PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
University Freres Mentouri -Constantine1
Faculty of Nature Sciences and Life
Department of Animal Biology

Thesis presented for the obtention of the degree of MASTER II
Domain: **Nature and life sciences**
Field: **Biological Sciences**
Option: **Cellular and Molecular Immunology**

N° d'ordre:
N° de série:

Titled

The effect of hot water and traditional yeast on liver inflammation induced by refined crystallize sugar

Presented by: Saidi Boutheina
Mosbah Lina

20/06/2023

Examination board:

President: ARIBI Boutheyna (MCB - UFMC1).
Supervisor: ZERIZER Sakina (Pr. UFM Constantine 1).
Examinator: MESSOUDI Saber (MCB - UFMC 1).

2022 - 2023


بسم الله الرحمن الرحيم

Acknowledgements

First and foremost, we are grateful to Allah Almighty who helped us complete this dissertation. We would like to extend our deep thanks to our supervisor

Pr. ***ZERIZER Sakina***

Thanks to her valuable advice, continuous efforts and patience with us throughout this period, we were able to produce and present this work.

To all the members of the jury, Dr. ***ARIBI Boutheyna*** *and Dr.* ***MESSOUDI Saber*** *we thank you for agreeing to judge this work, a special thanks to Dr.* ***BAHRIE Aid*** *for providing all kinds of assistance that we needed in the animal house.*

Finally, we extend our sincere thanks to all the work team that was with us and our friends and our family who have always supported and encouraged us during the realization of this thesis.

Dedication

After the grace of allah Almighty

I dedicate my graduation:

To my first role model and the beacon that lights my way, to the one who gave and continues to give me infinitely, to the one in whom I held my head high... Dear ***FATHER****, allah bless you for me.*

To the apple of my eye and the source of my joy and happiness, my allahheal you and protect you for me……my ***MATHER***

For those who stood by me and supported me through all the happy and sad times. My wonderful sister ***Souhaila****.*

To my dear sisters ***Sarah*** *and* ***Nabila.***

To my beloved brother, may Allah protect him for me.

To my deceased, whom my heart always remembers, may Allah have mercy on him and place him in his spacious gardens. My first defeat in life, my greatest loss, and my greatest pain in my heart.

To my closest and dearest friends in existence, ***Romaisa*** *and* ***Asala***

To the hidden shadow and my supporter in the graduation study, ***Zina****. May Allah protect you*

To my friend, ***Lina*** *and* ***Asma*** *my path in my university career, all thanks and appreciation to you.*

Bouthaina

Dedication

Thank Allah for giving me the privilege and the opportunity to study as I always wanted, and to be able to complete this journey and overcome its difficulties.

I dedicate this work to the person who made me who I am today.

*To my father **(Mosbah Mourad)**, my support, my hopes, my security and the most precious person I have.*

I pray to Allah to protect you from the misfortunes of life so that you remain a flame that illuminates my path.

*To the sweetest and most wonderful mother, my dear mom **(Kherief Saliha)** who accompanied me from the beginning of my path since I was 5 years old until I turned 23 years old, Mama, your prayers were a great support for me throughout my academic career.*

*I ask Allah to grant you good health and a long life. To the joy of our home, my dear sisters: the quiet **"Hana"** and the spoiled **"Maryam".***

All words of thanks are not enough for all the help and support you have given me. I wish you a better and brighter future.

*To those who are very dear to my heart, I hope that Allah will keep you healthy, well and smiling. To my companions in this work: **"Bouti", "Asma", "Zina", "Iman", "Shishi", "Lamis","Sako"**; I will always be with you, and I wish you all the best.*

***To all my loved ones and family members from my mom and dad's side, thank you for your support, especially during this time**.*

Lina

Table of content

Table of content

Table of content

List of abbreviations

ADP Adenosine Diphosphate

AGP Acid Glyco Protein

AIH Autoimmune Hepatitis

ATP Adenosine Triphosphate

C Control

C1q Complement Component 1q

CCL2 Chemokine Ligand 2

CCL3 Chemokine Ligand 3

CCR2 Chemokine Receptor 2

CCR7 Chemokine Receptor 7

CD4 Cluster of Differentiation

CRPC- Reactive Protein

CXCL12 Chemokine (c_xc motif) Ligand 12

DCs Dendritic Cells

DP Degree of Polymerization

ECs Endothelial Cells

EMP Embden-Meyerhof-Parnas

ESR Erythrocyte Sedimentation Rate

FC Crystallized Fragment

FCɣR Crystallized Factor Gamma Receptor

HBV Hepatitis B Virus

HCC Hepatocellular Carcinoma

HCV Hepatitis C Virus

HDL High-Density Lipoprotein

HFCS High Fructose Corn Syrup

HMGB1 High Mobility Group Box 1

Hs CRP High Sensibility of C-Reactive Protien

HW Hot Water

ICAM Intercellular Adhesion Molecule

IFN ɣ Interferon Gamma

IgG Immunoglobulin G

IL- α Interleukin Alpha

IL- β Interleukin Beta

IL-1 β Interleukin 1Beta

Mad CAM-1 Mucosal Addressin Cell Adhesion Molecules -1

mCRP Monomeric C-Reactive Protein

MI Myocardial Infarction

MMPs Matrix Metallo Protéinases

NAD^+ Nicotinamide Adenine Dinucleotide

$NADH^+$ Nicotinamide Adenine DinucleotideHydrogen

NAFLD Non Alcoholic Fatty Liver Disease

NASH Nonalcoholic Steatohepatitis

NETs Neutrophil Extracellular Traps

NF-κB Nuclear Factor-Kappa B

NK Natural Killer Cells

NO Nitric Oxide

PAI-1 Plasminogen Active Inhibitor-1

PAMPs Pathogen-Associated Molecular Patterns

PCH Phosphocholine

pCRP Pentameric C-Reactive Protein

PCT Procalcitonin

PGs Prostaglandins

PMNs Polymorphonuclear Cells

POLYOLS Poly Oligosaccharides

PRRs Pathogen Recognition Receptors

RA Right Atrium

RCA Right Coronary Artery

ROS Reactive Oxygen Species

RUQ Right Upper Quadrant

S Sugar

TANs Tumor Associated Neutrophils

TGF- β Transforming Growth Factor -Beta

Th1 Helper Cell Type 1

Th2 helper cell type 2

THP-1 Tamm-Horsfall Protein 1

TLRs Toll Like Receptors

TNF-α Tumor Necrosis Factor-Alpha

VAT Visceral Adipose Tissue

VCAM Vascular Cell Adhesion Molecule

VCAM-1 Vascular endothelial Cell Adhesion Molecular-1

VEGEF Vascular Endothelial Growth Factor

TY Traditional Yeast

List of figures

List of tables

Introduction

Sugar in many forms and permutations is ubiquitous, naturally accurring, and required for the most life forms on planet earth **(John and White, 2018)**. The term "sugar", as applied to human diets, is a collective term for several different chemical species. Thus, "table sugar" is essentially pure sucrose, whereas fruit juice, honey and syrups contain mixtures of sucrose, glucose and fructose, and often oligosaccharides of different size. These compounds are invariably combined as "sugars" **(Amoutzopoulos et al., 2020)**.

Excessive sugar intake is in associates with adverse health conditions, including obesity, metabolic syndrome, and inflammatory diseases **(Freeman et al, 2018)**. Recent evidence suggests that diets high in sugar (from sucrose and / or high fructose corn syrup “HFCS”) not only increases the risk for non alcoholic fatty liver disease (NAFLD), but also, non-alcoholic steatohepatitis (NASH) **(Jensen et al., 2018)**.

A healthy liver is crucial for maintaining overall health. However, liver is prone to various insults due to metabolic disorders, viral infections, hepatitis, cirrhosis, diabetes **(Bhondave et al., 2014)**. The prevalence of non-alcoholic fatty liver disease (NAFLD) has increased globally in recent years, becoming one of the leading causes of chronic liver disease in both developed and developing countries. In the western world, NAFLD affects 25%-30% of the general population because of fat rich foods and poor lifestyle **(Huanming et al., 2017)**.

Passive heating intervention have been linked to several positive health outcomes, such as improved vascular function and mental health, Weight loss, and enhanced insulin sensitivity **(Hoekstra et al., 2018)**.

Drinking enough water can support skin, muscle, and joint health .water helps the body’s cells absorb nutrients and fight infections drinking a few glasses of warm or hot water each day might offer even more benefits **(Baride et al., 2020)**.

Yeast have shown numerous beneficial effects on human health. Among these, probiotic effects are the most well know health effects including prevention and treatment of intestinal diseases and immuno modulatory effects **(Moslehi-Jenabian et al., 2010)**.

In the present research we aimed to:

- Evaluate the effect of hot water and traditional yeast on the weight and diet of mice induced by crystallize sugar.

- Evaluate the effect of hot water and traditional yeast on some parameters such as HDL-C, LDL-C, TG, CRP and blood sugar.
- Evaluate the effect of hot water and traditional yeast on GSH.
- Histological investigations of liver

Chapter I: sugar and inflammation

Sugar and inflammation

I. Sugar

I. 1. Definition of Sugar

Sugar is a conventional everyday concept used mainly in relation to sucrose (table sugar), as well as other water soluble simple carbohydrates with a sweet taste **(Saitkulov et al., 2022)**.

The most common sugar is sucrose, a crystalline tabletop and industrial sweetener used in foods and beverages **(Zaitoun et al., 2018)**.

The commercial sugar is the disaccharide sucrose white sugar **(Kamal and Klein, 2011)**.

The human body, and especially the brain, needs a constant supply of glucose, which ensures the effectiveness and efficiency of its work **(Saitkulov et al., 2022)**.

The majority of the natural sugars contain 6 or 12 carbon atoms in their molecules **(Kamal and Klein, 2011)**. The term dextrose is used to refer to glucose. Extrinsic or added sugar refers to sucrose or other refined sugars in soft drinks and incorporated into food, fruit drinks, and other beverages. Intrinsic or naturally occurring sugar refers to the sugar that is an integral constituent of whole fruit, vegetable, and milk products **(Howard and Wylie-Rosett, 2002)**.

Many food which contain high added sugar, provides energy but it is already poor in another nutrients, so that will affect the balance of intake nutrients like mineral, proteins and vitamins.

The increase of consuming sugar leads to several disease especially diabetes type 2, obesity, and cardiovascular disease **(Zaitoun et al., 2018)**.

Carbohydrates

Carbohydrates are the main source of energy that the human body ingests **(Asif et al., 2011)**. Carbohydrates are molecules consisting mainly of three basic elements of carbon, oxygen and hydrogen with the empirical formula (CH2O) **(Aldairi, 2020)**.

A prolonged lack of carbohydrates, the body begins to synthesize glucose from its own proteins, which reduces its protective ability against environmental factors **(Saitkulov et al., 2022)**.

I.2. Chemical structure of carbohydrates types

Structurally they are polyfunctional compounds. They contain two types of functional groups carbonyl and hydroxyl. They may be polyhydroxy aldehydes or polyhydroxy ketones **(Mondal, 2017)**. Carbohydrates with different, chemical structures, physical forms, particle sizes, and fiber contents induce distinct plasma glucose and insulin responses **(Jenkins et al., 1981)**.

The primary classification of dietary carbohydrate is based on chemistry, that is character of individual monomers, type of linkage (α or β) and degree of polymerization (DP) **(Cummings and Stephen, 2007)**.

Carbohydrates are classified into monosaccharides, disaccharides, oligosaccharides, polysaccharides (Figure 01) **(Asif et al., 2011)**. Simple carbohydrate refers to monocchacarides and disaccharides, complex carbohydrate refers to polysaccharides such as starch **(Howard and Wylie-Rosett, 2002)**.

A carbohydrate may be termed “complex” if it contains more than one type of monosaccharide building unit **(Seeberger, 2017)**.

I.2. 1. Simple carbohydrates

Monosaccharides

The most common naturally occurring monosaccharide is fructose found in fruits and vegetables **(Howard and Wylie-Rosett, 2002)**.

They are polyhydroxy aldehydes or polyhydroxy ketones which cannot be decomposed by hydrolysis to give simpler carbohydrates. e.g. fructose, Glucose, Galactose etc...(Figure 02).

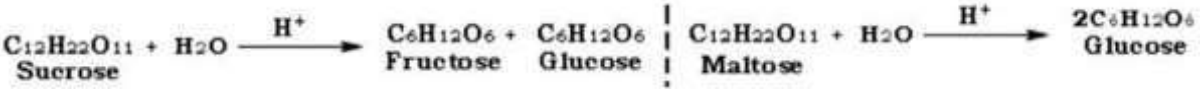
Sugars are also named according to their number of carbons, some of the most common types are trioses (three carbons), pentos (five carbons), and hexoses (six carbons) (Figure 03) **(Mondal, 2017)**.

Disaccharides

Disaccharides and starch are well known to constitute the major part of the carbohydrates present in our diet **(Dahlqvist and Borgstrom, 1961)**.

Common disaccharides are sucrose, found in sugar cane, sugar beets, honey, and corn syrup; lactose, found in milk products; and maltose, from malt (Figure 04) **(Howard and Wylie-Rosett, 2002)**.

They yield two monosaccharides molecules on hydrolysis. Which have molecular formula is C12H22O11 **(Mondal, 2017)**.



I.2. 2. Complex carbohydrates

Oligosaccharides

It represent carbohydrates that contain between 3 and 10 single sugar residues and are not relatively abundant in the diet when compared to other more common carbohydrates like those in the disaccharide category. Common oligosaccharides include raffinose, stachynose and verbascose (Figure 05) **(Ahnenet al., 2020)**.

Polysaccharides

Polysaccharides are essential macromolecules which almost exist in all living forms **(Mohammed et al., 2021)**. It is important component of higher plants, membrane of the animal cell and the cell wall of microbes **(Yu et al., 2017)**.

This term is typically used to denote any linear or branched polymer consisting of monosaccharide residues. The relationship of monosaccharides to polysaccharides is analogous to that of amino acids and proteins, or nucleotides and nucleic acids (polynucleotides) **(Seeberger, 2017)**.

They have higher molecular weight, which yield many monosaccharide molecules on hydrolysis. E.g. Starch, Dextrin, Cellulose, glycogen etc (Figure 06) **(Mondal, 2017)**.

I. 3. Uses of sugar

Although the main reason for the use of sugar is its sweet taste, sugar has many other functions in food technology. The most important among these are that added sugar in foods

acts as a sweetener, preservative, texture modifier, fermentation substrate, flavouring and colouring agent, bulking agent **(Koivistoinen and Hyvonen, 1985)**.

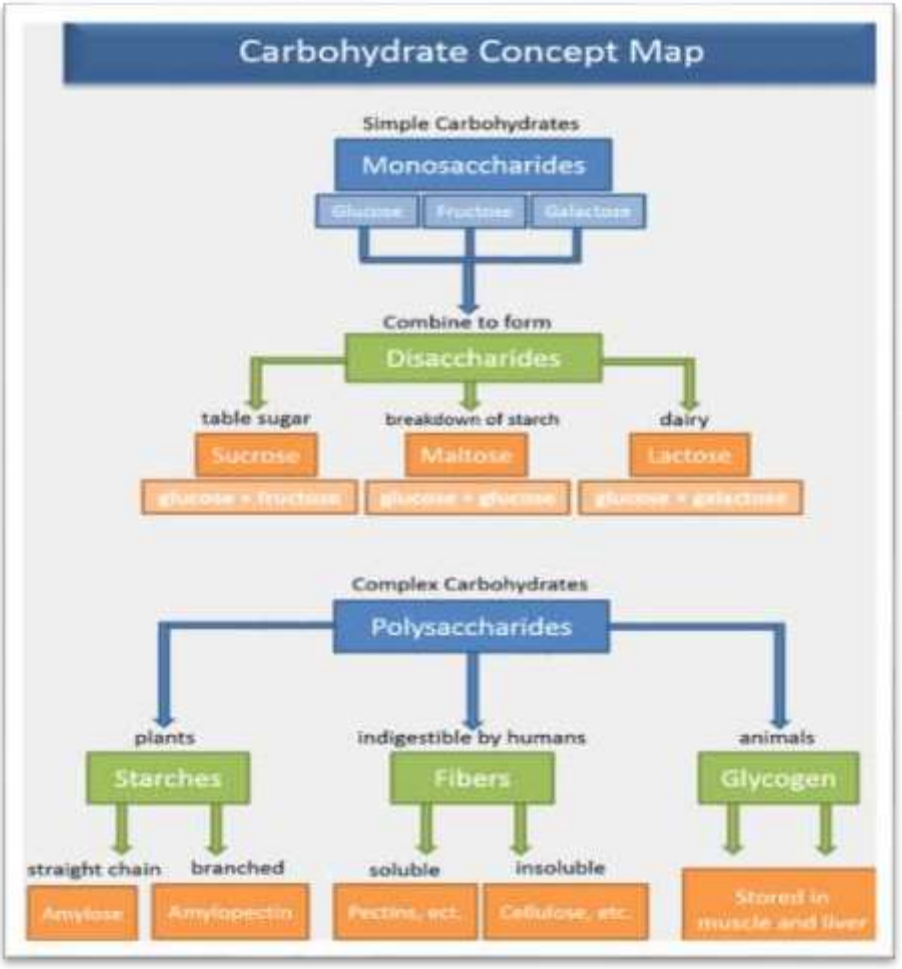


Figure 01: carbohydrate concept map (site web1).

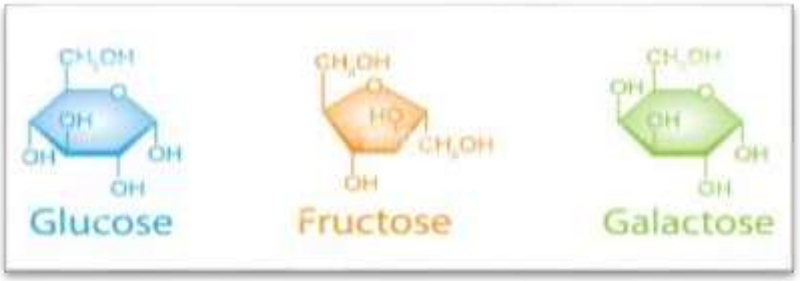


Figure 02: linear and ring structures of three common monosaccharides. All have the same molecular formula (C6H12O6), but they have different structures (red) and are therefore isomers of each other (site web 2).

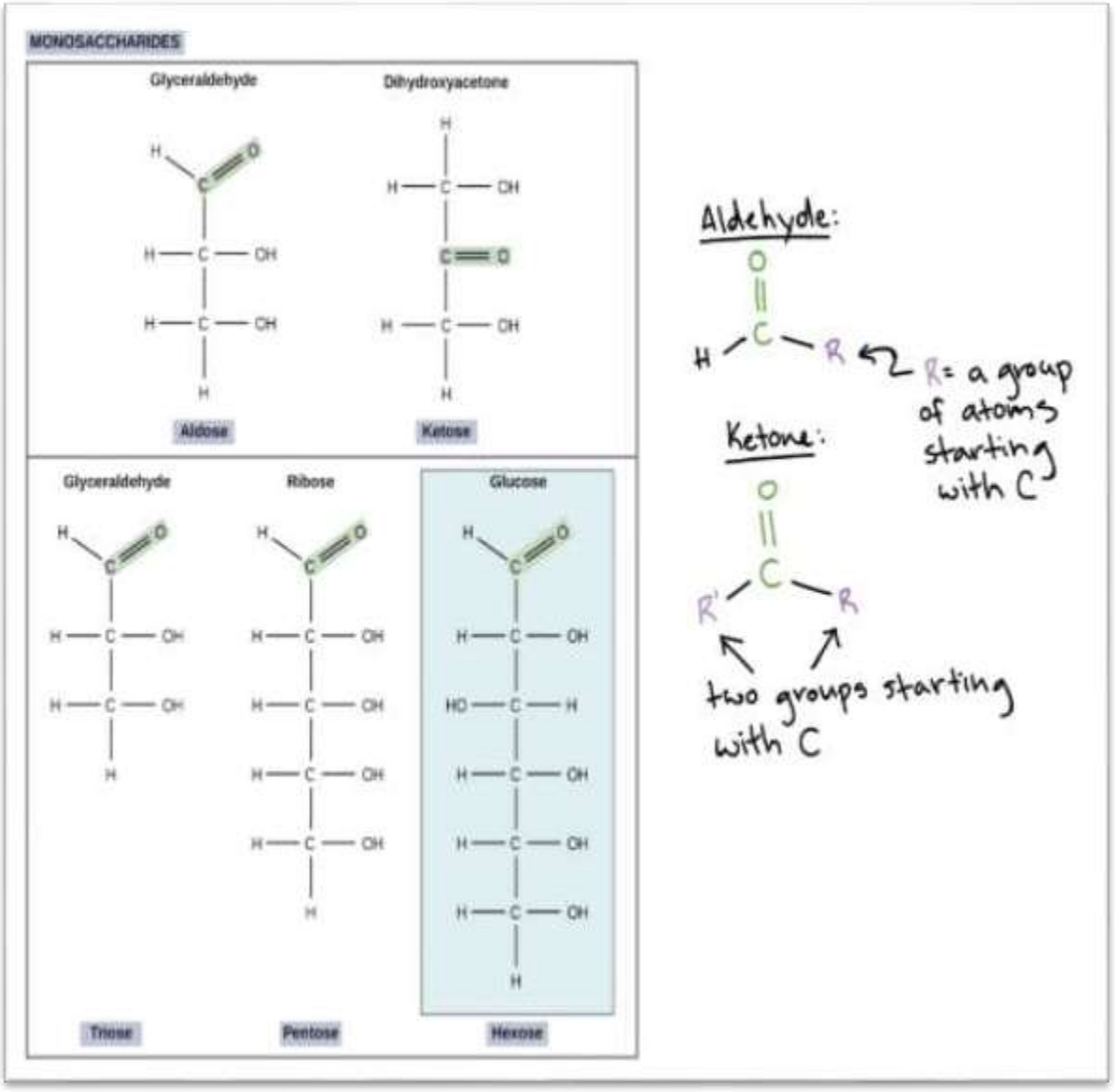


Figure 03: sugars are also named according to their number of carbons: some of the most common types are trioses (three carbons), pentoses (five carbons), and hexoses (six carbons).

Monosaccharides are classified based on the position of the carbonyl group and the number of carbons in the backbone (site web 3).

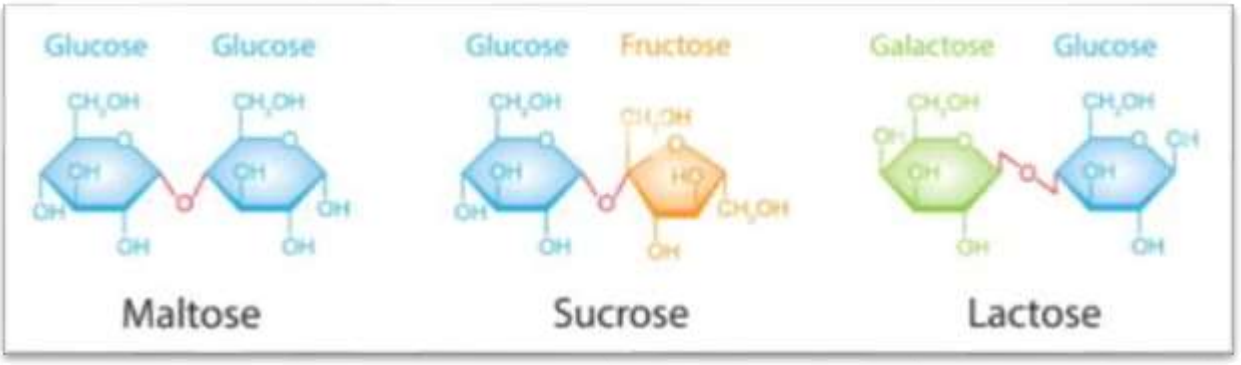


Figure 04: structures of the three common disaccharides. All contain glucose as one of their subunits, the difference between the three is the second subunit (site web 4).

