الشعبية
Democratic and People's Republic of Algeria
وزارة التعليم العالي والبحث العلمي
Ministry of Higher Education and Scientific Research
جامعة 8 ماي 1945 قالمة
University 8 May 1945 Guelma
Faculty of Nature and Life Sciences, Earth and Universe Sciences

Department of Biology

Field: Science of Nature and Life.

Sector: biological science.

Speciality/Option: applied biochimie.

Master's Thesis

Theme

Protective effect of spirulina against isoproterenol induced cardiotoxicity

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June 2023-2024

Acknowledgements

Thanks First of all , We thank Almighty God who has given us the strength, the courage and patience to do this job.

Our thanks and respect go to Ms. BOUSSENANE Nadia Hanene Lecturer at the University of Guelma, for accepting the presidency of the evaluation jury of this humble work.

We also express our thanks and consideration to Ms. MERABET Rym, Lecturer at the University of Guelma, for accepting to evaluate and review this modest work.

We would like to thank very warmly our supervisor Ms. BRAIK Asma, Lecturer at the University of Guelma, who granted us the honor of leading this work. Thank you for his availability, his patience. Its relevant advices have been a solid reference and comfort for us in all moments.

We thank Mr. GUEROUI Yassine, Dean of our faculty, for his support and all the facilities he has granted us to carry out this modest work.

Our thanks are also addressed to all the members of the laboratories and pet shop of our faculty, especially Ms. HIMER Ratiba, Ms Hanene and Mr GUERDI Mehdi for their continuous help during the practical realization of this work.

Finally, our thanks also go to all the people who have helped us from near or far for the realization of this thesis.

Thanks

Abstract

In recent years, the increase in cases of cardiovascular diseases (CVD), in particular myocardial infarction (MI), has become a global concern due to its high mortality rates, highlighting the need for more effective treatment with fewer side effects. Spirulina (*Arthrospira platensis*), a microalgae rich in phycocyanins, has the potential to prevent cardiovascular diseases, by offering protection against oxidative stress. The cardioprotective properties of spirulina were evaluated in a rat model of isoproterenol-induced myocardial infarction (ISO). The antioxidant activity of the spirulina was evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging test and the ferric reducing antioxidant power test (FRAP). Male Wistar rats received spirulina orally for 15 consecutive days (500 mg/kg/day), followed by a subcutaneous injection of ISO (150 mg/kg) for two consecutive days. Results indicate that spirulina effectively have an antioxidant activity *in vitro*. ISO-treated rats showed a significant increase in troponin level (TROP) and an hypercholesterolemia, as well as reduced cellular pool of reduced glutathione (GSH), and decreased enzymatic activities of glutathione peroxidase (GPx) and catalase (CAT) in cardiac tissue. A pre-treatment with spirulina has significantly protected the heart, restoring antioxidant statut and decreasing serum torponine and cholesterol levels. The histopathological analysis revealed various degrees of cardiac lesions in ISO-treated group, whereas the groups treated with spirulina presented milder lesions and provided good protection. These finding were comfirmed by the significante reduction of infarct size in spirulina-treated group using TTC staining method. Spirulina seems to be a promising substance for cardioprotection against ISO-induced myocardial infarction.

Keywords: Spirulina; Green-blue algae; Oxidative stress; Antioxidant activity; Isoproterenol; cardiotoxicity; Myocardial infarction

Résumé

Ces dernières années, l'augmentation des cas de maladies cardiovasculaires (MCV), en particulier d'infarctus du myocarde (IM), est devenue une préoccupation mondiale en raison de ses taux de mortalité élevés, soulignant la nécessité d'un traitement plus efficace avec moins d'effets secondaires. La spiruline (*Arthrospira platensis)*, une microalgue riche en phycociyanines, a le potentiel de prévenir les maladies cardiovasculaires, en offrant une protection contre le stress oxydatif. Les propriétés cardioprotectrices de la spiruline ont été évaluées chez le rat en utilisant un modèle d'infarctus du myocarde induit par l'isoprotérénol (ISO). L'activité antioxydante *in vitro* de la spiruline a été évaluée par le test de piégeage du radical libre 1,1-diphényl-2-picrylhydrazyl (DPPH) et le test du pouvoir réducteur du fer (FRAP). Des rats Wistar mâles ont reçu de la spiruline par voie orale pendant 15 jours consécutifs (500 mg/kg/jour), suivi d'une injection sous-cutanée d'ISO (150 mg/kg) pendant deux jours consécutifs. Les résultats indiquent que la spiruline possède effectivement une activité antioxydante *in vitro* inrterressante. Les rats traités par l'ISO ont montré une augmentation significative du niveau de troponine sérique (TROP) et d'hypercholestérolémie, ainsi qu'une réduction de la reserve cellulaire du glutathion réduit (GSH) et des niveaux d'activité réduites des glutathion peroxydase (GPx) et catalase (CAT) dans le tissu cardiaque. Un prétraitement à la spiruline a considérablement protégé le cœur, rétablissant l'activité antioxydante et diminuant les taux sériques de torponine et de cholestérol. L'analyse histopathologique a révélé divers degrés de lésions cardiaques dans le groupe ISO, alors que les groupes traités à la spiruline présentaient des lésions plus légères et offraient une bonne protection. Ces résultats ont été confirmés par la réduction significative de la taille de l'infarctus dans le groupe traité à la spiruline en utilisant la méthode de coloration TTC. La spiruline semble être une substance prometteuse pour la cardioprotection contre l'infarctus du myocarde induit par l'ISO.

Mots-clés : Spiruline ; Algues bleue-vertes ; Stress oxydant ; Activité antioxydante; Isoprotérénol ; cardiotoxicité; Infarctus du myocarde

الملخص

في السنوات الاخيرة ,اصبح ارتفاع حالات امراض القلب و الاوعية الدموية , و خاصة احتشاء عضلة القلب , مصدر قلق عالمي بسبب معدلات الوفيات المرتفعة , مما يؤكد على ضرورة علاج اكثر فعالية مع اثار جانبية اقل . سبيرولينا , منتج غني بالفيكوسيانين , يظهر امكانيات في الوقاية (الامراض القلبية الوعائية) بشكل عام من خلال توفير الحماية ضد الاجهاد التاكسدي. تم تقييم خصائص حماية القلب للسبيرولينا في نموذج من احتشاء عضلة القلب الناجم عن الأيزوبروتيرينول لدى الجرذان. تم تقييم النشاط المضاد للاكسدة للسبيرولينا من خلال اختبار تثبيط الجذر الحر (DPPH), و باختبار قوة اختزال الحديد (FRAP) , تم اعطاء ذكور الجرذان ويستار سبيرولينا عن طرق الفم لمدة 15 يوم متتالية (500مغ/كغ/يوم) متبوعة بحقن تحت الجلد من الايزوبروتيرينول (150 /مغ/كغ) لمدة يومين متتاليين . اشارت النائج الى ان السبيرولينا قامت بتثبيط فعال للجذر الحر و كذلك اختزال الحديد . أظهرت الجرذان التي عولجت بالايزوبروتيرينول زيادة ملحوظة في مستوى التروبونين (TROP) و فرط كوليسترول الدم . مع انخفاض مستويات الجلوتاثيون وانخفاض الأنشطة الأنزيمية للجلوتاثيون بيروكسيداز (GPx) والكاتلاز (CAT) في أنسجة القلب. أدى العلاج المسبق بالسبيرولينا إلى حماية القلب بشكل كبير، واستعادة الحالة المضادة للأكسدة وتقليل مستويات التوربونين والكوليسترول في الدم. كشف التحليل النسيجي المرضي عن درجات مختلفة من آفات القلب في المجموعة المعالجة بـ ISO، في حين قدمت المجموعات المعالجة بالسبيرولينا آفات أكثر اعتدالًا وقدمت حماية جيدة. تم تأكيد هذه النتائج من خلال التخفيض الكبير في حجم الاحتشاء في المجموعة المعالجة بالسبيرولينا باستخدام طريقة TTC يبدو أن السبيرولينا مادة واعدة لحماية القلب ضد احتشاء عضلة القلب الناجم عن ISO.

الكلمات المفتاحية: سبيرولينا؛ الطحالب الخضراء المزرقة؛ الاجهاد التأكسدي؛ النشاط المضاد للأكسدة؛ إيزوبروتيرينول؛ تسمم القلب؛ احتشاء عضلة القلب

Summary

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List of abbreviations

Abbreviation	Meaning
ADP	adenosine-diphosphoric acid
AMI	acute myocardial infraction
ASAT	Aspartate-Amino-Transferase
ASCVD	atherosclerotic cardiovascular disease
ATP	Adenosine Triphosphate
AxP	ascorbate Peroxidase
Cas-3	caspase-3
CAD	coronary artery disease
CAT	Catalase
CK	Creatine Kinase
CK-MB	Creatine Kinase myocardial band
COMT	catecho-O-methyltransferase
Cox-2	cyclooxygenase-2
DNA	Deoxyribonucleic Acid
DTNB	5,5-dithiodis-2-nitrobenzoic acid
DO	Optical density
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
GA	Gallic acid
GAE	Gallic acid equivalent
GPX	Glutathione peroxidase
GSH	Reduced Glutathione
HPLC	high performance liquid chromatography
H_2O_2	hydrogen peroxide
ISO	Isoproterenol
IsoP	Isoprostanes

IFN-γ	gamma interferons
IL-1β	interleukin-1 beta
IL-6	interleukin-6
LAD	Anterior interventricular artery
LDH	Lactate dehydrogenase
LDL	Low density lipoproteins
LPO	Lipid peroxidation
M	Molar
MDA	Malondialdehyde
Mg	milligram
MI	Myocardial infarction
mM	millimolar
MPO	Myeloperoxidase
MOA	monoamine oxidase
MYO	Myoglobin
Nacl	Sodium chloride
NO	Nitric oxide
NOS	nitric oxide synthase
˙OH	Hydroxyl
ONOO-	peroxynitrite
PBMCs	peripheral blood mononuclear cells
PrxR	peroxiredoxin
PUFAs	polyunsaturated fatty acids
QE	Quercetin equivalent
QRC	Quercetin
ROS	reactive oxygen species
SOD	Superoxyde Dismutase
SQ	Subcutaneous
TCA	2,4,6-trichloroanisole

TNF-α	tumor necrosis factor alpha
TOH	tocopherols
TTC	2,3,5-Triphenyltetrazolium chloride
VLDL	very low-density lipoprotein

Cardiovascular disease (CVD) is a group of heart disorders, the leading cause of nearly 18 million deaths annually, accounting for 31% of global deaths **(Virani et al., 2020)**. Myocardial infarction (MI), commonly known as a heart attack, is the primary form of CVD that causes morbidity and mortality globally **(Zhang et al., 2023)**.

MI is an acute disorder caused by an imbalance between oxygen demand and supply in the myocardium where coronary artery obstruction hinders blood flow to the heart muscle, resulting in heart muscle infarction and ischemic tissue **(Vilahur et al., 2022)**. MI development involves oxidative stress, inflammation, hyperlipidemia, and loss of plasma membrane integrity **(Pullaiah et al., 2021)**.

The MI models have paved the way for researching on experimental animals, an important part before applying on clinical trials. Currently, there are several ways to induce MI: *In vivo* and *ex vivo, in vitro* rodent models **(Martin et al., 2022)** and the chemical method involving the administration high dose of isoproterenol **(M. Siddiqui et al., 2016; Pipaliya and Vaghasiya, 2012)**. Using isoproterenol (ISO), a β-adrenergic agonist, is a non-invasive method, easy to apply, efficient to induce MI in experimental animals models, as it causes myocardial damage similar to human myocardial infarction **(Khalil et al., 2015)**.

Indeed, persistent β-adrenergic receptor stimulation with isoproterenol leads to the development of oxidative stress and lipid peroxidation, calcium overload, myocardial inflammation, thrombosis and platelet aggregation, which ultimately cause myocardial infarction **(Garg and Khanna, 2014)**.

Indeed, isoproterenol induces cardiac necrosis by increasing oxidative stress oxygen consumption associated with cytosolic calcium overload.

In addition to non-surgical angioplasty using stents for opening blocked coronary vessels **(Prete et al., 2024)**, therapeutic agents that are presently employed for the prevention and management of myocardial infarction are beta-blockers, antithrombotics, thrombolytics, statins, and calcium channel blockers **(Garg and Khanna, 2014)**. In spite of effective available interventions, the mortality rate of myocardial infarction is progressively increasing. Thus, there has been a regular need to develop effective therapies for the prevention and management of this insidious disease.

Nutraceuticals, a group of naturally occurring bioactive compounds with proven health benefits in addition to their nutritional value **(Carrizzo et al., 2020)**, have been a focus of these effective therapies **(Vilahur et al., 2018)**. Among them, microalgae have emerged as photosynthetic microorganisms capable of producing bifunctional molecules with various cytoprotective properties **(Costa et al., 2019)**.

Arthrospira platensis, a photosynthetic, microscopic filamentous blue-green microalga classified as cyanobacteria commonly known as "Spirulina", contains various compounds contributing to its antioxidant capacity, including Phycocyanobilin (PC), chlorophyll, carotenoids, phenolic compounds, polysaccharides, fatty acids, and vitamins **(Asghari et al., 2016)**.

Many research studies have demonstrated Spirulina's therapeutic functions, such as antioxidant, anti-inflammatory, hypolipidemic, antidiabetic, and brain-protective properties **(Chei et al., 2020; Deng and Chow, 2010a; Karkos et al., 2011)**. Research has also tested the cardioprotective effect of Spirulina **(Elmorsi et al., 2023)**.

In this context, our research investigates the impact of pretreatment with Spirulina (500 mg/kg/bw) on biochemical and histological changes during MI in rats induced by isoproterenol (150 mg/kg/bw).

Our dissertation is structured into two main sections.

The first part consists of a literature review focusing on theoretical concepts related to oxidative stress, Spirulina, and its role in cardioprotection. This review also covers the pathophysiology of myocardial infarction and the mechanism of MI induction by isoproterenol. The second part outlines the methodology used in conducting the study.

The second part describes the methodology based on the phytochemical analysis of spirulina followed by the evaluation of the effect of spirulina on isoproterenol-induced myocardial infarction in rats through *in vivo* study. In this section, obtained results are analyzed and discussed and the conclusion is also presented at the end of the thesis.

I. Myocardial infarction

1. Definition of myocardial infarction

Myocardial infarction (from Latin: *Infarctus myocardi*i, MI) is a term for an event of heart attack. MI occurs when blood stops flowing properly to a part of the heart, and the heart muscle is injured because of lack of oxygen supply. One of the coronary arteries which supplies blood to the heart develops a blockage due to an unstable buildup of plaques, white blood cells, cholesterol and fat. If the event becomes serious then it is called as "*acute myocardial infarction*" (AMI) **(Lu et al., 2015)**.

Myocardial infarction (MI) is sudden ischemia of the heart muscle tissue due to lack or obstruction of coronary blood flow, which leads to its damage**.** The heart muscle is anatomically fed by two major coronary arteries, the "right and left" (Figure 1). Each one terminates by small branches supplying the myocardium from the epicardial side. The left circumflex and left anterior descending "anterior interventricular" arteries form the main two terminal branches of left coronary artery. Although there is anastomosis between the small terminal branches of the coronary arteries, this anastomosis is insufficient if one of them is occluded**.** Obstruction or stenosis of any of them leads to a decrease in coronary blood flow and thus the development of MI. The MI is a leading cause of death in developed countries and has become a growing health problem in developing countries other than infectious diseases **(Hegazy et al., 2022)**.

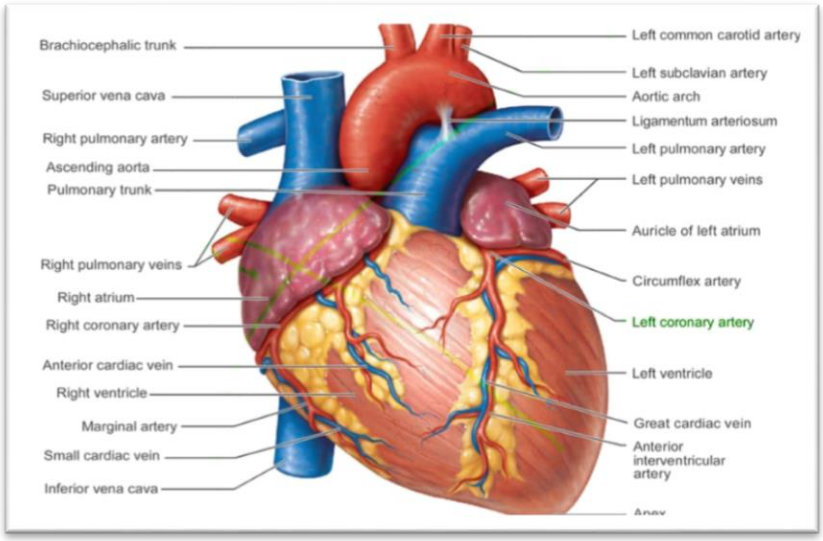


Figure 1: the anatomy of a heart **(Center, 2017)**.

2. Pathophysiology of myocardial infarction

Myocardial infarction (MI) or heart attack is the irreversible injury of myocardium due to prolonged ischemia and hypoxia. Atherosclerosis is the prominent cause of MI. Coronary atherosclerosis becomes complicated by subsequent rupture of Atherosclerosis plaque followed by occlusion of coronary artery via formation of a thrombus (Figure 2) **(Kumar et al., 2016)**.

The pathophysiological process can be summarized in the following steps.

- **Endothelial dysfunction**

Coronary endothelial dysfunction occurs before lesion formation and worsens as coronary artery disease (CAD) progresses. This dysfunction results from endothelial injury caused by factors such as oxidized low density lipoproteins (LDL), elevated blood glucose levels, hypertension, and increased oxygen-derived free radicals. Typically, there is a reduction in the release of endothelial nitric oxide (NO), which impairs vasomotion. Consequently, there is an increase in platelet and monocyte adhesion, as well as heightened smooth muscle cell proliferation**.** NO helps inhibit atherogenesis by promoting vasodilation and reducing platelet adhesion to the vascular endothelium **(Jebari-Benslaiman et al., 2022)**.

- **Plaque and necrotic core formation**

Atherosclerosis mainly leads to the formation of plaques through internal inflammation, fibrosis, necrosis and calcification. This process begins with the accumulation of LDL in the arterial endothelium, accompanied by intrinsic adaptive thickening. The LDLs then undergo modifications such as oxidation and aggregation. These modified LDLs trigger a chronic stimulation of innate and adaptive immune responses, leading to the differentiation of monocytes into phagocytes. These phagocytes engulf the lipid particles, forming foaming cells, characteristic of atherosclerotic plaques. This results in a leak of the endothelium **(Kowara and Cudnoch-Jedrzejewska, 2021)**.

- **Plaque angiogenesis and intervening hemorrhage**

New blood vessels form from the *vasa vasorum* within the vessel *adventitia*, providing an additional pathway for monocytes and immune cells to access the base of atheromatous plaques. These vessels are fragile, leading to bleeding and leakage of

erythrocytes and plasma proteins. This bleeding enlarges the plaque and encourages further inflammation. Additionally, the rupture of the fibrous cap of the plaque is another cause of bleeding **(Hegazy et al., 2022)**.

