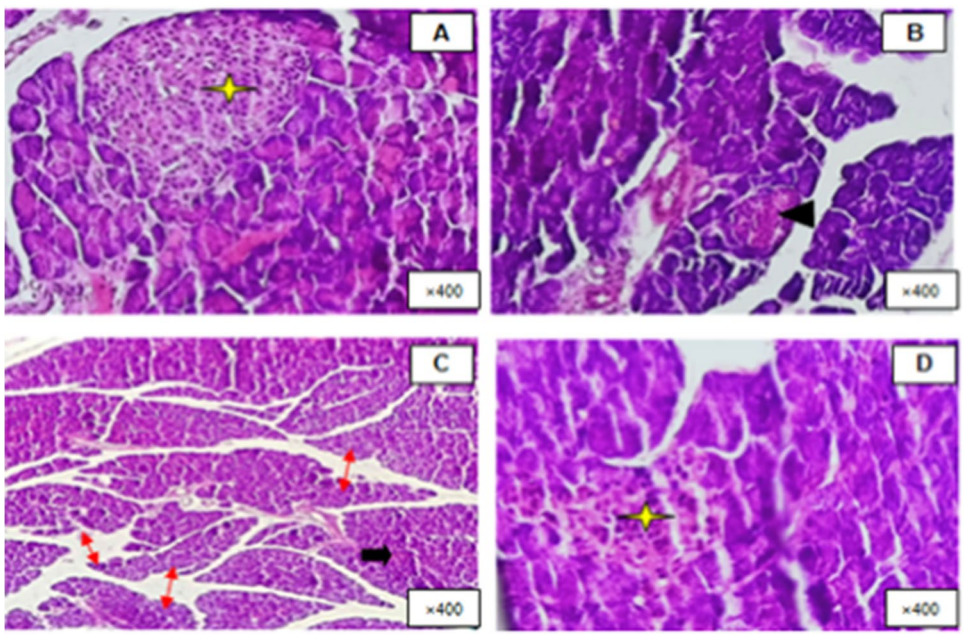

Fig. 7 Show hematoxylin–eosin–stained of pancreatic tissues from different experimental groups. **A** Section of the pancreas from non-diabetic group (ND) showing normal architecture of pancreatic islets (yellow star). **B** Section of the pancreas from Zn-sufficient diabetic rats (D) showing reduced *B-cells* size (black triangle). **C** Section of the pancreas from Zn-deficient diabetic rats (D-Zn) showing entirely lost β-cells and degeneration of acinar cell, thickening of the septa (double-headed red arrows). **D** Section of the pancreas from Zn-deficient diabetic rats treated with sesame oil (D-Zn + SO) indicating regeneration of pancreatic islets (yellow star). Optic microscopy

compounds which stimulate insulin secretion and promote the extra-pancreatic mechanism [46]. Hence, the presence of fat-soluble lignans, most importantly sesamin, sesamolin, and sesamol in sesame oil, may be responsible for the hypoglycemic effect [47]. Another property awarded to sesame oil is its high content of monounsaturated fatty acids. The later has the ability to improve insulin sensitivity and thus ameliorating glycemia [46]. Looking at the findings of OGTT, the oral administration of sesame oil at dose of 6% diet exhibited improvement in glucose tolerance in glucose fed normal mice, which reflects the effectiveness of sesame oil as a hypoglycemic agent through delaying carbohydrate digestion, thereby lowering blood glucose level [48].

The cholesterol and triglyceride levels were higher in both diabetic animals fed in an adequate or low dietary zinc diet. This might be due to the disturbance of lipid metabolism under diabetic conditions which led to suppress lipoprotein lipase activity in account of insulin deficiency [49] and the variation of zinc status exhibited lipid disorder through a highly significant elevation in total cholesterol and triglyceride. In other words, low nutritional zinc provoked catabolism of lipids due to an increase demand of energy [50]. However, the findings demonstrated the significant potential effect of sesame oil in lowering cholesterol and triglyceride levels in zinc-deficient diabetic rats. Sesamin, a major lignan of sesame oil, assumed to be responsible for the reduction of these parameters. To put it simply, sesamin is effective in lipid metabolism via inhibition of lipid synthesis and concomitant increase of fatty acid oxidation [51]. In this case, these effects are associated with down- and upregulation of several enzymes and transcription factors [52, 53]. In other words, these impacts provoke lowering of the activity and gene expression of enzymes and transcription factor involved in fatty acid synthesis and increase the activity and gene expression of hepatic fatty degradation transcription factor.

