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Dedication

I thank Allah almighty for having given me the privilege, the chance to study, follow the path of science and who granted me the ability to accomplish this work.

This thesis work is dedicated to my mother ***Hachou wassila*** *her never ending love., she have always loved and supported me.*

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KAOUTHER

Dédication

After the grace of allah

To my grandfather who once said to me before departing to the abode of truth:

"you will always succed"

and if they ask you from where you gained such confidence say:

“from my grandfather".

*To my parents **Zaimen Omar** and **Fekraoui Louiza** who have always been the solid wall and constant support , the warm refuge and dedicated heart , they have bequathed to me the ability to accepted challenges and hardships constantly strive for progress.*

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*To **my family** , to those who crafted wings for me to resist ,support me , and made me happy with all their capabilities , to the one who completed my joy ,I owe them everything .*

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ASMA

Dédication

I thank Allah almighty for giving me courage and will, in order to carry out this modest work that I dedicate:

To my parents, to whom I owe you all, you have been able to be there when it is necessary, I could never thank you for what you have done and done for me, if I am here today know that it is thanks to you.

To who the Rahman said:

(واخفض لهما جناح الذل من الرحمة وقل رب ارحمهما كما ربياني صغيرا)

To my mother, Source of wisdom, ambition and passion for all I am;

To my father, A pillar I had, have and will need until the last day of my life;

To my gazelle, From whom I learned to aim for the stars without carrying a gun; the best sister ever.

To my soulmate, who supported me body and soul, root of the brilliance of my mind, my source of perseverance;

To those who left us years ago;

To my partners, For their endless support, encouragement and dedication;

Finally, anyone who helped me from near or far in the development of this work.

RAZANE

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List of abbreviations

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AGP acid glyco protein

ATP adenosine triphosphate

C control

CCL3 chemokine ligand 3

CCR7 chemokine receptor 7

CRP C-reactive protein

CD crohn disease

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

Gi gastro-intestinal

HFCS high-fructose corn syrup

HDL high-density lipoprotein

HW Hot water

IBD inflammatory bowel disease

ICAM intercellular adhesion molecule-1

IFN ɣ interferon gamma

IL- α interleukin alpha

IL- β interleukin beta

IL-1 β interleukin 1 beta

IL-1 interleukin1

IL-10 interleukin 10

IL-12 interleukin 12

IL-13 interleukin 13

IL-17 interleukin 17

IL-2 interleukin 2

IL-4 interleukin 4

IL-5 interleukin 5

IL-6 interleukin 6

IL-8 interleukin 8

IL-9 interleukin 9

LDL low-density lipoprotein

mCRP monomeric C-reactive protein

MMPs matrix metallo protéinases

NAD+ nicotinamide adenine dinucleotide

NADH+ nicotinamide adenine dinucleotidehydrogen

NAFLD non alcoholic fatty liver disease

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 pollymorphonuclear cells

PRRs pathogen recognition receptors

ROS reactive oxygen species

S sugar

TANs tumor associated neutrophils

TGF- β transforming growth factor - beta

THP-1 tamm-Horsfall Protein 1

TLRs toll like receptors

TNF-α tumor necrosis factor-alpha

TY traditional yeast

VCAM vascular cell adhesion molecule

VCAM-1 vascular endothelial cell adhesion molecular-1

VEGEF vascular endothelial growth factor

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Introduction

Introduction

Sugars are a collective term for several different chemical species contains a mixture of variable compounds (**Birdem, et al., 2020)**. They are including on the term of carbohydrates a major source of energy diet, and divided into “monosaccharide, disaccharide “, “polyols”, “polysaccharides” **(Annie, et al., 2015).** Specially, “monosaccharide and disaccharide act as sweeteners in many foods, as well as natural preservative **(Kacper, et al., 2022).**
-It has been postulated that dietary sugar consumption contributes to increased inflammatory processes in humans, and that this may be specific to fructose alone, in sucrose or in high-fructose corn syrup (HFCS) **(Karen, et al., 2018).**