- **Fibrous cap rupture and thrombosis**

Plaque rupture occurs through a thin fibrous cap, often following intense exertion. Factors contributing to the rupture include an increase in free cholesterol and phagocyte infiltration within the plaque cap, as well as a reduction in its thickness. While plaque rupture can happen spontaneously, it can also be triggered by psychological, physical, or sexual stress, infections, or substance abuse . The mechanisms by which these triggers operate may involve elevated blood pressure and heart rate, which can precipitate plaque rupture, or enhanced coagulability and platelet thrombosis on an already vulnerable plaque **(Bentzon et al., 2014)**.

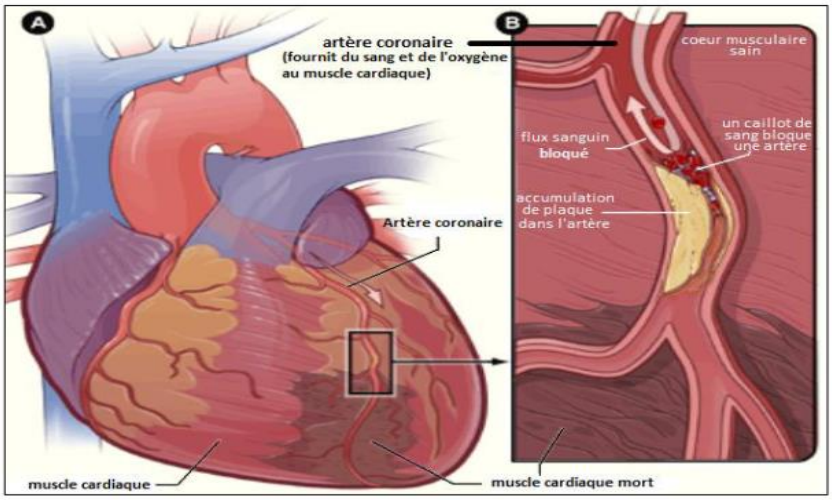


Figure 2: pathophysiology of MI **(Singh and Jat, 2021)**.

3. Assessment of myocardial damage

3.1. Hemodynamic parameters

Hemodynamics refers to the dynamics of blood flow within the cardiovascular system, which is a closed circulatory network responsible for delivering oxygen to all body tissues. It plays a crucial role in maintaining blood pressure and ensuring proper blood flow through the heart and blood vessels. Unlike purely theoretical fields such as mathematics or basic laboratory research, hemodynamics is a practical tool integral to daily clinical practice in cardiovascular medicine. Historically, it has been an essential method for systematically describing cardiovascular diseases through various

macroscopic parameters. These include systemic blood pressure, cardiac output, vessel blood flow rates, and peripheral vascular resistance. Such parameters are vital in clinical settings and are extensively used in environments ranging from outpatient clinics to intensive care units **(Itatani, 2015)**.

3.2. Biological blood markers

Ischemia caused by reduced coronary artery flow leads to the deterioration of ventricular function and myocardial necrosis. Consequently, troponin and enzymes like CK, LDH, ALT, AST, have long served as indicators for diagnosing acute myocardial infarction (AMI).We review the commonly used cardiac markers, including cTnI, cTnT, CK-MB, and myoglobin, which are frequently utilized for diagnosing AMI and assessing prognosis **(Aydin et al., 2019)**.

1.2.1. Non-enzymatic markers

- **Troponin complex**

Troponin is a complex molecule present in cardiac tissues. It confers calcium sensitivity on muscle actinomycin adenosine triphosphatase activity. It consists of three subunits withdistinct functions: (1) troponin C (cTnC), (2) cTnI, and (3) cTnT. Troponins are also present in skeletal muscle, and because the amino acid sequence of cTnC is similar to those of skeletal troponin, ctn cannot be used as a cardiac specific marker. However the isoforms of cTnI and cTnT differ significantly from skeletal muscle troponin **(Tiwari et al., 2012)**.

- **Myoglobine**

Myoglobin (MYO) is a low molecular weight heme protein (17 kDa) found in both cardiac and skeletal muscle, which limits its specificity as a cardiac marker. Despite this, it is one of the earliest markers released from infarcted myocardium **(Tiwari et al., 2012)**.

(MYO) is a small cytoplasmic protein that binds oxygen, found in both skeletal and cardiac muscle tissues. It is quickly released into the bloodstream, typically within 1 hour after myocardial injury onset, peaks at 4-12 hours, and returns to normal levels rapidly. However, MYO lacks specificity to cardiac tissue because it is also abundant in skeletal muscle. Therefore, MYO levels alone cannot reliably diagnose myocardial infarction but can be used alongside troponins or CK-MB. Serum MYO levels are more useful for ruling out myocardial infarction rather than confirming it **(Mythili and Malathi, 2015)**.

1.2.2. Nonspecific enzyme markers

- **Creatin kinase**

CK is an enzyme responsible for facilitating the reversible conversion of creatine and ATP into creatine phosphate and ADP **(Aydin et al., 2019)**. Since the early 1970s, and especially since the 1980s with the introduction of the ELISA technique, CK has become an essential laboratory parameter for detecting myocardial damage and AMI. However, the enzyme is also present in many other tissues, significantly reducing its specificity as a biomarker for myocardial damage. This challenge has been partially addressed through the use of the CK-myocardial band (CK-MB) isoform **(Tilea et al., 2021)**.

- **Lactate dehydrogenase (LDH)**

LDH, an enzyme involved in converting lactate to pyruvate, emerged as another promising indicator of AMI. LDH levels in the blood typically start to rise within 6–12 hours following AMI onset, peak within 1 to 3 days afterward, and return to normal within 8–14 days. Like AST, LDH is found in various tissues including the liver, kidneys, heart, red blood cells, lungs, and notably, skeletal muscle, resulting in LDH being a marker with limited specificity for cardiac injury **(Tilea et al., 2021)**.

- **L-aspartate-aminotransférase (ASAT)**

Aspartate aminotransferase (AST, EC 2.6.1.1) is an enzyme primarily located in the liver, with additional presence in red blood cells, heart cells, muscle tissue, and various other organs including the pancreas and kidneys **(Huang et al., 2006)**. In 1954, Ladue et al. showed a notable increase in AST levels approximately 3–4 hours after an AMI, marking the advent of enzyme-based AMI diagnosis. AST levels in the blood elevate within the initial 12–24 hours following an AMI, peak around 1–2 days after the acute event, and return to normal baseline levels within 10–14 days post-AMI **(Tilea et al., 2021)**.

3.3. Measurement of myocardial infraction size

Triphenyltetrazolium chloride (TTC) staining are the method used to determine experimental myocardial infarction size **(dos Santos et al., 2008)**. The mechanism by which tetrazolium salt reacts with cardiomyocytes is that TTC. stains the myocardium

red, due to complete dehydrogenase activity, while infarcted myocardium lacking dehydrogenase activity, remains unstained **(Khalil et al., 2006a)**.

3.4. Markers of cardiac cell damage

3.4.1. Parameters of oxidative stress

Biomarkers of oxidative stress can be divided into two main categories: molecules that undergo modifications as a result of interactions with reactive oxygen species (ROS) in the surrounding environment, and molecules belonging to the antioxidant system that respond to heightened redox stress **(Ho et al., 2013)**.

- **Isoprotanes**

Isoprostanes are non enzymatic, free radical– catalyzed isomers of cyclooxygenase-derived enzymatic products of arachidonic acid and are found in both plasma and in urine. Elevated F2 isoprostane levels have been documented in patients with a variety of risk factors. Furthermore, they localize within foam cells and atherosclerotic plaques **(Tsimikas, 2008)**.

- **Malondialdehyde**

MDA is produced naturally within the body through the peroxidation process of polyunsaturated fatty acids. It can bind with proteins and contribute to the development of atherosclerosis. Specifically, when MDA reacts with lysine residues, it forms lysine-lysine cross-links**.** These cross-links have been detected in the apolipoprotein B (apoB) sections of oxidized low-density lipoprotein (OxLDL), and are believed to hinder the interaction between OxLDL and macrophages, potentially exacerbating the progression of atherosclerosis **(Ho et al., 2013)**.

- **MPO**

Leucocytes play a central role in atherosclerotic plaque rupture. Myeloperoxidase in leucocytes may activate metalloproteinases and inactivate plasminogen activator inhibitor. Leucocytes also consume nitric oxide catalytically, causing vasoconstriction and endothelial dysfunction. Myeloperoxidase has been found in atheromatous plaques. Patients with chronic angina have circulating neutrophils with large quantities of MPO, which decrease substantially post-ACS**(Chan and Ng, 2010)**.

- **Oxidized low-density lipoprotein**

Low density lipoprotein is the major carrier of cholesterol to body tissues. Elevated LDL-cholesterol (LDLC) level is a well-recognized risk factor for CVD .The ingestion of OxLDL transforms macrophages into foam cells **(Trpkovic et al., 2015)**.

- **Nitrotyrosine**

The nitrification of the tyrosine protein is mediated by ROS such as peroxynitrite (ONOO-) and nitrogen dioxide (NO2), and results in the addition of a nitro group to sensitive tyrosine residues **(Ho et al., 2013)**.

3.4.2. Apoptosis and necrosis

Cell death is commonly categorized into two main types: apoptosis and necrosis. Apoptosis is portrayed as a deliberate, programmed cellular breakdown that occurs independently and doesn't trigger inflammation. On the other hand, necrosis is seen as an unintentional cell death caused by external factors, leading to the uncontrolled release of inflammatory substances from the cells. Because apoptosis is viewed as a carefully regulated process, its involvement in specific infections has been heavily studied **(Fink and Cookson, 2005)**.

3.4.2.1. Apoptosis

Apoptosis, also known as programmed cell death, is a fundamental process in various biological activities. Dysregulation of apoptosis, whether excessive or insufficient, is implicated in numerous diseases such as ischemia, neurodegeneration, autoimmune disorders, viral infections, and the development and regression of tumors. Multiple pathways within cells can trigger apoptosis. Among these, the intrinsic pathway is characterized by the central role of mitochondria, regulated by pro- and anti-apoptotic members of the Bcl-2 protein family (Figure 3). The extrinsic pathway involves cell surface receptors from the TNF-related family (such as TNF receptor, CD95/Fas, and TRAIL death receptors), their inhibitory counterparts, and cytoplasmic molecules like FADD or FLIP **(Jeong and Seol, 2008)** .Apoptosis which is characterized by cellular membrane hyper-permeabilization, nuclei fragmentation, chromatin condensation, and the formation of apoptotic bodies **(Zhu et al., 2021)**.

3.4.2.2. Necrosis

Unlike apoptosis, necrosis is not regulated by proteins or enzymes. The initial trigger signal for necrosis is the rupture of biofilms, such as cell membranes or mitochondria. It is accepted that the rupture of the cell membrane is the result of a decrease in intracellular osmotic pressure, favoring the entry of water into the cytoplasm **(Xu et al., 2018)**.

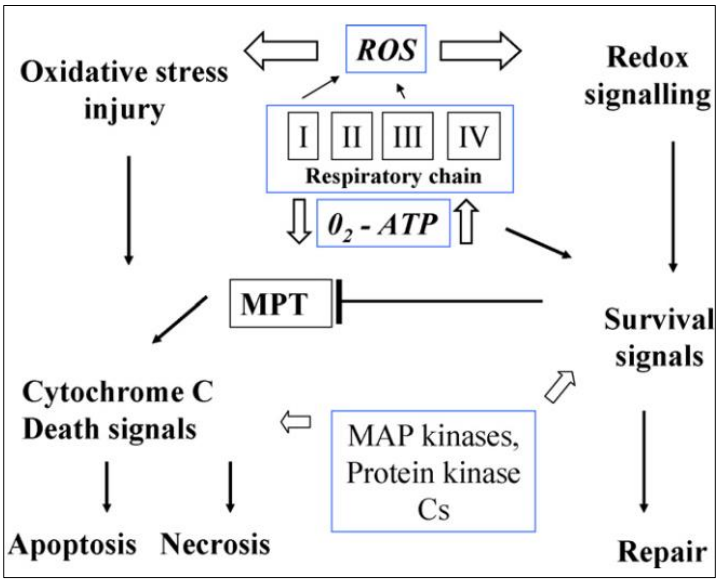


Figure 3: cell signalling inacute myocardial ischemia_death versus survival signals **(Ytrehus, 2006)**.

4. Experimental models of myocardial infarction

The use of animal models in cardiovascular disease research is crucial for understanding the mechanisms and developing therapeutic approaches. These models are essential tools in the early stages of drug development, offering deeper insights into disease pathophysiology. They also facilitate the creation of new methods to improve the diagnosis and treatment of cardiovascular diseases **(Kumar et al., 2016)**.

4.1. *In vivo* model

Experimental models aimed at studying myocardial ischemia in the integrated organism are important in human medicine given the limited possibilities for well-controlled human studies. Whether the investigator wants to be close to the situation in human medicine or studies the influence of extra-cardiac factors in ischemic heart

disease, the model of choice will be an *in vivo* model. This also includes development of new clinical methods, instruments and pharmacological agents **(Ytrehus, 2006)**.

A response similar to acute myocardial infarction (AMI) caused by atherosclerosis or thromboembolic events in otherwise healthy hearts can be induced by occluding a coronary artery in an experimental animal. This procedure can be carried out surgically by ligating a coronary vessel in an open chest configuration (Figure 4) or, less invasively, by catheter based techniques in closed-chest models including percutaneous balloon occlusion **(Sattler et al., 2019)**.

Most *in vivo* experimental models of myocardial ischemia have been established in the dog, pig, rabbit, rat or mouse **(Ytrehus, 2006)**.

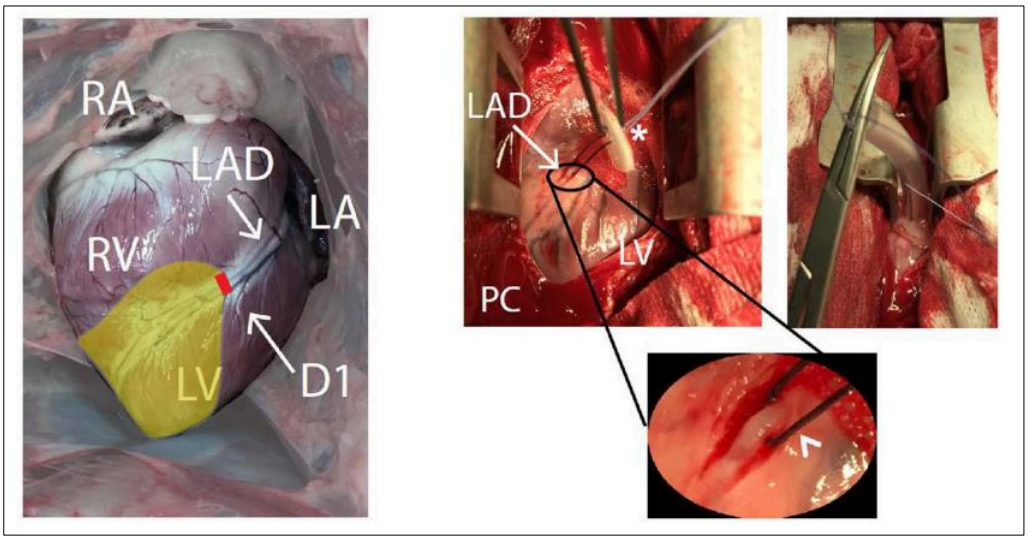


Figure 4: Open chest approach for MI **(Sattler et al., 2019)**.

Right (RA) and left atrium (LA), right (RV), and left ventricle (LV) as well as the left anterior descending artery (LAD) with its diagonal branch (D1) are visible. Pericardium (PC) is opened, allowing the dissection of the LAD, a silk snare with a counter bearing (asterisk) is placed around the artery (arrowhead, round agnification) and tightened for coronary occlusion.

4.2. *Ex vivo* model

Over the past 20–30 years, the use of isolated perfused hearts has steadily increased and they are now widely utilized for studying myocardial ischemia (Figure 5). The simplicity and relatively low cost of this technique generally allow for a greater number of experiments and the testing of more hypotheses compared to more complex *in vivo* models **(Ytrehus, 2006)**.

Ex vivo models are simple and reliable to understand the molecular mechanism, electrophysiology, mechanical activity, and target sites of novel pharmacological agents. *Ex vivo* studies are free from biological hindrance such as sex, age, and pharmacokinetic variables **(Kumar et al., 2016)**.

The isolated perfused rat heart is employed for biochemical tissue analyses, various functional measurements, and infarction size evaluation, along with the time-limited application of pharmacological probes, as the non-recirculating perfusion solution allows for washout **(Ytrehus, 2006)**.

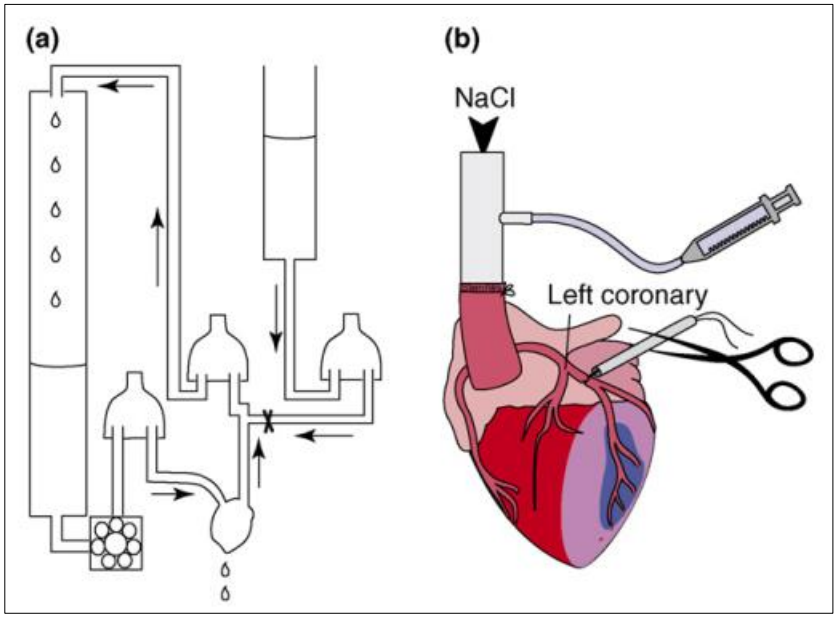


Figure 5: Isolated perfused hearts **(Ytrehus, 2006)**.

(a) *Isolated perfused hearts have been instrumental in understanding the basic principles of cardio-protection, in understanding cell signalling and last but not least in understanding time dependent metabolic changes in the heart muscle during limited oxygen supply.* ***(b)*** *Regional ischemia in the isolated perfused heart is obtained by ligation of the left coronary artery or one of its main branches depending on species Ischemic risk zone and the infarction are indicated by color difference (normally perfused myocardium: red and pink; ischemic risk zone: purple; infarction: blue).*

4.3. *In vitro* model : isolated cardiac cell

The *in vitro* systems allow for more precise control of experimental conditions and manipulations, offering several advantages over *in vivo* models for studying signaling pathways or conducting high-throughput drug screenings **(Oh et al., 2019)** .