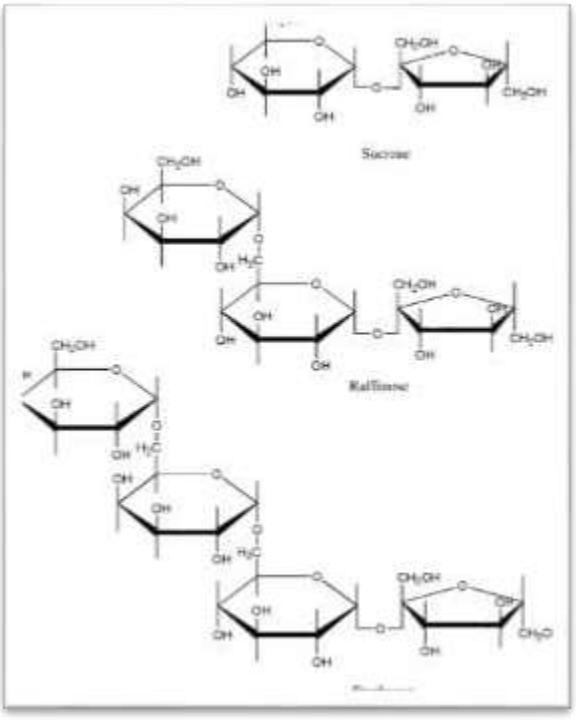


Figure 05: examples of the main soybean oligosaccharides, raffinose and stachyose, derived from sucrose, showing galactosyl residues linked to sucrose by α-(1, 6) bonds (site web 5)

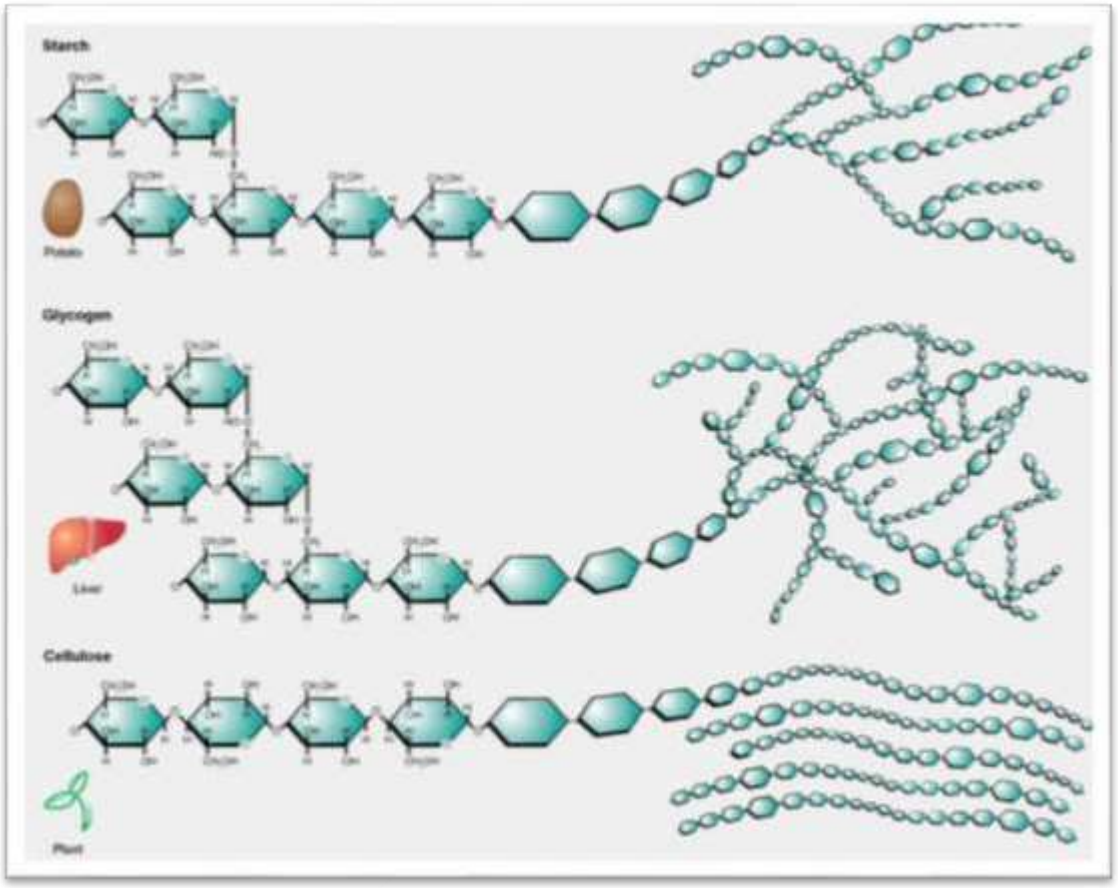


Figure 06: structures of the three common polysaccharide: starch, glycogen, cellulose (site web 6).

I.4.Sugar and inflammation

High sugar intake has long been recognized as a environmental risk factor for increased incidence of many non-communicable diseases, including obesity, cardiovascular disease, metabolic syndrome, and type 2 diabetes. It induce the increase of inflammatory mediators and certain pro-inflammatory cytokines in various tissues, which leads to insulin resistance and low-grade chronic inflammation **(Ma et al., 2022)**.

Fructose is recognized as a major mediator of NAFLD, as a significant correlation between fructose intake and the degree of inflammation and fibrosis **(Muriel et al., 2021)**.

Sugar stimulates the production of free fatty acids in the liver. When the body digests these free fatty acids, the resulting compounds can trigger inflammatory processes. Eating high levels of saturated fats, trans fats, and refined sugar are all risk factors for chronic inflammation **(Marengo, 2019)**.

II. Inflammation

II. 1. Definition

Inflammation is a complex process, comprising many events, initiated by tissue damage caused by endogenous factors (such as tissue necrosis or bone fracture) as well as exogenous factors. These include various types of damage such as mechanical injury (e.g, cut), physical injury (e.g, burn), chemical injury (e.g, exposure to a corrosive chemical), biological injury (e.g, infection by microorganisms), and immunologic injury (e.g, hypersensitivity reactions) **(Binjamini et al., 1996)**. It is also important for the development of many complex diseases and disorders including autoimmune diseases, metabolic syndrome, neurodegenerative diseases, cancers, and cardiovascular diseases **(Murakami and Hirano, 2012)**.

Inflammation is an evolutionarily conserved physical process, affecting any part of the body in which the immune system senses an infection or injury. The five classic signs of inflammation are redness, heat, swelling, pain, and loss of function (Figure 07) **(Hawiger and Zienkiewicz, 2019)**.

Inflammation represents a fundamental biological process that stands at the foreground of a large number of acute and chronic pathological conditions **(Lugrin et al., 2013)**.

Inflammation involving the innate and adaptive immune systems is known to be the protective immune response for maintaining tissue homeostasis by eliminating harmful stimuli, including damaged cells, irritants, pathogens **(Zhao et al., 2021)**.

II. 2. Markers of inflammation

Markers of inflammation are used to detect acute inflammation that might indicate a specific disease and also to assess treatment response. Raised levels of inflammatory markers can indicate the probability of infections, autoimmune conditions, and cancers. Where levels are normal, certain conditions can be ruled out. Although they are valuable for indicating diseases, inflammatory markers are not specific enough to allow diagnosis of serious underlying disease **(Watson et al., 2012)**.

The most common inflammatory markers are C-reactive protein, erythrocyte sedimentation rate, and pro-calcitonin. Although other markers of inflammation are useful in certain circumstances (Table 1).

Table 1: inflammatory markers.

Marker	Application
C-reactive protein (CRP)	It is a biological mark, used as an early indicator to detect infections, tissue injury or acute infection at an early stage **(Boncler et al., 2019)**.
Erythrocyte sedimentation rate (ESR)	Is a common hematology test that may indicate and monitor an increase in inflammatory activity within the body caused by one or more conditions such as autoimmune disease, infections or tumors, and determine the presence of increased inflammatory activity and pulmonary tuberculosi **(Bull et al., 1993)(Bray et al., 2016)**.
Procalcitonin (PCT)	Marker of bacterial infection, severe viral infection, pancreatitis, tissue trauma, and certain autoimmune disorders. Useful in the diagnosis of sepsis **(Meisner, 2014)**.
Serum amyloid A	Acute phase protein released in response to inflammation or infection. Concentration increases dramatically during acute infection and injury **(Targońska and Majdan, 2014)**.
Cytokines	Small proteins including interleukins, chemokines, interferons, and tumor necrosis factors with varying roles in inflammation and immunity. They are released in a number of paracrine, autocrine, or endocrine pathways and have been implicated in a variety of infections and immune system-affecting disorders by both proinflammatory and anti-inflammatory mechanisms. Cytokines which have proinflammatory effects include interferon IFNγ, interleukin IL-17, IL-1β, and tumor necrosis factor TNFα, and those with anti-inflammatory effects include IL-10, IL-4, and IL-1 **(Monastero and Pentyala, 2017)**.

Marker	Application
Alpha-1-acid glycoprotein	AGP glycoforms are very useful in the detection of intercurrent infections in the course of rheumatoid arthritis, systemic lupus erythematosus, or myeloblastic leukaemia, and in the detection of secondary infections in human immunodeficiency virus infected individuals and differentiation between various forms of trophoblastic disease **(Mackiewicz and Mackiewicz, 1995)**.
Plasma viscosity	The plasma viscosity is rising in the presence of proteins produced in reponse to infection or inflammation (erythrocyte sedimentation rate, C- reactive protein, and platelet) **(Lobo et al., 1992)**.
Ceruloplasmin	One of the main proteins in metabolism and distribution of copper in blood serum, and appears to act as an antioxidant. Positive-phase protein, meaning that its level changes in acute and chronic inflammation **(Adamczyk et al., 2016)**.
Hepcidin	Regulator of iron metabolism produced by the liver. Iron deficiency can be indicated by reduced hepcidin levels. Levels of hepcidin are often abnormally high during inflammation, such as during sepsis or in patients with IBD **(D'angelo, 2013)**.
Haptoglobin	Acute phase protein induced by inflammation, which can bind hemoglobin and act as an antioxidant **(Wang et al., 2001)**.

II. 3. Stages of inflammation

In response to tissue injury, a multifactorial network of chemical signals initiates and maintains a response designed to treat injured tissue and repair it **(Sherwood, 2004**). By stimulating and transporting leukocytes of neutrophils, monocytes, and macrophages directed from the venous system to sites of damage, tissue mast cells also play an important role **(Coussens and Werb, 2002)**. This process includes several stages:

II. 3. 1. Vasodilatation

The purpose of the vasodilatory response is to facilitate the local delivery of soluble mediators and inflammatory cells. Inflammation ind uced vasodilatation is mediated primarily by nitric oxide (NO) and vasodilatory prostaglandins **(Sherwood, 2004)**.

Activation of members of the selectin family of adhesion molecules (L-P- and E-selectin) that facilitate rolling along the endothelium of blood vessels.

Release of signals and molecules that activate, stimulate and regulate leukocyte integration by cytokines and inflammatory mediators such as tumor necrosis factor-α(TNF-α).

Fixation of neutrophils to the vascular endothelial surface by tight adhesion through a4b1 and a4b7 integrins associated with vascular endothelial cell adhesion molecule-1 (VCAM-1) and MadCAM-1 **(Coussens and Werb, 2002)**.

II. 3. 2. Recruitment of inflammatory cells

Translocation of cells such as neutrophils, monocytes and macrophages through the endothelium to sites of infection by extracellular proteases, such as matrix metalloproteinases (MMPs) **(Coussens and Werb, 2002)**.

A family of chemical cytokines, called chemokines, have the ability to chemically attract specific groups of leukocytes. Activated neutrophils increase their level of Fc receptor expression allowing the increased uptake and phagocytosis of pathogens **(Thacker, 2006)**.

II. 3. 3. Tissue remodeling and resolution

Resolution occurs after a successful host response. Complete bacterial phagocytosis. Resolving inflammation depends upon apoptosis as well as timely and adequate removal of acute inflammatory cells by macrophages. During apoptosis, neutrophils and eosinophils undergo surface changes enabling phagocytes to recognize and ingest them. The apoptotic process is modulated through extracellular signaling **(Moldoveanu et al., 2022)**.

Tissue remodeling includes tissue metaplasia, granulation, angiogenesis and fibrosis, and roles of prostaglandins (PGs) in these processes have been reported **(Aoki and Narumiya, 2012)**.

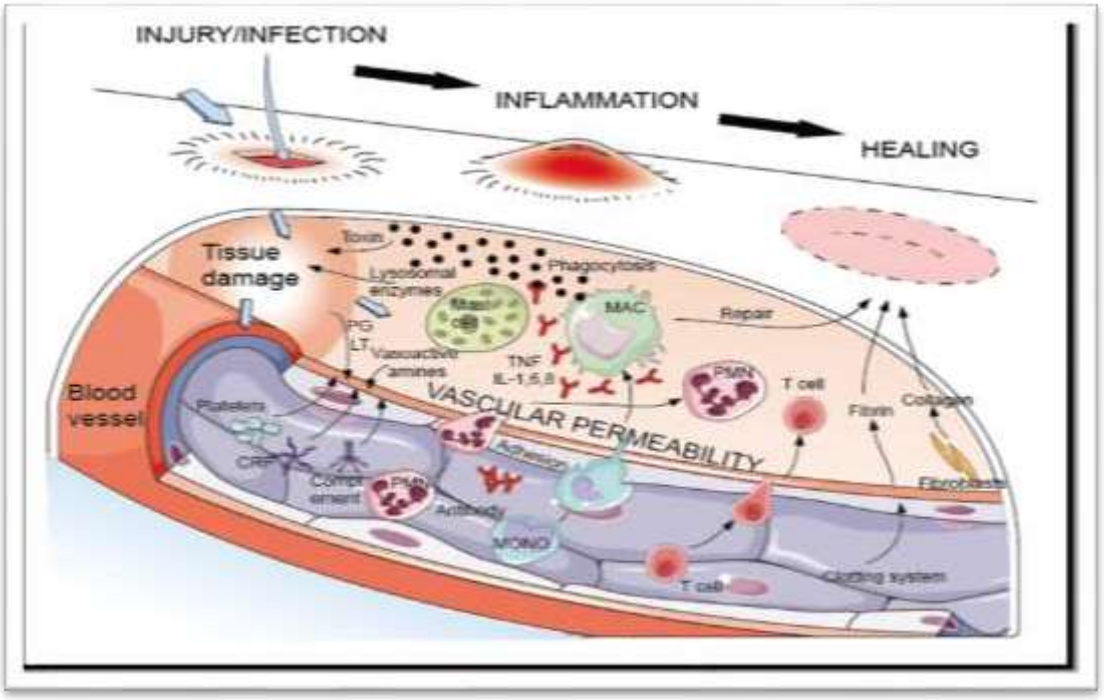


Figure 07: showing signs of inflammation and the stages of response (site web 7).

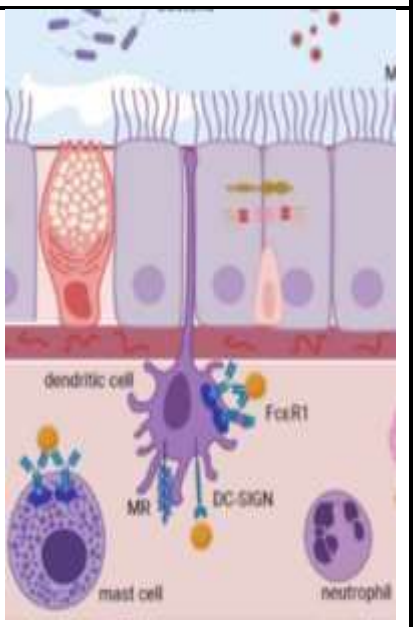
II. 3. 4. Cells of inflammation

The inflammatory response involves a highly coordinated network of many cell types. Activated macrophages, monocytes, and other cells mediate local responses to tissue damage and infection. At sites of tissue injury, damaged epithelial and endothelial cells release factors that trigger the inflammatory cascade, along with chemokines and growth factors, which attract neutrophils and monocytes, and other cells such as lymphocytes (natural killer cells [NK cells], T cells, and B cells), mast cells and dendritic cells (Table 2).

Table 2: shows some of the cells involved in inflammation, as well as their functions and structures.

Cells	Functions	cell structures
mast cells	Participate in the induction and/or propagation of certain inflammatory diseases, through selective release of mediators. Secrete numerous vasoactive and pro-inflammatory mediators such as histamine, serotonin, TNF, kinins and proteases stored in secretory granules **(Theoharides et al., 2012)**. In addition, a number of cytokines (e.g. IL-1, 2, 5, 6, 8, 9, 13, and TNF) and vascular endothelial growth factor (VEGF) are synthesized de novo and released several hours after stimulation **(Mukai et al., 2018)**. It has role in innate or acquired immunity, bacterial infections, as well as in autoimmunity. Superactivate T cells through TNF **(Theoharides et al., 2012)**.	
Macrophages cells	**M1:** macrophages are characterized by efficient producers of toxic effector molecules (ROS and NO) and inflammatory cytokines (IL-1β, TNF, IL-6); participate as inducers and effector cells in polarized Th1 responses. **M2:** repond to stimuli (IL-4 and IL-13; alternative inflammation) and (immune complexes, FcγR/TLR triggering), and (IL-	

	10, TGF-β, glucocorticoids; deactivation). Take part in polarized Th2 responses, allergy, parasites clearance, dampening of inflammation, tissue remodeling, angiogenesis, immunoregulation **(Italianiand Boraschi, 2014)**.	
Dendritic cells	It is a part of the innate immune system in to sense and respond to external pathogenic stimuli via PRRs such as TLRs, and secretion of type I interferons. Migration to specific destinations, and the release of cytokines and chemokines **(Szabo et al., 2018)**. This migration depends on CCR7 from peripheral tissues to lymphoid tissues for host defense against pathogens and immune tolerance of harmless self- or nonself-antigens **(Liu et al., 2021)**.	
Monocyte cells	Have the ability to differentiate into monocyte-derived macrophages, and thus It has the ability to phagocytosis and antigen presentation, innate response/immune responses and migration, secretion of cytokines, secretion of TNF-α, IL-1β, IL-6 and CCL3 upon TLR stimulation and regulation of apoptosis, differentiation **(Kapellos et al., 2019)**.	

Neutrophils cells	The role of neutrophils is phagocytosis, degranulation, and the release of nuclear material in the form of neutrophil extracellular traps (NETs). Respond to multiple signals by producing several cytokines and other inflammatory factors that influence and regulate inflammation and also the immune system such as IL-1α, IL-1β, IL-6, IL-10, and TNF-α **(Rosales, 2018)** **(Wright et al., 2010)**.	
Epithelial cells	Epithelial cells derived from airway, intestinal and ocular mucosal sites actively participate during inflammatory processes. They express adhesion and co-stimulatory molecules in response to different cytokines and/or chemokines, and they also secrete several cytokines/chemokines that contribute to inflammation. Can play roles as non-professional antigen presenting cells in the recruitment and activation of lymphoid cells **(Enríquez et al., 2008)**.	

II. 4. Types of inflammation

Inflammation can be divided into two categories according to the durating of the disease: acute and chronic inflammation.

II. 4. 1. Acute inflammation

Tissue damage due to trauma, microbial invasion, or noxious compounds can induce acute inflammation. It starts rapidly, becomes severe in a short time and symptoms may last for a few days for example cellulitis or acute pneumonia. Subacute inflammation is the period between acute and chronic inflammation and may last 2 to 6 weeks **(Pahwa et al., 2022)**.

The prototypical acute inflammatory response, characterized by local vasodilation, extravasation of leukocytes, and release of multiple plasma components, has been particularly well worked up in the field of invasion by microorganisms. Activation of an acute inflammatory response is a fundamental requirement to eradicate threats to the host organism such as bacterial or viral infections **(Feehan and Gilroy, 2019)** and these processes are mediated largely by the detection of so-called pathogen-associated molecular patterns (PAMPs) **(Erridge, 2008)**.

Initiation of inflammation, is mediated by resident immune cells via pathogen recognition receptors (PRRs) such as Toll-like receptors (TLRs), leading to the synthesis of soluble mediators such as proinflammatory cytokines, which activate downstream proinflammatory signalling **(Feehan and Gilroy, 2019)**.

II. 4. 2. Chronic inflammation

Chronic inflammation is a risk factor for a broad diseases such as hypertension, diabetes, atherosclerosis, and cancer **(Sanada et al., 2018)**.

Chronic inflammation is characterized by the simultaneous occurrence of destruction and healing of tissues **(Zhao et al., 2021)**.

The main infiltrating immune cells in chronic inflammation sites are macrophages and lymphocytes **(Moldoveanu et al., 2022)**. If the pro-inflammatory stimulus is not eliminated during the acute inflammation process **(Zhao et al., 2021)**, characterised by high plasma levels of numerous pro-inflammatory cytokines IL-1β, IL-6, TNF-α and CRP **(Rehman and Akach, 2016)**. It will lead to chronic inflammation, autoimmunity, tissue fibrosis, and necrosis. The persistence of inflammatory factors and damage to tissues are the key factors of chronic inflammation **(Zhao et al., 2021)**.

The underlying purpose of chronic inflammation is to clear necrotic debris produced during the acute inflammatory process, to provide defense against persistent infections, and to heal and repair the damage. Destruction of the normal tissue architecture results in scarring **(Thacker, 2006)**.

II. 5. C-reactive protein

II. 5. 1. Definition

C-reactive protein (CRP) is a homo-pentameric classical acute phase inflammatory protein. In 1930, it was initially discovered by Tillet and Francis during the investigation of the sera of patients suffering from the acute condition of Pneumococcus infection. It was the

named for its reaction capacity (for precipitation) with the bacterial cell wall somatic capsular (C)-polysaccharide of Streptococcus pneumoniae **(Tillet et al., 1930)**.

CRP is a highly sensitive marker of inflammation and tissue damage, and levels can rise to more than 500 mg/liter in a variety of acute or chronic inflammatory conditions **(Tall, 2004)**. It is a major acute phase protein whose concentration may increase more than 1,000-fold in severe inflammatory states **(Pathak and Agrawal, 2019)**.

CRP is synthesized primarily in liver hepatocytes but also by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes **(Sproston and Ashworth, 2018)**.

It is one of the most important proteins that is rapidly produced during an acute-phase response upon stimulation by IL-6, TNF-α, and IL-1-β originating at the site of inflammation or pathology **(Vermeire et al., 2004)**.

Nehring et al. (2017), reported that CRP has both pro-inflammatory and anti-inflammatory properties. It plays a role in the recognition and clearance of foreign pathogens and damaged cells by binding to phosphocholine, phospholipids, histone, chromatin, and fibronect.

II. 5. 2. Structure of C-reactive protein

CRP is a pentameric protein composed of five identical non-covalently bound subunits of 206 amino acid residues with a molecular weight of ~23 kDa. CRP binds to phosphocholine (PCh) in a Ca2+-dependent manner. There are five PCh-binding sites, one located on each subunit (Figure 08) **(Pathak and Agrawal, 2019)**. The calcium ions are important for the stability and binding of ligands **(Sproston and Ashworth, 2018)**.

Other ligands include histones, chromatin, and small nuclear ribonucleoproteins **(Vermeire et al., 2004)**.

Each protomer has been found by x-ray crystallography to be folded into two antiparallel sheets with a flattened jellyroll topology similar to that of lectins such as concanavalin A each protomer has a recognition face with a phosphocholine binding site consisting of two coordinated calcium ions adjacent to a hydrophobic pocket **(Thompson et al., 1999)**.

The loss of the pentameric structure of CRP results in modified or monomeric CRP (mCRP), which is a naturally occurring form of CRP and it is a tissue-based rather than a serum

based molecule. mCRP is less soluble than CRP and tends to aggregate (Figure 09) **(Shrivastava et al., 2015)**.

Pentameric C-reactive protein (pCRP) can undergo protomer dissociation into mCRP in the absence of Ca2+ or upon binding to lysophosphatidylcholine monolayers or altered cell membranes. Or on the surface of activated platelets and apoptotic monocytic THP-1 cells **(Wu et al., 2015)**.

II. 5. 3. The role of C-reactive protein

The main biologic function of CRP is determined by its ability to recognize pathogens and damaged cells of the host and to mediate their elimination by recruiting the complement system and phagocytic cells **(Volanakis, 2001)**.

It is directly amplifies and facilitates innate immunity, it increases LDL uptake into macrophages and enhances the ability of macrophages to form foam cells. It also binds the phosphocholine of oxidized LDL. CRP activates macrophages to secrete tissue factor, a powerful procoagulant, which can lead to disseminated intravascular coagulation and ultimately to thrombosis during inflammatory states **(Shrivastavaet al., 2015)**.