Both hyperglycemia and excessive sugar intake disrupt the intestinal barrier **(Javier, et al.,2018),**thus increasing gut permeability and causing profound gut micro biota dysbiosis, which results in a disturbance in mucosal immunity that enhances infection susceptibility **(Javier et al., , 2018).**

as well as is known the intestinal mucosal barrier is the layer of intestinal epithelium ,and mucous layer that protects the human body from the luminal contents of the gut **(Matthew, et al., 2021).**Where the intestinal health is related to the richness and diversity of intestinal microorganisms **(Qiangjun, et al., 2019).**

Furthemore , high-fat diets are not only associated with metabolic inflammation but also with intestinal inflammation **(Stefani, et al., 2019),** thus pathophysiology of intestinal inflammation is multifactorial **(Mike, et al., 2008).**

The drinking hot water had a favorable impact on intestinal movement **(Jothi, 2020),** drinking hot water helps to break down food faster than drinking cold or warm water. It reduces the risk of constipation by supporting regular bowel movements **(Deborah & Zawn, 2017)**

while the use of sourdough fermentation could be considered as an adjuvant to enhance the recovery from intestinal inflammation of coeliac patients at the early stage of the gluten-free diet **(Maria, et al., 2012).**

In the present research we aimed to:

- Evaluate the impact of hot water and traditional yeast on the weight and dietary of rats.

- Examine the influence of hot water and traditional yeast on the excessive intake of refined sugar by analyzing the levels of T-Ch, HDL-c, LDL-c, TG, and liver enzymes (ASAT and ALAT).
- Evaluate the impact of hot water and traditional yeast on inflammation caused by the high consumption of refined sugar by measuring C-reactive protein levels (CRP).
- Analyse the correlation between excessive intake of refined sugar and the intestinal inflammation by the histological investigation on the colon.

Chapter I: sugar and inflammation

Chapter I

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 **(Margaret 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 **(Barbara and Judith, 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)**.simple carbohydrates are molecules consistingnantly of three basic elements of carbon, oxygen and hydrogen with the empirical formula CH_2O **(Aldairi et al., 2008)**.

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

Structurally they are poly-functional compounds. They contain two types of functional groups- carbonyl and hydroxy. They may be poly-hydroxy aldehydes or poly-hydroxy 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 chains come in different lengths **(Raven et al., 2014)**.Carbohydrates are classified into monosaccharide, disaccharides, oligosaccharides, polysaccharides (Figure2)**(Asif et al., 2011)**.Simple carbohydrate refers to monocchacaride 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

Monosaccharide:

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

They are poly hydroxy aldehydes or polyhydroxy ketones which cannot be decomposed by hydrolysis to give simpler carbohydrates.e.g. Fructose, Glucose, Galactose etc...(Figure 3).

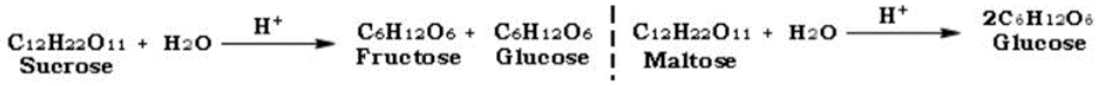
Sugars are also named according to their number of carbons, some of the most common types are trioses (three carbons), pentos (five carbons), and hexoses (six carbons) (Figure4) **(Mondal, 2017)**.

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 5) **(Howard and wylie-Rosett,2002)**.

They yield two monosaccharide molecules on hydro-lysis.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 04) **(Ahnen et 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., 2018)**.

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

They have higher molecular weight, which yield many monosaccharide molecules on hydrolysis. E.g. Starch,dextrin, Cellulose, glycogen etc (Figure 05) **(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 et al., 1985)**.