Commonly cardiomyocytes are isolated from cultured embryonic cells or adult cells by different techniques such as a langendroff perfusion method and enzymatic digestion method. Enzymes such as collagen type II, proteases and pancreatin are commonly used enzymes for digestion method. Isolated myocytes are placed in a perspex chamber super fused with physiological salt solution (PSS), and bubbled with 95% O_2 and 5% CO_2 **(Kumar et al., 2016)**.

Advantages of this method includs the ability to manipulate and replicate disease mechanisms using advanced molecular biological techniques, thereby enhancing our understanding of disease pathology **(Janczewski et al., 2003)**.

4.4. Chemical Method-Isoproterenol Induced MI Model

Isoproterenol (ISO)-induced acute myocardial infarction is a well-established non-surgical animal model in rats. Chemically, ISO is known as L-β-(3,4-dihydroxyphenyl) α-isopropylamino ethanol hydrochloride, containing a catechol nucleus. This model offers several advantages over surgical methods, including a low mortality rate, simplicity, and the absence of surgical procedures, thus reducing the risk of post-surgical infections. While rats are commonly preferred for this model, other species such as mice and rabbits have also been reported to be used. Administration of a single subcutaneous dose of ISO at 85-150 mg/kg body weight on two consecutive days has been found to induce similar metabolic, biochemical, and morphological changes to those observed in humans **(Kumar et al., 2016).**

II. Isoproterenol induced myocardial infraction

1. Definition and structural

Isoproterenol (ISO) [1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride], is a synthetic catecholamine and β-adrenergic agonist which has been documented to produce severe stress in the myocardium resulting in the myocardial infarction, if administered in supramaximal doses. It produces myocardial necrosis which caused cardiac dysfunction, increased lipid peroxidation along with an increase in the level of myocardial lipids, altered activities of the cardiac enzymes and antioxidants **(Patel et al., 2010)**.

2. Physicochemical properties of isoproterenol

The molecular formula of isoproterenol is $C_{11}H_{17}NO_3HCl$ (Figure 6). It has a molecular weight of 247.72 g/mole. Isoproterenol hydrochloride is a racemic compound. The pH is adjusted between 2.5 and 4.5 with hydrochloric acid or sodium hydroxide. It must be stored in unopened ampoules, protected from lighti at 4°C **(Tubergen, 2023)**.

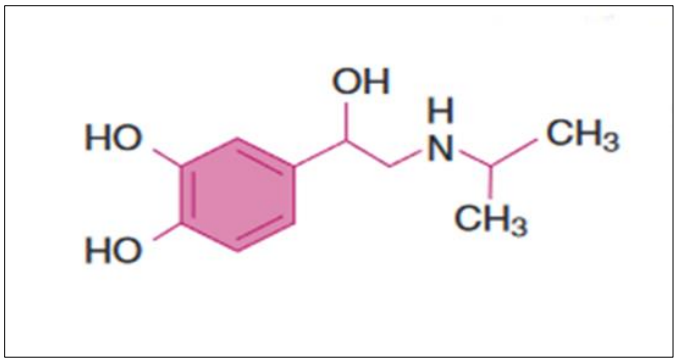


Figure 6: Chemical structure **(Ebert, 2013)**.

3. Pharmacokinetics of isoproterenol

3.1. Absorption

Isoproterenol can be absorbed systemically through the sublingual mucosa, but it is absorbed more consistently when administered parenterally or as an inhaled aerosol. It is minimally metabolized by catecho-O-methyltransferase (COMT) and is resistant to degradation by monoamine oxydase **(M. A. Siddiqui et al., 2016)**.

3.2. Distribution

The half-life of isoprenaline hydrochloride is short, lasting only a few minutes after intravenous administration and up to 2 hours following subcutaneous administration. Isoproterenol is not efficiently metabolized by monoamine oxydase (MOA) and is not taken up by sympathetic neurons **(Gordon et al., 2006)**.

3.3. Metabolism

Isoproterenol is quickly metabolized, mainly in the liver, into 3-O-methyl-ISO by the intracellular enzyme catechol-O-methyltransferase. Another metabolic pathway involves the removal of isoproterenol from the blood by extraneuronal peripheral tissues with uptake mechanisms, such as the heart, smooth muscles, and fat cells, where it is subsequently metabolized by catechol-O-methyltransferase **(Reyes et al., 1993)**.

3.4. Elimination

The primary metabolite after intravenous administration is 3-O-methylisoprenaline, which has been noted to exhibit weak beta-adrenergic blocking activity, along with its conjugates. These metabolites are eliminated through the kidneys and excreted in the urine and bile, either in their free or conjugated forms. Additionally, isoproterenol can be excreted unchanged in the urine or conjugated by sulfatase and glucuronidase enzymes present in the liver **(Reyes et al., 1993)**.

4. Mechanism of isoproterenol-induced myocardial damage

ISO is given subcutaneously at a dose of 85–100 mg/kg/day for two consecutive days, with a 24-hour interval between doses for acute toxicity studies. For subchronic studies, it is administered over a period of 21–28 days. Rodents are the preferred species for this model, with male albino Wistar rats and Swiss mice being commonly used **(Allawadhi et al., 2018)**.

This substance Causes a myocardial damage similar to the one observed in AMI in humans. Among several mechanisms proposed to explain the ISO-induced myocardial harm, we cite first an unbalance between oxygen supply to and demand from cardiomyocytes inwardly, which is related to myocardial hyperfunction. Secondly, it is also claimed that there is an elevation of Ca^{2+} overcharge inside the cell. In Addition, that ion is related to the activation of the adenylate Cyclase enzyme and the depletion of ATP levels on the course of the events. There is also an oxidative stress augmentation because of several metabolic products originated from isoproterenol (Figure 7) **(Lobo Filho et al., 2011a)**.

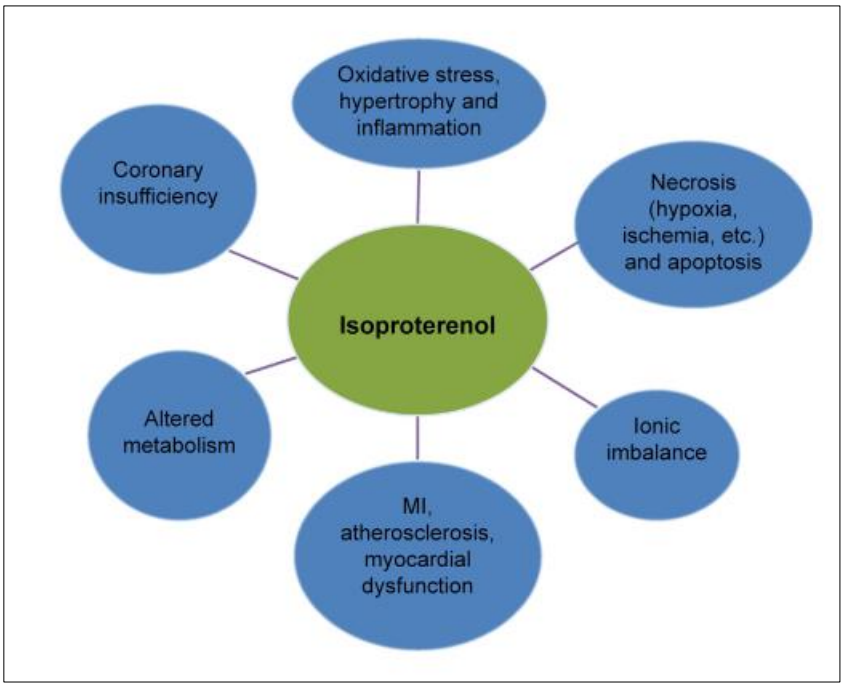


Figure 7: Physiological effect of isoproterenol in the myocardium **(Wong et al., 2017)**.

4.1.ISO-mediated oxidative stress

The signaling mechanisms linked to β-adrenergic receptor overactivation have been investigated using animals treated with isoproterenol (ISO). Studies have shown that ISO treatment causes myocardial oxidative stress **(Davel et al., 2014)** .

Oxidative stress is generated due to ROS and imbalanced antioxidant defense mechanisms. ROS are generated by an activated nicotinamide adenine dinucleotide phosphate oxidase, xanthine oxidase, autooxidized catecholamines, increased angiotensin-II and aldosterone levels as well as released proinflammatory cytokines. Further, Additionally, calcium-dependent NADPH oxidases (NOXs) are another major source of ROS, such as hydrogen peroxide (H_2O_2) and superoxide anion (O_2) **(Yu et al., 2021)**.

The increase in oxidative stress corresponds to the elevated neutrophil count, which in turn, leads to increases in the release of leukotrienes, reactive oxygen species (ROS) and hydrolytic enzymes which further aggravate the myocardial injury [12]. On the other hand, the levels of antioxidants, most notably catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPx), are significantly reduced **(Wong et al., 2017)**.

4.2. ISO-mediated calcium overload

ISO administration also produces an ionic imbalance. Ca^{2+} ions are overloaded as a result of Exaggerated direct β-receptor stimulation by isoproterenol leading to activation adenylate cyclase enzyme activation, as well as the depletion of ATP levels (Figure 8) **(Garg and Khanna, 2014)**.

The accumulation of Ca^{2+} ions negatively affected the mitochondrial membrane potential, inducing the formation of ROS and free radicals, which activate the calcium- and magnesium-dependent endonuclease and damage the DNA by fragmentation, eventually leading to ischemic injury and cellular apoptosis **(Wong et al., 2017)**.

Increase of myocardial Ca^{2+} content will result in myofilament over-stimulation, increase of contractile force and oxygen requirement as well as excessive adenosine triphosphate (ATP) breakdown; each of these factors contribute to cardiac muscle cell injury **(Garg and Khanna, 2014)**.

4.3. ISO-mediated lipid peroxidation

Isoproterenol, when oxidized, generates quinones that interact with oxygen to produce superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2). The generation of superoxide radical leads to the release and reduction of iron from tissue ferritin, along with the subsequent formation of hydrogen peroxide and hydroxyl radical ($^{\bullet}OH$) which, in the presence of iron, forms highly reactive hydroxyl radicals and causes protein, lipid, and DNA damage and increased MI size **(Chattopadhyay et al., 2003a)**. In addition, excessive formation of free radicals may result in the loss of function and integrity of myocardial membranes. These free radicals may attack polyunsaturated fatty acids (PUFAs) within the membranes, forming peroxyl radicals. These radicals can then attack adjacent fatty acids, causing a chain reaction of lipid peroxidation (LPO). The lipid hydroperoxide end products are also harmful and may contribute to further tissue and organ damage **(Khalil et al., 2015b)**.

4.4. ISO-mediated necrosis and apoptosis

ROS and free radicals activate calcium- and magnesium-dependent endonucleases, causing DNA damage through fragmentation, ultimately leading to ischemic injury and cellular apoptosis. Myocardial infarction (MI) involves both necrosis and apoptosis. Isoproterenol (ISO) administration leads to the downregulation of the anti-apoptotic protein Bcl-2 and increased expression of the pro-apoptotic protein Bax. Additionally, there is an upregulation of the caspase-3 (Cas-3) enzyme and an increase in the number of TUNEL-

positive cells. The physiological and morphological changes induced by ISO in the heart closely resemble those observed in human MI **(Wong et al., 2017) (Garg and Khanna, 2014)**.

4.5. ISO-mediated inflammation

Inflammation is a complex cellular process triggered by ROS in response to stress and is reported in the majority of coronary heart diseases during the initiation and progression of atherosclerotic plaque formation. NF-κB, a transcription factor, plays a crucial role in inflammation. Elevated ROS levels during MI activate NF-κB, leading to the expression of various proinflammatory genes, including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (Cox-2), tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6). Thus, ischemic stress induces cytokine release during myocardial infarction, leading to inflammatory responses (Figure 8) **(Viswanadha et al., 2020)**.

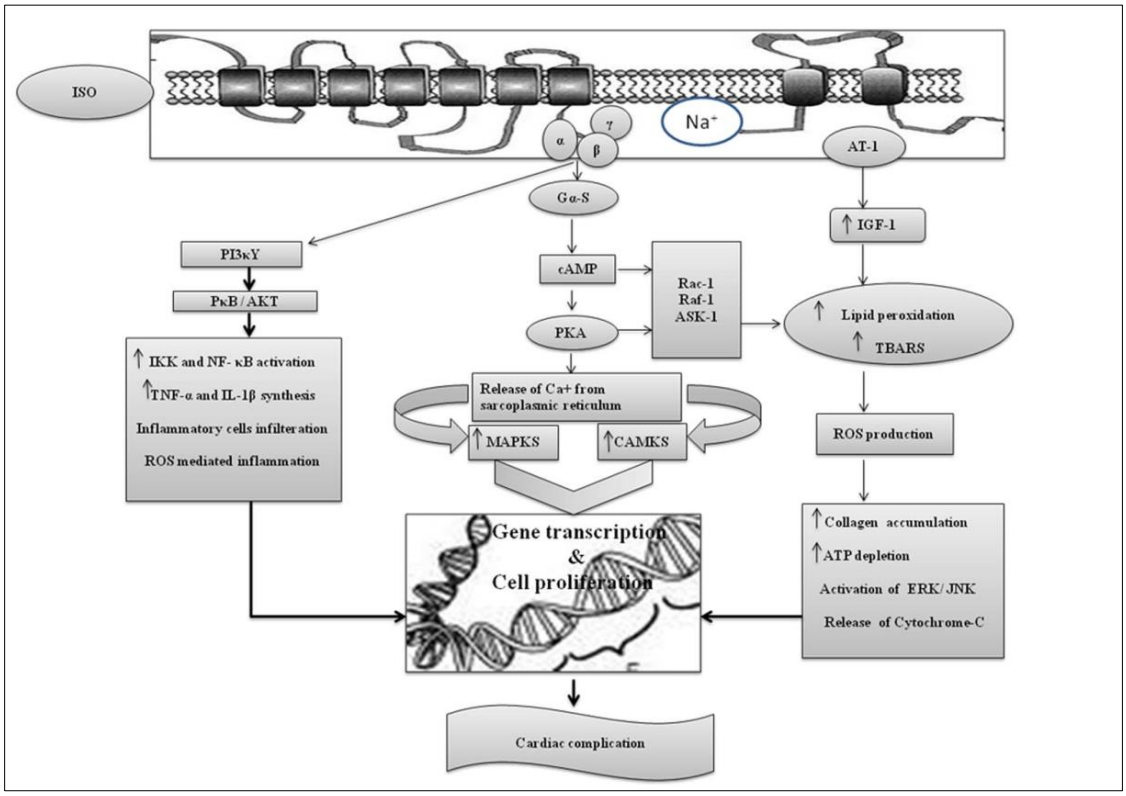


Figure 8: β -adrenergic receptor over activation mediated cardiac toxicity through oxidative stress, Calcium overload and inflammatory cascades **(Garg and Khanna, 2014).**

5. Pharmacological interventions to attenuate ISO-induced MIs

There are several pharmacological interventions that stop the progression of myocardial injury and can be used to improve toxicity in ISO-treated animals **(Garg and Khanna, 2014)**.

5.1. Antioxidative agents

Quinidine Na^+ channel blocker possessing free radical scavenging properties, enhances the antioxidant defense system by increasing the activity of SOD and catalase. Dhalla and colleagues conducted experiments illustrating that free radical scavenging enzymes serve as the primary cellular defense against oxidative stress by breaking down $O_2^{\bullet-}$ and H_2O_2 before they combine to form more reactive hydroxyl radicals **(Dhalla et al., 2000)**.

5.2. Calcium antagonists

Calcium antagonists exert multiple effects aimed at counteracting excitation-contraction coupling, leading to vasodilation and decreased myocardial contractility. Additionally, they restrict calcium entry into cardiac and smooth muscle cells, thereby lowering adenosine triphosphate breakdown and reducing myocardial oxygen demand. Dexrazoxane likely decreases mortality rates in isoproterenol-treated animals by mitigating myocardial calcium overload and ameliorating histological damage, along with improving peripheral hemodynamic alterations **(Garg and Khanna, 2014)**.

5.3.Lipid-lowering agents

Emerging lipid treatments target the reduction of atherosclerotic cardiovascular disease (ASCVD) events by decreasing atherogenic particles like LDL and Lp (a) (lipoprotein[a]), as well as triglyceride (TG)-rich VLDL (very-low-density lipoprotein). They also aim to lower the risk of pancreatitis in individuals with markedly elevated TG-rich lipoproteins, mainly chylomicrons. Conversely, there is less excitement about increasing HDL (high-density lipoprotein) **(Hegele and Tsimikas, 2019)**.

6. Natural product as myocardial infraction protection

Natural products of animal, plant and mineral origins , have been a reliable source of new chemical entities to treat various disorders**.** Only a minute proportion of the 500,000 existing plant species have been investigated for bioactivities. More than 2000 of the registered plants in the system have been scientifically proven to help with cardiovascular diseases**.** Most of these plant extracts contain several types of secondary metabolite, which

would mean that each extract manifests multiple overlapping actions (such as antioxidant or anti-inflammatory) to exhibit cardioprotection (Figure 9) **(Wong et al., 2017)**. Including Curcumin, Citrus limon (sinapic acid), Coriandrum sativum, Propolis**.**

6.1. Curcumin

Medicinal plants offer significant benefits in treating cardiovascular disease due to Curcumin, a polyphenol compound extracted from the turmeric plant's rhizome. It has anti-inflammatory, antioxidant, antifibrotic, and antitumor properties **(Ali et al., 2018; Rashid et al., 2017)**. Experimental research has shown that curcumin exhibits potent antioxidant effects. It works by reducing the formation of peroxides in blood vessels, lowering vascular resistance, restoring vascular reactivity, and hindering the onset and progression of hypertension (**Nakmareong et al., 2011**). Additionally, curcumin can suppress H/R-induced apoptosis and autophagy in H9c2 cardiomyocytes by upregulating Bcl-2 and inhibiting the expression of Bax, BECN1, BNIP3, and SIRT1 **(Huang et al., 2015)**. Through the inhibition of PI3K-AKT-mTOR signal transduction, promotion of BECN1 and Bcl-2 dissociation, prevention of FOXO1 acetylation, and reduction of oxidative stress, curcumin regulates autophagy, thereby safeguarding vascular endothelial cell function and blood pressure control **(Han et al., 2012)**.

6.2. Citrus limon (sinapic acid)

Sinapic acid is a compound derived from cinnamic acid, characterized by specific chemical substitutions in its structure. It is commonly found in plants. Lemon (Citrus limon L.) contains the highest amount of sinapic acid among natural sources. Studies have shown that pretreatment or co-administration of sinapic acid at a specific dose can protect against heart damage induced by certain chemicals. Sinapic acid has demonstrated the ability to reverse the harmful effects caused by these chemicals **(Roy and Prince, 2013)**, such as elevated cardiac troponin levels, heart weight, and abnormal electrocardiogram readings. It also helps in restoring cholesterol levels and enzyme activities back to normal **(Roy and Prince, 2012)**.