CRP upregulates the expression of adhesion molecules in endothelial cells (Ecs) that can attract monocytes to the site of injury **(Pfutzner et al., 2010)**.

Davis et al. (2012), reported that CRP increases PAI-1 expression and activity. PAI-1 is a protease inhibitor that regulates fibrinolysis by inhibiting tissue plasminogen activator. Increased PAI-1 indicates lowered fibrinolysis and thus leads to atherogenesis.

CRP also indirectly affects specific immune response, during atherogenesis, through the increase of IL-12 production from macrophages, with the subsequent induction of CD4 + T lymphocytes differentiation and Interferon gamma production **(Calabro et al., 2012)**.

II. 5. 4. Methods for measuring C-reactive protein

Initially, high-sensitivity quantification methods were based on ELISA, utilized in several population studies despite its cumbersome routine use in clinical laboratories. As a consequence, more accessible methods, such as immunonephelometric techniques and, more recently, automatized immunoluminometry and immunoturbidimetry, have been implemented, improving the sensitivity of the quantification even in cases of very low concentrations. Additionally, these are inexpensive techniques, an important aspect regarding its routine use in clinical practice **(Salazar et al., 2014)**.

II. 5. 5. C-reactive protein and inflammation

The main role of CRP in inflammation tends to focus around the activation of the C1q molecule in the complement pathway leading to the opsonization of pathogens **(Sproston and Ashworth, 2018)**. It can also initiate cell-mediated pathways by activating complement as well as to binding to Fc receptors of IgG (Figure 10) **(Pradhan et al., 2001)**.

Pentameric C-reactive protein (pCRP) induces the upregulation of cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin via NF-κB upregulation **(Thiele et al., 2015)**.

CRP binds to Fc receptors with the resulting interaction leading to the release of proinflammatory cytokines **(Du, 2000)**.

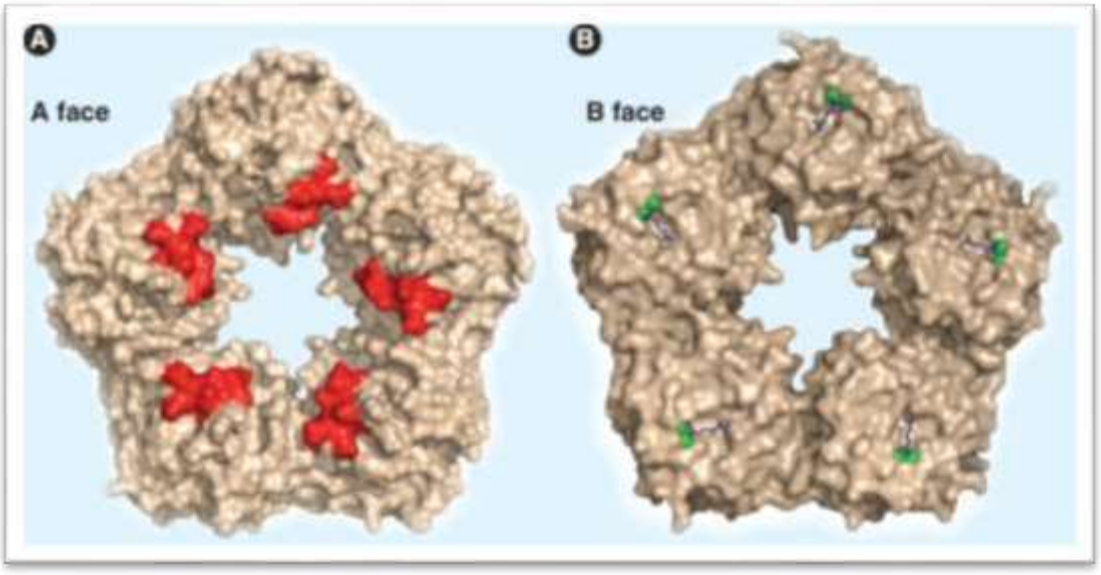


Figure 08: pentameric structure of C-reactive protein (CRP). (A) Space - filling model of the A face (receptor and C1q binding) of the CRP pentamer with the ridge helix in red. (B) Spacefilling model of the CRP molecule showing a phosphocholine molecule (blue) with the two calcium molecules (green) located in the binding site of each protomer on the B face (ligand - binding face) **(Peisajovich et al., 2008)**.

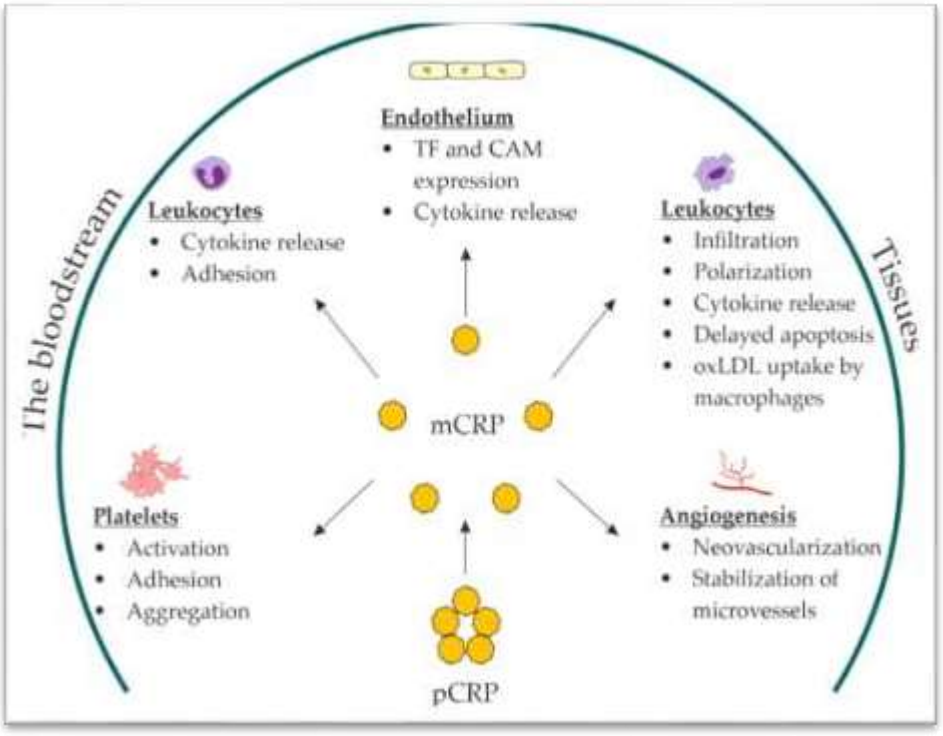


Figure 09: proposed roles for mCRP in atherosclerosis. mCRP, monomeric C-reactive protein; pCRP: pentameric C-reactive protein. TF: tissue factor. CAM: cell-adhesion molecules. oxLDL: oxidized low-density lipoproteins **(Melnikov et al., 2023)**.

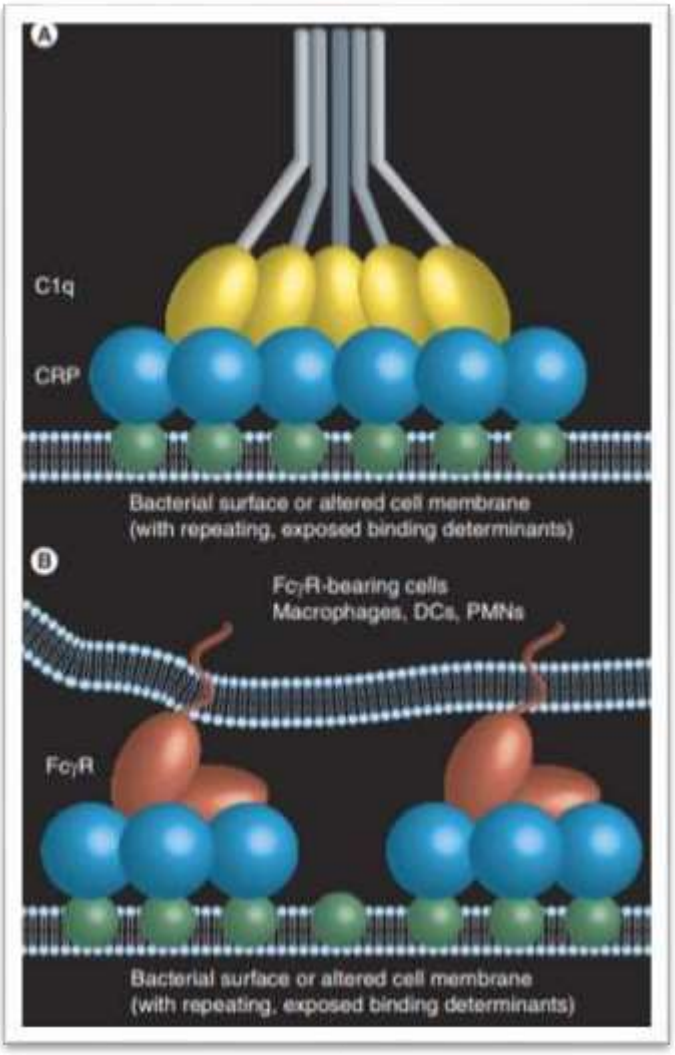


Figure 10: CRP bound to bacterial or altered cell surfaces. CRP binds to a surface on its B-face phosphocholine-binding site, leaving the A face exposed. This allows each pentamer to bind either (A) one of the six globular heads of C1q leading to the activation of the classical complement cascade or; (B) FcγR on the surface of macrophages, DCs or PMNs. The type of FcγR helps to determine the downstream effect of this binding. CRP: C-reactive protein; DC: Dendritic cell; FcγR: Fcy receptor; PMN: Polymorphonuclear cell (neutrophil) **(Peisajovich et al., 2008)**

Chapter II: sugar and liver inflammation

Chapter II: Sugar and liver inflammation

II. 1. Anatomy of liver

Definition

The liver is about 2% of body weight in the adult, which amounts to approximately 1400 g in females and 1800 g in males (Figure 11) **(Sibulesky, 2013).**

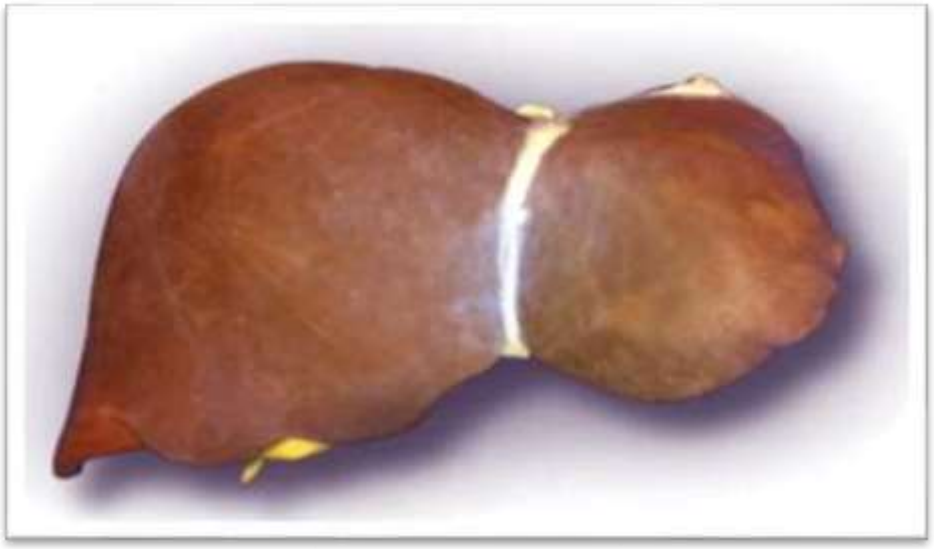


Figure 11: normal liver **(Koyama and Brenner, 2017)**.

The liver is a fascinating organ that possesses many unusual features, both anatomical and function **(Mahadevan, 2020)**.

The liver is found inferior to the diaphragm and occupies the majority of the right upper quadrant (RUQ) of the abdomen (Figure 12) (**Vernon et al., 2022)**.

Its domed upper surface relates entirely to the diaphragm while its postero-inferior, or visceral, surface rests against the abdominal oesophagus, stomach, upper duodenum, hepatic flexure of the colon, right kidney and suprarenal gland, as well as carrying the gall bladder (Figure 13) (Figure 14) **(Ellis, 2011)**.

The liver is composed of several cell types that not only interact with each other but also are adapted to perform specific functions. The principal cell type is the hepatic parenchymal cell, generally referred to as the hepatocyte, which accounts for 60% of the total cell population and 80% of the volume of the organ (Figure 14) **(Abdel-Misih and Blomston, 2010)**.

The liver receives its blood supply from two sources: 80% is delivered by the portal vein, which drains the spleen and intestines: the remaining 20%, the oxygenated blood, is delivered by the hepatic artery (Figure 15) **(Sibulesky, 2013**).

The liver is completely covered by visceral peritoneum, with the exception of the bare area, which is where the liver is in contact with the diaphragm **(Sieroslawska, 2022**).

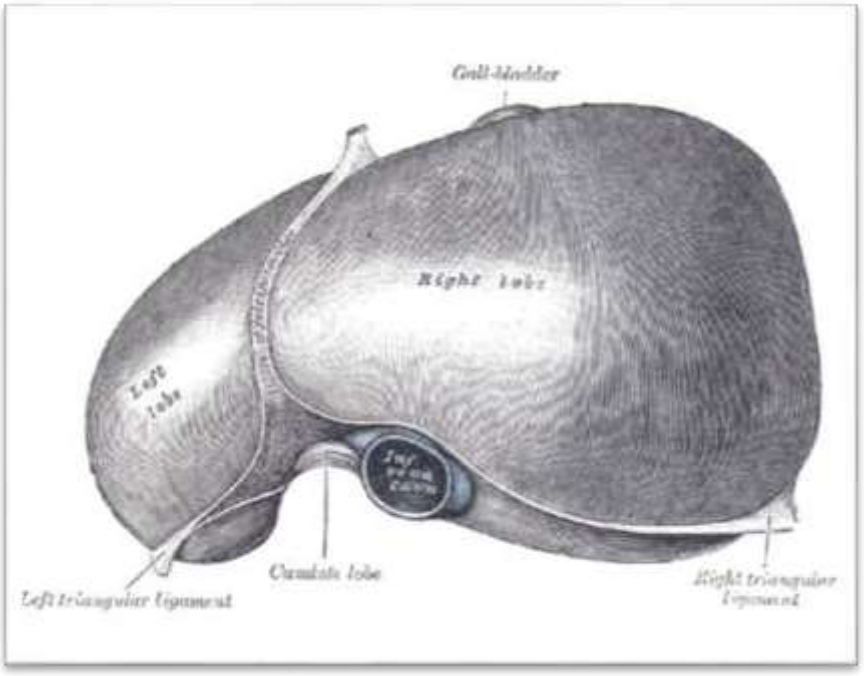


Figure 12: superior surface of the liver **(Sibulesky, 2013**).

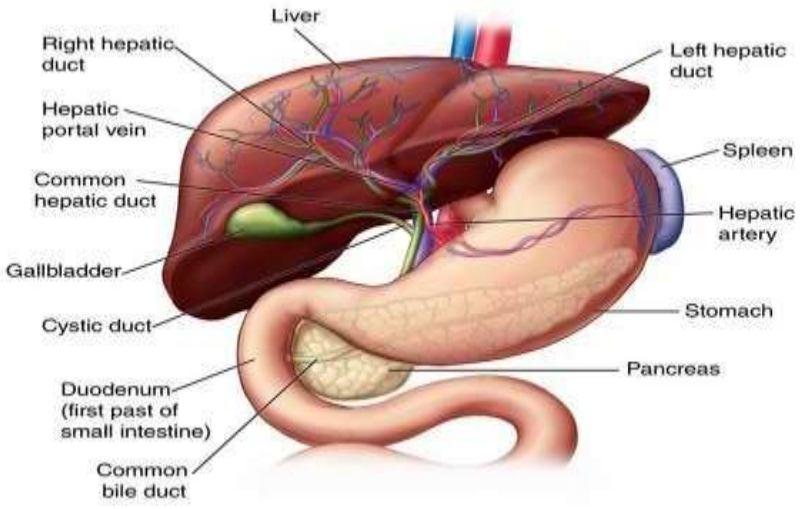


Figure 13: anatomy of the liver (site web 8).

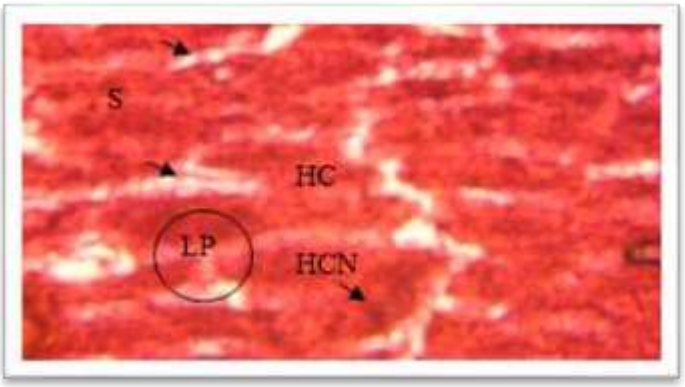


Figure 14: histological section of normal liver.

Heamtoxylin eosin (x100). S: sinusoid, LP: liver parenchyma, HCN: hepatocyte nucleus, HC: hepatocyte **(Benmebarek et al., 2013)**.

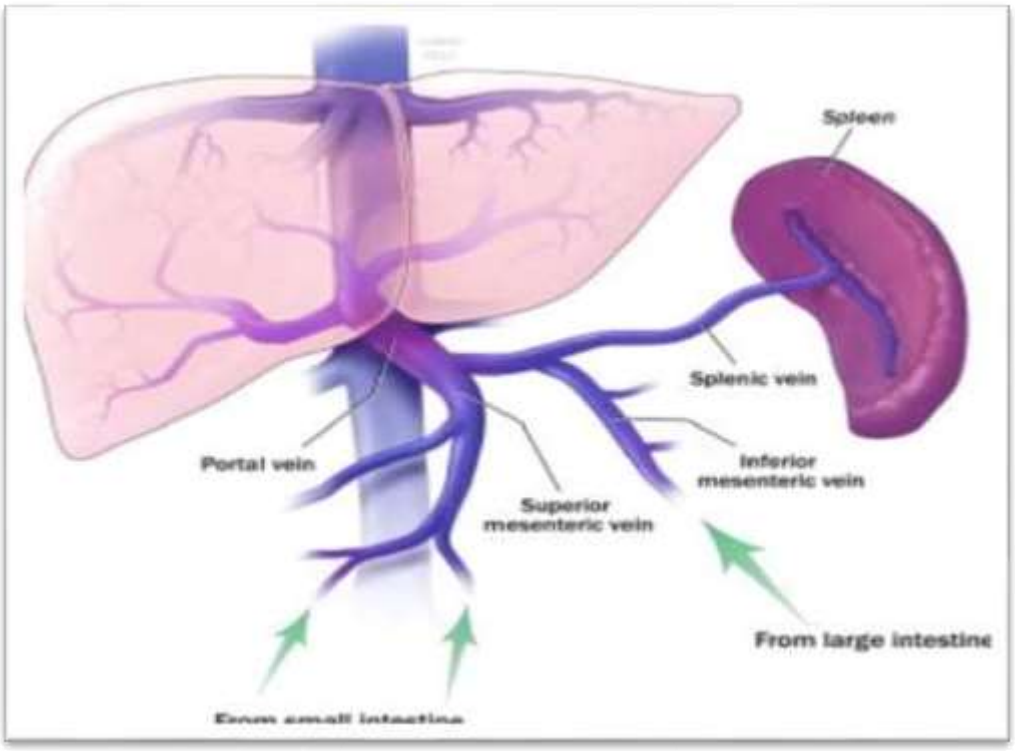


Figure 15: portal venous drainage **(Sibulesky, 2013**).

II. 2. Function of liver

The liver has a number of important functions in innate and adaptive immunity **(Parkerand Picut, 2005)**.

The liver is a major handler of protein and amino acid metabolism as it is responsible for the majority of proteins secreted in the blood, the processing of amino acids for energy, and disposal of nitrogenous waste from protein degradation in the form of urea **(Elijah et al., 2017)**.

II. 3. Liver inflammation

Inflammation is one of the most characteristic features of chronic liver disease of viral, alcoholic, fatty, and autoimmune origin. Inflammation is typically present in all disease stages and associated with the development of fibrosis, cirrhosis, and hepatocellular carcinoma **(Seki and Schwabe, 2014)**.

Autoimmune hepatitis (AIH) is a severe liver disease that arises in genetically predisposed male and female individuals worldwide **(Sucher et al., 2019)**. Viral hepatitis is the most common form (**Lewis, 2022)**.

In both acute and chronic inflammation, a variety of immune and non-immune cells in the liver is involved in the processes resulting in either regeneration or fibrosis **(Tanaka and Miyajima, 2016)**.

II. 4. Symptoms of liver inflammation

Symptoms of an inflamed liver can include: Feelings of fatigue, nausea, vomiting and pain in the abdomen **(Robinson, 2022)**.

II. 5. Cause of liver inflammation

- Hepatocyte steatosis is a component of metabolic syndrome and insulin resistance (**Koyama and Brenner, 2017)**.
- The most common cause for chronic hepatic inflammation in humans is infection with HBV or HCV. The spread of HBV and HCV has resulted in approximately 500 million people with persistent virus infections, some of which will lead to liver cancer causing the strong rise in hepatocellular carcinoma (HCC) incidence **(Boege et al., 2011)**.
- Alcohol use disorder, including fatty liver disease and alcoholic cirrhosis (**Lewis, 2022)**.
- Decreased blood flow to the liver **(Lewis, 2022)**.
- Hemochromatosis, a disorder of excess iron in the body **(Lewis, 2022)**.
- Hepatic steatosis may be benign or progress to hepatocyte injury and the initiation of inflammation, which activates immune cells (Figure 16) **(Koyama and Brenner, 2017)**.

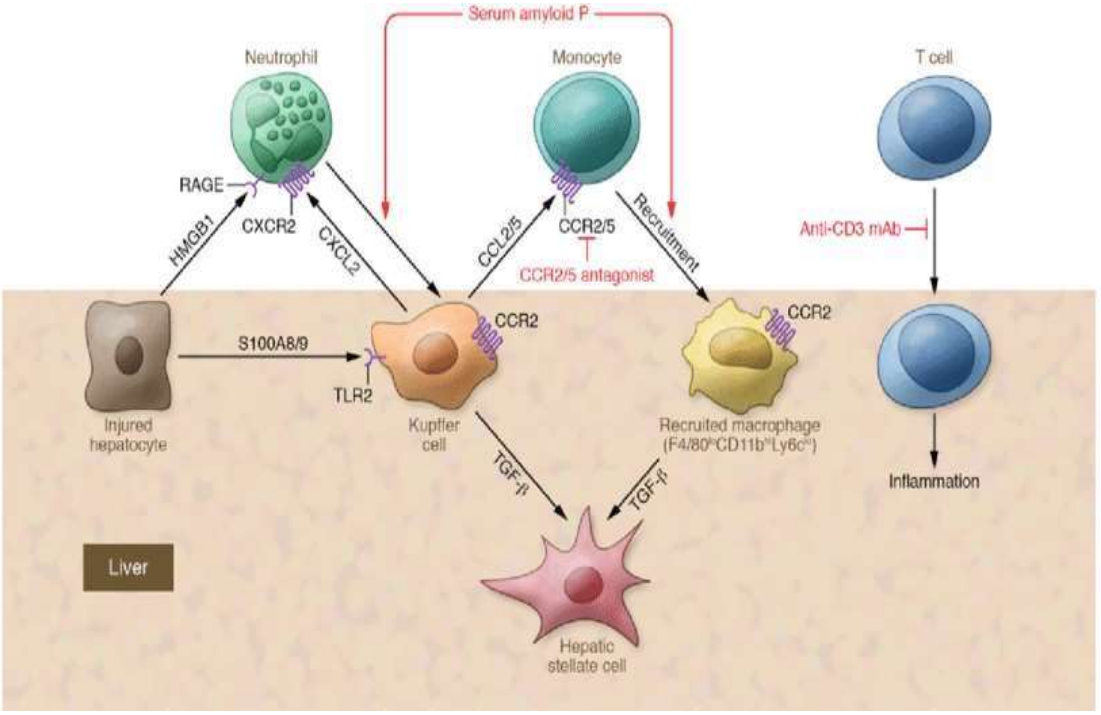


Figure 16: interaction of immune cells and the liver (**Koyama and Brenner, 2017)**.