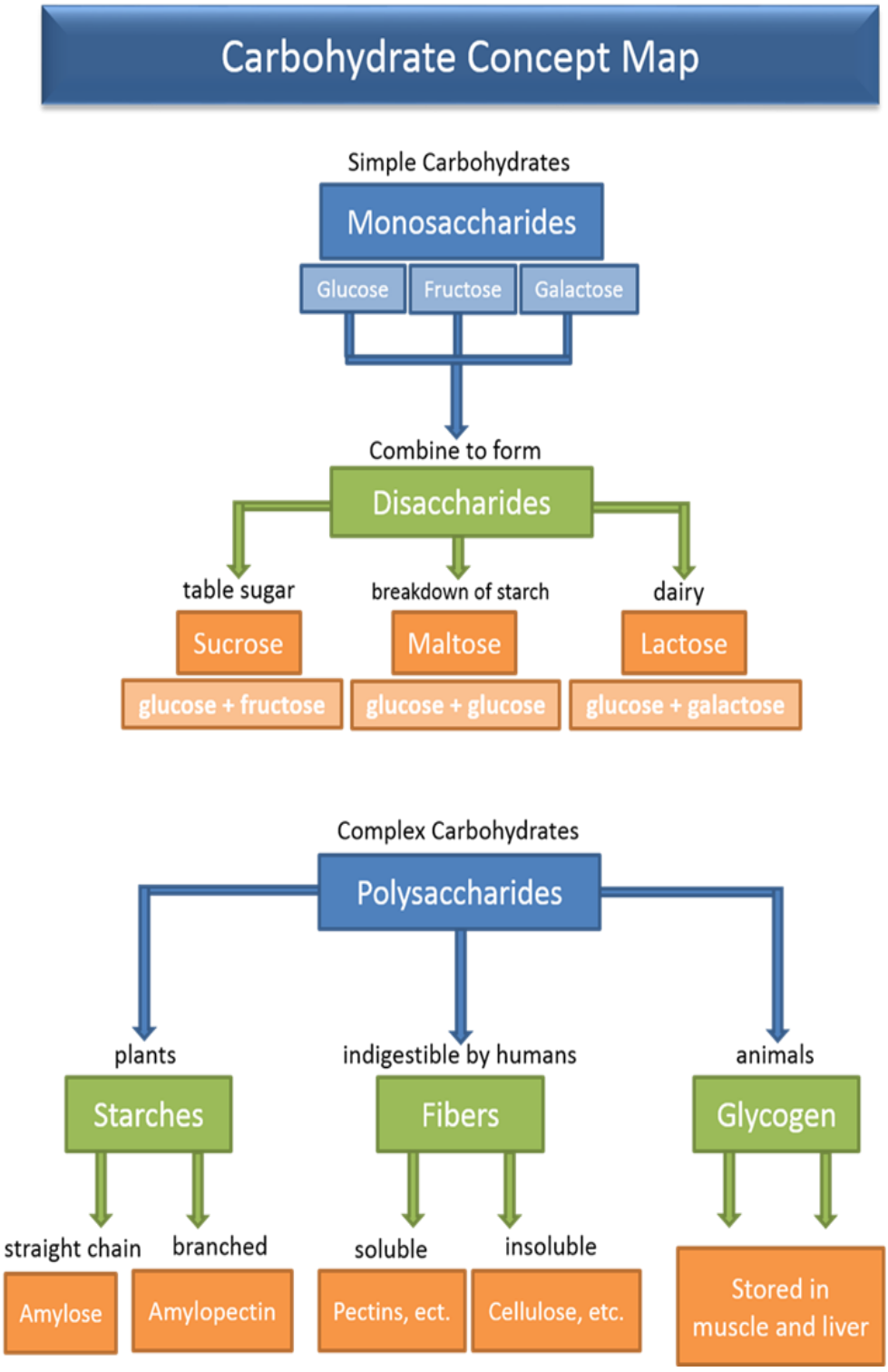


Figure 01: carbohydrates map (site web 01).