Coriandrum sativum is an herb from the Apiaceae family, with its leaves and seeds being beneficial for medicinal purposes. Various extracts of the plant have shown therapeutic potential in conditions like CVDs, diabetes, and urinary disorders. A methanolic extract has demonstrated a cardioprotective effect in a rat model of ISO- induced cardiac damage

The rich polyphenolics in the extract play a crucial role in preventing oxidative damage by scavenging the ROS generated by ISO, highlighting its significance in cardiac protection **(Patel et al., 2012)**.

6.3. Propolis

Propolis is a resinous substance gathered by bees from plant exudates and buds, to which substances produced by the bee's metabolism are added. It exhibits various cardiovascular protective properties due to its active components, particularly its phenolic compounds such as chrysin, quercetin, pinocembrin, and luteolin **(Braik et al., 2019)**. These phenolic compounds in propolis reduce the activity of cyclooxygenases, reactive oxygen species, and nitric oxide (NO), which are also linked to the antioxidant properties of propolis **(Zullkiflee et al., 2022)**. These polyphenols impact the production of NO in the vascular endothelium, leading to blood vessel dilation and the activation of genes that promote cardiovascular protection **(Braakhuis, 2019)**. propolis shows cardioprotective effects and antioxidant properties against oxidative stress induced by isoproterenol by scavenging cytotoxic radicals **(Ahmed et al., 2017a)**.

6.4. Spirulina

Spirulina has been recognized as a superfood and nutraceutical due to its high nutritional value and associated health benefits **(Pérez-Alvarez et al., 2021)**. Several studies have demonstrated the modulation of markers related to various cardiovascular diseases through the consumption of spirulina. Both *in vitro* and *in vivo* experiments have been conducted to elucidate the molecular mechanisms underlying these positive effects. For instance, research on the effects of Spirulina platensis extract on cardiac injury markers during isoproterenol-induced myocardial infarction in rats has shown a reduction in biomarker levels (Prasesti et al., 2023a). In another study, oral supplementation with spirulina for 10 days prior to inducing ST-elevation myocardial infarction (STEMI) led to a 64% decrease in infarct size, an 18% increase in myocardial salvage, and a 30% improvement in cardiac function compared to controls **(Vilahur et al., 2022)**. These positive outcomes have been attributed to spirulina's components such as phycocyanin, gamma-linolenic acid, B vitamins, and potent antioxidants **(Sokary et al., 2024)**. Further investigations are needed to fully understand the mechanisms behind its protective effects and its potential for clinical application.

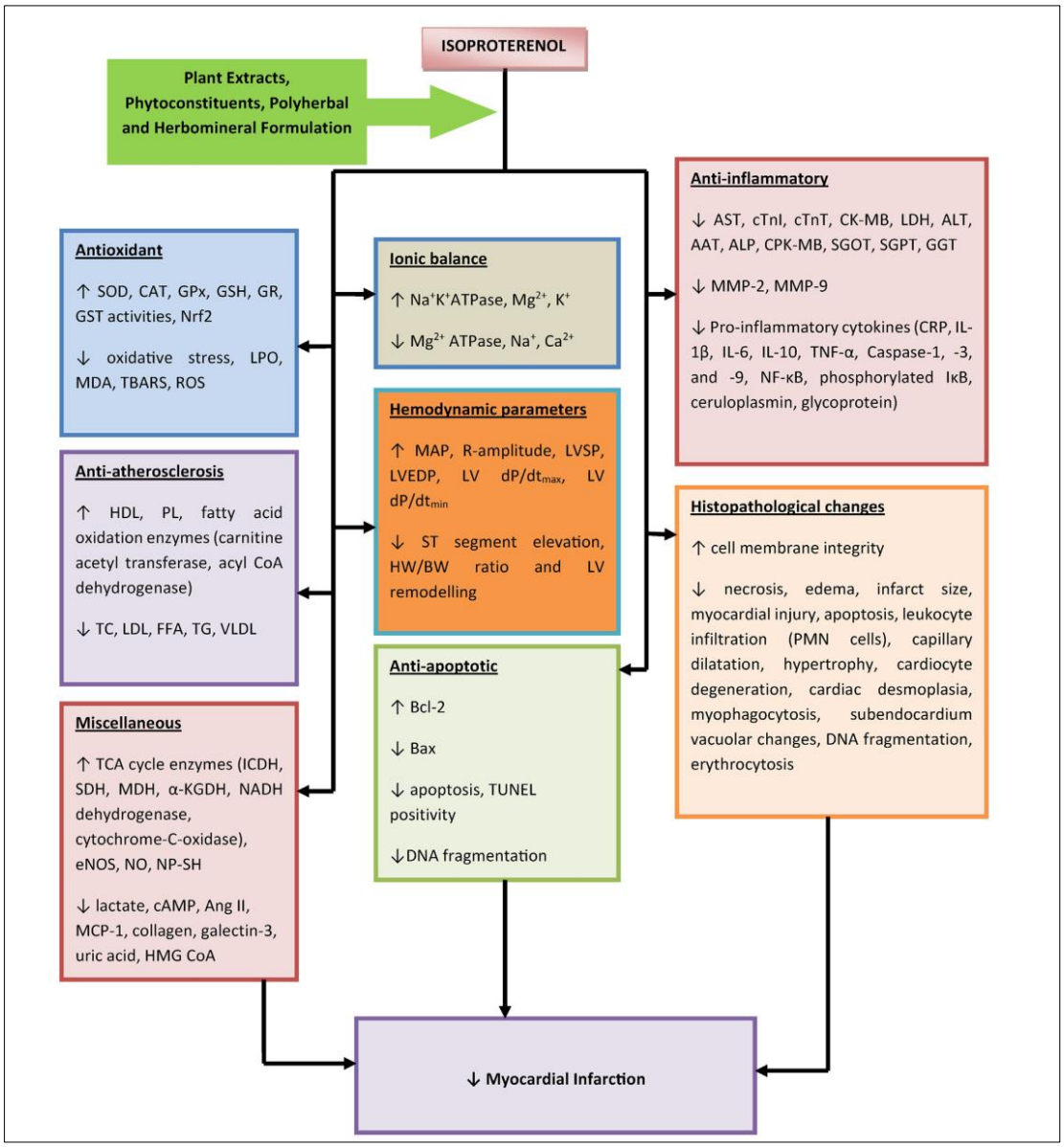


Figure 9: Natural product as myocardial infraction protection**(Wong et al., 2017)**.

Flow diagram indicating amelioration of biochemical parameters and signaling molecules involved in cardiac remodeling events associated with myocardial infarction when treated with plant extracts, phytoconstituents, polyherbal and herbomineral formulations.

III. Spirulina: Natural antioxidant

1. Definition and origin

Arthrospira platensis called Spirulina, is a spiral cyanobacterium of photo-autotrophic blue-green color. It is a prokaryotic organism that shares with plants the ability to perform photosynthesis. From mineral compounds, water, and light energy captured thanks to their chlorophyll, they transform carbon dioxide and release oxygen **(Chorus and Welker, 2021)**.

Spirulina's photosynthetic pigment system is made up of blue phycocyanine, chlorophyll and carotenoids. Some spirulina contain phycoerythrin, another pigment that gives this micro-algae a red or pink colour **(Hajati and Zaghari, 2019)**.

The name "Spirulina" comes from the latin word meaning "*helix*" or "*spiral*" referring to the organism's shape. Spirulina naturally thrives in high-salt, alkaline water reservoirs in subtropical and tropical regions such as America, Mexico, Asia, and Central Africa **(Ohmori and Ehira, 2014)**.

Spirulina takes the form of a filament, with an average length of 250 μm when it has 7 turns and a diameter of around 10 μm **(Cruchot, 2008)** . Generally, it is presented in different forms, "spiral", "wavy", and "straight"(figure10). This particularity of form is in direct relation with the ecological conditions encountered in their habitat **(Charpy et al., 2008).**

Arthrospira platensis exhibits distinctive physiological traits,One notable feature of *A. platensis* is the vigorous gliding movement of its filaments (trichomes), which involves rotation along their length. This gliding is a form of self-propulsion across solid or semi-solid surfaces, occurring without the assistance of any visible flagella or other structure **(Ohmori and Ehira, 2014)**.

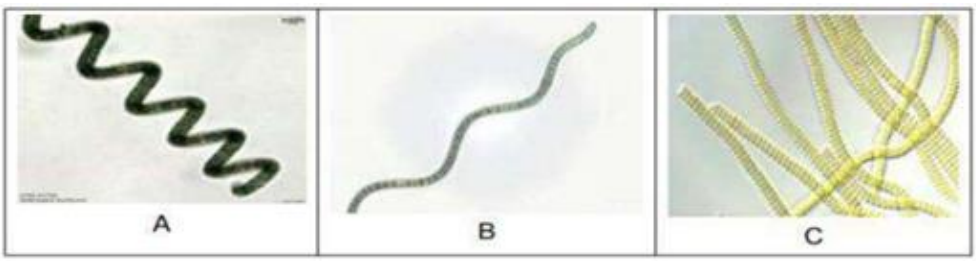


Figure 10: Different microscopic aspects of spirulina **(Charpy et al., 2008)**.

(A) spiral shape; **(B)** wavy shape; **(C)** straight shape

Spirulina can be packaged in the form of powder, tablets **.**It is well-known for its use as a food or dietary supplement and is a significant part of the diet in tropical regions. The Aztecs regularly consumed Spirulina. Lake Chad is notable for its residents'use of Spirulina, which they harvest from natural populations for food . However, current taxonomy suggests that the name "Spirulina" is not accurate for the strains used as food supplements, and there is consensus that Arthrospira is a distinct genus, comprising over 30 species, including *A. platensis*, *A. maxima*, and *A. fusiformis* . These Arthrospira species are edible and possess high nutritional and potential therapeutic values. *A. platensis* has become a significant industrial organic resource, employed as a health supplement, a source of β-carotene, and a natural colorant. Moreover, the hydrogenase in its cells renders this cyanobacterium beneficial for clean, sustainable energy production **(Ohmori and Ehira, 2014)**.

2. Composition

Spirulina is considered a high quality nutritional food source, particularly due to its high digestibility **(Bellahcen et al., 2013)**. The use of analytical techniques (spectroscopy, chromatography, etc.) has made it possible to identify and measure all the components of spirulina with a high degree of precision **(Sall et al., 1999)**.

The composition of spirulina varies according to the growing conditions, harvesting period, geographical origin, harvesting, drying, grinding and packaging processes, as well as the amount of sunlight **(Manet, 2016)**.

Spirulina is made up of 60-70% protein, 15% carbohydrates, 6% lipids, 7% minerals and 3-6% water **(Figure) (Niangoran, 2017)**.

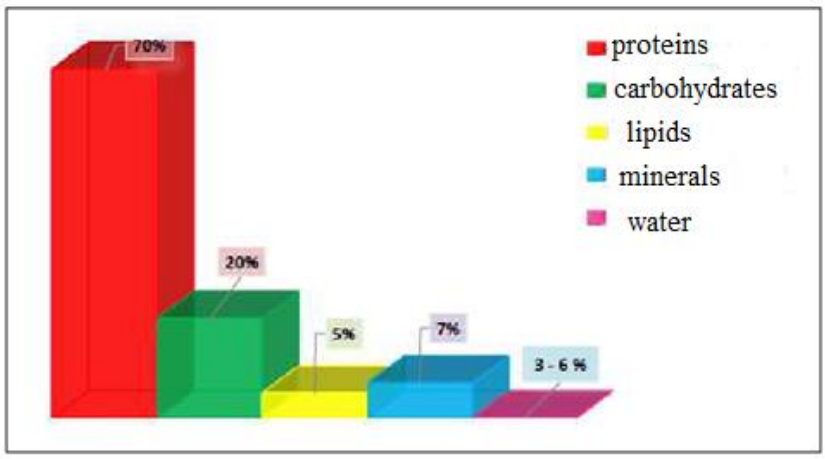


Figure 11: Average Chemical Composition of Spirulina **(Sonia and Abderrezek, 2023)**.

2.1. Proteins

From a qualitative point of view, spirulina proteins are complete and therefore of high quality, as they contain all the essential amino acids. They are virtually 100% bio-assimilable, which means that the body can use almost all of them. This is part of the nutritional richness of this micro-algae **(Karleskind, 2019)**.

Spirulina has a high protein content, with fluctuations of 10-15% depending on when it is harvested. As light levels increase, so do protein levels. It is 10 to 11% of the wet mass, which corresponds to 60 to 70% of its terrestrial matter. This is much higher than the protein content of fish (25%), soya (35%), milk powder (35%) and cereals (14%) **(Sonia and Abderrezek, 2023)**.

2.2. Carbohydrates

Spirulina contains mainly carbohydrates in the form of glycogen and rhamnose. The presence of glucose, fructose, sucrose and a few polyols such as glycerol, mannitol and sorbitol is very limited **(Niangoran, 2017)**.

2.3. Lipids

As a general rule, spirulina contains only 6 to 8% lipids in its dry weight, but this percentage can rise to 11% depending on the extraction methods used or the strain of spirulina used. The balance between saturated and polyunsaturated fatty acids is present in the total lipid composition. It is divided into two distinct parts: a saponifiable part (or fatty acids) (83%) and a non-saponifiable part (17%) **(Sonia and Abderrezek, 2023)**.

2.4. Minerals

All the essential minerals are present in spirulina (7% of dry weight). Depending on the pH and composition of the growing environment, spirulina absorbs minerals to a greater or lesser extent, resulting in varying levels **(Sonia and Abderrezek, 2023)**.

Spirulina contains essential and familiar minerals and trace elements such as iron, zinc, magnesium, calcium, phosphorus and potassium **(Niangoran, 2017)**.

As for iron, its consumption is two to three times higher than that of vegetables or meat. The iron in spirulina does not occur naturally, but is chelated with amino acids to make it easier to absorb **(Sonia and Abderrezek, 2023)**.

Spirulina is particularly popular with vegetarians, sports enthusiasts, pregnant women and growing adolescents because of its high iron content (a mineral found mainly in foods of animal origin such as meat, offal and fish) **(Michka, 2005)**.

2.5. Vitamins

Spirulina contains a wide range of vitamins, which help our bodies to function properly, such as vitamin B complexes where only vitamins B5 and B8 are absent. These compounds play a role in the energy production process and the proper functioning of the nervous system. It is important to highlight the remarkable amount of vitamin B12, which is by far the most difficult vitamin to find in a vegetarian diet, as no common plant contains it (spirulina is 4 times richer than raw liver, which was long considered the best source of vitamin B12) **(Jourdan, 2006)**. There is also a large amount of beta-carotene, also known as provitamin A, which is converted by humans into vitamin A and is beneficial for vision. The development of cells and the proper functioning of the nervous system **(Miranda et al., 1998)**.

2.6. Enzymes

Spirulina also contains numerous enzymes, such as SOD (superoxide dismutase), which plays a crucial role in the fight against oxidation and cell ageing **(Ahounou, 2018)**.

The bioavailability of SOD is of great importance due to the absence of cellulose in the spirulina membrane **(Manet, 2016)**.

2.7. The pigments

The color of spirulina is influenced by three main pigments: chlorophyll, phycocyanin and β-carotene **(Sguera, 2008)**.

2.8. Chlorophyll

The amount of chlorophyll in dry mass is 1%, which is responsible for its green hue **(Manet, 2016)**.

Sometimes its composition, which is very similar to that of mammalian haemoglobin, gives it the name “green blood”. This dye plays a role in restoring acid-base balance, improving heart function, controlling intestinal transit and increasing red blood cell levels **(Casal, 2019)**.

2.9. Phycocyanin

Phycocyanin is present in spirulina. It is a pigmented protein called "complex" which has a blue tint. It is precisely this protein that gives spirulina its bluish hue. Phycocyanin has many medicinal and therapeutic properties. In fact, it acts effectively against everyday immune attacks and also against more serious illnesses such as the effects of radioactivity and chemotherapy. Overall, it preserves our DNA and our liver. Finally, it manages the generation of stem cells **(Karleskind, 2019)**.

2.10. Beta-carotene

Vitamin A, the orange pigment that is a precursor to beta-carotene, is also present in large quantities in spirulina. It plays a crucial role in cell renewal and the immune system **(Charpy et al., 2008).** Its multiple antioxidant properties also help to reduce the risk of cancer, promote wound healing and protect the skin from external aggression **(Azouz Amani, 2023)**.

3. Biological properties of spirulina

As demonstrated above, Spirulina platensis is distinguished by its rich and varied composition. Until recently, attention to spirulina was mainly limited to its nutritional benefits. Today, however, many researchers are exploring the possible therapeutic properties of this micro-organism, such as its antioxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetic and anti-obesity activity **(Shao et al., 2019)**.

3.1. Antioxidant activity

Oxidative stress is associated with diseases, like blood pressure diabetes, heart disease and cancer**.** Numerous studies have shown that using Spirulina can effectively reduce stress both in lab settings and in living organisms. These protective effects are attributed to compounds like phycocyanins, beta-carotene and other essential nutrients, in Spirulina **(Wu et al., 2016)**.

Spirulina has the capability to prevent cell damage through containing both enzymatic and non-enzymatic antioxidant defense system that counteract the effects of Reactive Oxygen Species (ROS) and protect the cells from their deleterious actions under normal and stress conditions . Moreover, Spirulina can be used for production of some antioxidant compounds due to its high contents of carotenoids, tocopherols (TOH), ascorbic acid, glutathione (GSH)

and chlorophyll derivatives as non-enzymatic defense system while the enzymatic defenses system represented by superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), peroxiredoxin (PrxR) and ascorbate Peroxidase (AxP) **.**The chemical constituents of Spirulina demonstrate its antioxidant and anti-lipid peroxidation properties. Its antioxidant activity can be attributed to the presence of two key phycobiliproteins: phycocyanin and allophycocyanin, which primarily act against superoxide radicals. Phycocyanin has the ability to separate free radicals, such as alkoxyl, hydroxyl and peroxyl radicals. Similarly, research by Kurd and Samavati (2015) showed that polysaccharides from *S. platensis* exhibited strong *in vitro* scavenging activities against DPPH and hydroxyl radicals **(Farag et al., 2016)**.

3.2. Anti-inflammatory and immunomodulatory activity

Spirulina is of particular biological interest because of its high protein and fatty acid content, particularly omega 3 and 6, which cannot be synthesised by the body. These fatty acids are precursors of prostaglandins, molecules that have an anti-inflammatory and immunostimulant action within the body **(Charpy et al., 2008)**.

Phycocyanin inhibits the production of pro-inflammatory cytokines such as TNF-α (Tumor Necrosis Factors α), suppresses the expression of cyclooxygenase 2 (COX-2), the main mediator of inflammation, and reduces the production of prostaglandin E. Phycocyanin also stimulates the production of red and white blood cells**.** β-carotene or provitamin A, another element present in spirulina, could be responsible for its anti-inflammatory activity. The production of prostaglandin E is inhibited, as is the expression of COX-2, TNF-α and IL-1 β (Interleukin 1 β) **(Sonia and Abderrezek, 2023)**.

The activation of macrophages, the activity of T cells and the activity of naturally destructive cells (NK) are enhanced by this. Under this method, gamma interferons (IFN-γ) would be released, which could eventually render the viruses inactive **(Charpy et al., 2008)**.