The creation of a CXCL12-like intravascular chemokine gradient promotes neutrophil migration to areas of injury. Necrotic hepatocytes release HMGB1 to recruit neutrophils.CCR2 and its ligand CCL2 regulate monocyte infiltration into the liver. Serum amyloid P inhibits profibrotic macrophages, reduces neutrophil adherence, stimulates complement system, promoting phagocytosis of cell debris **(Koyoma and Brenner, 2017)**.

Chapter III: hot water and traditional yeast

Chapter III: Hot water and traditional yeast

III. 1. Hot water

III. 1. 1. Definition

Water is an essential compound for the existence of life as we know it **(Mottl et al., 2007)**.

It is the most important constituent of all living organisms (70% of the total mass and 99% of all molecules) **(Giudice et al., 2009)**.

It is of fundamental importance for human life and plays an important role in many biological and chemical systems **(Ludwig, 2001)**.

Water is the principal chemical constituent of the human body. Total body water represents 50% to 70% of body weight. Variability in total body water is primarily due to differences in body composition. Lean body mass is about 73% water and fat body mass is 10% water. Total body water is distributed into intracellular fluid and extracellular fluid compartments, which contain about 65% and 35% of total body water, respectively **(Sawka et al., 2005)**.

It is the medium in which all life occurs. Biological molecules are bathed in it, and the interactions of proteins, nucleic acids and membranes with water shape their structures and functions **(Raschke, 2006)**.

III.1.2. Structure

Water molecules are V-shaped with molecular formula H2O. Which is symmetric (point group C2) with two mirror planes of symmetry and a two-fold rotation axis. The hydrogen atoms may possess parallel or antiparallel nuclear spin (Figure 17) **(Xiao, 2014)**.

The structure is affected by the temperature and the external pressure. A rise in the temperature of a liquid causes its expansion and a decrease of its density, whereas rising pressures above ambient cause an increase in the density (Figure 18) **(Marcus, 2009)**.

Other basic properties of water are its size, shape and polarity. Water is a very polar molecule with the ability to make strong electrostatic interactions with itself, other molecules and ions **(Sharp, 2001)**.

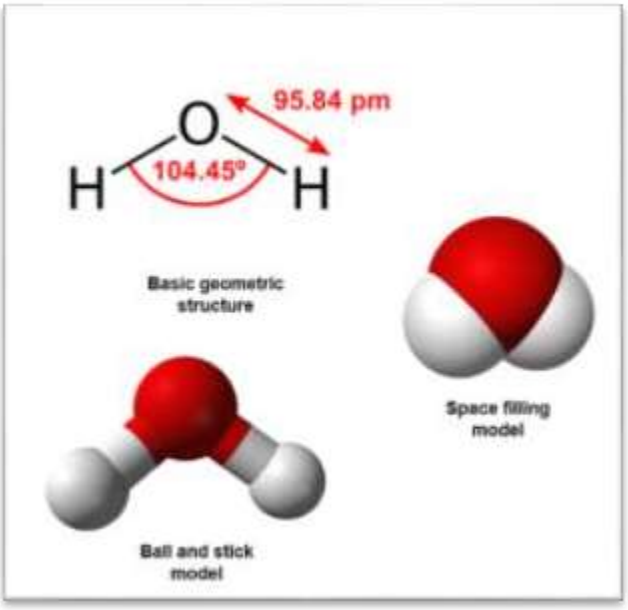


Figure 17: water molecules (site web 9).

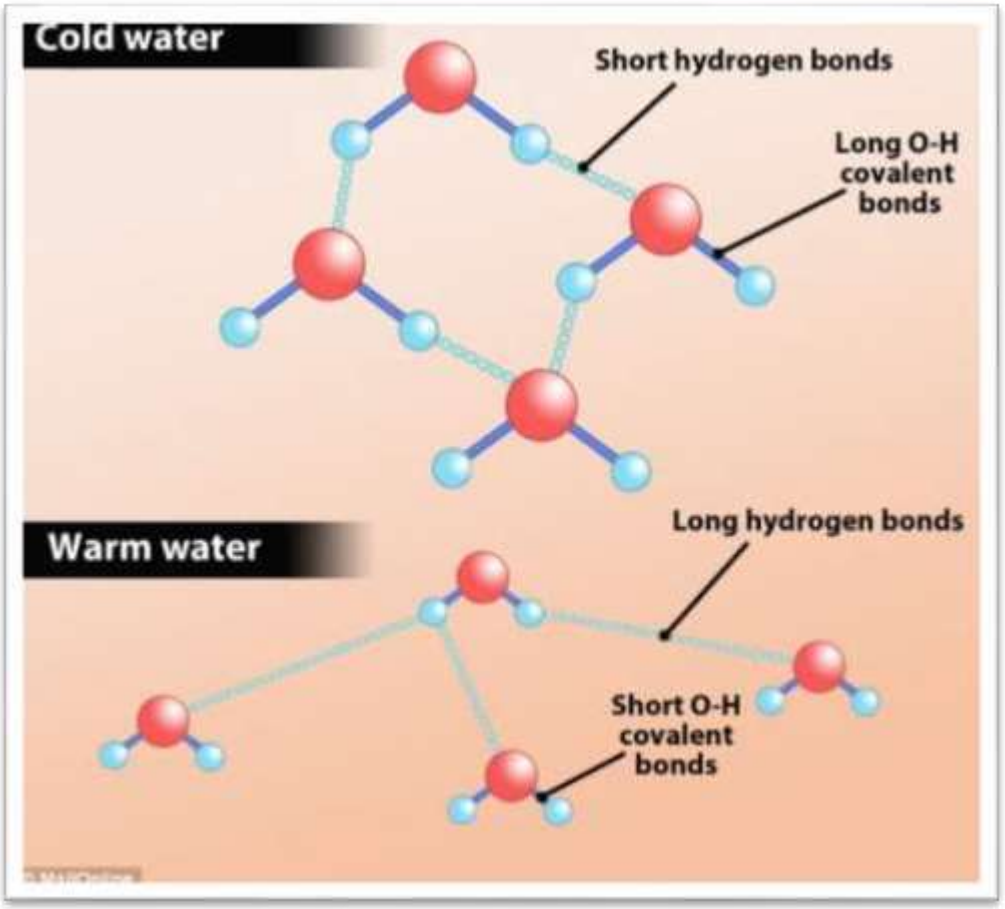


Figure 18: cold and warm water structure (site web 10).

3. Hydrotherapy

During the 1800s, Sebastian Kneipp, the 'founder of hydrotherapy', wrote extensively about the healing effects of water. His research was immediately recognized by healthcare professionals **(Hall et al., 2008)**.

Hydrotherapy is derived from the Greek word "Hydro" meaning water, and "Therapia" meaning healing. It means water healing. It is controlled aquatic exercise in heated sanitized water. Any treatment in water **(Bahadorfar, 2014)**.

Hydrotherapy is the external or internal use of water in any of its forms (water, ice, steam) for health promotion or treatment of various diseases with various temperatures, pressure, duration, and site **(Mooventhan and Nivethitha, 2014)**.

Three types of hydrotherapy can be used: neutrotherapy, thermotherapy and cryotherapy the difference being the varying temperature of the water **(Devkate et al., 2016)**.

These health benefits come from the mechanical and thermal effects of water interacting with the body. It includes the use of physical water properties, specifically temperature and pressure, and sometimes the delivery of minerals or herbal treatments to manipulate the body's flow of blood, the endocrine system and associated neural systems in order to treat the symptoms of certain diseases **(Bahadorfar, 2014)**.

Table 03: techniques and uses of hydrotherapy **(Chowdhury et al., 2021)**.

Techniques of hydrotherapy	Kneipp system, hydro-massage, colon hydrotherapy, baths and showers, cold foot bath, heating compress, hip bath, steam bath, steam inhalation, and full immersion bath.
Uses of hydrotherapy	For pain relief, for circulation, for immunity, stress, complexion, arthritis, back, pain, hydrotherapy for acne, insomnia, joint pain, headaches, colonic hydrotherapy for stomach problems, sciatica, hydrotherapy for sleep disorders, labor, temperature regulation.

III. 1. 4. Benefits of drinking hot water

Drinking hot water leads to healthier digestion and consuming it daily leads to help in body detoxification and helps in improving blood circulation , prevents from ageing and clears skin and prevents premature ageing like wrinkles **(Subaraman et al., 2020)**.

It can relieve pain for patients with various conditions **(Bender et al., 2005)**. It can also improve their sensory perception by blocking the nociception signals **(Yamazaki et al.,2000)**. Additionally, it can help nourish the body and reduce the effects of lactic acid and other chemicals in the body **(Fam, 1991)**.

Prevent various diseases, symptoms and allergies and improve brain memory **(Alhadjri, 2010)**.

Consumption of hot beverages stimulates the overall physiological process faster than normal rate. It will be beneficial to patients who have arthritis **(Subaraman et al., 2020)**.

III. 2. Traditional yeast

III. 2. 1. Definition

Sourdough" is one of the oldest forms of cereal fermentation utilized primarily for baking purposes and it has been proven to be perfect for upgrading the shelf life, texture, palatability, and nutritional values of wheat and rye breads. Its main function is to leaven the dough to produce more aerated bread **(Behera and Ray, 2015)**.

It is dough that tastes sour due to the high levels of acids produced intentionally or unintentionally by microorganisms or by the addition of acid. The bread produced from such dough is called sour bread **(Amr and Alkhamaiseh, 2022)**.

Yeasts are responsible for the aroma and flavor of many fermented foods and beverages such as bread, soy sauce, cheeses, beer, wine, and sake **(Punyauppa-path et al., 2022)**.

Yeasts are unicellular fungi with a typical vegetative growth by budding or fission **(De et al., 2016)**.

Yeasts species

The most widespread yeast species in sourdough are "*Saccharomyces cerevisiae*, *kazachstania humilis* (previously named *Candida humilis*), *kazachstania exigua*, *pichia kudriavzevii*, and *torulaspora delbrueckii*" **(Carbonetto et al., 2020)**.

III. 2. 2. Types of sourdough starter

The term wild yeasts refer to strain coming from the direct environment, three types of sourdough starter can be distinguished which influence the microbiology of the mature sourdough (Figure 19) **(De et al., 2021)**.

The utilization of different types of flour during fermentation of sourdough also leads to the diversity of wild yeasts species **(Lau et al., 2021)**.

Type I *sourdough starters* are most commonly used in artisanal bakeries and are usually kept at ambient temperature (20–30 °C), though they can be refrigerated when not in use or at regular intervals **(Calvert et al., 2021).**

3. Formulation yeasts

Sourdough starter can be considered as a mixture of water and flour fermented by yeasts and bacteria (Figure 19) **(Albagli et al., 2023)**.

Wild yeasts consume the carbohydrates in the flour and produces alcohol and carbon dioxide gas as the primary by products (bubbles) **(Bunning et al., 2022)**.

In sourdough fermentation, yeast and lactic acid bacteria work together to form the natural flora **(Kezer et al., 2022)**.

Sourdough yeasts fermented the flour sacharides (maltose, sucrose, glucose, and fructose) via the embden-meyerhof-parnas (EMP) pathway into pyruvate, thereby generating both ATP and reducing power (NADH+ H+), and further convert pyruvate into ethanol and carbon dioxide (alcoholic fermentation), thereby regenerating the cofactor NAD+ consumed in the upper part of the EMP pathway (Figure 20) **(De et al., 2021)**.

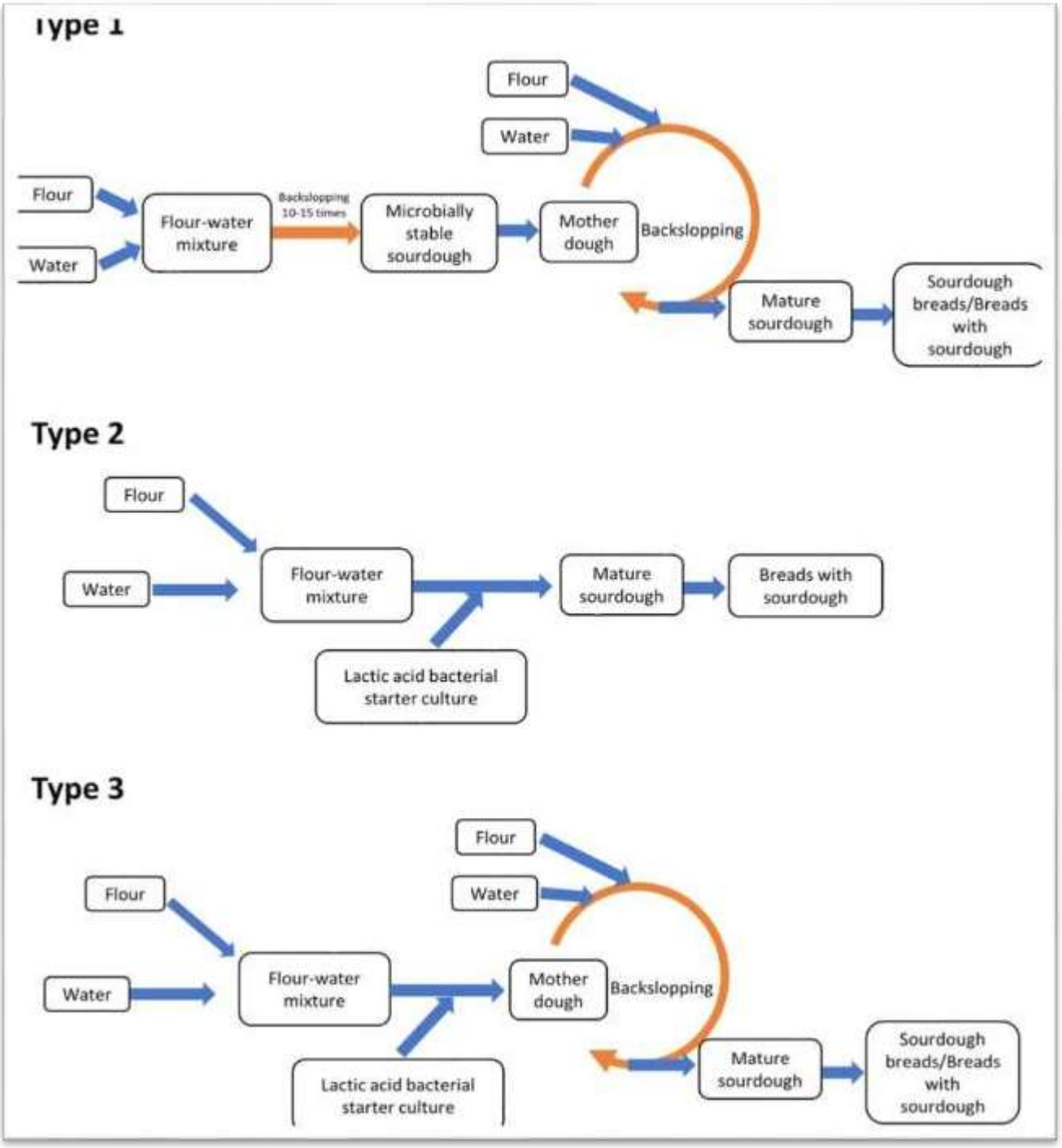


Figure 19: types of sourdough starter: various types of sourdough production processes based on the way of inoculation of the flour-water mixture **(De et al., 2021)**.

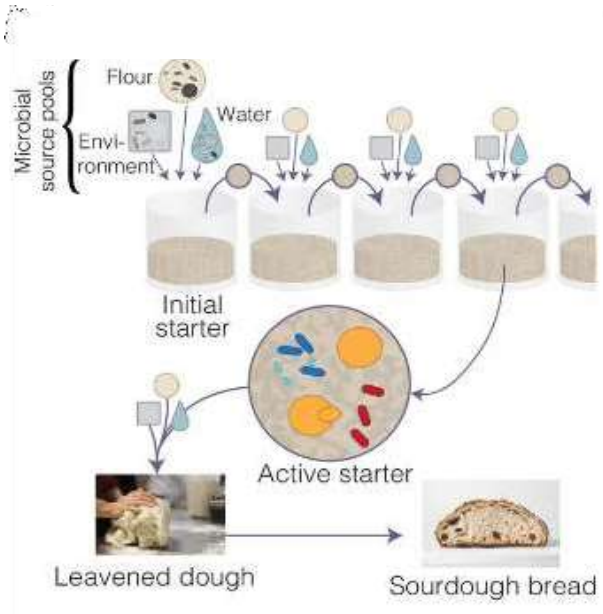


Figure 20: sourdough starters in bread making **(Landis et al., 2021)**.

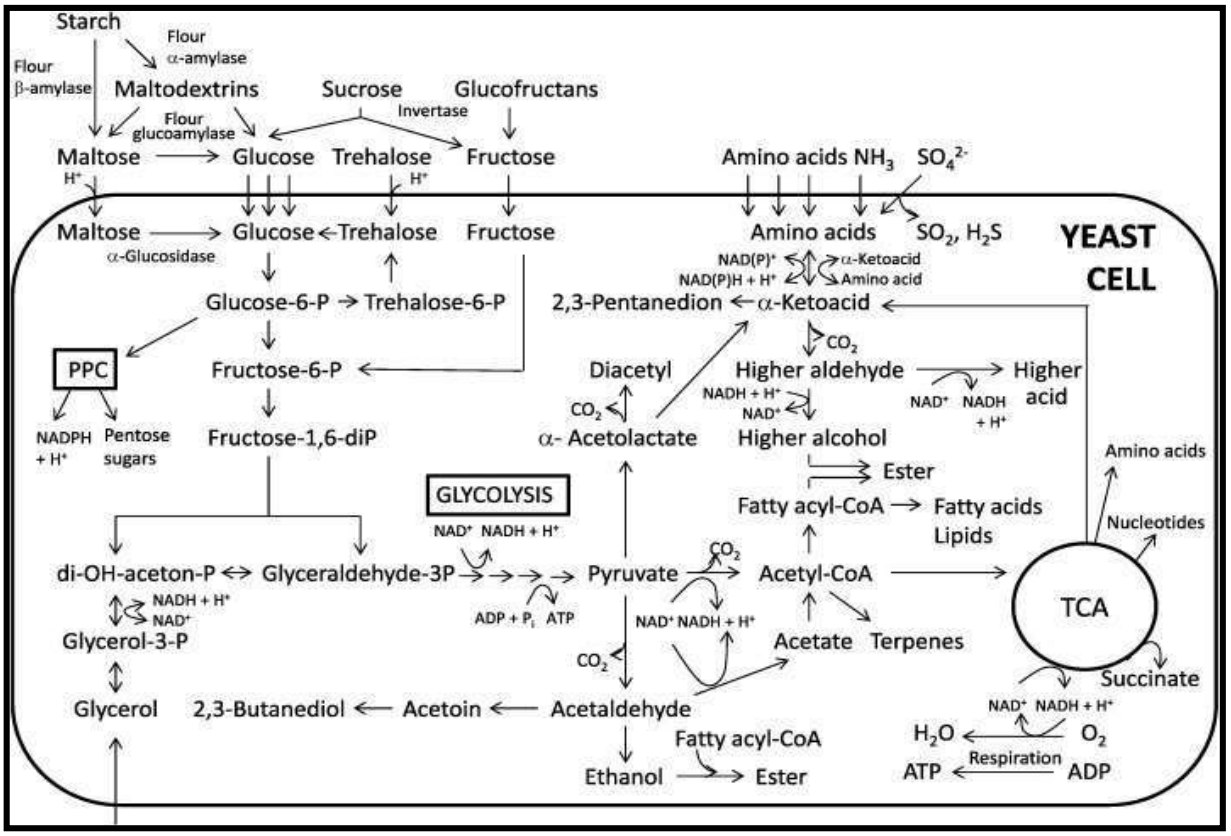


Figure 21: overview of the metabolism of yeasts in a sourdough matrix **(De et al., 2021)**.

III.2. 4. Benefits of traditional yeast

Excellent source of vitamins, minerals, and high-quality protein, it support the immune system and reduce inflammation resulting from bacterial infection. It may also be helpful in treating diarrhea **(Marengo, 2023)**.

Good for your gut: The fermentation process for sourdough bread can lead to an increased number of prebiotic- and probiotic-like properties, which help improve gut health **(Ball, 2022)**.

Can lead to better digestion: even though sourdough bread is not gluten-free, a 2021 review in the journal Foods found that sourdough consumption might help improve the digestion of gluten. The fermentation process for sourdough alters the enzymes in the wheat and might potentially help counteract adverse reactions to gluten **(Ball, 2022)**.

It may promote weight loss: the high prebiotic profile in sourdough improves digestion and absorption of nutrients, which promotes glucose regulation and metabolism, causing one to burn fat and lose weight **(Blackwood, 2022)**.

Good for nerve function: dry yeast has thiamine, riboflavin, vitamin B6 and folate, which promote nerve health **(Marengo, 2023)**.

Promotes healthy aging: whole grains and bread, like sourdough bread, are a staple of the mediterranean diet. Some research has pointed out it could also be a crucial food to help promote healthy aging. A 2019 review in the journal Nutrients found that fermented grain-based products, like sourdough, have antioxidant, anti-hypertensive, anti-diabetic and FODMAP-reducing qualities **(Ball, 2022)**.

Provides vital nutrients during pregnancy: dry yeast is an excellent source of folate. Folate can help reduce the risk of birth defects and optimize foetal growth and development **(Marengo, 2013)**.

Material and methods

IV. Material and methods

IV. 1. Materials

IV. 1. 1. Chemical products

Chemical products used in our study are:

Chloroform, NaCl 0.9%, formalin 10%, dithiobis-2-nitrobenzoic acid (DTNB), sulfo-salicylic acid (0.01M), Bovine Serum Albumin (BSA), orthophosphoric acid (85%), Tris Ethylene Di-amine Tetra Acetic acid (EDTA, 0.02M), Tris buffered saline (TBS), different concentrations of ethanol (60%, 70%, 95% and 96%), HCl, NaOH, NaCl, butanol, xylene, paraffin and glycerin, acetic acid, hematoxylin eosin, NaH2PO4, Na2HPO4, Coomassie Brilliant Blue G-250 , Tris –EDTA solution.

IV. 1. 2. Equipments

Precision weighing balances (readability 0.01g) to determine the weight of the mice, precision weighing balances (readability 0.0001g) to determine the quantity of sugar and yeast, , heating magnetic stirrer, pH meter, centrifuge, spectrophotometer, oven, microtome, vortex mixer, eppendorf tubes, paraffin molds, tissue cassettes, lithium heparin tubes, small bottles (Figure 22).

Figure 22: materials used during experimental study.

IV. 1. 3. Choice of treatment

In this research we have used hot water at 50°C proposed by ALHAJRI (2010) and ALHAJRI (2020). The concentrations of crystallize sugar of (200g/65kg) and yeast (50g/65kg) were used in this study.

IV. 1. 4. Animals

In this study we have used 36 mice Albino *Mus musculus* obtained from the animal house at University frères Mentouri-Constantine1 (Algeria).

IV. 2. Methods

IV. 2. 1. Treatment of mice

The study was carried out on a group of 36 adult male Albino *Mus Musculus* mice, aged between 2 to 3 months and weighing between 28-41g. After obtaining the animals, they were separated and housed in plastic cages covered with wire mesh coated with anti-rust paint, with a layer of sawdust placed at the bottom of each cage, and replenished daily. They were placed under standard laboratory conditions of temperature, humidity, and light and free access to water and diet. Animals were acclimated to laboratory conditions for a week prior to the experiment.

Animals were divided into six groups of similar mean body weights and fed for 21 days with control and experimental diet as shown down:

Group control (C, G1): was fed with standard diet and drunk water at room temperature.

Group sugar (S, G2): was fed with standard diet rich in sugar and drunk water at room temperature.

Group hot water (HW, G3): was fed standard diet and drunk hot water at fifty degrees.

Group yeast (TY, G4): was fed by standard diet rich with traditional yeast and drunk water at room temperature.

Group sugar + hot water (S+HW, G5): was fed on standard diet rich with traditional yeast and drunk hot water at fifty degrees.

Group sugar + yeast (S+TY, G6): group was fed on standard diet rich in sugar with traditional yeast and drinking water at room temperature (Table 04). The diet and weight were measured every day at the same time during 21days of treatment.