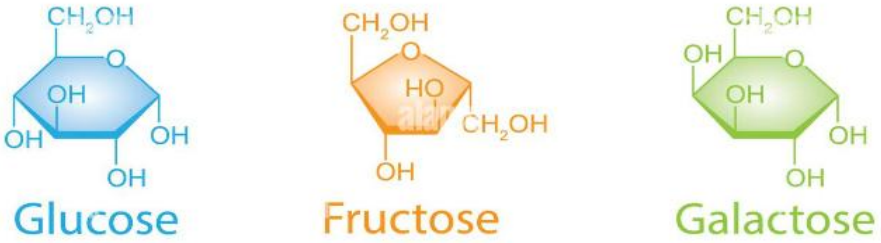


Figure 02: linear and ring structures of three common monosaccharide. All have the same molecular formula (C6H12O6), but they have different structures (red) and are therefore isomers of each other (site web 02).

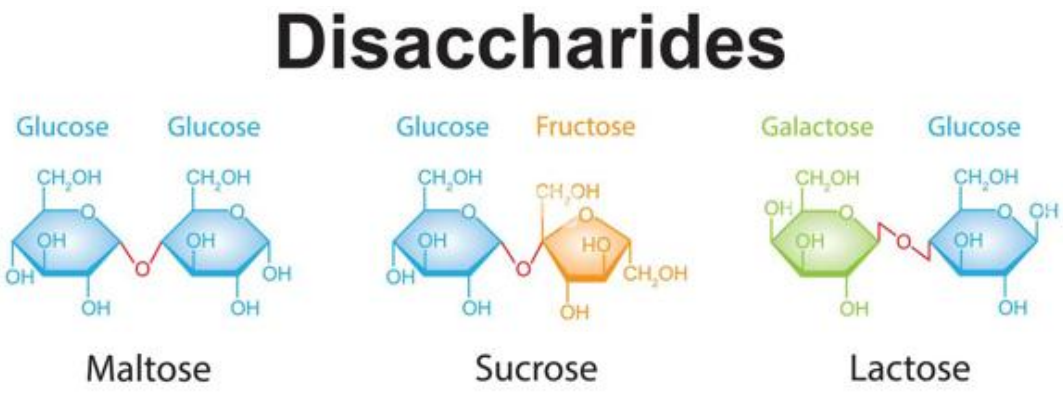


Figure 03: the structures of the three common disaccharides. All contain glucose as one of their subunits; the difference between the three is the second subunit.

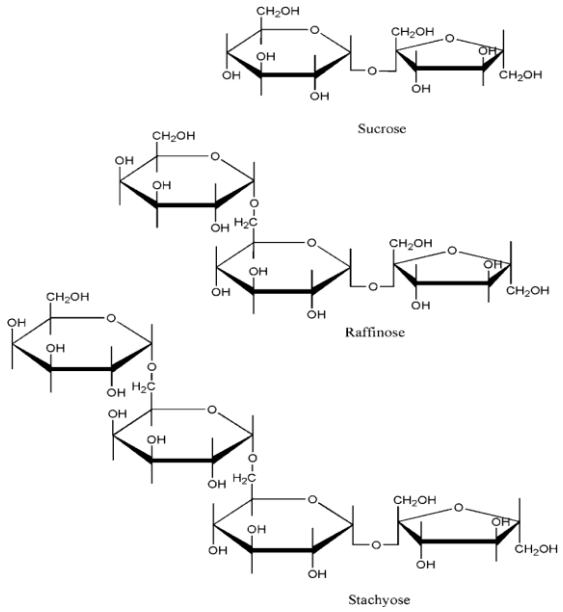


Figure 04: oligosaccharides, liaisons glycosidiques, 8-10 monosaccgarides (3)