Studies on animals have demonstrated the potent immune system stimulant properties of spirulina. It accomplishes this through boosting macrophage phagocytic activity, NK cell accumulation in tissues, cytokine and antibody production stimulation, and T and B cell activation and mobilization**.** Spirulina has pronounced immune-suppressive properties**.** (employed human peripheral blood mononuclear cells (PBMCs) to assess spirulina's immunomodulatory effects. They demonstrated that Spirulina enhanced PBMC production of

IL-1β, IL-4, and interferon (IFN)-γ by about 2.0, 3.3, and 13.6 times basal levels respectively Phycocyanin from Spirulina platensis significantly increased the production of IFN-γ in PBMCs, in contrast to their finding that phycocyanin decreased the production of IL-4. These variations might result from the Spirulina extracts additional active ingredients **(Wu et al., 2016)**.

4. Effect of spirulina on cardiovascular system

Oxidative stress and inflammation both play a role in the development of cardiovascular diseases, such as atherosclerosis, cardiac hypertrophy, the heart failure and hypertension. Spirulina contains several active compounds, such as phycocyanin and β-carotene, which have powerful properties as antioxidants and anti-inflammatory. The antioxidant and anti-inflammatory properties of phycocyanin firstly described in 1998 and confirmed by numerous subsequent studies. Phycocyanin has the ability to separate free radicals, such as alkoxyl, hydroxyl and peroxyl radicals. It also decreases the production of nitrate, slows down the expression of the unspeakable nitric oxide synthase (iNOS) and inhibits the peroxidation of microsomal lipids **(Deng and Chow, 2010b)**.

The cardiovascular benefits of using Spirulina are described in many articles. According to a review published in 2009, several studies suggest that Spirulina (Arthrospira) could have a beneficial effect in the prevention of cardiovascular diseases. A decrease in blood pressure and lipid concentrations in the blood, in particular triacylglycerols and cholesterol with low lipoprotein density, has been observed following the oral consumption of Spirulina. It has also been shown that spirulina indirectly modifies the levels of total cholesterol and high lipoprotein-cholesterol density lipoproteins **(Prete et al., 2024b)**.

The pigment phycocyanin in spirulina, which structurally resembles the pigment bilirubin, is believed to contribute to spirulina's protective effect against CVDs. Bilirubin, found in bile, is known for its potent antioxidant properties, which help prevent oxidative stress and the formation of radical byproducts in plasma proteins and aromatic amino acid residues .Studies have demonstrated that spirulina supplements positively affect various metabolic and cardiovascular health markers in humans, including triglycerides, total cholesterol, LDL, very low-density lipoprotein (VLDL), fasting blood glucose, and blood pressure, without causing adverse side effects **(AlFadhly et al., 2022)**.

Our study aims to assess the cardioprotective effects of spirulina against Isoproterenol-induced myocardial infarction in male albino Wistar rats. Experiments of this study were carried out between the biochemistry laboratory and the animal house of the 8 Mai 1945 University and the anatomopathological service of Ibn Zohr Hospital, Guelma.

I. In vitro experiments on spirulina

1. Biological material

1.1. Presentation and solubility

To select the optimal sample, two varieties of commercial Spirulina obtained from the Algerian market were procured.Quality assessments was conducted to discern the most appropriate choice for our research.

The best spirulina is grown in high-quality water without heavy metals and dried at low temperatures through ventilation.which guarantees low temperature drying that preserves its nutrients.

Spirulina's sample is a blue-green powder contained in capsules

Figure 12: Spirulina **(original photo).**

1.2.Solubility

Spirulina is a type of blue-green microalgae that is rich in various bioactive compounds, including proteins, vitamins, minerals, and pigments like phycocyanin. The solubility and extraction of these compounds from spirulina can be influenced by the choice of solvent.

Based on the search results provided:

Phycocyanin, the main antioxidant pigment in spirulina, can be effectively extracted using water as a green solvent through a maceration method **(Stunda-Zujeva et al., 2023)**. The maceration method involves soaking the dried spirulina powder in water for an extended period (e.g. 48 hours) at room temperature, followed by centrifugation to obtain the phycocyanin-rich extract **(Aderemi, 2020)**.

Other solvents that have been explored for extracting bioactive compounds from spirulina include:

- Ethanol/water mixtures (e.g. 50:50 v/v): Ethanol-water mixtures can effectively extract antioxidant compounds and pigments from spirulina, especially when combined with pre-treatment techniques like pulsed electric field (PEF) **(Martí-Quijal et al., 2021)**.

- Natural deep eutectic solvents (NADES) like glucose/glycerol: NADES have been shown to be effective in extracting bioactive pigments from spirulina, including phycocyanin, chlorophyll, and carotenoids **(Martins et al., 2023)** .

- Phosphate-buffered saline (PBS) and calcium chloride (CaCl2) solutions**(Aderemi, 2020)**: These aqueous solutions can also be used to extract antioxidant compounds from spirulina, though they may be less effective than ethanol or water-based solvents**.**

In summary, water, ethanol/water mixtures, and natural deep eutectic solvents appear to be the most promising solvents for extracting the key bioactive compounds, especially phycocyanin, from spirulina **(Aderemi, 2020; Martins et al., 2023; Martí-Quijal et al., 2021; Stunda-Zujeva et al., 2023)**.

2. Methods

2.1.Quality assessment of spirulina

This test is designed to check the phycocyanin content in spirulina, which is a key indicator of its quality **(Grover et al., 2021)**.

To perform a quality test for spirulina, we follow these steps:

To prepare spirulina for analysis, begin by weighing out 1 gram of the substance using a precise scale. Next, dissolve the 1 gram of spirulina in 250 ml (25 cl) of water, ensuring complete dissolution. Allow the mixture to rest for 24 hours to facilitate the settling of insoluble components, while the water-soluble phycocyanin remains suspended. Post the

waiting period, observe an opaque mixture displaying a distinctive purple-red glow when illuminated, indicative of phycocyanin, a notable pigment in spirulina. Assess the quality of the spirulina based on the intensity of color and the presence of phosphorescence .

Figure 13: Quality of Spirulina test **(original photo)**

2.2. Phytochemical screening

The ethanolic extract of spirulina was subjected to preliminary phytochemical screening.

- **Preparation of Spirulina Extract**

A 10 mg dose of spirulina powder was soaked in 10 ml of ethanol for 10 minutes at room temperature. The extract was obtained by filtering the mixture with filter paper, resulting in a green-colored extract.

2.2.1. Alkaloids test

The Spirulina platensis extract was treated with diluted Hydrochloric acid (HCl) and filtered. The filtrate was treated with.Few drops of Mayer's reagent. The presence of green color or white precipitate indicates the presence of alkaloid **(Mane and Chakraborty, 2018)**.

2.2.2. Carbohydrates test

The extracts were treated with 3 ml of alpha naphthol in alcohol and Conc. Sulphuric acid was carefully added to the side of the test tubes. The formation of a violet ring at the junction of two liquids indicated the presence of carbohydrates **(Rahoof Iqbal, 2020)**.

Fehling's Test: To the sample Fehling`s solution A and B were added and heated for two minutes. The appearance of a reddish-brown color indicated the presence of reducing sugars.

2.2.3. Glycosides test

3 mL of chloroform and 10% ammonium solution were added to 2 mL of the Spirulina platensis extract. The formation of Pink color indicates the presence of glycosides **(Mane and Chakraborty, 2018)**.

2.2.4. Saponin test

Identification of saponin was done using a foam test. Spirulina extract was diluted into 9 ml of water and then heated for 5 minutes. The solution then was agitated vertically for 10 seconds. The agitation process will result in the formation of a foam layer on top of the solution. Afterward, one drop of 2N hydrochloric acid was added to the solution. The presence of saponin can be confirmed by the formation of foams (at least 1 cm high) that remain on the top of the solution for at least 10 minutes and do not subside with the addition of 2 N HCl **(Yuniati et al., 2020)**.

2.2.5. Flavonoid test

The presence of flavonoids in the ethanol extract of S. platensis was identified using the Willstatter test. This test uses magnesium strips and a hydrochloric acid-ethanol mixture as the test reagent. One milliliter of S. platensis ethanol extract was put into a test tube. Subsequently, 0.1 mg of magnesium and 0.4 ml of amyl alcohol (a mixture of 37% hydrochloric acid and 95% ethanol with a similar volume) were added into the test tube. A positive result can be seen in the color change of the solution, which will turn dark yellow or orange **(Yuniati et al., 2020).**

2.2.6. Phenols test

The identification of phenolic substances in this study was done using $FeCl_3$ solution as the reagent. One milliliter of Spirulina ethanol extract was mixed with 2 drops of 5% $FeCl_3$ in a test tube. The presence of phenols can be identified by the formation of green or greenish-blue color **((Rahoof Iqbal, 2020)**.

2.2.7. Terpenoids test

2 mL of chloroform along with concentrated Sulphuric acid were added to 0.5 ml of the Spirulina platensis extract. The formation of a reddish-brown color at the interface indicates the presence of terpenoids **(Mane and Chakraborty, 2018)**.

2.3. Phycocyanin Content

- **Extraction**

A water extraction of 4% of spirulina was carried out. Suspension in water was prepared in darkness. The resulting solution underwent decantation followed by centrifugation (9000 rpm/15 min) at 4°C. The supernatant was then collected, which underwent a dilution (dilution factor 100) with water. The optical density of the solution, using Uv-visible spectrophotometer (JENWAY 630S), was subsequently measured at, 652 nm, 620 nm. The calculation of the phycocyanin content (mg or µg/mg of spirulina) **(LAFRI et al, 2017)**.

was carried out according to the following formula**.**

Phycocyanin %: 1.873 × (Abs620 – 0.474×Abs652)

2.4.Dosage of polyphenols and flavonoids

2.4.1. Polyphenols content determination

The Folin-Ciocalteu method was used to determine the total polyphenols in the ethanol extract of spirulina **(Li et al., 2007)**.

Principle

The Folin-Ciocalteu reagent comprises a blend of heteropoly acids, phosphomolybdic acid, and phosphotungstic acid **(Agbor et al., 2014)**. This procedure involves transferring electrons from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes in an alkaline environment, resulting in the formation of blue complexes. These complexes can be quantified using spectroscopy at approximately 765 nm **(Ainsworth and Gillespie, 2007)**.

Operating procedure

In a test tube, 200 µl of 0.1 mg/ml spirulina (Prepared in ethanol with proper dilution) solution was added to 1 ml of Folin-Ciocalteu reagent diluted 10 times. After mixing, the solution was incubated for 5 minutes, followed by adding 800µl of 7.5% sodium carbonate Na_2CO_3 before further incubating the reaction mixture for 90 minutes at room temperature and in the dark. The blank was prepared in the same manner, replacing Sp with ethanol. A dilution series starting from gallic acid (0.005, 0.01, 0.025, 0.05, 0.075, 0.1mg/ml) was used to generate a standard curve, following the same testing protocol. Absorbance measurements

were taken using a spectrophotometer (JENWAY 630S) at 765 nm, with each measurement performed in triplicate. The results are expressed as mg equivalent of gallic acid per gram of spirulina (mg Eq AG/g SP).

2.4.2. Flavonoids content determination

The total flavonoid content in the ethanolic extract of spirulina is determined using the method developed by **(Bahorun et al., 1996)**.

Principle

The method relies on the oxidation of the 4 and 5 carbons of flavonoids by a 2% $AlCl_3$ reagent, resulting in the formation of a yellow complex that absorbs light at 430nm **(Tine et al., 2019)**.

Operating procedure

1 ml of the 1 mg/ml spirulina ethanolic extract solution was mixed with 1 ml of 2% AlCl3 methanolic solution, followed by stirring and incubation for 10 minutes in the dark at room temperature. A blank was prepared in the same way, substituting the spirulina ethanolic extract with 1 ml of ethanol. The absorbance was then measured at 430 nm using a UV-visible spectrophotometer (JENWAY 630S). To create a calibration curve, a stock solution of quercetin at 0.1 mg/ml (a reference flavonoid) was prepared, and dilutions (0.005, 0.01, 0.025, 0.05, 0.075, 0.1mg/ml) were made. Each measurement was conducted in triplicate.

The results are expressed in milligrams equivalent of quercetin per gram of extract weight (mg Eq QRC/g SP).

2.5. Antioxidant activity evaluation

2.5.1. Free radical scavenging activity (DPPH test)

Principle

DPPH (2,2'-diphenyl-1-picrylhydrazyl) is a synthetic radical that displays a deep violet hue when oxidized (Molyneux, 2003a). The reduction of this radical by protons from antioxidants leads to the vanishing of the violet color, which diminishes based on the concentration of compounds in the extract capable of counteracting this radical. The rate of color fading is assessed through spectrophotometry in comparison to a control (lacking extract) using the subsequent formula:

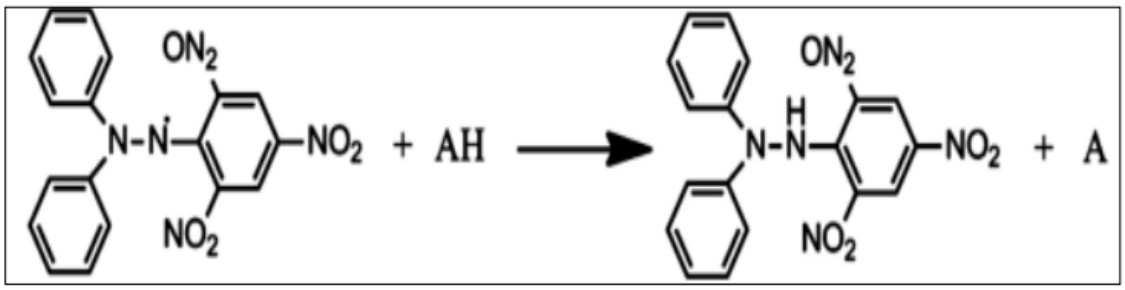


Figure 14: Free radical DPPH: AH is an antioxidant donating molecule and A is a formed free radical **(Sirivibulkovit et al., 2018).**

Operating procedure

The spirulinas's capacity to capture free radicals was assessed through a straightforward colorimetric technique **(Koleva et al., 2002)**.

Different concentrations of spirulina extract (0.001 – 1 mg/ml) were combined with an ethanol solution of DPPH (4× 10-5 M). In aluminum-covered test tubes, 1.5 ml of the ethanol solution of DPPH was added to 0.05 ml of each dilution of spirulina extract (0.001, 0.0025, 0.005, 0.0075, 0.01, 0.025, 0.05, 0.075, 0.1 mg/mL). The reaction mixture was kept in the dark at room temperature for 30 minutes. A control was prepared under the same conditions by substituting spirulina with ethanol. The absorbance of the reaction medium was measured at 517 nm. All procedures were conducted in triplicate. Dilutions of quercetin and ascorbic acid, serve as the positive control and matching the dilutions of spirulina were evaluated using the same method.

Inhibition percentage (%) = (Abs Control - Abs Sample / Abs Control) x 100

Studying the variation of antioxidant activity based on the concentration of the extracts aids in identifying the concentration corresponding to 50% inhibition (IC_{50}) (50% inhibitory concentration), also known as EC_{50} (Effective concentration50) which is graphically determined.

2.5.2. Ferric reducing antioxidant power (FRAP test)

Principle

The reducing ability of phenolic extracts relies on the conversion of Fe3+ in the compound K3Fe (CN)6 to Fe^{2+} (Prussian blue) when an antioxidant capable of donating electrons is present, resulting in the blue color **(Zhong and Shahidi,2015)**.

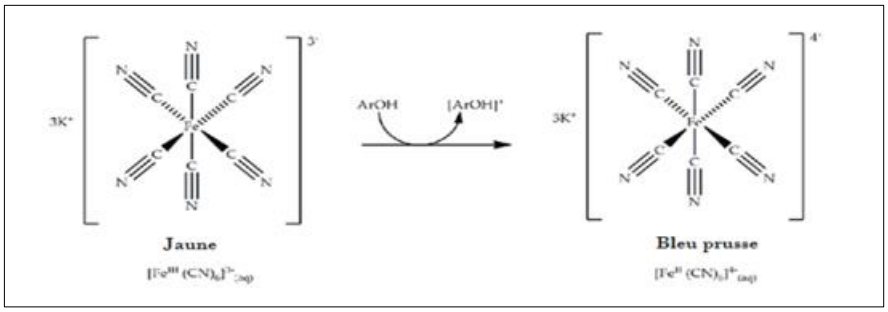


Figure 15: Potassium ferricyanide reduction mechanism **(Bibi Sadeer et al., 2020).**

Operating procedure

The FRAP test was performed following the method outlined by **(Aparadh et al., 2012).** In test tubes, 500 µl of the sample at various concentrations, 1 ml of phosphate buffer solution (0.2 M, pH = 6.6), and 1 ml of potassium ferricyanide solution $K_3Fe(CN)_6$ (1%) were added. The mixture was stirred and incubated at 50°C for 20 minutes, then 1 ml of trichloroacetic acid (10%) was included to halt the reaction. The tubes were centrifuged at 3000 rpm for 10 minutes. Subsequently, 1.5 ml of distilled water and 0.1 ml of a solution of iron trichloride ($FeCl_3$) (0.1%) were added to 1.5 ml of supernatant. The mixture was stirred again and incubated for 10 minutes. The absorbance was then measured at 700 nm. The absorbances were converted to mmol FeII/mg of spirulina quercetin using a standard curve of different concentrations of $FeSO_4$ ($7H_2O$) (0.1, 0.2, 0.4, 0.6, 0.8, 1 mM) prepared under the same conditions.

II. Evaluation of the cardioprotective effect of spirulina *in vivo*

1. Animal material

This experimental study was conducted on adult male rats, weighing between 200 and 400 g, obtained from the Pasteur Institute of Algeria.

The rats were housed in polypropylene cages filled with wood chip bedding, with Eight rats per cage. The cages were cleaned and inspected daily by the researcher. The rats were fed a standard diet and had unrestricted access to water. A 12-hour light/dark cycle was maintained. Before the experiment was carried out, rats were adapted to their laboratory condition within 15 days **(Pham et al., 2023)**.

2. Methods

2.1. Experimental protocol

After the adaptation period, the rats were selected according to their weight and divided into four groups of seven rats each and were treated as follows:

Control group (C): Rats of this group received orally distilled water and subcutaneously 0.9% NaCl daily for 15 days.

Spirulina group (SP): Rats of this group received spirulina by gavage at 500 mg/kg for 15 days.

Isoproterenol group (ISO): Rats of this group received 0.9% NaCl daily by subcutaneous (sc) route for 15 days followed by an SC injection of isoproterenol (150 mg/kg) on the 14th and the 15th day at 24-hour interval.

Isoproterenol treated group (ISO+SP): Rats of this group received spirulina by oral gavage at a dose of 500 mg/kg for 15 days followed by an s.c. injection of isoproterenol (150mg/kg) on the 14th and the 15th day at 24-hour interval.

2.2. Sacrifice and Collecting of Blood and Organs

After the experiment and 48 hours post the initial isoproterenol dose administration, the animals were fasted overnight (12 h) with unrestricted water access. Subsequently, the rats were weighed, sacrificed, and placed in a supine position for the chest incision. The

beating heart was quickly excised, then rinsed in 0.9% NaCl at 4°C and weighed., and then preserved in a freezer at -20°C until being homogenized.