IV. 2. 2. Blood and tissue sampling

After 21 days of treatment, blood samples were collected after fasting the animals from retro orbital plexus into heparin tubes by using glass capillaries and the blood was taken directly to the analysis laboratory (EL AMINE laboratory Constantine Algeria).

After the blood samples collection, the animals were sacrificed. Then, the liver removed and rinsed with saline solution (0.9%), and fixed in formalin 10%, and the rest of liver are stored in the freezer without rinsing them with a saline solution at-20°c for the dosage of the antioxidant (GSH).

Table 04: treatment of mice for 21 day.

Experiment al group	Treatment	Number of animals	Duration of experiment	Daily dose
G 1 (C)	Normal water Standard diet	6	21	125ml / day 120 g / day
G 2 (S)	Normal water Standard diet+ Sugar	6	21	125ml / day 120 g / day 200g/65kg/ day
G 3 (HW)	Hot water Standard diet	6	21	125ml / day 120 g / day
G 4 (TY)	Normal water Standard diet + Yeast	6	21	125ml / day 120 g / day 50g/65kg/ day

G 5 (S+HW)	Hot water Standard diet Sugar	6	21	125ml / day 120 g / day 200g/65kg/ day
G 6 (TY+S)	Normal water Standard diet+ Sugar Yeast	6	21	125ml / day 120 g / day 200g/65kg/ day 50g/65kg/ day

IV. 2. 3. Biochemical investigation

A- The blood analysis

The blood analysis was carried on some parameters such as blood sugar, creatinine, T-ch, TG, HDL-C, LDL-C, CRP, ASAT and ALAT.

B- Determination of antioxidant

1- Tissue homogenate preparation

0,5g of the liver was homogenized in 2ml of TBS (Tris 50 mM, NaCl 150 mM, pH 7.4). The homogenates were centrifuged at 9000 g for15 min at 4˚C. The supernatant was keeped in the freezer at -20C until the determination of protein and reduced glutathione concentrations.

2- Reduced glutathione measurement

Liver homogenate sample (0.8ml) was deproteinized with (0.2ml) of 5-sulfosalicylic acid solution (0.25%) and was allowed stand on ice for 10 min. Following centrifugation at 1000 tours/mn) during 5minutes to remove the precipitated protein. (0.5ml) of supernatant was mixed with 1 ml Tris/EDTA buffer (pH 9.6) and (0.025 ml) of DTNB-reagent (0.01M 5, 5'dithiobis-2- nitrobenzoic acid) and left at room temperature for 5 min. Then the absorption was measured at 412 nm using a spectrophotometer (SHIMADZU UV-1280) against the blank reaction (Figure 32).

3- Protein determination

We have measured the protein concentration by the method of Bradford (1976), using bovine serum albumin as a standard. Where the absorbance is proportional to the protein present in the solution.

A 0.1 mL liver homogenate sample was mixed with 5 mL of Bradford's reagent and left for 5 min. Then the absorbance was measured at 595 nm using a spectrophotometer in comparison with blank reaction.

The protein concentration in the test sample is determined from the calibration graph (Figure 23) (Table 07).

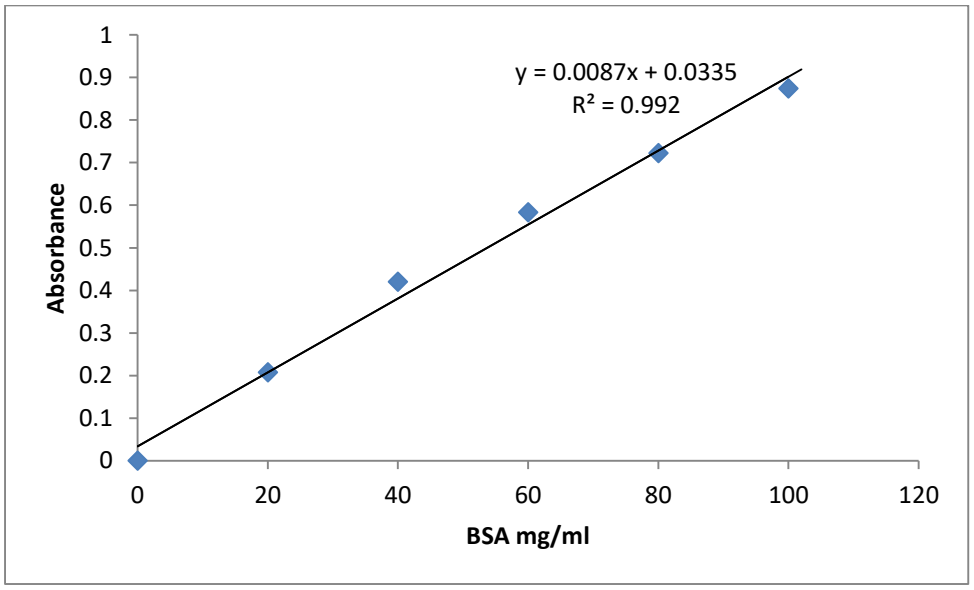


Figure 23: calibration graph of bovine serum albumin.

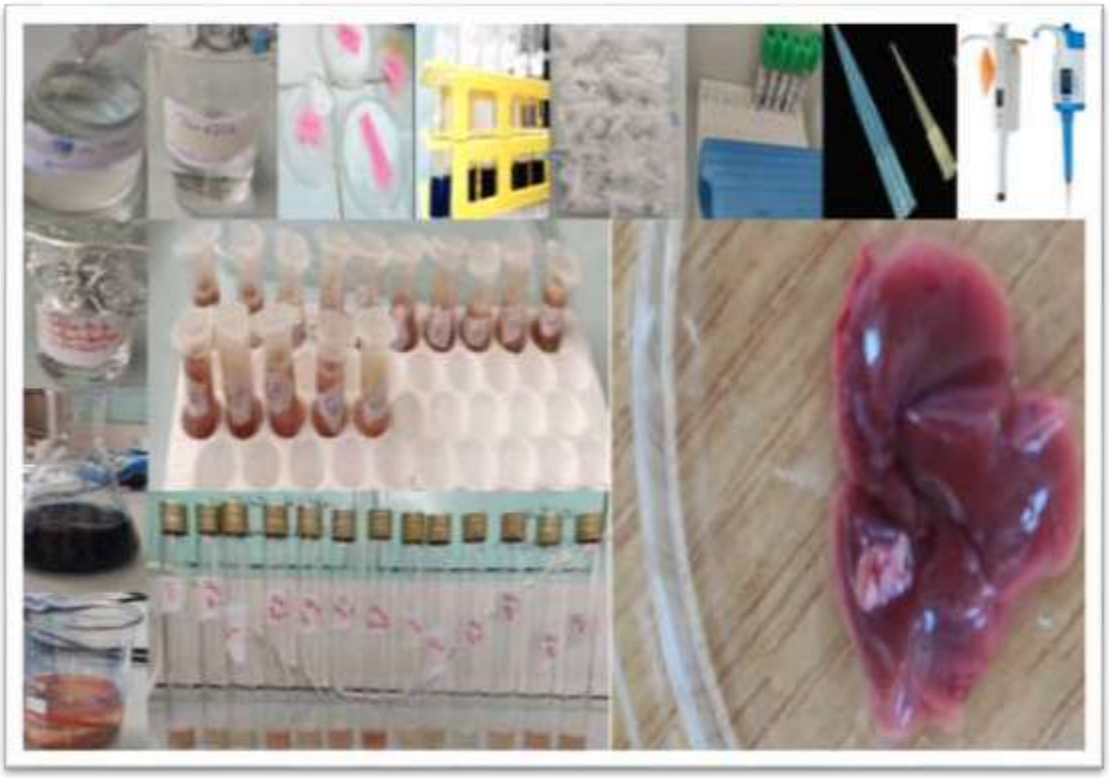


Figure 24: materials and solutions used in protein and reduced glutathione determinations

IV 2. 4. Preparation of histological sections

Fixation

The liver was fixed in the formol 10% solution as shown before.

Dehydration

Dehydration was performed through a series of ethanol solution baths:

- First bath: 60% ethanol (3 x 20 minutes)
- Second bath: 75% ethanol (3 x 20 minutes)
- Third bath: 96% ethanol (3 x 20 minutes)

Samples were kept in small bottles with butanol for 3 days. Then they were cleared in xylene for 10 min with two exchanges.

Insertion into paraffin

In the next step, the organs were immersed in paraffin at 60°C for 2 hours in two exchanges then were placed into paraffin molds and into tissue cassettes. After that the cut tissue was made with a thickness of 5 µm using a microtome.

Colouring stage

We placed the samples in two xylene baths for 10 minutes each.

Samples were placed in ethanol baths with decreasing concentrations:

- First bath: 96% ethanol 5 min
- Second bath: 75% ethanol 5 min
- Third bath: 60% ethanol for 5 minutes

The samples were placed in hematoxylin for 4 minutes, and then washed with tap water. After that the sample coloured with eosin for 5minutes, after this time the samples were washed with tape water.

The samples immersed in ethanol for 1 minute, then placed in two xylene baths for 2 minutes each.

Samples are placed on the heating plate at 37°C. After this stage, the samples are ready for viewing under a microscope.

IV. 2. 5. Statistical analysis

The values obtained were expressed as mean ± SEM and subjected to statistical analysis using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test (PRISM 5). $P < 0.05$ values were considered as significant different.

Results and discussion

V. Result and discussion

V. 1. Results

V. 1. 1. Weight and food consumed

Weight

Our results demonstrated that the weight is increased during the first week (33.06g±1.61) and the third week (36.80g±0.99) respectively in the group control, in the group of mice treated with hot water in the first week was (33.01g±0.71) and in the third week was (33.17g±0.74) and in the group administrated with crystallize sugar in the first and the third week was (35.81g±0.41) (36.07g±1.44) respectively, in the group administered with crystallized sugar and treated with hot water (33.60g±0.99) (34.11g±3.01). On the other hand, the weight is also increased in the groups administered with yeast in the first and third week (30.50g±1.74) (33.13g±2.32), and in the group administered with crystallized sugar and treated with traditional yeast (29.27g±2.15) (31.60g±1.98) (Figure 25).

Food

Our results demonstrated that the food consumed by mice is increased during the first and third week (60.14g±33.57) (83.14g±19.74) respectively in the group control, in the group of mice treated with hot water in the first week was (63.57g±18.97) and in the third week was (82.71g±29.09) and in the group administered with crystallize sugar in the first and third week (42.57±28.05) (99.57g±18.54) respectively. On the other hand, the food consumed by mice is increased in group administered with crystallized sugar and treated with hot water (80.14g±18.30) (92.42.83g±28.98) and in the group administered with yeast in the first and third week (84.14±19.11) (76.41±42.05), however the food consumed is decreased in group administered with crystallized sugar and treated with traditional yeast (68.71±29.43) (42.83±41.03) between the first and third week (Figure 26).

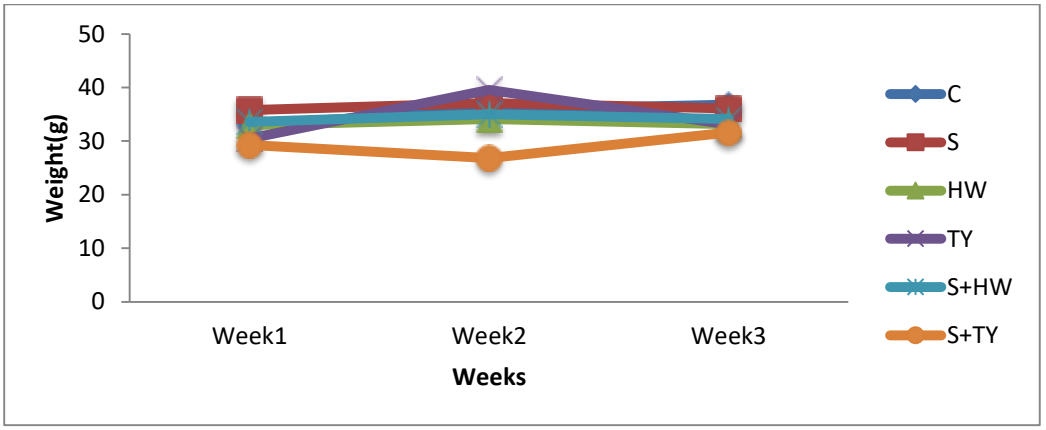


Figure 25: effect of crystallize sugar and hot water and traditional yeast on the weight in mice during 21 days.

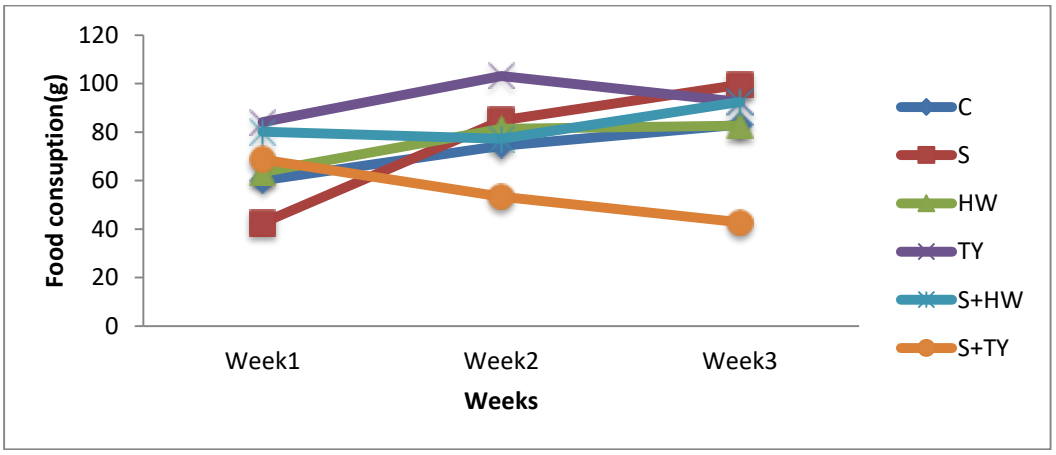


Figure 26: effect of crystallize sugar, hot water and traditional yeast on the food consumed by mice during 21 days.

V. 1. 2. Biochemical analysis

Blood sugar

The (Figure 27) showed that there is a difference between groups very highly significantly. C (0.45 g/L±0 .13), H (0.94g /l±0.2), S (1.15g /l±0.38), S+HW (0.54g/l±0.19), TY (1.81g/l±0.10), S+TY (1.66g/l±0 .37) P<0.0001.

The tukey test demonstrated that the level of blood sugar is increased very highly significantly in groups (S) and (TY+S), and P<0.001 respectively and highly significantly in group (TY) P<0.01. On the other hand we obtained that the level of blood sugar in groups of animals treated with hot water is increased significantly P<0.05 and P>0.05 respectively when compared to the group control.

The concentration of sugar in group of animal treated with hot water is decreased but not significantly when it is compared to the group of (S) P>0,05

Lipids status

Total cholesterol

The data showed that there is a difference between the concentration of T-ch but not significantly P> 0.05.

The tukey test showed that the concentration of total-cholesterol is increased but not significantly in the groups administered with crystallized sugar (1.44g/l±0.20) and S+HW (1.68g/l±0.20) and the group administered with traditional yeast (1.50g/l±0.18) when it is compared to the group C (1,40g/l ±0.24) P> 0.05.

The total cholesterol is decreased but not significantly in groups treated with Hot water (1.23g/l±0.38) and in the group treated with crystallize sugar and traditional yeast when compared to the control group P>0.05 (Figure 28).

Triglyceride

The data showed a difference very higly significantly in the values of triglycerides in groups administered with crystallized sugar (S) (0.62g/l±0.13) and administered with crystallized sugar and treated with hot water (S+H) (0.68g/l±0.13), (S+TY) (0.55g/l±0.23), group (H) treated with hot water (0.84g/l±0.34), control group (0.68g/l±0.12) and the group administered with traditional yeast (1.25g/l±0.16) (Figure 29) P≤0,001.

The tukey test showed that the concentration of TG in group (TY) is increased higly significantly when compared to groups (C and S) P≤0,01

HDL-C

The data showed a difference in the values of HDL-C in groups (S) were (1.15g/l±0.16) and (S+HW) (1.40g/l±0.18) and (S+TY) (0.87g/l±0.28) (HW) (1.05g/l±0.31) and control (1.26g/l±0.24) and (TY) (1.30g/l±0.14) but not significantly P>0.05 (Figure 30).

LDL-C

The data showed a difference in the values of LDL-C in groups (C) were (0.06g/l±0.03), (TY) (0.08g/l±0.06), (S) (0.18g/l±0.14), (HW) (0.15g/l±0.07), (S+HW) (0.16g/l±0.04) and (S+TY) (0.11g/l±0.09) but not significantly P>0.05 (Figure 31).

CRP

The (Figure 32) showed that there is a difference very highly significantly in the values of CRP between groups P<0.0001. The values were (S) (0.09mg/l±0.06), (S+HW) (0.41mg/l±0.34) and (HW) (0.12mg/l±0.05), (Traditional yeast) (0.08mg/l±0.06) and STY (0.12mg/l±0.08) when compared to the group (C) (0.74mg/l±0.21).

The turkey test demonstrated that the values of CRP in (S) (HW) (TY) are decreased very highly significantly P<0.001 and it is decreased in group (S+HW) and (S+TY) significantly and highly significantly when compared to the control group P<0.05 and P<0.01 respectively.

Creatinine

Our data obtained showed that the concentration of creatinine is differ in all groups but not significantly P>0.05.

The turkey test demonstrated that the level of creatinine in group (S) (2.97mg/l±1.06) increase but not significantly when it is compared to the control group (2.17mg/l±1, 47).

We obtained that the concentration of the creatinine is decreased in group treated with hot water (H) (1.74mg/l±0.59) and group treated with traditional yeast (TY) (1.60mg/l±0.54) but not significantly P>0.05 (Figure 33).

Reduced glutathione

The concentration of reduced glutathione is decreased in groups (HW) (0.016nmol/mg protein), (TY) (0.01 nmol /mg protein), (HW+S) (0.013 nmol /mg protein) and (S+TY) (0.0045

nmol /mg protein). When compared to the (C) and (S) (0.17nmol /mg protein) (0.16 nmol /mg protein) respectively (Figure 34).

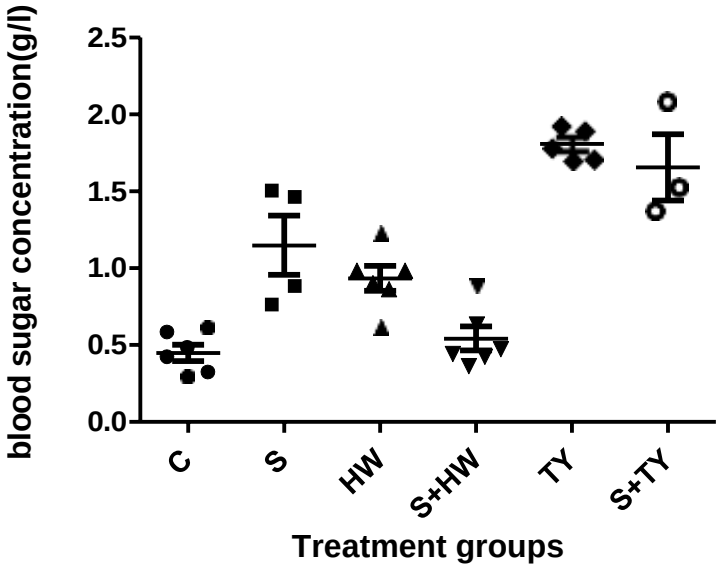


Figure 27: effect of high consumption of crystallize sugar, hot water and traditional yeast on fasting blood sugar in mice.

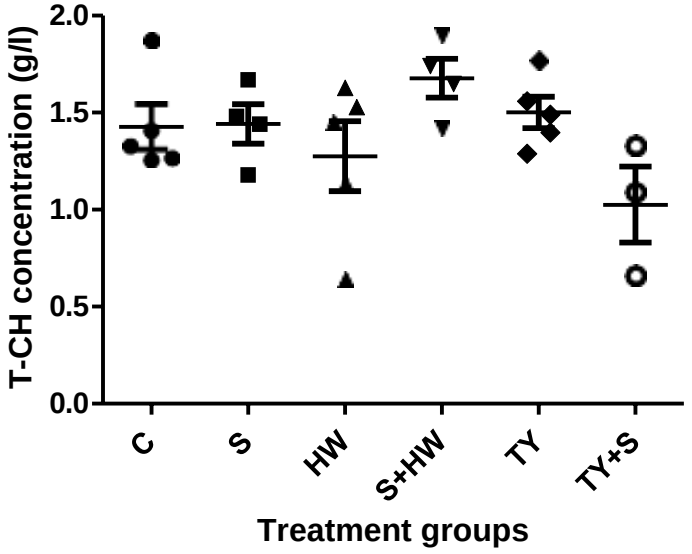


Figure 28: effect of high consumption of crystallize sugar, hot water and traditional yeast on T- cholesterol in mice.

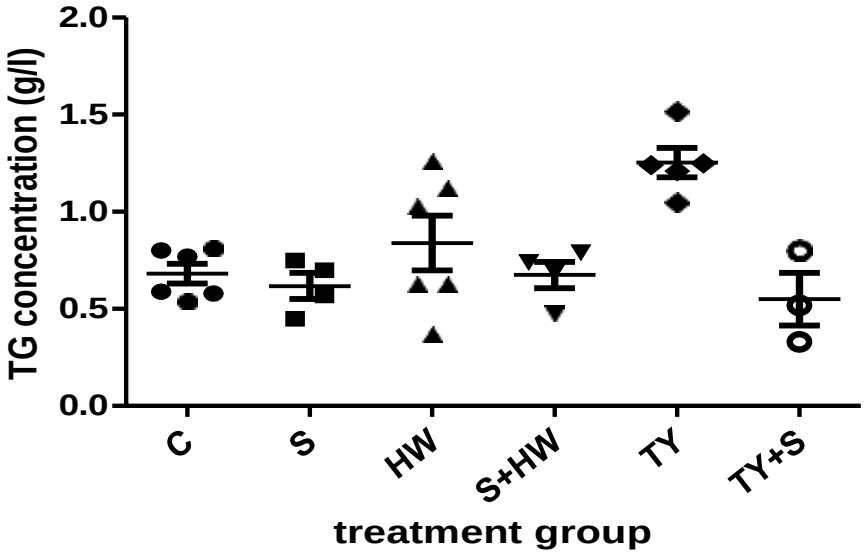


Figure 29: effect of high consumption of crystallize sugar, hot water and traditional yeast on triglyceride in mice.

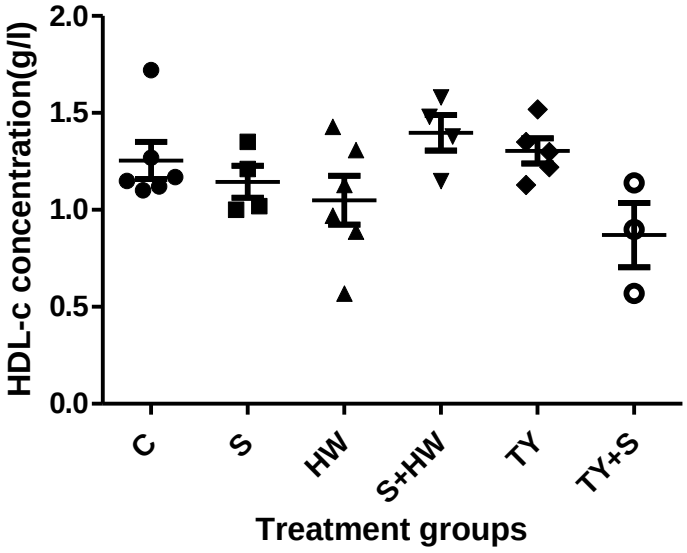


Figure 30: effect of high consumption of crystallize sugar, hot water and traditional yeast on HDL-C in mice.