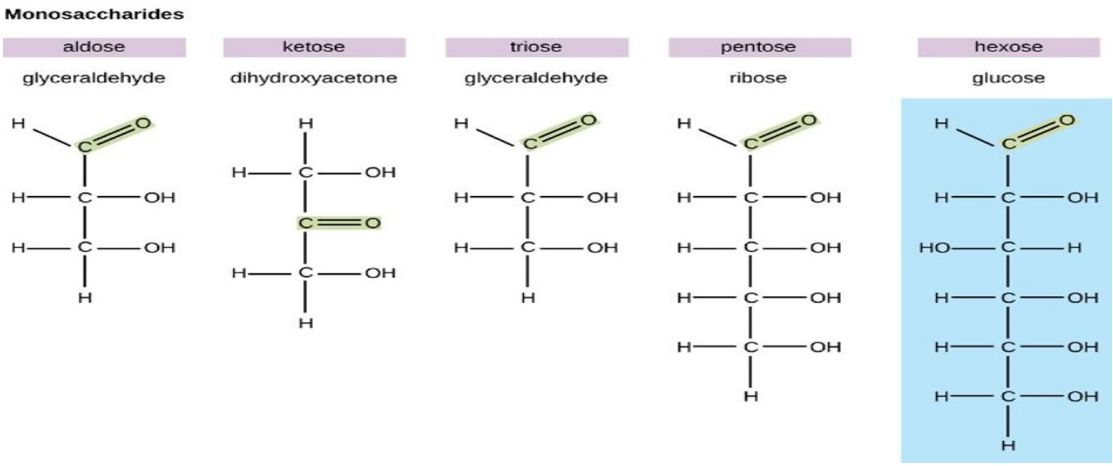


Figure 05: monosaccharides are classified based on the position of the carbonyl group and the number of carbons in the backbone (4).

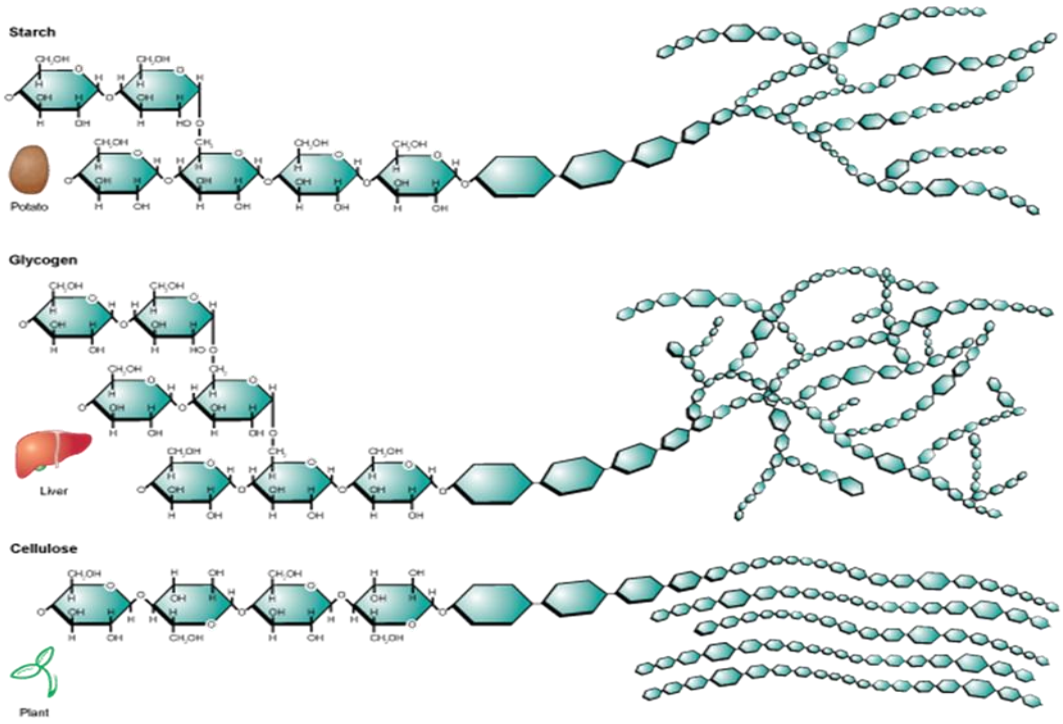


Figure 06: the structures of the three common polysaccharide: starch, glycogen, cellulose (site web 05)

I.4.Sugar and inflammation

High sugar intake has long been recognized as an environmental risk factor for increased incidence of many non-communicable diseases; including obesity, cardiovascular disease, metabolic syndrome, and type 2 diabetes. it induces 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 8) **(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 **(Zhaol 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 and Hamilton, 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 01)**.