2.3. HBI herat body index

By calculating the relative organ body weight, the proportion of an organ's weight is assessed about the overall body weight, providing insights into organ development and the potential effects of ISO on organ size. It depends on the relation between organs and body weight **(Pham et al., 2023)**.

The formula can be expressed as:

HWI was computed as HWI = heart weight (HW) body weight (BW) × 100

2.4.Evaluation of cardiac biochemical markers

2.4.1. Blood sampling

The blood was taken from beating hearts and was placed in heparinized tubes then centrifuged (SIGMA 2-16KL) at 4000 rpm for 5 min. the plasma obtained is recovered and used for the determination of biochemical parameters (Trop,lipidic profil).

2.4.2. Lipidic profil

The plasma cholesterol levels of the four groups were estimated using commercial kits (ELITech). These measurements were carried out by an automated analyzer (SELECTRA PRO-S).

2.4.3. Troponin I dosage

The troponin I released into the infusion solution was also measured using the VIDAS high-sensitivity troponin I assay based on the Enzyme-Linked Fluorescent Assay (ELFA) technique.

2.5. Evaluation of oxidative stress parameters in cardiac tissue

2.5.1. Preparation of the cytosolic fraction

The cytosolic fraction was prepared following the methods outlined by **(Mnafgui et al., 2021)**. The rat heart sample was rinsed with 0.9% NaCl and then weighed. The remaining heart tissues were cut into small pieces and homogenized in phosphate buffer

(0.1 M, pH 7.4, 4 °C) containing KCl (1.17%) using an ULTRA-TURRAX homogenizer. The homogenate was centrifuged at 10000 rpm for 30 minutes at 4°C.

The resulting supernatant was fractionated and stored at -20°C for the assessment of oxidative stress parameters.

2.5.2. Evaluation of antioxidant statut

2.5.2.1. Reduced glutathione level

Principle

Reduced glutathione (GSH) is a dominant intracellular antioxidant present in various organizations. It is composed of a gamma-glutamyl-cysteineglycine tripeptide **(Narayanankutty et al., 2019)**.

The determination of reduced glutathione (GSH) is carried out by the Ellman colorimetric method **(Gergel' and Cederbaum, 1997)** , which is based on the oxidation reaction consisting of cutting the 5,5-dithiols-2-nitrobenzoic acid (DTNB) molecule with GSH which releases 2-nitro-5-mercapto-benzoic acid (NMB**) (Bahlil et al., 2020)** having an absorption peak at 412 nm, thus indicating a yellow coloration resulting from the reaction **(Vuolo et al., 2022)**.

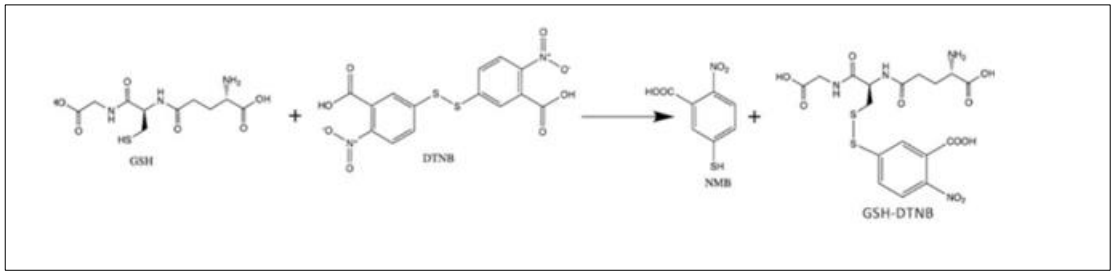


Figure 16: Réaction du glutathion (GSH) avec le réactif d'Ellman / DTNB ; Acide 2-nitro-5-mercaptobenzoïque **(Jîtcă et al., 2021).**

Operating procedure

The determination of the GSH was carried out by spectrophotometry according to the method described by **(Lahouel et al., 2004)**.

1 g of cardiac tissue was homogenized in 5% TCA and then centrifuged at 1000 rpm, for 30 minutes. 200 µl of the supernatant was added to 1700 µl of phosphate buffer (0.1 M,

pH = 7.4), then 100 µl of DTNB (0.01 M) was added .After an incubation of 5 minutes, the reading of the absorbances is made at 412 nm against the white prepared under the same conditions by replacing the homogenate with distilled water.

A standard range of GSH with different concentrations (from 0.125 to 1 mM) was made by carrying out the same previous steps to determine the GSH concentrations.

The optical density is read at 412 nm. The concentrations are deduced from the glutathione standard range and the results are expressed in µmoles of glutathione/g of cardiac tissue.

2.5.2.2.Glutathione peroxidase enzymatic activity (GSH-Px)

Principle

The enzymatic activity of glutathione peroxidase (GSH-Px) was measured by the method **(Flohé and Günzler, 1984)**. This method is based on the reduction of hydrogen peroxide (H2O2) in the presence of reduced glutathione (GSH), the latter is transformed into (GSSG) under the influence of GSH-Px.

Operating procedure

400 µl of the GSH (0.1 mM) is added to 200 ml of the supernatant.Then, 200 µl of the TBS buffer solution (Tris 50 mM NaCl 150 mM pH 7.4). After incubation for 5 min in a water bath at 25°C, 200 µl of $H_2\ O_2$: (1.3 mM) is added to initiate the reaction and left to react for 10 minutes. Then 1 ml of TCA (1%) is added to stop the reaction, the mixture is placed on ice for 30 minutes. And is centrifugated for 10 minutes at 3000 rpm, 2.2 ml of TBS buffer solution is added to 480 µl of the supernatant, then 0.32 ml of DTNB (1.0 mM), it mixed, and the optical densities at 412 nm after 5 min **(Boutolleau et al., 1997 ;Behnisch- Cornwell et al., 2020)**.

$$GPx = \frac{DOec \times DO\ et \times 5}{DOet \times cp} 0.04$$

DO ec: The enzyme activity of GPx in the presence of glutathione (GSH).

DO et: The enzyme activity of GPx in the absence of glutathione (GSH).

DO et: The enzyme activity of GPx in the presence of glutathione (GSH) and the inhibitor.

Cp : The concentration of the inhibitor.

This formula is a mathematical representation of the inhibition of GPx enzyme activity by an inhibitor in the presence of glutathione (GSH).

2.5.2.3. Catalase enzymatic activity (CAT)

Principle

The measurement of the catalase activity (CAT) was carried out using the method of Clearance which is based on the degradation of hydrogen peroxide (H_2O_2) measured at 240 nm **(Greenwald, 2018).**

Operating procedure

To 1 ml of phosphate buffer (0.1 M, PH 7.4), 950 µl of the freshly prepared H_2O_2 solution (0.019M) were added, 25 µl of homogenate was added last, the mixture thus prepared in a quartz tank was placed in the spectrophotometer for kinetic monitoring (for 2 min) of the disappearance of the H_2O_2 at 240 nm.

The result of the enzymatic activity is expressed in IU/g of proteins/g of tissue, and obtained in the formula below:

$$\textbf{CAT activity} = \left(\Delta T \times \log.\frac{DO1}{DO2}\right) \times \frac{FD}{\varepsilon \times L \times C\ proteins}$$

DO1: Absorbance at 0 minutes.

DO2: Absorbance at 1 minute.

ΔT: Time interval in minutes.

FD: Dilution factor (Vt/Vs) where Vt: Total volume of reaction medium, Vs: Supernatant volume.

ε: Molar extinction coefficient of H2O2 (43.6 M-1 cm-1).

L: Length of the cuvette used (L = 1 cm).

C protein : Protein concentration (mg/g of tissue).

2.5.2.4. Protein dosage

Principle

The protein assay is carried out according to the Biuret method using a commercial kit ready to use. In an alkaline medium, copper ions (Cu^{2+}) interact with the binds peptides of proteins to form a blue-violet complex whose color intensity is proportional to the protein concentration; thus, a colorimetric assay can be carried out at 540 nm **(Ndibualonji et al., 2017)**.

Operating procedure

1 ml of Gornall's reagent is added to 20 µl of the cytosolic fraction. The mixture is incubated at room temperature for 10 min. The absorbance at 540 nm is measured by a spectrophotometer. The optical density of the standard (BSA solution) is measured in the same conditions. The white represents the Gornall reagent alone. The protein concentration of the samples is calculated according to the following ratio:

$$[\mathbf{Proteins}] = \left(\frac{DO_E - DO_B}{DO_S - DO_B}\right) \times n$$

Where:

n= 5 g/dL or 50 g/L

$\boldsymbol{DO_E}$ **:** absorbance of the sample.

$\boldsymbol{DO_B}$ **:** absorbance of White.

$\boldsymbol{DO_S}$ **:** absorbance of the standard (Annex1)

The protein concentration is expressed in g of protein/g of cardiac tissue which will be used to express the enzymatic activity of the catalase.

2.6. Measurement of myocardial infarction size (TTC staining)

Principle

The infarcted area was visually identified using Triphenyl chloride Tetrazolium (TTC). The TTC is converted to triphenyl-tetrazolium formazan (TTF), resulting in a brick-red color in the healthy myocardium where dehydrogenase activity is present. In

contrast, the infarcted myocardium, lacking enzymatic activity, appears as a pale uncolored area. Therefore, the TTC staining enables clear differentiation between the normal and ischemic areas **(Kakimoto et al., 2013)**.

Operating procedure

The method used is adapted from the two methods of **(Khalil et al., 2006b)** and **(Csonka et al., 2010)**.

After the sacrifice the heart is removed, rinsed with NaCl 0.9%. The heart was frozen for 30 min, then sliced with a heart cutting die, sliced transverse by 2 mm, the slices were weighed and incubated at 37°C in a 1% TTC solution. The slices moistened with TTC have been dried to avoid reflections when taking a photo. Apical and basal views of all slices were photographed. The slices were put in 10% formaldehyde and then photographed in the same way.

The calculation of the infarct volume in rat's heart using the TTC (Triphenyltetrazolium Chloride) protocol was performed by The area of the infarct is measured using image processing software (ImageJ, Version 1.44p). The sum of the infarcted area of the individual slice was measured and divided by the total area of that slice to obtain the fraction of the slice measured on percent.

2.7. Histopathological Analysis

For histopathological examination hearts were quickly removed, cleaned with a saline solution, and conserved in a 10 % formalin solution. Sections of the heart tissue embedded in paraffin were prepared with a thickness of 5 µm each. These sections were treated with hematoxylin and eosin (H&E) staining and underwent histopathological examination **(Mert et al., 2018 ; Islam et al., 2020)**.

Steps of histological sections preparation are described below:

In the tissue collection process, the tissue is first fixed in 10% formalin to maintain its structural integrity. Following fixation, the tissue undergoes dehydration using a series of ethanol solutions (70%, 80%, 90%, and 100%) for1-2 hours. Subsequently, it is cleared with xylene or toluene for 1-2 hours to eliminate the ethanol content. The tissue is then infiltrated with a paraffin wax solution for 1-2 hours to replace the clearing agent. Moving on to the embedding step, the tissue is placed in paraffin wax in a mold, ensuring proper centering and even distribution of the wax. After allowing the wax to cool and harden

completely, the tissue is sectioned using a microtome into thin sections typically measuring 5-10 μm in thickness. These sections are then mounted on glass slides using a mounting medium. Following this, the sections are stained with Hematoxylin and Eosin (HE) to visualize the general tissue structure and cellular morphology, as well as special stains like Masson's trichrome, Gomori's trichrome, or Van Gieson's stain to highlight specific tissue components or structures. Finally, the stained sections are examined under a light microscope to observe the tissue morphology and identify any histopathological changes.

2.8.Statistical Analysis

All data were expressed as mean ± S.E.M., statistical analysis was performed with Graph Pad Prism 8.0 software. Differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-test. To statistically interpret the differences between the results of the biochemical parameters a $p<0.05$ was considered statistically significant.

I. In vitro experiments on spirulina

1. Quality assessment of spirulina

Figure 17: Spirulina sample 1

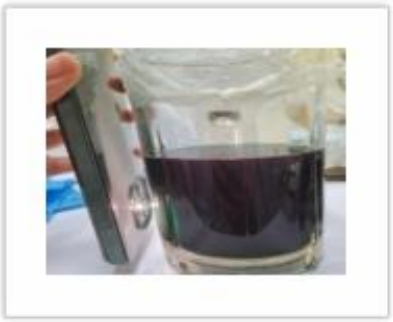


Figure 18: Spirulina sample 2

Concerning the quality assessement of commercial spirulina samples, we have noticed that light does not pass through one of the two samples (sample 2) compared to sample 1. Therefore, we can choose sample 2 for the rest of tests to be carried out

2. Phytochemical screening

The chemical screening results are obtained from methanol extract of spirulina. The phytochemical screening allowed us to highlight the presence of secondary metabolites at the level of spirulina. The detection of these chemical compounds is based on solubility tests of the constituents. Table groups the results of the phytochemical tests carried out on the spirulina.

Table 1: Results of the phytochemical screening of spirulina(+) : positif test

Tests	**Sample extracted (methanol)**
Alkaloids	+
terpenoids	+
tannins	+
saponins	+
flavonoids	+
phenols	+
Carbohydrate	+
Lipid	+

According to the results obtained from phytochemical screening, it is found that the studied spirulina has a molecular diversity as to the secondary metabolites. The screening carried out lead to note the presence of the five major chemical groups, tannins, flavonoids, sterols and steroids and saponosides, alkaloids. **(Anbarasan et al., 2011)** reported the presence of the same classes of chemical families found at the spirulina level.

This was confirmed by **(Abdellatif et al., 2021)** in his study on the detection of secondary metabolites of spirulina, where the results showed the presence of saponins, alkaloids and flavonoids and other compounds in spirulina.

Thus, we can say that the presence of flavonoids, phenols and saponins in spirulina, which are antioxidants known for their anti-radical effect **(Mane and Chakraborty, 2018)**.

The presence of the main secondary metabolites in spirulina suggests an activity photosynthetic and energetic, comparable to that of plants.

3. Determination of the phycocyanin content

Spirulina platensis is an excellent source of phycocyanin. The phycocyanin would have a antitumor activity. It would induce a mechanism of apoptosis (self-destruction) of the cells cancer cells **(Li et al., 2006)**, would have an antioxidant activity and a hepato-protective role. In addition, the high content of this pigment could be of great industrial interest **(Gershwin and Belay, 2007)**. Phycocyanins constitute about 15-25% of the dry weight of microalgae **(Romay et al., 2003)**.

Spirulina has a dark green-blue color because it is rich in a polypeptide bright blue called phycocyanin.In our study we relied on the water extraction method to estimate the phycocyanin value. By the following equation Concentration of phycocyanin (mg/ml) = $1.873\times(DO_{620} - 0.474\times DO_{652})$. Spirulina content was estimated at 0,188 ±0,03 (mg/ml of spirulina). This result is very close to the value of **(LAFRI et al, 2017)** which had results between 0,19 mg /ml for the strain algerian and between 0,2 mg/ml for the Tunisian strain.

Compared with the results obtained by **(LAFRI et al, 2017)** when he extracted the phycocyanin in different ways, it is found that by the freezing extraction method (0.06 mg/ml for the Algerian strain and 0.10 mg/ml for the Tunisian strain). These results are

lower compared to ours. Also, the two-phase aqueous extraction gave concentrations (0.39 mg/ml for the Algerian strain and 0.41 mg /ml for the Tunisian strain), shows higher values compared to our results. Whereas **(Antelo et al., 2010**) have obtained by the double-phase aqueous extraction method a phycocyanin concentration estimated at 1.11 mg/ml. these results are higher than ours. These differences in values could be related to the extraction method, cultivation conditions, climate, mother strain, or to the drying means.

4. Determination of polyphenols

Phenolic compounds are also recognized for their antioxidant properties, which means that they can help protect cells from damage caused by free radicals and oxidative stress. By acting as antioxidants, they can contribute to the prevention of certain chronic diseases such as cardiovascular diseases, cancer and neurodegenerative diseases **(Colla et al., 2007)**.

Based on the calibration curve shown in the figure, obtained from increasing concentrations of gallic acid (mg/ml), the level of polyphenols present in the spirulina extract is evaluated at 230 ± 65.2 mg GA Eq /g of spirulina using the following formula (Abs = 11.593 [AG] + 0.08; R^2=0.99).

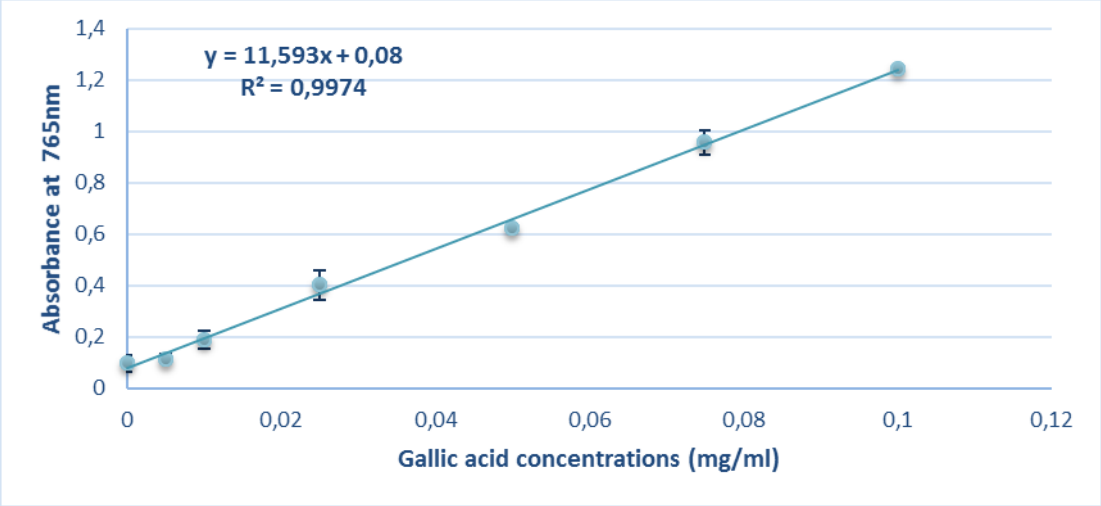


Figure 19: Calibration curve for the determination of polyphenols concentration.

The results of our study indicating that spirulina has a higher concentration of total polyphenols (230 ± 65.2 mg GA Eq /g of spirulina) compared to those found by **(LAFRI et al, 2017)** which had results between 4 and 22 mg /g for the strain algerian and between 6 and 25 mg / g for the Tunisian strain. It is also comparable to those found by **(Machu et al., 2015)** who have determined a low content (43.2 mg GA Eq/g), using water extraction

distilled (80 ° C. for 10 min in a water bath with constant stirring). By the way **(Abu Zaid et al., 2015)** showed a content of compounds total phenolics varying between 26.75 and 40.45 mg/g for Spirulina platensis.