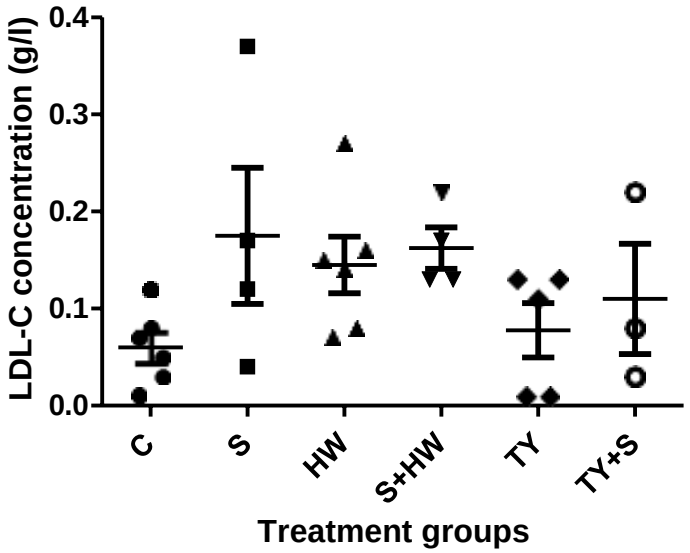


Figure 31: effect of high consumption of crystallize sugar, hot water and traditional yeast on LDL-C in mice.

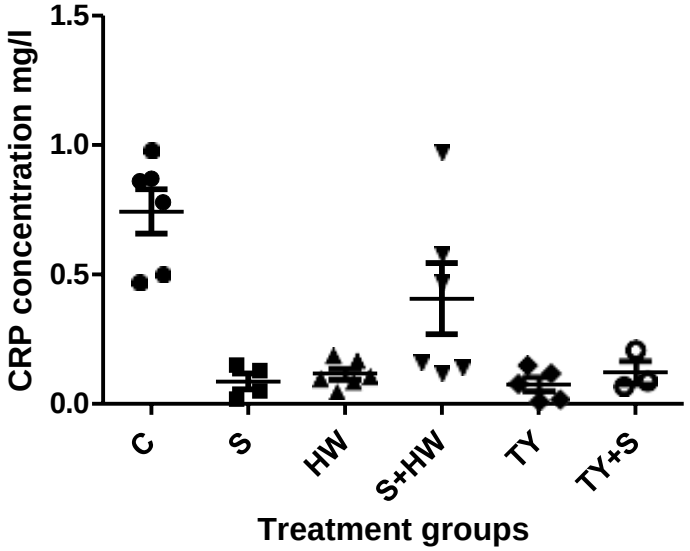


Figure 32: effect of high consumption of crystallize sugar, hot water and traditional yeast on CRP in mice.

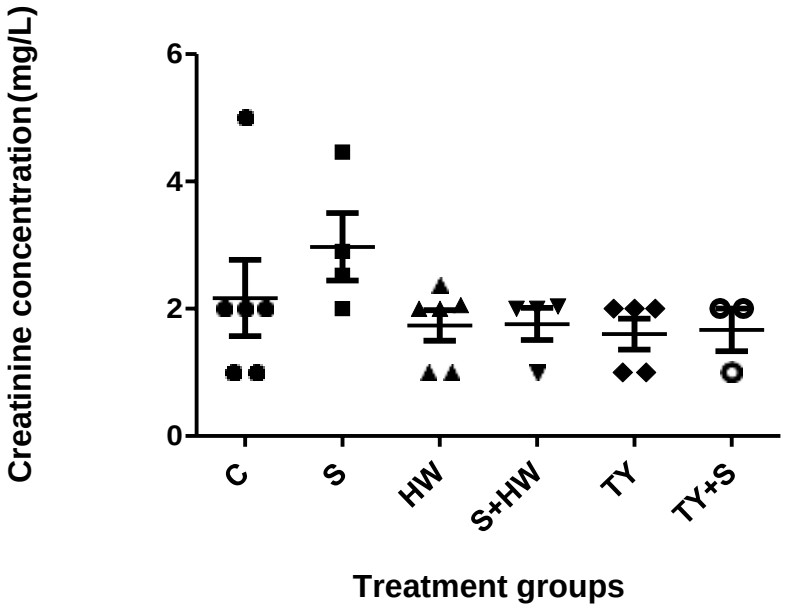


Figure 33: effect of high consumption of crystallize sugar, hot water and traditional yeast on creatinine in mice.

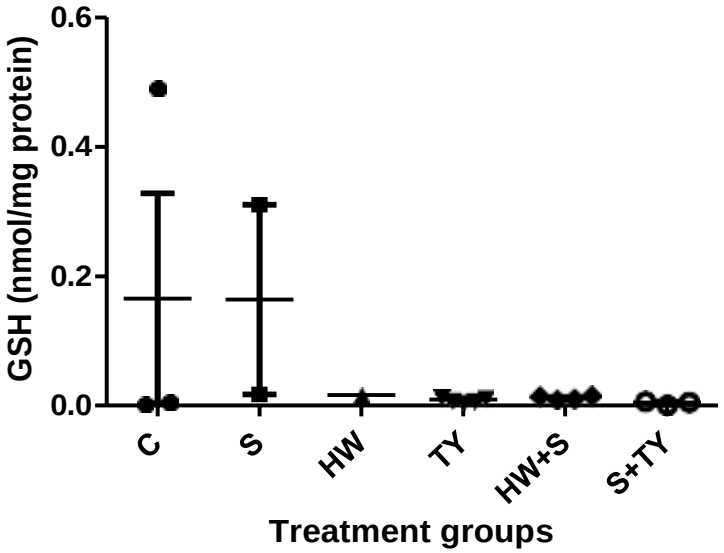


Figure 34: effect of high consumption of crystallize sugar, hot water and traditional yeast on GSH in mice.

ASAT and ALAT

The data showed that there is a difference significantly between groups in the level of the liver enzyme ASAT $P<0.05$.

The turkey test demonstrated that ASAT is decreased significantly in animal treated with hot water (195.05UI/L±39.02) and group treated of traditional yeast (110.74UI/L±36.57) significantly when compared to the control group $P<0.05$.

For the second enzyme, the ALAT there is a difference between groups (C) (144.8UI/L±107.98), (S) (46.55UI/L±15.25), (HW) (53.16UI/L±20.83), (S+HW) (41.10UI/L±13.70), (TY) (44.07UI/L±11.29), and (TY+S) (129.18UI/L±11.67) but not significantly $P>0.05$ (Figure 35, 36).

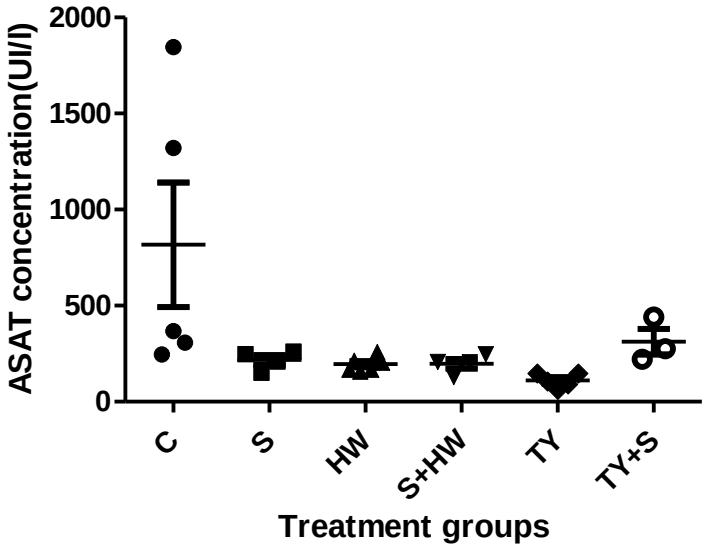


Figure 35: effect of high consumption of crystallize sugar, hot water and traditional yeast on ASAT in mice.

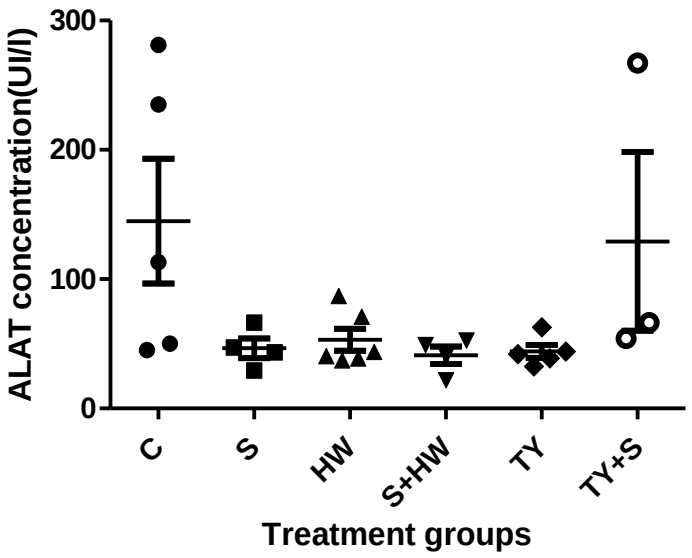


Figure 36: effect of high consumption of crystallize sugar, hot water and traditional yeast on ALAT in mice.

V. 1.3.Histological study

Microscopic observations

We observed in the liver of group fed with crystallize sugar, hypertrophy of hepatocytes nuclei (Figure 37), destruction of membranes hepatocytes cells (Figure 38), and sinusoid dilatation (Figure 39). In contrast to the other groups we detected that the liver parenchyma was intact (Figure 40, 41, 44).

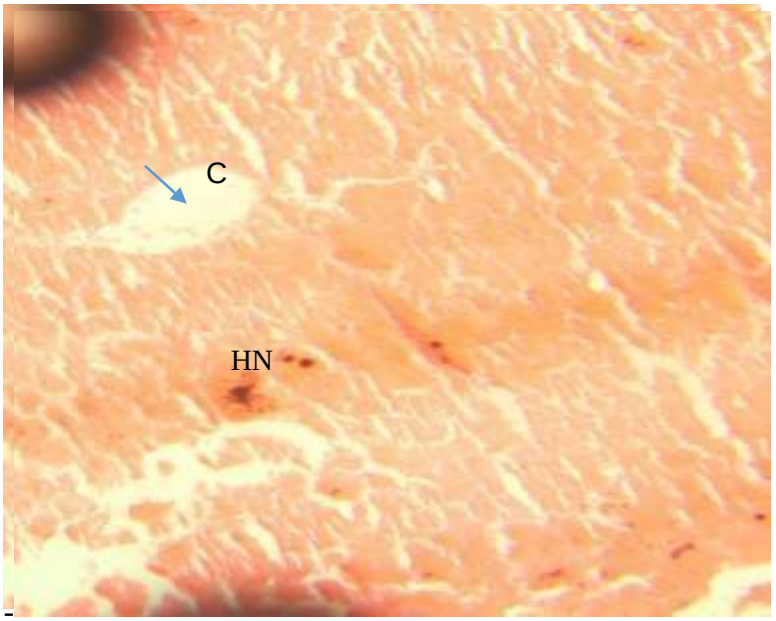


Figure 37: histological section of liver in group fed with crystallize sugar (S) during 21 days. Hematoxylin eosin (x40).

CV: central vein, S: sinusoid, H: hepatocyte, HN: hypertrophy of nucleus

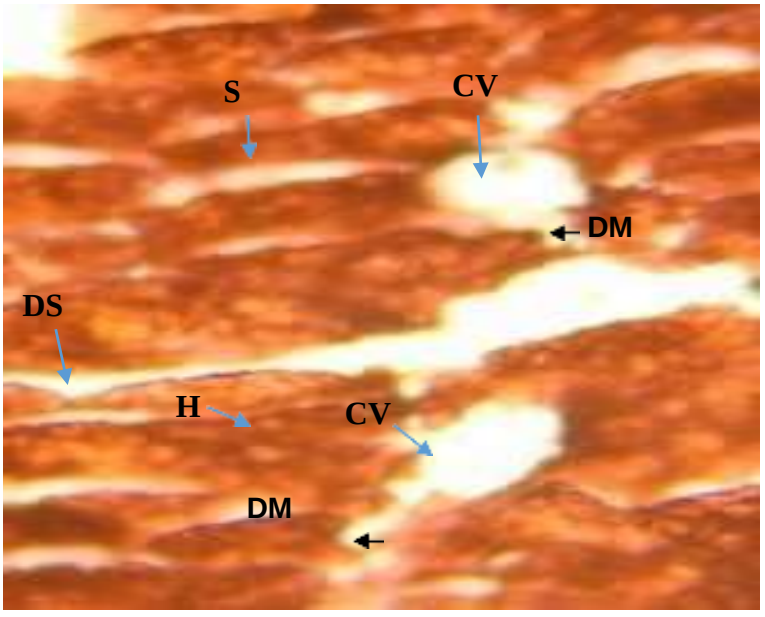


Figure 38: histological section of liver in group fed with crystallize sugar (S) during 21 days. Hematoxylin eosin (x100).

CV: central vein. H: hepatocytes, DMC: Destruction of membrane cells, DS: dilatation of sinusoid

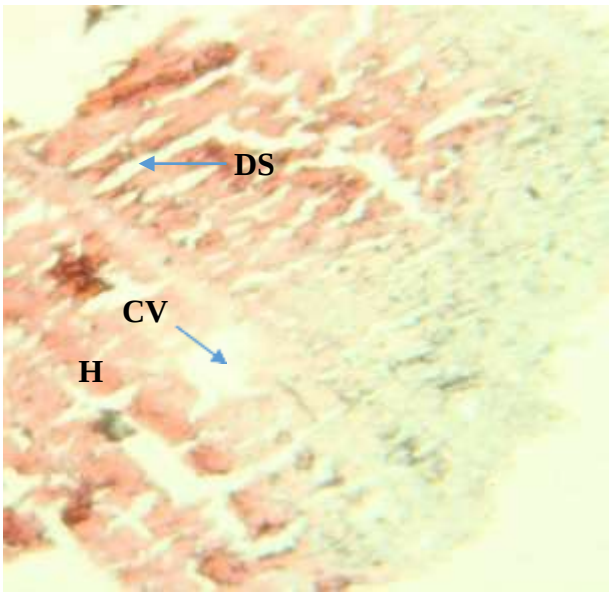


Figure 39: histological section of liver in group fed with crystallize sugar (S) during 21 days. Hematoxylin eosin (x100).

CV: central vein, DS: dilatation of sinusoid, H: hepatocytes.

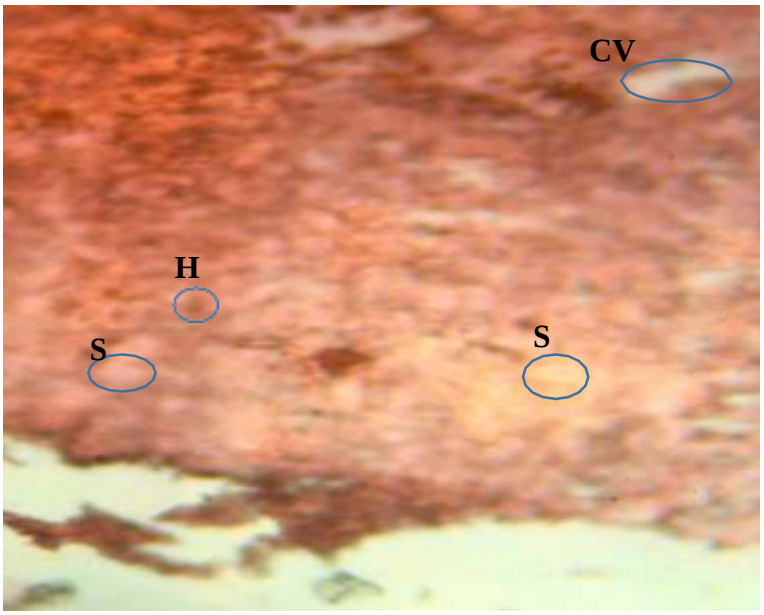


Figure 40: histological section of liver in group treated with hot water (H) during 21 days. Hematoxylin eosin (x100).

H: hepatocytes. S: sinusoid. CV: central vein.

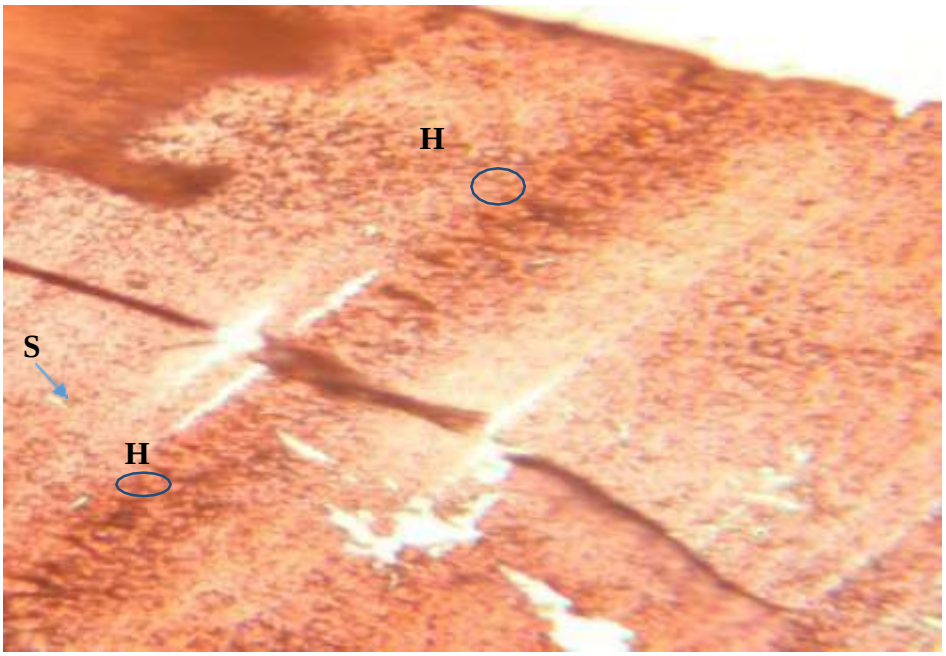
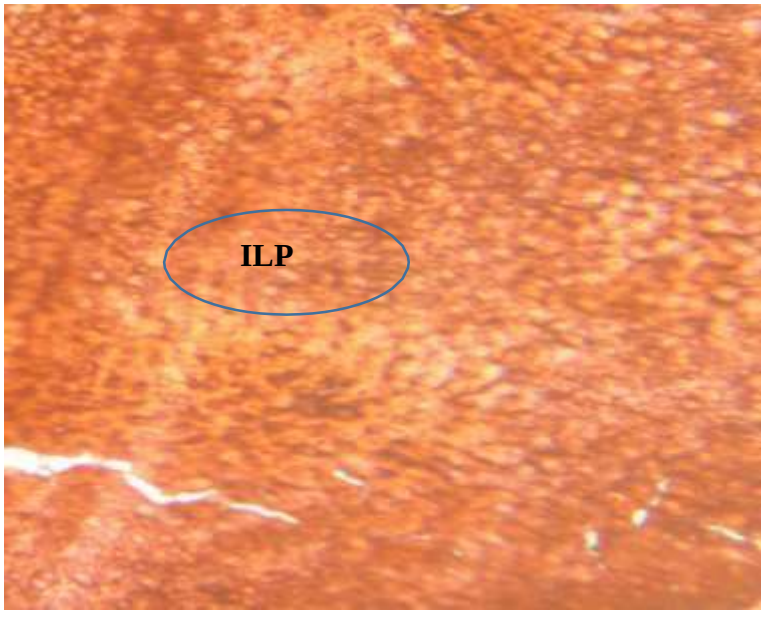


Figure 41: histological section of liver in group treated with traditional yeast (TY) during 21 days. Hematoxylin eosin (x100).

H: hepatocytes. S: sinusoid.



I

Figure 42: histological section of liver in group fed with crystallize sugar and treated with hot water (S+HW) during 21 days. Hematoxylin eosin (x100).

ILP: Intact liver parenchyma.

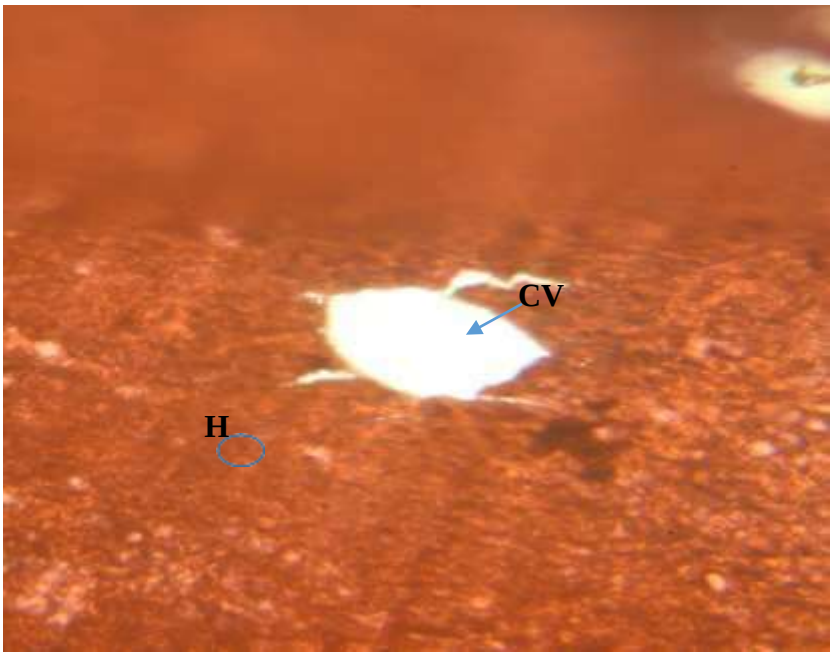


Figure 43: histological section of liver in group fed with crystallize sugar and treated with hot water (S+Hw) during 21 days. Hematoxylin eosin (x100).

CV: central vein. H: hepatocytes

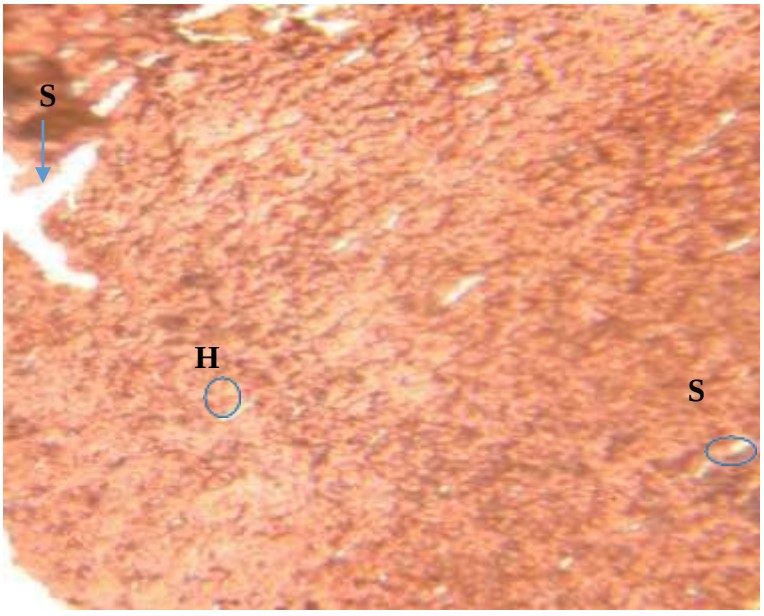


Figure 44: histological section of liver in group fed with crystallize sugar and treated with hot water (S+Hw) during 21 days. Hematoxylin eosin (x100).

S: sinusoid. H: hepatocytes

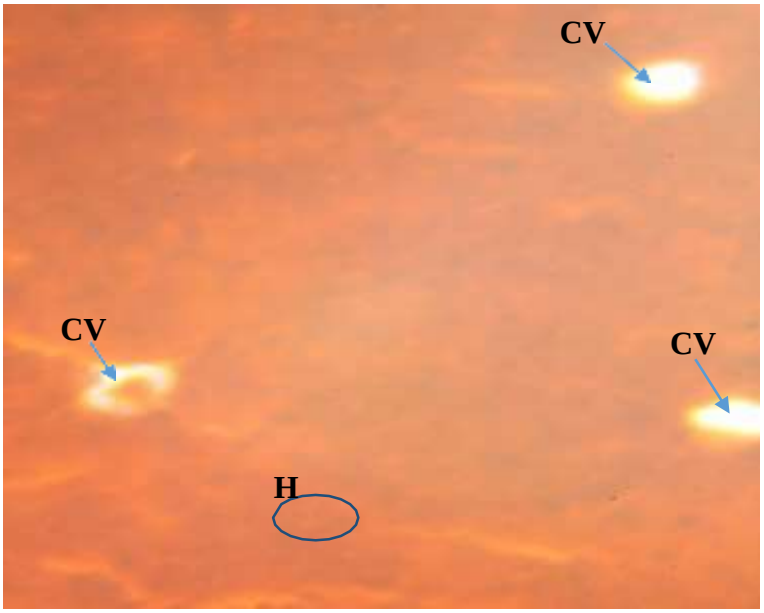


Figure 45: histological section of liver in group fed with refined crystallize sugar and treated with traditional yeast (S+TY) during 21 days. Hematoxylin eosin (x100).