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 tuberculosis **(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 diagnoses of sepsis **(Meisner, 2014)**.
Serumamyloid 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 pro-inflammatory and anti-inflammatory mechanisms. Cytokines which have pro-inflammatory 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)**.
Alpha-1-acid glycoprotein	AGP glycoforms are very useful in the detection of intercurrent infections in the course of rheumatoid arthritis, systemic lupus

	erythematous, or myeloblasticleukaemia, and in the detection of secondary infections in human immunodeficiency virus infected individuals and differentiation between various forms of trophoblastic disease **(Mackiewiczand Mackiewicz, 1995)**.
Plasma viscosity	The plasma viscosity is rising in the presence of proteins produced in response 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 **(Angelo, 2013)**.
Haptoglobin	Acute phase protein induced by inflammation, which can bind hemoglobin and act as an anti-oxidant **(Wang et al., 2001)**.

II.3. Stages of inflammation

In response to tissue injury, a multi-factorial 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, 2017)**.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 induced vasodilatation is mediated primarily by nitric oxide (NO) and vasodilatory prostaglandins **(Sherwoodand Toliver-kinsky, 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 metallo proteinases (MMPs) **(Coussens and Werb, 2002)**.

A family of chemical cytokines, called chemokines, has 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)**.

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

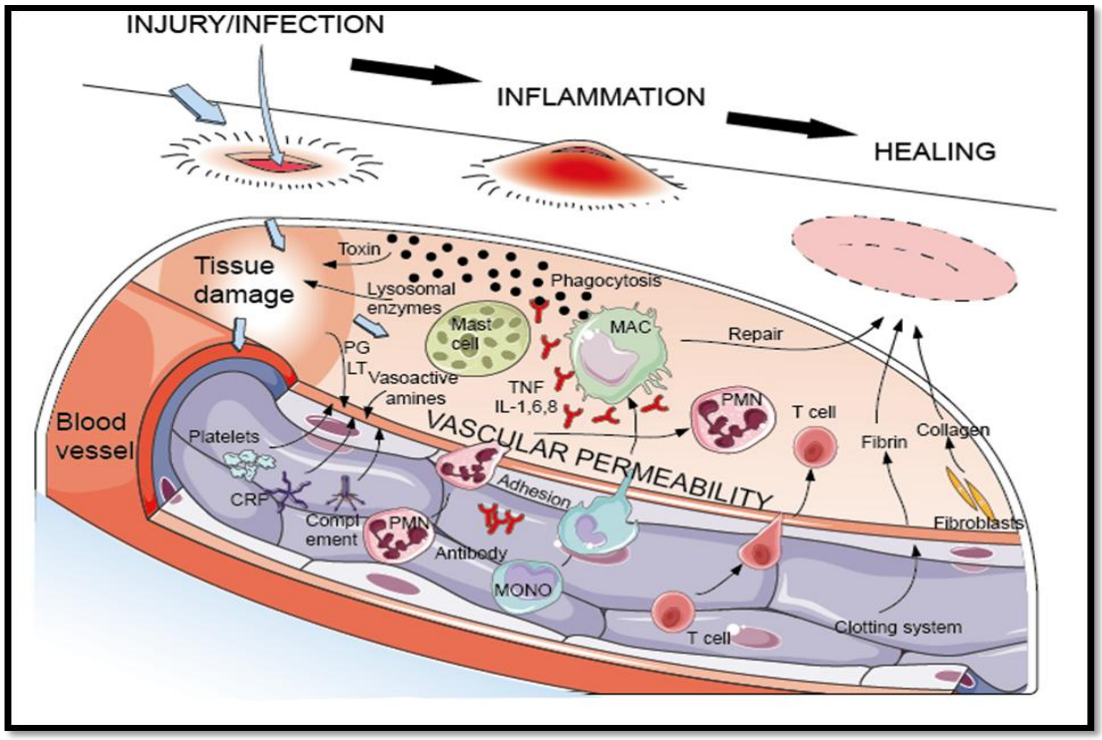
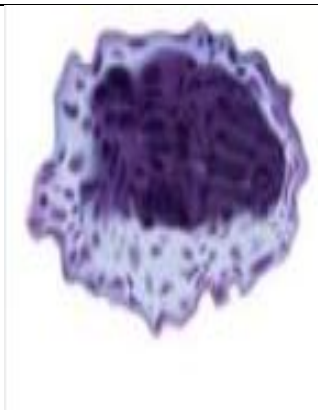