Higher and more important contents have been recorded of our spirulina compared to the study by **(Aouir et al., 2017)**, who made a comparison between different Spirulina strains from different countries; **Algeria, Chad and USA**, where they showed that S. platensis in fresh form Hiri-Tamenrasset (HTAM) revealed a total polyphenol content of 67.52 mg Eq AG/g dry weight followed by Spirulina in dry form; Hawaiian, Spirulina pacifica (HSP) (48.93 mg Eq AG/g), Spirulina in dry form Hiri-Tamanrasset (HTM) (45.22 mg Eq AG/g), Spirulina in dried form The Chad Lake Chad Spiral (19.61 mg Eq AG/g). The difference between the total phenolic contents of algae can be explained by several influential factors, such as geographical origin or area of cultivation, seasonal, physiological and environmental variations **(Machu et al., 2015)**, The nature of the solvent used in the extraction (water, methanol or ethanol), the strain to be used, and the temperature as a significant parameter in the production of biomass **(Dahloum et al., 2014)**. Therefore, the richness of spirulina in polyphenols indicates that it can play a very important role in the neutralization of free radicals and in the minimization of cell damage.

5. Dosage of flavonoids

Flavonoids are considered to be very powerful antioxidant agents because of their structure, relating in particular to the position of the hydroxyl groups on the aromatic nuclei, and the ability of the aromatic compounds to support electronic delocalization. In recent years, particular attention has been paid to the antioxidant properties of flavonoids **(Chae et al., 2013)**.

Based on the calibration curve shown in figure 21 obtained from increasing concentrations of quercetin (QRC) (mg / ml), the level of flavonoids present in spirulina is evaluated at 16,09 ± 3,06 mg of equivalent QRC/g of spirulina using the following formula (Abs= 32.362 [QRC] + 0.0163; R^2=0.99).

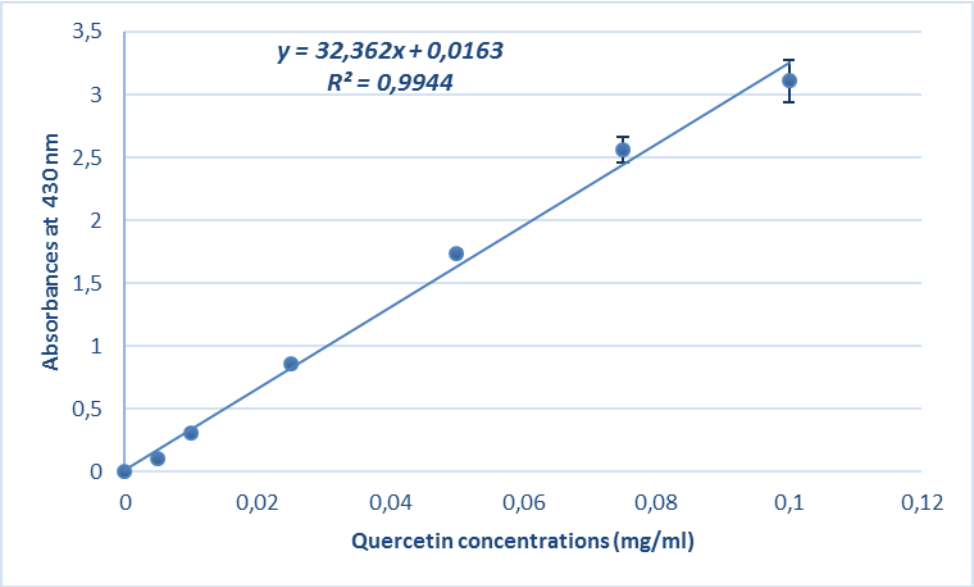


Figure 20: Calibration curve for the dosage of flavonoids.

The amount of flavonoids found in this present work (16,09±3,06mg EQ/g of Spirulina) is significantly higher than that found by **(adb el baky et al., 2009)**, which obtained a contained in flavonoids of (2.9 mg /g), which have been extracted by cold maceration in methanol 70%. Lower results were obtained by **(LAFRI et al, 2017)**, with a flavonoid level of 2,12 mg EQ /g for the Algerian strain and 2.9 mg EQ /g for the Tunisian strain.

According to **(LAFRI et al, 2017)**, The concentrations of the flavonoids obtained by different extraction methods (glycerol extraction for the Algerian strain 3.4 mg/g and for the Tunisian strain 4.5 mg/g; solvent extraction for the Algerian strain 2.12 mg/g and for the Tunisian strain 2.9 mg/g; extraction by freezing for the Algerian strain 1.878 mg/ g and for the Tunisian strain 2.1). These values show a low content of flavonoids compared to our spirulina which shows 16 ± 3.06 mg EQ/g of spirulina. This variation in the content of spirulina flavonoids is probably due to several factors such as extraction method which have already been mentioned, the geographical area, the harvesting season, the environmental conditions.

Based on the results obtained and the comparisons with the results of studies however, it indicates that this level of flavonoids may contribute to spirulina's ability to trap free radicals.

6. Measurement of antioxidant activity

Various assays have been used to assess the antioxidant activity of Spirulina extracts and compounds. Most of the *in vitro* assays are based on the measurement of radical scavenging activity **(Huang et al., 2007)** while rat models **(Mohan et al., 2006)** and human subjects are often used in *in vivo* studies. The present study used a combination of both chemical (DPPH FRAP) and rat-model to assess the antioxidant activity of Spirulina extract **(Lu et al., 2006)**.

6.1. DPPH test

DPPH is an organic free radical, always used as a reagent to evaluate the anti-radical activity of antioxidants **(Molyneux, 2003b)**.

Results of the antioxidant capacity of spirulina measured using the radical scavenging method DPPH$^{\bullet}$ is illustrated in the figure 21.

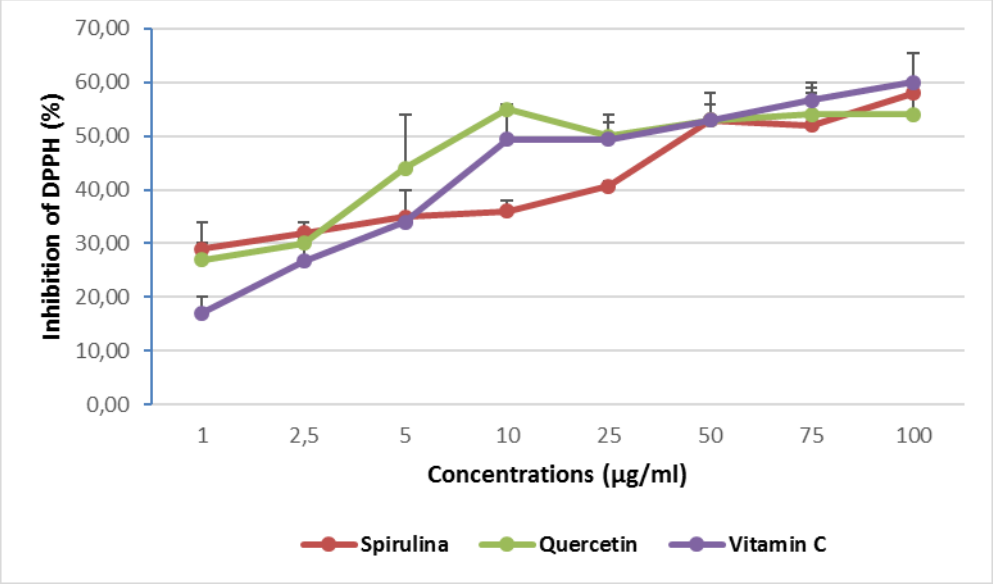


Figure 21: Trapping Percentage of DPPH radical by spirulina, quercetin and Vit C.

At concentrations of 0.001 to 0.01 mg/mL, spirulina presents a low scavenging capacity ranging from 29±1% to 36±2%, unlike QRC and Vit C which have a high scavenging capacity with 27±7% to 55± 1% and 17±3% to 49.33± 6.22% respectively in the same concentration range.

In addition, the anti-radical activity of the aqueous extract of spirulina evaluated by the power of DPPH trapping is close to that of vitamin C reaching 58% ± 2 at 0.1 mg/mL and 60% ± 5.33 at 0.1 mg / ml, compared to 54% ± 4 at 0.1 mg/mL for the QRC. In addition, the amount of spirulina necessary to inhibit 50% of the DPPH$^{\bullet}$ (IC_{50}), as

indicated in the table 02, was 0.0439 ± 0.00 mg/ mL, which is higher than the IC_{50} of quercetin (0.0250 ± 0.00 mg/mL) and vit C (0.0295 ± 0.00 mg/mL).

The IC_{50} is inversely related to the antioxidant capacity of a compound, because it expresses the amount of antioxidant required to reduce the concentration of the free radical by 50%. The lower the IC_{50} value indicates the greater the antioxidant activity of a compound **(Laib, 2012)**. Therefore, spirulina has lower antioxidant activity compared to Vit C and QRC, since : IC_{50} Spirulina C >IC_{50} Vit C > IC_{50} Quercetin

Table 2: Percentages of inhibition of spirulina and quercetin and vitamin C.

Sample	**Spiulina**	**Vit C**	**Quercetin**
IC_{50} mg / ml	0.0439 ± 0.00	0.0295 ± 0.00	0.0250 ± 0.00

By comparison of the anti-radical activity of our spirulina with those of **(Rajamanickam, 2011)** and **(Shalaby and Shanab, 2013)** recorded a value of IC_{50} respectively 1.444 mg/ml, 1.823 mg/ml possessing a weaker anti-radical effect than our spirulina. According to **(LAFRI et al, 2017)** who used the freezing extraction method for both Algerian and Tunisian strains respectively (0.01% and 0.015%). The scavenging power of free radicals of our spirulina is weak

The study of **(Chu et al., 2010)** showed that the antioxidant activity of phycocyanin was less than Spirulina extract and vitamin C. The extract might contain other constituents (e.g. phenolic compounds) which plays an important role in the antioxidant activity.

Spirulina contains a set of pigments such as carotenes, and xanthophyll phytopigments, which, together with phycocyanin, seem to be linked to its activity antioxidant **(Jaeschke et al., 2021)**. The chemical structure and the polarity of the antioxidant are decisive for its ability to scavenge free radicals. Synergistic but also antagonistic effects have been observed in model solutions which contain several functional compounds with anti-radical activity **(Popovici et al., 2009)**.

6.2. FRAP test

The FRAP test is considered one of the best, simplest, oldest and most reliable approved tests that studies the effectiveness of antioxidants in terms of their ability to reverse various free radicals, and is usually used to study the extent of the ability of extracts to inhibit the oxidation process **(Katalinic et al., 2006)**. The FRAP assay mainly evaluates the ability of antioxidants to reduce Fe^{3+} to Fe^{2+}**(Benzie and Strain, 1996)**.

The antioxidant power of spirulina measured by the FRAP method is illustrated in the histogram shown in the figure made from the standard curve of FeSO4-7H2O.

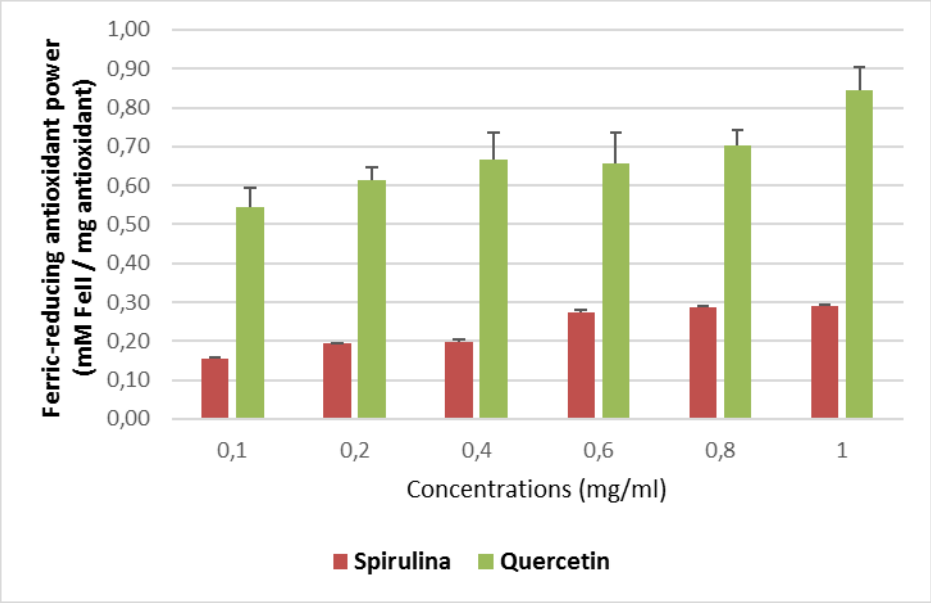


Figure 22: Antioxidant activity of spirulina and quercetin by the FRAP test.

According to the histogram illustrated in Figure 22, we observed that spirulina shows a low reducing power in all concentrations compared to that of quercetin. On the other hand, the reducing power of quercetin increases in proportion to the concentration. At concentrations equal to 0.1, 0.2, 0.4,0.8, 1 mg/mL.

It is clear that our spirulina has a low reducing power compared to standard (quercetin). In comparison with the study conducted by **(LAFRI et al, 2017)**, The highest concentration is obtained by the glycerol extraction method for the two strains (Algerian and Tunisian) with respective values of 0.6 mg/g and 1.4 mg/g. On the other hand, the lowest concentrations are obtained by the methods extraction by water (0 ,2 mg/g, 0.4 mg/g) respectively. From the results we have obtained, we find that this difference is due to the extraction method.

II. Evaluation of the cardioprotective effect of spirulina *in vivo*

1. HBI heart body index

The proportion of an organ's weight is assessed about the overall body weight, providing insights into organ development and the potential effects of ISO on organ size **(Pham et al., 2023)**.

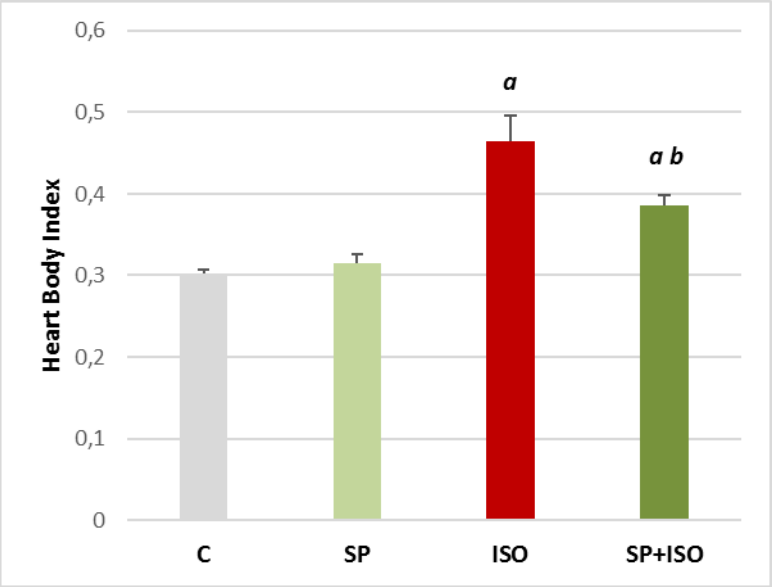


Figure 23: Variation of the heart/body weight ratios in the different rats.

The results were analyzed using a one-way ANOVA followed by Tukey post hoc test. The results are expressed as Mean ± SEM. ***a****: significant difference vs control group.* ***b****: significant difference vs ISO group. P≤0,05 indicates a significant change.*

According to the results expressed in the figure 23, a considerable and significant increase in the ratio heart/body weight was observed in the ISO group compared to the control group (0,302 ± 0.005). However, the group treated with SP + ISO shows a significant decrease in the heart/body weight ratio compared to the ISO group. On the other hand heart/body weight ratio in the SP+ISO group is almost similar to that of the control rats.

The elevation in the heart-to-body weight ratio signifies an indicator of cardiac hypertrophy **(Sharma et al., 2023).**

Isoproterenol, when administered at 150 mg/kg concentration, induces structural and functional modifications in the heart, leading among other changes and damages (reduction in antioxidant defenses, cellular damage, impaired cardiac function, and eventually heart failure) to a significant increase in heart weight and heart weight to body weight ratio.

Previous research has shown that the augmented heart weight in response to isoproterenol treatment is primarily due to fluid retention, inflammation in the intramuscular space, extensive cardiac cell death, and subsequent infiltration of inflammatory cells. Despite no notable change in overall body weight **(Li et al., 2012)**. the pathological alterations induced in the heart by isoproterenol led to an elevation in the heart-to-body weight ratio **(Mi et al., 2023)**.

Spirulina offers potential mechanisms to counter isoproterenol-induced cardiac hypertrophy. This is attributed to the presence of bioactive compounds in Spirulina, including phycocyanin, known for their anti-inflammatory, antioxidant, and cardioprotective properties. Additionally, Spirulina is believed to influence key signaling pathways involved in cardiac hypertrophy regulation, such as the PI3K/Akt, AMPK, and mTOR pathways crucial for physiological cardiac growth control. In summary, research findings suggest that Spirulina has the capacity to prevent the increase in the heart weight to body weight ratio induced by isoproterenol, indicating its protective role against pathological cardiac hypertrophy. This protective mechanism is likely linked to the various bioactive compounds in Spirulina, which can modulate signaling pathways associated with cardiac remodeling **(Ahmadi et al., 2019)**.

2. Evaluation of cardiac biochemical markers

2.1. Lipid profile evaluation

Lipids play an important role in cardiovascular diseases, not only in hyperlipidemia and the development of atherosclerosis, but by modifying the composition, structure and stability of the cellular membranes **(Shaik et al., 2012)**.

From the figure 24, it is obvious that the cholesterol level in rats treated with Isoproterenol is high compared to that in rats in the control group (0.46 ± 0.01 g/L) but the diffence was not statistically significant. However, spirulina pretreatment decrease ($p>0.05$) the total cholesterol compared to ISO group. The cholesterol level in rats treated with spirulina only (0.40 ± 0.03 g/L) was lower than that of rats in the isoproterenol group.

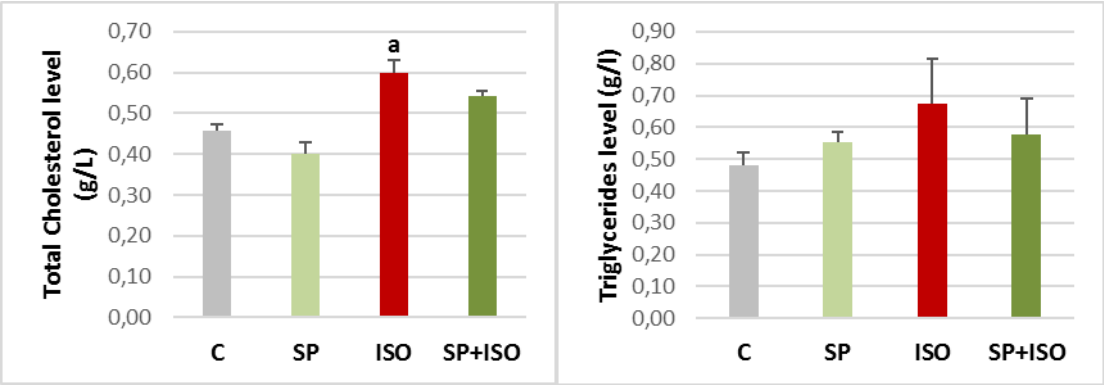


Figure 24: Representation of lipid profil in rats of the different groups.