CV: central vein, H: hepatocyte.

V. 2. Discussion

Sweeteners derived from fructose; sucrose (high fructose syrup) have the potential to cause a metabolic, disorder non-alcoholic fatty liver disease (NAFLD) and hyperinsulinemia has a critical role in the imbalance of the cellular pathway, daily fructose consumption increases hepatic inflammation and hepatocyte ballooning **(Yu et al., 2011)**.

The aim of our research is to clarify the effects of a diet high in crystallize sugar on some biochemical parameters (CRP, Total Cholesterol, Triglyceride, HDL-C LDL-C, blood glucose and creatinine) and on the histological structure of the liver in mice and to examine the effect of hot water and traditional yeast on the abnormalities caused by the high-crystallized sugar diet.

V. 2. 1. Body weight and food

In our research we detected that the weight of mice are slightly increased in group fed with refined crystallize sugar. This result is in agreement with the work of Abderrahmane et al.(2022) who detected an increase in the weight of animals fed with refined crystallize sugar. For instance we have detected an increase of weight in the group treated with hot water. Our result is not agrees with the work of Abderrahmane et al. (2022) who reported that the weight in group of mice is decreased in the group of animals treated with hot water during 21 days of treatment.

Also the weight of mice is increased in group of mice fed with high crystallize sugar and treated with hot water. Our result is agrees with those of Abderrahmane et al. (2022) who reported that the weight of animal is slightly increased in the group administered with crystallize sugar and treated with hot water.

The quantity of diet consumed by mice is increased in the groups administered with refined crystallize sugar, treated with hot water, and control group. Same results are obtained by Abderrahmane et al. (2022) who obtained the food consumed by mice is increased in the three groups, control group fed with standard diet, group treated with hot water and group fed with crystallize sugar during 21days of experimental study.

We detected in our study that the group fed with refined sugar and treated with hot water is consumed more food. Our result is not agrees with the result of Abderrahmane et al. (2022) who reported that animals fed with refined sugar and treated with hot water is consumed less quantity of food during the experimental study of 21days.

On the other hand, the quantity of food taken by animals is decreased in group fed with diet rich with traditional yeast and crystallize sugar this is an original work never done before.

V. 2. 2. Biochemical investigations

Our study showed that the treatment with hot water could decrease the concentration of blood glucose when compared to the group treated with sugar but it is higher than the control group. There is a benefit of drinking hot water on the stimulation of insulin which converts the glucose to glycogen in the liver. Our result is agrees with Abderrahmane et al. (2022) who found that the blood sugar is decreased in mice fed with refined sugar and treated with hot water.

Diabetes mellitus is a non-communicable disease that occurs in both developed and developing countries. This metabolic disease affects all systems in the body, including the liver. Hyperglycaemia, mainly caused by insulin resistance, affects the metabolism of lipids, carbohydrates and proteins and can lead to non-alcoholic fatty liver disease, which can further progress to non-alcoholic steatohepatitis, cirrhosis and, finally, hepatocellular carcinomas **(Mohamed et al., 2016)**.

Accumulating evidence suggests that lipotoxicity mediated by hepatic free cholesterol (FC) overload is a mechanistic driver for fibrosis, characteristic of nonalcoholic steatohepatitis (NASH), in many animal models and also in some patients with NASH **(Horn et al., 2022)**.

We detected in this research, mice administered with refined crystallize sugar (200g/kg) have shown a decrease in the values of lipids (TG, HDL-C) .Abderrahmane et al. (2022) reported that the levels of TG, HDL-C are increased in mice treated with crystallize sugar (50g/65kg). The concentration of T-CH is increased in group S this result is agree with the result of Abderrahmane et al. (2022). On the other hand we detected an increase in the low density lipoprotein (LDL-C) in group S our result is in accordance with the previous work of Abderrahmane et al. (2022). By the high density lipoprotein is increased in group TYand decreased in TY+S; this result is original never done before and need more investigation.

Because the liver does not serve as a storage depot for fat, the steady state concentration of hepatic triglycerides is low under physiological conditions. Nevertheless, there is considerable trafficking of both triglycerides and fatty acids into and out of the liver in response to feeding and fasting **(Kawano and Cohen, 2013)**.

High-density lipoprotein cholesterol (HDL-C) is a protein-rich lipoprotein that has been shown to have a role in reverse cholesterol transport, which essentially transports excess cholesterol from peripheral vessels to the liver for disposal, thereby reducing the risk of severe disease **(Harshavardhan et al., 2021)**.

Serum total, LDL and HDL cholesterol level in patients with cirrhosis is inversely correlate with severity of cirrhosis **(Ghadir et al., 2010)**.

C-reactive protein (CRP) is an acute inflammatory protein that increases up to 1,000-fold at sites of infection or inflammation **(Sproston and Ashworth, 2018)**.

The c-reactive protein is decreased in the group fed with crystallize sugar and the group treated with hot water. This results are same obtained by Abderrahmane et al. (2022). On the other hand the CRP is decreased in the group treated with traditional yeast.

The creatinine levels are increased in the group fed with crystallize sugar and decreased in the other groups (HW, S+HW, TY and TY+S).

Findings suggest that in the chronic kidney disease (CKD) population, increased sugar sweetened beverages (SSB) intake was associated with a higher risk of mortality and indicated a stratified association with dose. Plain water and unsweetened coffee/tea might be possible alternatives for SSBs to avert untimely deaths **(Cai et al., 2022)**.

From our results we found that the liver enzymes (ASAT) are decreased in groups of the experimental study (HW, S+HW and TY) for ALAT in groups (S+HW and TY) when compared to the group fed with crystallize sugar.

But the levels of ALAT and ASAT are increased in group of animal fed with crystallize sugar and traditional yeast where 3 mice are died before the end of the experiment. The hot water could detoxify the body from the toxin decreasing the liver enzymes in the plasma. But the ASAT and ALAT are affected by adding sugar and traditional yeast to mice this work is original and for this reason more study is needed in this part.

Glutathione (GSH), a naturally occurring thiol tripeptide of γ-glutamyl-cysteinyl-glycine, plays a vital role in cellular redox reactions and is involved in the inhibition of melanin synthesis, protection from reactive oxygen species, and cell detoxification **(Lee et al., 2020)**.

Where an increase in the level of prooxidants, notably reactive oxygen species, relative to that of antioxidants in cells and tissues results in “oxidative stress”, which potentially leads to oxidative damage to important biological components such as DNA, proteins, and lipids.

The change in the ratio of reduced (GSH) to oxidized glutathione (GSSG) has been reported to be diagnostic of various diseases such as cancers, neurodegenerative diseases, and cardiovascular diseases **(Ngamchuea et al., 2017)**.

Our results demonstrated that Glutathione reduced was lower in the groups of hot water, traditional yeast, sugar + hot water and sugar +traditional yeast compared to the groups control and sugar. Our results are agree with the work of Abderrahmane et al. (2022) who reported that GSH levels are increased in group of mice fed with crystallize sugar and decreased in groups control and hot water. More analysis needs to prove this results by measuring the glutathione oxidized and the dosage of GSH in the plasma.

V. 2. 3. Histological study

We have detected some alterations caused by the administration of high dose of crystallize sugar such as destruction of cell membrane and observation of some hypertrophy nuclei hepatocytes cells, our results are agree with the results obtained by Aklil et al. (2018) who observed a hypertrophy of nuclei in liver of animals administered with diet rich with L-methionine during 21 days. However we detected corrections of these alterations in the liver of mice concerning the groups treated with hot water and traditional yeast. Our result is agrees with those obtained by Boufedeche et al. (2018) who obtained that the hot water at 50°C could ameliorated the alterations of liver rats.

Conclusion and future work

The goal of this study was to induce high level of blood sugar by administration of high dose of crystallize sugar (200 g/65kg/day) during 21 days in an *in vivo* animal, therefore evaluate the protective and preventive effect of hot water at 50°C and traditional yeast on lipids profile, marker of inflammation and structural disorders of liver.

The current study has shown that food rich in high dose of crystallize sugar caused some metabolic disorders manifested by hyperglycemia and hyperlipedemia (T-cholesterol) and dyslipoproteinemia (increase in LDL-C and decrease of HDL-C). In addition, histology observations showed destruction of cell membrane and hypertrophy of hepatocyte nucleus. Meanwhile, a treatment with traditional yeast and hot water at 50°C was effective in preventing the increase of these metabolic disorders and liver damages.

Based on the findings of this study our future work and perspectives can evaluate many topics:

1- Evaluate the effect of drinking hot water and traditional yeast on blood sugar in human and rats.

2- Evaluate the effect of drinking hot water and traditional yeast in rat liver inflammation induced by high crystallize sugar.

3- Evaluate the antioxidants proteins and enzymes in animals fed on diet rich with crystallize sugar, treated with hot water and traditional yeast.

References

Reference

Abderrahmane K., Seraoui R., Nasri B. (2022). Interaction between hot water and high consumption of crystallize sugar in cardiovascular disease. Thesis submitted for the degree of Master II. *University des frères Mentouri Constantine1 (Algeria)*.

Abdel-Misih S. R. Z., and Bloomston M. (2010). Liver Anatomy. *Surgical Clinics of North America*, 90(4): 643–653. Doi: 10.1016/j.suc.2010.04.017

Adamczyk-Sowa M., Sowa P., Mucha S., Zostawa J., Mazur B., Owczarek M., and Pierzchała K. (2016). Changes in serum ceruloplasmin levels based on immunomodulatory treatments and melatonin supplementation in multiple sclerosis patients. *Medical Journal of Experimental and Clinical Research*, 22: 2484–2491.

Ahnen R. T., Mottet R., Omolo M., and Slavin J. (2020). Carbohydrates. Present Knowledge in Nutrition. *Present Knowledge in Nutrition*, 1: 37-50. ISBN: 9780128198421

Albagli G., Finotelli P. V., Ferreira T. F., Priscilla F. F., and Amaral P. F. F. (2023). Toward sourdough microbiome data: a review of science and patents. *Foods Journal*, 12(2): 1- 21.

Aldairi A. F. (2020). Evaluation of various methodologies used in purification of biologically active carbohydrates derived from marine Life. *Biomedical Journal of Scientific & Technical Research*, 27(4): 20919-20827.

AlHadjri F. (2010). The miracle and wonders of treatment from hot water: hot water miracle. 1-108.

AlHajri F. (2020). Corona- virus (COVID) out break and the last treasure. A*uthor House™ UK Bloomington*, USA.

Amoutzopoulos B., Steer T., Roberts C., Collins D., and Page P. (2020). Free and added sugar consumption and adherence to guidelines: the UK National Diet and Nutrition survey. *Nutrients*, 12(2): 393.

Amr A. S., and Alkhamaiseh A. M. (2022). Sourdough use in bread production. *Jordan Journal of Agricultural Sciences*, 18(2): 81-98.

Aoki T., and Narumiya S. (2012). Prostaglandins and chronic inflammation.

Pharmacological-sciences, 33(6): 304-311.

Asif H. M., Akram M., Saeed T., Khan M. I., Akhtar N., Riaz U. R., Ali Shah S. M., Khalil A., Shaheen G. (2011). Carbohydrates. *International research journal of biochemistry and bioinformatics*, 1(1): 1-5.

Bahadorfar M. (2014). A Study of hydrotherapy and its health benefits. *International Journal of Research*, 1(8): 294-305.

Baride P. M., Joshi D. A., Galisin V., Bavage S., and Bavage N. B. (2020) . Benefits of warm water. *World journal of pharmaceutical research*, 9(6): 443-460.

Behera S. S., and Ray R. C. (2015). Sourdough Bread. CRC Press, Boca raton, USA. Cristina M. Russell. 53-67.

Benmebarek A., Zerizer S., Laggoune S., and Kabouche Z. (2013). Effect of Stachys mialhesi de Noé on the inflammation induced by hyperhomocysteinemia in cardiovascular diseases. *Der Pharmacia Lettre*, 5(2):212-223.

Bender T., Karagulle Z., Balint G. P., Gutenbrunner C., Balint P. V., and Sukenik S. (2005). Hydrotherapy, balneotherapy, and spa treatment in pain management. *Rheumatology International*, 25: 220–224.

Bhondave P. D., Devarshi P. P., Mahadik K. R., and Harsulkar A. M. (2014). "Ashvagandharishta"prepared using yeast consortium from woodfordia fruticosa flowers exhibit hepatoprotective effect on CCL4 induced liver damage in wistar rats. *Journal of Ethnopharmacology*, 151: 183-190.

Binjamini E., Sunshine G., and Leskowitz S. (1996). Immunology a short course. Wiley-Liss, Ine. 605 Third Avenue, New York, NY10158-0012: 25-27.

Boege Y. T., Reisinger F., and Heikenwalder M. (2011). Chronic liver Inflammation and hepatocellular carcinoma: persistence matters. *Swiss medical weekly*, 141(1920): 13197.

Boncler M., Wu Y., and Watala C. (2019). The multiple faces of C-reactive protein-physiological and pathophysiological implications in cardiovascular disease. *Molecules*, 24(11): 1-18. Doi: 10.3390/molecules24112062

Boufedeche S., Grendou Y., and Merikhi.F. (2018). Effet de l'eau chaude sur les maladies cardiovasculaires induites par l'hypercholestérolémie chez les rats. Thèse Master II. *Université des frères Mentouri Constantine1 Algeria*.

Bray C., Bell L. N., Liang H., Haykal R., Kaiksow F., Mazza J. J., and Yale S. H. (2016). Erythrocyte Sedimentation Rate and C-reactive Protein Measurements and Their Relevance in Clinical Medicine. *Wiscosin Medical Journal*, 115(6): 317-321.

Bull B. S.,Caswell M., Ernst E., Jou J M., Kallner A., KoepkeJ A., Lewis S M., Lowe G D., Rampling M W., and Stuart J. (1993). ICSH recommendations for measurement of erythrocyte sedimentation rate. International Council for Standardization in Haematology (Expert Panel on Blood Rheology). *Journal Clinical Pathology*, 46(3):198-203.

Bunning M., Shackelton E., Pennington A., and Clark C. (1908). Understanding and Selecting Sourdough for Health Benefits. *Colorado State University Extension*, 1-5.

Cai X. Y, Nan-Hui Z, Yi-Chun C, Shu-Wang G. (2022). Sugar-sweetened beverage consumption and mortality from chronic kidney disease: results from the US. National Health and Nutrition Examination Survey. *Clinical Kidney Journal*, 15(4): 718-726.

Calabro P., Golia E., and Yeh E T. (2012). Role of C-reactive protein in acute myocardial infarction and stroke: possible therapeutic approaches. *Current Pharmaceutical Biotechnology*, 13(1): 4-16.

Calvert M. D., Madden A. A., Nichols L. M., Haddad N. M., Lahne J., Dunn R. R., Erin A., and McKenney E. A. (2021).A review of sourdough starters: ecology, practices, and sensory quality with applications for baking and recommendations for future research. *PeerJournal*, 9(11389): 1-37.

Carbonetto B., Nidelet T., Guezenec S., Perez M., Segond D., and Sicard D. (2020). Interactions between *Kazachstania humilis* Yeast Species and Lactic Acid Bacteria in Sourdough. *Microorganisms*, 8(2): 240.

Chowdhury R. S., Islam M. D., Akter K., Sarkar M. A. S., Roy T., and Tajdit R. S. M. (2021). Therapeutic aspects of hydrotherapy: a review. *Bangladesh Journal of Medicine*, 32(2): 138-141.

Coussens L. M., and Werb Z. (2002). Inflammation and cancer. *Nature*, 420: 860–867.

Cummings J. H., and Stephen A. M. (2007). Carbohydrate terminology and classification.

European Journal of Clinical Nutrition, 61: 5-18.

D'angelo G. (2013). Role of hepcidin in the pathophysiology and diagnosis of anemia. *Blood Research*, 48: 10-15.

Dahlqvist A., and Borgstrom B. (1961). Digestion and absorption of disaccharides in man.

Biohemical Journal, 81: 411.

Davis F J., Vidyasagar S., and Maiya G A. (2012). C-reactive protein and coronary heart disease - risk marker or risk factor? *Journal of Clinical Science Research*, 1: 178-186.

De V. L., Comasio A., and Kerrebroeck S. V. (2021). Sourdough production: fermentation strategies, microbial ecology, and use of non-flour ingredients. *Food Science and Nutrition*, 63(15): 2447-2479.

De V. L., Harth H., Kerrebroeck S. V., Leroy F. (2016). Yeast diversity of sourdoughs and associated metabolic properties and functionalities. *International Journal of Food Microbiology*, 239(19): 26-34.

Devkate G. V., Tate S. S., Deokate S. B., Bhujba A. S., Tupe A. P., and Patil R. N. (2016). Hydrotherapy anew trend in disease treatment. *Human Journals*, 5(2): 117-135.

Du W. C. T. (2000). Function of C-reactive protein. *Annals of Medicine*, 32(4): 274–278.

Elijah T., Gannon M., and Wasserman D. H. (2017). The liver.*Biologia actual*, 27(25): 1147-1151. Doi: 10.1016/j.cub.2017.09.019

Ellis H. (2011). Anatomy of the liver. *Surgery (Oxford)*, 29(12): 589-592.

Enríquez A., Calder V., Gao J., Galatowicz G., García-Vázquez C., Fernández I., Stern

M. E., Diebold Y., and Calonge M. (2008). Cytokine responses by conjunctival epithelial cells: An *in vitro* model of ocular inflammation. *Cytokine*, 44(1): 160-167.

Erridge C. (2008). The roles of pathogen-associated molecular patterns in atherosclerosis.

Trends in cardiovascular medicine, 18(2): 52-56.

Fam A. G., and Rheumatol J. (1991). Spa treatment in arthritis: A rheumarologist's view.

Journal of Rheumatology, 18: 1775–1777.

Feehan K. T., and Gilroy D. W. (2019). Is resolution the end of inflammation?. *Trends in molecular medicine*, 25(3): 198–214.

Freeman C., Zehra A., Ramirez V., Wiers C. E., Volkow N. D., Wang G-J. (2018). Impact of sugar on the body, brain, and behavior. *Frontiers in Bioscience-Landmark*, 23(12): 2255-2266. Doi: 10.2741/4704.

Ghadir M. R., Riahin A. A., Havaspour A, Nooranipour M., and Habibinejad A. A.(2010). The relationship between lipid profile and severity of liver damage in cirrhotic patients. *Journal of Polymers and the Environment*, 10(4): 285–288.

Giudice D. E., Elia V., and Tedeschi A. (2009). The role of water in the living organisms.

Neural Network World, 19(4): 355-360.

Hall J., Swinkels A., Briddon J., and McCabe C. S. (2008). Does aquatic exercise relieve pain in adults with neurologic or musculoskeletal disease? A systematic reviewand meta-analysis of randomized controlled trials. *Archives of physical medicine and rehabilitation*, 89: 873–883.

Harshavardhan R. B., Priya N., Anoop K. K., Krishnapriya S., GreeshmaC. R., and Venu P. R.(2021). Role of high-density lipoprotein cholesterol (HDL-C) as a clinical predictor of decompensation in patients with chronic liver disease (CLD). *International Journal of Hepatology*, Doi: 10.1155/2021/1795851

Hawiger J.,and Zienkiewicz J. (2019). Decoding inflammation, its causes, genomic responses, and emerging countermeasures. *Scandinavian journal of immunology*, 90(6): 1-32.

Huanming X ., Junnin J., Yubao X., Shi Meijic S., Guangjun T., Pengtoa Z., Chaozhen Z., Hwjun C., Chanyuan X. U., and Xiao C. L. (2017). Colon hydrotherapy plus traditional chinese Medicine to treat nonalcoholic fatty liver disease: a pilot study. *Journal of Traditional Chinese Medicine*, 37(3): 393-396.

Hoekstra S. P., Bishop N. C., Faulkner S. H., Bailey S. J., and Leicht C. A. (2018). Acute and chronic effects of hot water immersion on inflammation and metabolisme in sedentary, overweight adults. *Journal of Applied Physiology*, 125(12): 1661-2018.

Horn C. L., Morales A. L., Savard C., Farrell G. C., and Ioannou G. N. (2022). Role of Cholesterol-Associated Steatohepatitis in the Development of NASH. *Hepatology Communications*, 6(1): 12–35.

Howard B. V., and Wylie-Rosett J. (2002). Sugar and cardiovascular disease. *Circulation*, 102(4): 523-527.

Italiani P and Boraschi D. (2014). From monocytes to M1/M2 macrophages: phenotypical vs. functional differentiation. *Frontiers in Immunology*, 5(514): 1-22.

Jenkins D. J., Wolever T. M., Taylor R. H., Barker H., Fielden H., Baldwin J. M., Bowling A. C., Newman H. C., Jenkins A. L., and Goff D. L. (1981). Glycemic index of foods:a physiological basis for carbohydrate exchange. *The American Journal of Clinical Nutrition*, 34(3): 362-366.

Jensen T., Abdelmalek M. F., Sullivan S., Nadeau K. J., Green M., Roncal C., Nakagawa T., Kuwabara M., Sato Y, Kang D-H., Tolan D. R, Laura G. Sanchez-Lozada , Hugo R. Rosen H. R., Lanaspa M. A., Diehl A. M., and Johnson R. J. (2018). Fructose and sugar: A major mediator of non-alcoholic fatty liver disease. Journal of Hepatology, 68(5): 1063-1075. PMID: 29408694

John R., and White, J. R. (2018). Sugar. *Clinical Diabetes*, 36 (1): 74-76.

Kamal M. A., and Klein P. (2011). Determination of sugars in honey by liquidchromatography. *Saudi journal of biological sciences*, 18(1): 17-21.

Kapellos T. S., Bonaguro L., Gemünd I., Reusch N., Saglam A., Hinkley E. R., and Schultze J. L. (2019). Human Monocyte Subsets and Phenotypes in Major Chronic Inflammatory Diseases. *Frontiers in immunology*, 10(2035): 1-13.

Kawano Y., and Cohen D. E. (2013). Mechanisms of hepatic triglyceride accumulation in non- alcoholic fatty liver disease.*Journal of Gastroenterology*, 48(4): 434–441.

Kezer G. (2022). Functional Perspective on sourdough bread. *Agriculture - Food Science and Technology*, 10(8): 1410-1414.

Koivistoinen P., and Hyvönen L. (1985). The use of sugar in foods. *International Dental Journal*, 35(3): 175-9.

Koyama Y., and Brenner D. A. (2017). Liver inflammation and fibrosis. *Journal of Clinical Investigation*, 127(1): 55-64.

Landis E. A., Oliverio A. M., McKenney E. A., Nichols L. M., KfouryN., Biango-Daniels M., Shell L. K., Madden A. A., and Shapiro L. (2021). The diversity and function of sourdough starter microbiomes. *eLife Sciences*, 10(6): 1-24.

Lau S. W., Chong A. Q.,Nyuk Ling Chin N. L., Rosnita A. Talib R. A., and Kadir Basha R. (2021). Sourdough microbiome comparison and benefits. *Microorganisms*, 9(7): 1355.

Lee Y., Kumar S., Kim S. H., Seong K. Y., Lee H., Kim C., Jung Y. S., and Seung Y. Y. (2020). Odorless glutathione microneedle patches for skin whitening. *Pharmaceutics*, 12(2): 100.

Liu J., Zhang X., Cheng Y., and Cao X. (2021). Dendritic cell migration in inflammation and immunity. *Cellular and molecular immunology*, 18: 2461-2471.

Lobo A. J., Jones S. C., Juby L. D., and Axon A. T. (1992). Plasma viscosity in inflammatory bowel disease. *Journal of clinical pathology*, 45(1): 54-57.

Ludwig R. P. (2001). Water: From clusters to the bulk. *Angewandte chemie*, 40(10): 1808-1827.

Lugrin J., Rosenblatt-Velin N., Parapanov R., and Liaudet L. (2013). The role of oxidative stress during inflammatory processes. *Biological Chemistry*, 395(2): 203-230.