The current study revealed a significant increase of GOT and GPT activities in zinc-sufficient diabetic group. It has been indicated that increased levels of transaminases activities might be due to insulin insufficiency [54] that is responsible for the elevated gluconeogenesis and ketogenesis action of GOT and GPT in providing new source supplies of glucose such as amino acids [55]. Hence, the biochemical disruptions noticed in diabetic rats fed in low-zinc diet seem to be in conformity with histological alteration of pancreatic *B-cells*. In other words, the histological alteration showed a decrease in the number and size of *B-cells*, in contrast to what the diabetic sufficient zinc group displayed, whereas the elevation of transaminases in deficient zinc diabetic group confirms the work of Greeley and Sandstead [56], who found evidence of decreased oxidation of the carbon chain of alanine when zinc was restricted and led to alanine accumulation in blood. Interestingly, the group treated with sesame oil recovered the *B-cells*, GOT, and GPT activities.

A decrease in serum lactic dehydrogenase and amylase was found in diabetic rats fed zinc-deficient diet. The decline in the activity of these enzymes is undoubtedly due to the reduction of zinc concentration [57]. Briefly, both lactic dehydrogenase and amylase are metalloenzymes, which need zinc as a co-factor for their activities [58, 59]. Nevertheless, the findings indicated that some symptoms associated with zinc deficiency such as the reduction in Zn-dependent enzymes activities rectified with sesame oil supplementation. This means that sesame oil has antioxidative capacity [60], in addition to its anti-hyperglycemic and zinc conservation efficacy.

In this experiment, high level of liver MDA in diabetic conditions along with decrease of reduced glutathione level, catalase, SOD, and GST activities was observed. This could probably be linked to increased oxidative stress caused by the impairment of antioxidant defense system [61]. It is known that zinc is an important element for various enzymes and proteins, which are involved in antioxidant defense [62], so its deficiency induces an increased free radical generation and depletion of antioxidant capacity [63]. According to the results obtained, an increase in MDA concentration had been also demonstrated in zinc deficiency diabetic rats; this indicates the deleterious effect of zinc deficiency in increasing lipid peroxidation [64]. Besides, a remarkable depletion of glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), as well as glutathione-S-transferase (GST) activities, was noticed in those rats with dietary zinc deficiency. The decline of GSH might be linked to the higher consumption of glutathione in the scavenging free radicals and higher oxidative damage in zinc-deficient rats, whereas the depletion of liver antioxidant enzymes activity in diabetes state might be due to promoting reactive oxygen species generation through glucose autoxidation and non-enzymatic glycation of protein [8]. Nevertheless, treatment with sesame oil showed a high decrease in MDA level and an increase of GSH concentration, SOD, CAT, and GST activities. This could be due to the capability of sesame oil to inhibit lipid peroxidation or reduce membrane damage [51]. In other words, the effective antioxidant activity of the sesame oil was depending on its richness in lignans and tocopherols. In this regard, many studies suggest that the antioxidant and radical scavenging activity of this oil is attributed to the lignans [65]. Despite that, a wide range of phenolic compounds such as flavonoids, tannins, and phenolic acids was also reported to possess antioxidant properties [66]. It is apparent that the bioactive compounds of sesame oil could prevent the complications from ROS by promoting the stability of cellular membrane as well as by controlling the generation of radicals, either by scavenging them or enhancing their decomposition. Alternatively, it is probably assumed that the

improvement in the endogenous antioxidant enzymes is due to reduced utilization of these enzymes as sesame is a good source of exogenous antioxidants. In addition, the reason for the significant increase of GSH level may be a result of the richness of sesame oil in vitamin E [67].

Conclusion

This study demonstrates the potentiality of sesame oil in ameliorating growth rate, zinc status, carbohydrate metabolism, and oxidative stress biomarkers as a result of deficient zinc severity and diabetes. In this sense, the antidiabetic and antioxidant effects might be due to the bioactive compounds of this oil. Further practical investigations are required to assess the effectiveness of this oil and its bioactive compounds.

Acknowledgements Authors thank Mr. Boulezaz Kamel, head of factory unit, ONAB, El-Harrouche, for the element composition of diet supply; Mr. Belhoshette Saif-eddine, founder of the Nature Touch Center, for oil extraction, Constantine, Algeria; and members of Algiers Pasteur Institute for providing rats.

Author Contribution ZK formulated the present hypothesis. ZK and AB were responsible for writing the report. AB and AMB were responsible for the analysis of the data.

Funding This work was supported by the research project under the number D01N01UN230120190003, funded by the Ministry of Higher Education, Algeria.

Data Availability Not applicable

Declarations

Ethics Approval Experimental studies were handled in accordance to the protocol approved by the Institutional Animal Ethical Committee of Badji Mokhtar University, Annaba.

Consent to Participate Not applicable

Consent to Publish Not applicable

Conflict of Interest The authors declare no competing interests.

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