The results were analyzed using a one-way ANOVA followed by Tukey post hoc test. The results are expressed as Mean ± SEM. ***a****: significant difference vs control group.* ***b****: significant difference vs ISO group. P≤0,05 indicates a significant change.*

Concerning triglycerides, it is noted that the TG level is higher in rats treated with isoproterenol compared to that in rats of control group, while the SP+ISO group showed a slight decrease compared to the ISO group.

It was observed in this study that the ISO administration caused a significant increase in the level of cholesterol and triglycerides in serum. When the rats were treated with spirulina, a decrease in both levels of cholesterol and TG was noted

According to our results, the administration of a dose of spirulina (500mg/kg), It has a positive effect on the level of fats disturbed by isoproterenol.

Elevated LDL and low HDL cholesterol, causes myocardial membrane damage **(Thomes et al., 2010)**. Cholesterol is a major component of the atherosclerotic plaque that is associated with MI. The increased myocardial cholesterol content observed in ISO induced myocardial infarcted rats is because of increased uptake of LDL-c from the blood by myocardial membranes **(Roy and Stanely Mainzen Prince, 2013)**. Whereas HDL inhibits the uptake of LDL from arterial wall and facilitates the transport of cholesterol from peripheraltissues to the liver, where it's catabolised and excreted from the body **(Saravanan et al., 2012)**. in addition to , triglyceride were significantly elevated. These changes in lipid concentrations might be due to enhanced lipid biosynthesis**(Al-Yahya et al., 2013)**.

Spirulina platensis effect on lipoprotein lipase activity and hepatic triglyceride lipase. The presence of novel protein C-phycocyanin (C-PC) present in Spirulina platensis may involve in the inhibition cholesterol. It is found that the aqueous extract of Spirulina

platensis may inhibit the intestinal absorption of dietary fat by inhibiting pancreatic lipase activity **(Han et al., 2006)**.

2.2. Troponin I assessment

Troponins (cTnI) are highly specific and sensitive laboratory markers of myocardial insult and are considered as the gold standard for biochemical detection of myocardial injury. In addition cardiac troponin were proposed as marker of cardiac cell death, it's a protein was now widely used and established as the guideline recommended marker in order to assist in the diagnosis of acute myocardial infarction **(Boeddinghaus et al., 2018)**.

In our results, administration of isoproterenol has increased 3 folds the troponin level in comparison with the value found in the control group (1.23 ±0.34 µg/ml), this difference is statistically significant ($p<0.05$). However supplementation with spirulina (500 mg/kg) shows a considerable diminution about 2 folds of its high level in ISO group ($p>0.05$).

The biochemical profile in ISO- treated rats showed abnormalities as evidenced by a remarkable elevation in the levels of troponin- T in plasma. The observed elevation of these cardiac specific indictators might be related to deterioration in cell membrane integrity, as has been reported previously **(Khdhiri et al., 2021)**.

Our result shows that the administration of Isoproterenol led to a remarkable increase in troponin levels. This is in line with previous studies **(Prasesti et al., 2023)** who evaluated the perspective-cardioprotective effects of spirulina platensis extract, bitter melon fruit extract (*Momordica charantia*), and their combination. The levels of troponin generated by isoproterenol were dramatically reduced due to the presence of Spirulina. which have antioxidants like β-carotene and phycocyanin may play a role in this.

3. Evaluation of antioxidant statut

3.1. Assessment of catalase activity

Catalase, in particular, is considered one of the most important antioxidant enzymes. It is found in nearly all living organisms. In mammalian cells, the highest catalase activity is found in the liver and kidney mitochondria as well as in the cytoplasm of red blood cells. Having one of the highest turnover numbers known, catalase protects cells from ROS-induced oxidative damage. One catalase molecule can decompose millions of hydrogen

peroxide (H_2O_2) molecules into harmless products, oxygen, and water **(Panahi et al., 2019)**.

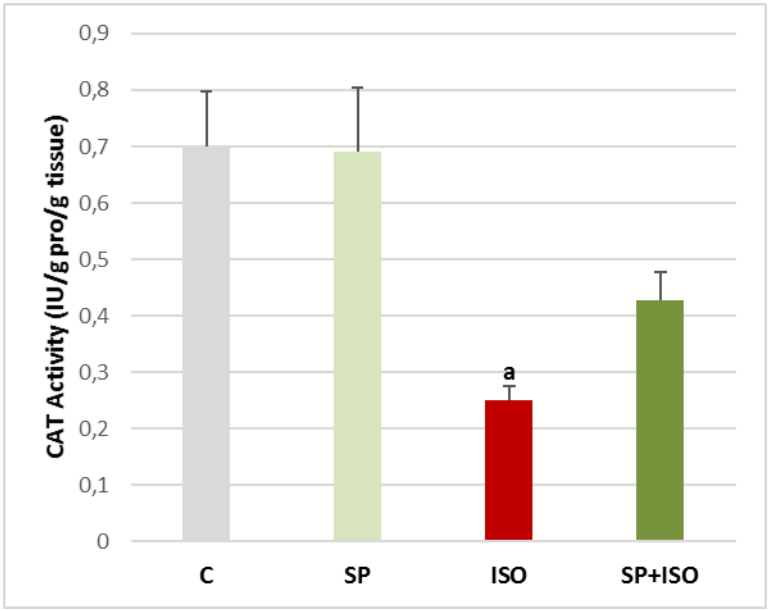


Figure 25: Variation of catalase activity in the rats of the different groups.

The results were analyzed using a one-way ANOVA followed by Tukey post hoc test. The results are expressed as Mean ± SEM. ***a****: significant difference vs control group.* ***b****: significant difference vs ISO group. P≤0,05 indicates a significant change.*

As is shown in the figure 25, the activity of the catalase in the ISO group is reduced considerably ($p<0.05$) in comparison with the control group. The rats having undergone pretreatment with spirulina at a dose of 500 mg/kg showed a no significant difference in catalase activity compared to the normal rats. And on the other hand the groups SP + ISO demonstrates increase the catalase activity compared to the ISO group.

In our study, the induction of MI by isoproterenol resulted in an important reduction of the catalase activity in the heart tissue, this result agrees with the results obtained by **(Tiwari et al., 2009),(Chattopadhyay et al., 2003)**. The decrease in catalase activitye is due to excessive generation of $^{\bullet}O_2^-$ leading to the inactivation of the enzyme $^{\bullet}O_2^-$ is small enough to gain access to the hemes of catalase and might convert the resting enzyme to ferro-oxy state (compound III) which is known to be inactive **(Chattopadhyay et al., 2003)**.

During myocardial infarction, superoxide radicals generated at the site of damage, modulates SOD and CAT resulting in the loss of activity and accumulation of supperoxide radical, which damages myocardium **(Saravanan and Prakash, 2004)**.

In addition, the pretreatment with spirulina 500 mg/kg prevented the decrease in catalase activity in the cardiac tissue following the administration of isoproterenol. spirulina platensis extract has the ability to strengthen the body's antioxidant system at significant concentrations by activating the enzyme catalase **(Teimourpour et al., 2020)**.

3.2. Assessment of reduced glutathione and glutathion peroxidase activity

GSH plays a multifactorial role in the antioxidant defense mechanism **(Sathishsekar and Subramanian, 2005)**. It is a direct scavenger of free radicals, a co-substrate necessary for the activity of GPx and that of glutathione-s-transferase **(Ravi et al., 2004)** and participates in the regeneration of oxidized vitamin E. Therefore, changes in GSH levels can be considered a particularly sensitive indicator of oxidative stress **(Taleb-Senouci et al., 2009)**.

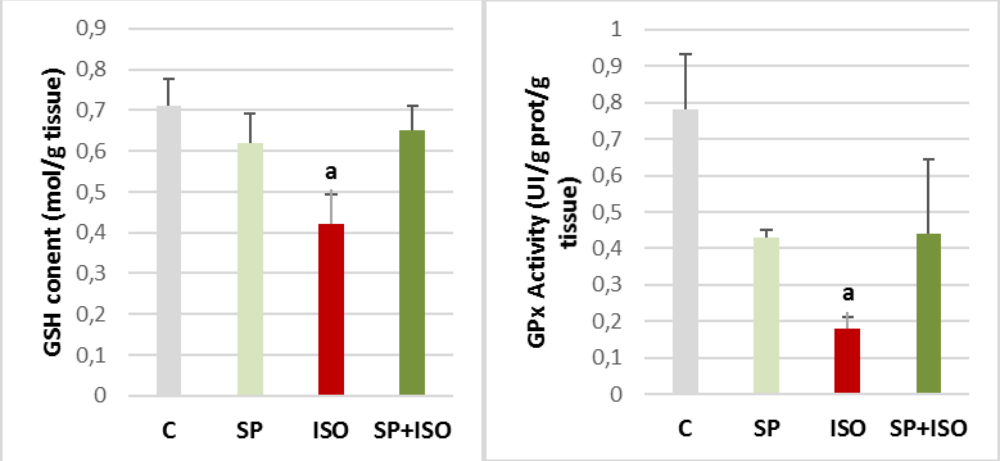


Figure 26: Variation in GSH levels and GPx activities in the rats of the different groups.

The results were analyzed using a one-way ANOVA followed by Tukey post hoc test. The results are expressed as Mean ± SEM. ***a****: significant difference vs control group.* ***b****: significant difference vs ISO group. P≤0,05 indicates a significant change.*

As shown in the figure 26, the levels of GSH and GPx activity decreased in the rats of the group treated with isoproterenol, compared to the control group. On the other hand, rats pretreated with spirulina represents an increase ($p>0.05$) in the level of GSH in comparison with the isoproterenol group.

Based on our research findings, exposure to ISO has been associated with alterations in the levels of glutathione within the myocardial tissue, showing decreased concentrations in the infarcted group compared to the simulated group, which is consistent with existing literature **(Ahmed et al., 2004)** **(Lobo Filho et al., 2011b)**. The decline in glutathione

depletion in rats exposed to ISO is attributed to its utilization in scavenging free radicals, as well as its involvement in repairing oxidized proteins and participating in the detoxification of peroxides facilitated by GPx. These results suggest that reduced glutathione levels may act as a primary defense mechanism against the oxidative stress induced by this beta-adrenergic agonist.

Reactive oxygen species (ROS) are produced during the early phases of acute myocardial infarction (AMI), and glutathione plays a role in neutralizing hydrogen peroxide radicals, with a decrease in glutathione levels observed during this period. Glutathione plays a crucial role in safeguarding the myocardium against damage caused by free radicals, and a decrease in its levels could impede recovery following ischemic episodes **(Stanely Mainzen Prince et al., 2009)**.

Pre-treatment with spirulina 500 mg/kg slightly prevented a major decrease in GSH level in cardiac tissue. Spirulina platensis maintained the activity of cellular antioxidant enzymes total GPx, GPx-Se, and GReductase and increased the levels of reduced glutathione in cells. Intriguingly, the antioxidant capacity of S. platensis could be enhanced when exposed to additional environmental stress **(Abd El Baky et al., 2009).**

Spirulina therapy increased glutathione stages and shielded the interest of cell antioxidant enzymes which includes glutathione peroxidase (GPX) and oxidized glutathione reductase (GR). Potential antioxidant spirulina was confirmed by these data. According to an *in vitro* study **(Taleb-Senouci et al., 2009)** spirulina was found to be effective against cardiovascular diseases, owning to their inhibitory effect on the production of ROS, therefore , it is urgent to widely screen the bioactive components of Spirulina with special biological functions **(Zheng, 2020)** . The antioxidant activities of Spirulina may be attributed to the presence of potent antioxidant components including β-carotene, vitamin C, vitamin E, zeaxanthin, diatoxanthin, echinenone, xanthophyll and phycocyanin, which can protect against oxidative damage **(El-Hady et al., 2022)**.

4. Measurement of the size of the infarction

The infarct size increases significantly 4 fold ($p<0.05$) in rats treated with ISO compared to the control group (4.98 ± 0.83%). However, pretreatment with spirulina decreases significantly 3 fold infarct size ($p<0.05$) compared to the ISO group. This

pretreatment reduces the infarct size to a point where it aligns with the control group ($p>0.05$).

The cardioprotective effects of Spirulina were further confirmed by direct observations on myocardial infarction in heart tissues using TTC staining. TTC serves as a proton acceptor for various pyridine nucleotide-linked dehydrogenases and cytochromes, which are essential components of the inner mitochondrial membrane and the electron transport chain. Specifically, TTC forms a red formazan precipitate with LDH in viable myocardial tissue, while the infarcted myocardium does not stain. We also noted the presence of pale patches in cardiac tissue slices of rats treated with ISO, indicating areas of necrosis due to the lack of TTC reduction. Interestingly, pre-administration of Spirulina to ISO-treated rats protected the heart tissues from infarction, thus demonstrating protection against cardiac necrosis.

According to results obtained by **(Alam et al., 2022) (Ma et al., 2020)** ISO increases the generation of ROS which induced myocardial necrosis leading to the increase of size infraction **(Alam et al., 2022)**.

Interestingly, pre-administration of Spirulina to ISO-treated rats protected the heart tissues from infarction, thus demonstrating protection against cardiac necrosis. and because spirulina rich in c-phycocyanin reduced MI related to myonecrosis, interstitial oedema and inflammatory infiltaration in the heart muscle **(Blas-Valdivia et al., 2022)**. Also spirulina play an important role in decreasing of size of infraction **(Vilahur et al., 2022)**.

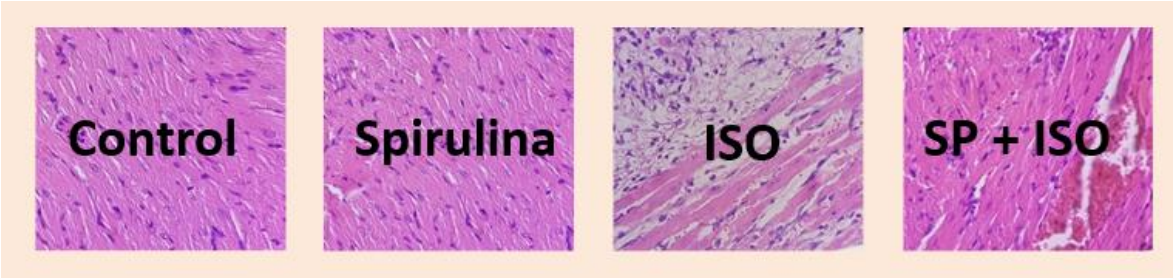
5. **Histopathological Analysis**

The histological examination of cardiac tissue is a crucial aspect of assessing the protective effect of Spirulina against isoproterenol-induced myocardial infarction in rats. The study aimes to evaluate the cardioprotective effect of Spirulina platensis against isoproterenol-induced myocardial infarction in rats. This examination involves the microscopic analysis of the heart tissue to identify any changes or damage caused by the isoproterenol-induced myocardial infarction and to evaluate the effectiveness of spirulina in mitigating these changes.

The table 3 depict the impact of spirulina on myocardial infarction induced by ISO. The photomicrographs of the control groups show clear integrity of the longitudinal myocardial cell membrane near the epicardium. The myocardium is composed of cross-

striated muscle cells (cardiomyocytes) with a centrally located nucleus. The cardiomyocytes are arranged in spirals or longitudinally attached to the cardiac skeleton, which may consist of connective tissue. The MI photomicrographs reveal that this condition leads to interstitial edema with extensive necrosis of cardiac muscle fibers showing varying degrees of vacuolar changes and areas of inflammatory infiltration; however, the Spirulina treatment reduces the edema, necrosis, and inflammatory response.

Table 3: Histopathological observation of heart in different groups using H&E staining (× 40).



The histological changes observed in the heart tissue of the isoproterenol-treated rats included:

- Myocardial Cell Damage: The myocardial cell membrane was found to be damaged, indicating cell death or necrosis.
- Inflammation: Inflammation was observed in the heart tissue, which is a common response to tissue damage.
- Fibrosis: Fibrosis, or the formation of scar tissue, was observed in the heart tissue, which can lead to cardiac dysfunction.

The administration of spirulina extracts was found to reduce the histological changes observed in the heart tissue. Specifically:

- Attenuation of Myocardial Cell Damage: Spirulina platensis was found to attenuate the damage caused by the myocardial infarction, as evidenced by the reduction in LDH and CK levels.
- Reduced Inflammation: Spirulina extracts were found to reduce inflammation in the heart tissue, as evidenced by the reduction in the levels of inflammatory markers.
- Prevention of Fibrosis: Spirulina extracts were found to prevent the formation of fibrosis in the heart tissue, which can lead to cardiac dysfunction.

The results of the histological examination showed that the myocardial cell membrane was clearly intact in the normal rat (negative/sham control group). However, in the isoproterenol-treated rats, the integrity of the myocardial cell membrane was altered, indicating myocardial damage that was improved by the study of **(Kołodzińska et al., 2022) (Kannan and Quine, 2011)**. The administration of Spirulina extracts significantly attenuated the histopathological changes in the cardiac tissue, indicating a protective effect against myocardial infarction.

In conclusion. The histological examination of cardiac tissue is a valuable tool in assessing the protective effect of spirulina against isoproterenol-induced myocardial infarction in rats. The results of this study suggest that spirulina extracts, can attenuate the damage caused by the myocardial infarction and reduce the levels of cardiac injury markers. These findings support the potential therapeutic use of spirulina in the prevention and treatment of myocardial infarction.

The present study was carried out with the aim of evaluating the protective effect of spirulina against isoproterenol-induced myocardial infarction (MI). The key findings can be summarized as follows:

- Isoproterenol induce cardiac injury mediated by oxidative stress and leads to cell death and cardiac tissue necrosis.

- Spirulina's cardioprotective effects against MI is attributed to its antioxidant activity insured by its bioactive compounds, phycocyanines, carotenoids, and phenolic compounds.

- Spirulina leads to the improvement of cardiac injury marker by reducing troponine leakage in the blood and thus, indicating a protective effect against MI.

- Spirulina ameliorates lipid profile and reduces significantly the LDL cholesterol amount wich confers to spirulina a hypolipidic activity and prevent the formation of atherosclerosis plaque.

- Spirulina improved the endogenous antioxidants activities of the myocardium. It exerted antioxidant effects on MI due to its richness in polyphenols and its ability to scavenge free radicals, which may lead to improved cardiac function and alleviate myocardial damage.

- Spirulina prevent necrosis and reduces significantly the size of the infarcted area in the myocardium.

The available evidences suggest that spirulina supplementation may have protective effects against myocardial infarction. These results revealed new perspectives in the development of an innovative therapeutic target for the prevention of myocardial infarction and also the use of spirulina as a prophylactic measure. It remains to be said that it would be wise to complete this study with:

- Extending the duration of treatment with spirulina, in order to reach a definite conclusion regarding the preventive effect of spirulina extract on myocardial infarction.

- Testing the curative effect of spirulina by administering it after inducing MI in rats.

- Testing the effect of spirulina using *in vivo* modele of induction of MI by ligating the LAD coronary artery wich is the best experimental method closest to the real situation of heart attack in humans.

- Verifying whether the cardioprotection provided by spirulina is due to the synergistic effect of all of its compounds or not, by testing the isolated effect of each pure constituent on MI.

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