Mackiewicz A., and Mackiewicz K. (1995). Glycoforms of serum Alpha 1-acid glycoprotein as markers of inflammation and cancer. *Glycoconjugate journal*, 12: 241-427.

Mahadevan V. (2020). Anatomy of the liver. *Surgery (Oxford)*, 38(8): 427-431. Doi: https://doi.org/10.1016/j.mpsur.2014.10.004

Marcus Y. (2009). Effect of ions on the structure of water: structure making and breaking. *ACS Chemicalreviews*, 109(3): 1346-1370.

Meisner M. (2014). Update on procalcitonin measurements. *Clinical Chemistry*, 34(4): 263-273.

Melnikov I., Kozlov S., Saburova O., Avtaeva Y., Guria K., and Gabbasov Z. (2023). Monomeric C-Reactive protein in atherosclerotic cardiovascular disease: Advances and perspectives. *International Journal of Molecular Sciences*, 24(3): 2079.

Mohamed J., Nafizah A. H. N., ZariyanteyA. H., and Budin S. B. (2016). Mechanisms of diabetes-induced liver damage. *Sultan Qaboos University Medical Journal*, 16(2): e132–e141.

Moldoveanu B., Otmishi P., Jani P., Walker J., Sarmiento X., Guardiola J., Saad M., and Jerry Y. (2022). Inflammatory mechanisms in the lung. *Journal of Inflammation Research*, 2: 1-11.

Monastero., and Pentyala. (2017). Cytokines as biomarkers and their respective clinical cutoff levels. *International Journal of Inflamation*, 2017: 1-11.

Mondal S. (2017). Chemistry of carbohydrates. *Pharm v sen applied biochemistry*, 1-20. Doi:10.13140/RG.2.2.32509.67040

Mooventhan A., and Nivethitha L. (2014). Scientific evidence-based effects of hydrotherapy on various systems of the body. *North American Journal of Medical Sciences*, 6(5): 199-209.

Moslehi-Jenabian S., Lindegaard L., and Lene. (2010).Beneficial effects of probiotic and food Borne yeasts on human health. Nutrients, 2(4): 437-449.

Mottl M. J. Glazer B. T., Kaiser R. I., and Meech K. J. (2007). Water and astrobiology. *Geochemistry*, 67(4): 253-282.

Mukai K., Tsai M., and Galli S. J. (2018). Mast cells as sources of cytokines, chemokines and growth factors. *Immunological reviews*, 282(1): 121-150.

Murakami M., and Hirano T. (2012). The molecular mechanism of chronic inflammation development. *Frontiers in Immunology*, 3: 1-2. Doi: 10.3389/fimmu.2012.00323

Muriel P., López-Sánchez P., and Ramos-Tovar E. (2021). Fructose and the liver. *International Journal of Molecular Sciences*, 22(13): 6969.

Nehring SM., Goyal A., and Patel BC. (2017). C reactive protein. *Stat Pearls Publishing*, Treasure Island (FL). PMID: 28722873

Ngamchuea K., Christopher B. M., and Richard G. C. (2017). Rapid method for the quantification of reduced and oxidized glutathione in human plasma and saliva. *American Chemical Society*, 85(5): 2901-2908. Doi: 10.1021/acs.analchem.6b04186

Pahwa R., Goyal A., and Jialal I. (2022). Chronic Inflammation. In: StatPearls. Treasure Island (FL): StatPearls. PMID: 29630225

Parker G. A., and Picut C. A. (2005). Liver immunobiology. *Sage Journals*. 33(1): 52-62.

Pathak A., and Agrawal A. (2019). Evolution of C-reactive protein. *Frontiers Immunology*, 10(943): 1-12. Doi: 10.3389/fimmu.2019.00943

Peisajovich A., Marnell L., Mold C., and Du W. C. T. (2008). C-reactive protein at the interface between innate immunity and inflammation. *Immunology*, 4(3): 379-390.

Pfützner A., Schöndorf T., Hanefeld M., and Forst T. (2010). High-sensitivity C-reactive protein predicts cardiovascular risk in diabetic and nondiabetic patients: effects of insulin-sensitizing treatment with pioglitazone. *Journal Diabetes Scince Technology*, 4: 706-716.

Pradhan A. D., Manson J. E., Rifai N., Buring J. E., Ridker P. M. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Journal American Medical Association*, 286(3): 327-334.

Punyauppa-path S., Kiatprasert P., Punyauppa-path P., Rattanachaikunsopon P., Khunnamwong P., Limtong S., and Srisuk N. (2022). Distribution of kazachstania yeast in thai traditional fermented fish (Plaa-Som) in Northeastern Thailand. *Journal of Fungi*, 8(10): 1029.

Raschke T. M. (2006). Water structure and interactions with protein surfaces. *Current Opinion in Structural Biology*, 16(2): 152-159.

Rehman K., and Akach M. S. H. (2016). Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *Journal of Biomedical Science*, 23(87): 1-18.

Rosales C. (2018). Neutrophil: A cell with many roles in inflammation or several cell types?.

Frontiers Physiology, 9:113.

Saitkulov F. E., Azimov I. O., Ergasheva M. A., Jo H. O., and Shaxabiddinova R. S. (2022). Carbohydrates are the main source of energy in the body. *Tashkent State Agrarian University*, 7: 68-71.

Salazar J., Martínez M. S., Chávez M., Toledo A., Añez R., Torres Y., Apruzzese V., Carlos S., Rojas J., and Bermúdez V. (2014). C-reactive protein: Clinical and epidemiological perspectives. *Cardiology Research and Practice*, 2014: 1-10.

Sanada F., Taniyama Y., Muratsu J., Otsu R., Shimizu H., Rakugi H., and Morishita R. (2018). Source of chronic inflammation in aging. *Frontiers in CardiovascularMedicine*, 5(12): 1-5.

Sawka M. N., Cheuvront S. N., and Carter R. (2005). Human Water Needs. *Nutrition Reviews*, 63(6): 30-39.

Seeberger H. P. (2017). Monosaccharide diversity. Cold spring harbor laboratory press, Cold Spring Harbor (NY). Doi: 10.1101/glycobiology.4e.2

Seki E., and Schwabe R. F. (2014). Hepatic inflammation and fibrosis: functional links and key pathways. *Hepathology*, 61: 1066-1079.

Sharp K. A. (2001). Water: Structure and Properties. Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pennsylvania, USA17. DOI:10.1038/npg.els.0003116

Sherwood E. R. (2004). Mechanisms of the inflammatory response. *Best Practice and Research Clinical Anaesthesiology*, 18(3): 385-405.

Shrivastava A. K., Singh H. V., Raizadaand A., andSingh S. K . (2014). C-reactive protein, inflammation and coronary heart disease. *The Egyptian Heart Journal*, 67(2): 89-97. Doi: https://doi.org/10.1016/j.ehj.2014.11.005

Sibulesky L. (2013). Normal liver Anatomy. *Clinical liver disease*, 2(1): 1-3. Doi: 10.1002/cld.124

Sproston N. R and Ashworth J. J. (2018). Role of C-reactive protein at sites of inflammation and infection. *Frontiers in Immunology*, 13(9): 754.

Subaraman M., Devi R. G., and Jothi P. A. (2020). Knowledge and awareness about benefits of drinking hot water- a survey. *PalArch's Journal of Archaeology of Egypt / Egyptology*, 17(7): 1630-1638.

Sucher E., Sucher R., GradistanaC., Brandacher G., Schneeberger S., and Berg T. (2019). Autoimmune hepatitis immunologically triggered liver pathogenesis_ diagnostic and therapeutic strategies. *Journal of Immunology Research*, 2019: 19.

Szabo A., Gogolak P., Koncz G., Foldvari Z., Pazmandi K., Miltner N., Poliska S., Bacsi A., Djurovic S., and Rajnavolgyi E. (2018). Immunomodulatory capacity of the serotonin receptor 5-HT2B in a subset of human dendritic cells. *Science Report*, 8: 1765.

Tall A. R. (2004). C-reactive protein reassessed.*New England Journal of Medicine*, 350(14): 1450-1452.

Tanaka M., and Miyajima A. (2016). Liver regeneration and fibrosis after inflammation.

Inflammation and Regeneration, 36(19): 1-6.

Targońska S., and Majdan. (2014). Serum amyloid A as a marker of persistent inflammation and an indicator of cardiovascular and renal involvement in patients with rheumatoid arthritis. *Mediators of Inflammation*, 2014: 1-7.

Thacker E. (2006). Lung inflammatory responses. Veterinary Research, 37 (3): 469-486.

Theoharides C. T., Alysandratos K. D., Angelidou A., Delivanis D. A., Sismanopoulos N., Zhang B., Asadi S., Vasiadi M., Weng Z., Miniati A., and Kalogeromitros D. (2012). Mast cells and inflammation. *Biochimica et Biophysica Acta*, 1822(1): 21-33.

Thiele J. R., Zeller J., Bannasch H., Stark G. B., Peter K., and Eisenhardt S. U. (2015). Targeting C-reactive protein in inflammatory disease by preventing conformational changes. *Mediators of Inflammation*, 2015: 9.

Thompson D., Pepys M. B., and Wood S. P. (1999). The physiological structure of human C-reactive protein and its complex with phosphocholine. *Structure*, 7:169 -177

Tillett W. S., and Francis T. (1930). Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. *The Journal of experimental medicine*, 52: 561-571.

Vermeire S., Van Assche G., and Rutgeerts P. (2004). C-Reactive protein as a marker for inflammatory bowel disease. *Frontiers*, 10(5): 661–665.

Wang Y. W., Kinzie1 E., Franklin G. B., Sai K. L., and Baumann1 H. (2001). Haptoglobin, an inflammation-inducible plasma protein. *Redox Report*, 6: 379-385.

Watson J, Round A, Hamilton W. (2012). Raised inflammatory markers. *British medical journal*, 344: 454.

Wright H. L., Moots R. J., Bucknall R. C., and Edwards S. W. (2010). Neutrophil function in inflammation and inflammatory diseases. *Rheumatology*, 49(9): 1618-1631.

Wu Y., Lawrence A., Potempa., El Kebir D., and János G. F. (2015). C-reactive protein and inflammation: conformational changes affect function. *Biological Chemistry*, 396(11): 1181-1197.

Xiao Feng Pang. (2014). Water: molecular structure and properties. USA Hackensack, NJ 07601, UK London WC2H9HE: Singapore.

Yamazaki F., Endo Y., Torii R., Sagawa S., and Shiraki K. (2000). Continuous monitoring of change in hemodilution during water immersion in humans: Effect of water temperature. *Aviation, Space, and Environmental Medicine*, 71: 632-639.

Yang B., Glenn A. J., Liu Q., Madsen T., Allison M. A., Shikany J. M., Manson J. E., Hang

K. K. C., Wen-Chih W., Li J., Liu S., and Kenneth L. (2022). Added Sugar, Sugar-Sweetened Beverages, and artificially sweetened beverages and risk of cardiovascular disease:

findings from the women's health initiative and a network meta-analysis of prospective studies. *Nutrients*, 14(20):4226.

Yu S., Li C., Ji G and Zhang L. (2021). The Contribution of dietary fructose to non-alcoholic fatty liver disease. *Frontiers in Pharmacology*, 12. Doi:10.3389/fphar.2021.783393

Yu Y., Shen M., Song Q., and Xie J. (2017). Biological activities and pharmaceutical applications of polysaccharide from natural resources: A review. *Carbohydrate Polymers*, 183: 91-101.

Zaitoun M., Maissam Ghanem M., and Harphoush S. (2018). Sugars: Types and their functional properties in food and human health. *International Journal of Public Health Research*, 6(4): 93-99.

Zhao H., Wu L., Yan., Chen Y., Zhou M., Wu Y., and Li Y. (2021). Inflammation and tumor progression: signaling pathways and targeted intervention. *Signal Transduction and Targeted Therapy*, 6(263): 1-46.

Reference site web

Ball J. (2022). Is sourdough bread healthy?. *Eating well*,

https://www.eatingwell.com/article/7904008/health-benefits-of-sourdough-bread-according-to-a-dietitian/

Blackwood M. (2022). Health benefits of sourdough bread. *Healthier steps*,

https://healthiersteps.com/health-benefits-of-sourdough-bread/

Lewis S. (2022). Liver Inflammation Guide. *Healthgrades*,

https://www.healthgrades.com/right-care/liver-conditions/liver-inflammation

Marengo K. (2019). Does sugar cause inflammation in the body?. *Medical News Today*.

https://www.medicalnewstoday.com/articles/326386

Marengo K. (2023). What are the benefits of nutritional yeast?. *Medical news today*,

https://www.medicalnewstoday.com/articles/323245

Robinson J. (2022). Remedies for inflamed Liver. *Webmd*,

https://www.webmd.com/a-to-z-guides/remedies-

inflamed-liver

Sieroslawska A. (2022). Liver Anatomy. *Kenhub*,

https://www.kenhub.com/en/library/anatomy/liver

1. https://www.nicepng.com/ourpic/u2e6y3e6w7e6a9a9_cho-map-polysaccharide-concept-map/
2. https://www.shutterstock.com/fr/image-vector/chemical-illustration-monosaccharides-glucose-fructose-galactose-2211684449
3. https://cdn.kastatic.org/ka-perseus-images/8a30b4ae79dc16796372df9286f45ed91daaa9e6.png
4. https://www.shutterstock.com/fr/image-vector/chemical-illustration-disaccharides-maltose-sucrose-lactose-2211686407
5. https://www.researchgate.net/profile/Angelo-Miguel/publication/283078312/figure/fig7/AS:614041743532042@1523410368355/Examples-of-the-main-soybean-oligosaccharides-raffinose-and-stachyose-derived-from.png
6. https://content.byui.edu/file/a236934c-3c60-4fe9-90aa-d343b3e3a640/1/module3/images/polysaccharides2.png
7. https://nutrition.org/inflammation-what-is-it-and-how-can-my-diet-and-behavior-affect-it/
8. https://www.hopkinsmedicine.org/health/conditions-and-diseases/liver-anatomy-and-functions
9. https://study.com/learn/lesson/properties-water-structure-density-molecules.html
10. https://fos.cmb.ac.lk/blog/freezes-faster-warm-water-cold-water/

Summary

When eating meals, the body gets enough energy from food, and the liver stores excess sugars, including glucose in the form of the most complex sugar, glycogen, to release it when needed.

Excessive consumption of sugars for long periods leads to many diseases, including high blood sugar and triglycerides, which lead to fatigue, hepatitis, and various diseases, including cirrhosis and non-alcoholic fatty liver disease, which leads to elevated liver enzymes and affects hormones.

In the present study, we evaluated *in vivo* the interaction of high consumption of refined crystallize sugar (200mg/kg) , hot water at 50°C and the traditional yeast on the liver during 21 days of experimental study in mice. This was evaluated by using the detection of blood sugar, lipids, CRP, creatinine, GSH concentrations and histological investigations of the liver.

The findings indicated that using hot water at a temperature of 50°C had the ability to lower the levels of CRP, T-ch levels which help to decrease the inflammation and also we detected a decrease in creatinine, ALAT and ASAT. The consumption of traditional yeast reduces CRP, creatinine and liver enzymes and increase the concentration of HDL-C. We have observed ameliorations on the liver tissue by the treatment with yeast and hot water.

We concluded that hot water at a temperature of 50°C and consumption of traditional yeast can be regarded as a natural preventative measure for reducing liver inflammation.

Keywords: inflammation, liver, reduced glutathione, lipids profile, ASAT, ALAT

Résumé

Lors des repas, le corps, reçoit suffisamment d'énergie des aliments et le foie stocke les sucres en excès, y compris le glucose sous la forme du sucre le plus complexe, le glycogène, pour le libérer en cas de besoin.

Une consommation excessive des sucres pendant de longues périodes entraîne de nombreuses maladies, notamment une glycémie élevée et des triglycérides, qui entraînent une fatigue, hépatite et diverses maladies, notamment la cirrhose et la stéatose hépatique non alcoolique, qui entraîne une élévation des enzymes hépatiques et affecte les hormones.

Dans notre étude, nous avons évalué *in vivo* l'interaction du sucre cristallisé et de l'eau chaude à 50°C et de la levure traditionnelle sur le foie pendant 21 jours de traitement chez les souris.

Ceci a été évalué en utilisant la détection de la glycémie, des lipides, de la CRP, de la créatinine, des concentrations de GSH et des investigations histologiques du foie.

Les résultats ont indiqué que l'utilisation d'eau chaude à une température de 50 °C avait la capacité d'abaisser les niveaux de CRP, les niveaux de T-ch qui aident à diminuer l'inflammation et aussi nous avons détecté une diminution de la créatinine, ALAT et ASAT. La consommation de la levure traditionnelle réduit la CRP, la créatinine et les enzymes hépatiques et augmente la concentration de HDL-C. Nous avons observé des améliorations sur le tissu hépatique par le traitement à la levure et à l'eau chaude.

Nous avons conclu que l'eau chaude à une température de 50°C et une consommation de la levure traditionnelle peut être considérée comme une mesure préventive naturelle pour réduire l'inflammation hépatique.

Mot clés: inflammation, le foie, glutathione réduit, profil lipidique, ASAT, ALAT

الملخص

عند تناول وجبات الطعام ، يحصل الجسم على الطاقة الكافية من الطعام ، ويقوم الكبد بتخزين السكريات الزائدة ، بما في ذلك الجلوكوز على شكل السكر الأكثر تعقيدًا ، الجليكوجين ، لإفرازه عند الحاجة.

يؤدي الإفراط في تناول السكريات لفترات طويلة إلى الإصابة بالعديد من الأمراض ، منها ارتفاع نسبة السكر في الدم والدهون الثلاثية التي تؤدي إلى الإرهاق والتهاب الكبد وأمراض مختلفة منها تليف الكبد ومرض الكبد الدهني غير الكحولي ، مما يؤدي إلى ارتفاع إنزيمات الكبد و التاثير على الهرمونات.

في هذه الدراسة ، قمنا بتقييم أثر استهلاك السكر والماء الساخن عند 50 درجة مئوية مع الخميرة التقليدية على أمراض القلب والأوعية الدموية خلال 21 يومًا في الفئران. تم تقييم ذلك باستخدام الكشف عن الجلوكوز في الدم ، الدهون ، الجلوثاثيون المختزل' الكريلتنين'البروتين سي التفاعلي و فحص قطاعات نسيجية على الكبد.

أشارت النتائج إلى أن استخدام الماء الساخن عند درجة حرارة 50 درجة مئوية له القدرة على تخفيض مستويات بروتين سي التفاعلي و الكولسترول الكلي مما يساعد على تقليل الالتهاب واكتشفنا أيضًا انخفاضًا في الكرياتينين و ALAT و ASAT.

استهلاك الخميرة التقليدية تقلل من بروتين سي التفاعلي والكرياتينين وأنزيمات الكبد و تعمل على زيادة تركيز HDL-C. لاحظنا تحسنًا في النسيج الكبدي من خلال العلاج بالخميرة والماء الساخن.

وهكذا توصلنا إلى أن الماء الساخن عند درجة حرارة 50 درجة مئوية و استهلاك الخميرة التقليدية يمكن ان يكون واقيا لالتهاب الكبد

الكلمات المفتاحية :التهاب' الكبد ' الجلوتاثيون المختزل 'الليبيدات' انزيمات كبدية

Annex

Annex

Treatment dose calculation

- Sugar given dose (200g/Kg)

200g ⟶ 65000g

X g ⟶ average weight of mice (g)

200g: the amount of sugar consumed by a person weighing 65Kg (65000g).

65000g or 65Kg: weight of person.

X g: the amount of sugar consumed at mice.

- Yeast given dose (50g/Kg)

50g ⟶ 65000g

X g ⟶ average weight of mice (g)

50g: the amount of yeast consumed by a person weighing 65Kg (65000g).

65000g or 65Kg: weight of person.

X g: the amount of yeast consumed at mice.

Preparation of the solutions:

- **Preparation of NaCl 0.9%**

0.9g NaCl ⟶ 100 ml distilled water.

- **Preparation of 10% formalin**

10 ml formalin (37%) + 27 ml distilled water.

- **preparation of ethanol**

Ethanol 25%: 25ml ethanol + 71ml distilled water.

Ethanol 60%: 60ml ethanol + 36ml distilled water.

Ethanol 75%: 75ml ethanol + 21ml distilled water.

Ethanol 96%: Used with the same focus.

- **Preparation of DTNB**

0.04g DTNB ⟶ 10ml ethanol (96%).

- **Preparation of TBS**

3.028g Tris + 4.383g NaCl ⟶ 500ml distilled water ⟶ pH= 7.4 (modified by HCl).

- **Preparation of Bradford**

0.19g Coomassie brilliant Blue G-250 ⟶ 50ml ethanol ⟶ 100ml ortophosphoric acid + 850ml distilled water.

- **Preparation of sulphosalic acid**

0.25g Sulphosalic acid ⟶ 100ml distilled water.

- **Preparation of Tris EDTA**

- 6.06g tris + 0.96g EDTA ⟶ 125ml distilled water ⟶ pH= 9.6

- **Preparation of Bouin alcohol**

1g picric acid ⟶ 45ml ethanol (25%) + 26ml formalin (37%) + 7ml acetic acid ⟶ 22ml distilled water.

- **Preparation of bovine serum albumin (BSA)**

5 mg BSA⟶ 5 ml distilled water

- **Preparation of hematoxylin**

1 g hematoxylin ⟶ 10 ml distilled water

- **Preparation of eosin**

2 g eosin ⟶ 100ml distilled water

- **Preparation of gelatin**

0.5 g gilatin ⟶ 100 ml distilled water

Table 05: composition of water used in the experimental study.

Composition	Mg/litre
Calcium	4.6
Magnesium	3.75
Potassium	1
Sodium	29
bicarbonates	48.8
Sulfates	10
Chlorides	30
Nitrates	9
Nitrites	0.06
R.S à 105 c°	140
pH	6.87

Table 06: composition of standard diet (ONAB).

Composition	Amount in g / kg	Percentage %
Corn	620	62
Soja	260	26
Phosphate	16	1,6
Limestone	9	0,9
Cellulose	10	1
Minerals	10	1
Vitamins	10	1

Table 07: calibration graph of BSA.

Tube	**1**	**2**	**3**	**4**	**5**	**6**
Distilled water (ml)	100	80	60	40	20	0
BSA (µl)	0	20	40	60	80	100
Bradford (ml)	5	5	5	5	5	5
DO	0	0.207	0.420	0.583	0.722	0.874

Academic year: 2022-2023	**Presented by :** Saidi Boutheina Mosbah Lina

The effect of hot water and traditional yeast on liver inflammation induced by refined crystallize sugar

Thesis presented for the obtention of the degree of master II

Summary

When eating meals, the body gets enough energy from food, and the liver stores excess sugars, including glucose in the form of the most complex sugar, glycogen, to release it when needed.

Excessive consumption of sugars for long periods leads to many diseases, including high blood sugar and triglycerides, which lead to fatigue, hepatitis, and various diseases, including cirrhosis and non-alcoholic fatty liver disease, which leads to elevated liver enzymes and affects hormones.

In the present study, we evaluated *in vivo* the interaction of high consumption of refined crystallize sugar (200mg/kg) , hot water at 50°C and the traditional yeast on the liver during 21 days of experimental study in mice. This was evaluated by using the detection of blood sugar, lipids, CRP, creatinine, GSH concentrations and histological investigations of the liver.

The findings indicated that using hot water at a temperature of 50°C had the ability to lower the levels of CRP, T-ch levels which help to decrease the inflammation and also we detected a decrease in creatinine, ALAT and ASAT. The consumption of traditional yeast reduces CRP, creatinine and liver enzymes and increase the concentration of HDL-C. We have observed ameliorations on the liver tissue by the treatment with yeast and hot water.

We concluded that hot water at a temperature of 50°C and consumption of traditional yeast can be regarded as a natural preventative measure for reducing liver inflammation.

Keywords : inflammation, liver, GSH, lipids profile, ASAT, ALAT

Laboratoire de recherche : Université des frères Mentouri-Constantine 1, laboratory of Immunology

President: ARIBI Boutheyna	(MCB - UFMC1).
Supervisor: ZERIZER Sakina	(Pr. UFM Constantine 1).
Examinator: MESSOUDI Saber	(MCB - UFMC 1).