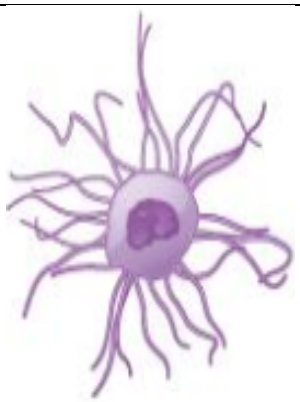
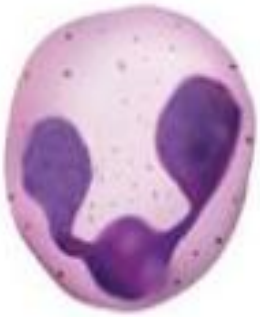
Figure 07: showing signs of inflammation and the stages of response **(Dimitratos, 2018)(6)**.

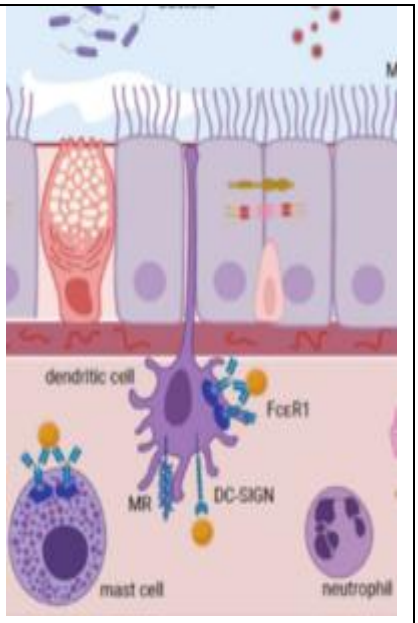
II.3.4. Cells of inflammation

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

Cells	Functions	cell structures
mastcells	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 **(Theoharis 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. Super activate T cells through TNF **(Theoharis 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:** respond 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, immune-regulation **(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 interferon. 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 non-self-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)(Wrightet al., 2010)**.TANs are pro-inflammatory and anti-tumorigenic.	

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 duration 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 vasodilatation, extravasations of leukocytes, and release of multiple plasma components, has been particularly well worked up in the field of invasion by micro-organisms. 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 pro-inflammatory cytokines, which activate downstream pro-inflammatory signaling **(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)**, characterized 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 then 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 are 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 **(Vermine et al., 2004)**.

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 **(Nehring et al. 2017)**.

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 07) **(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 An 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 10) **(Shrivastava et al., 2014)**.

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 **(Shrivastava et al., 2014)**.

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

Reported that CRP increases plasminogen activator inhibitor-1(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 (**Davis et al. 2012)**.

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)**.

Hs-CRP correlates with extent of atherosclerosis, and high triglyceride and BMI is closely associated with high hs-CRP levels in patients with dyslipidemia **(Swastini et al., 2019)**.

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 09) **(Pradhan et al., 2001)**.

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 pro-inflammatory cytokines **(Du, 2000)**.

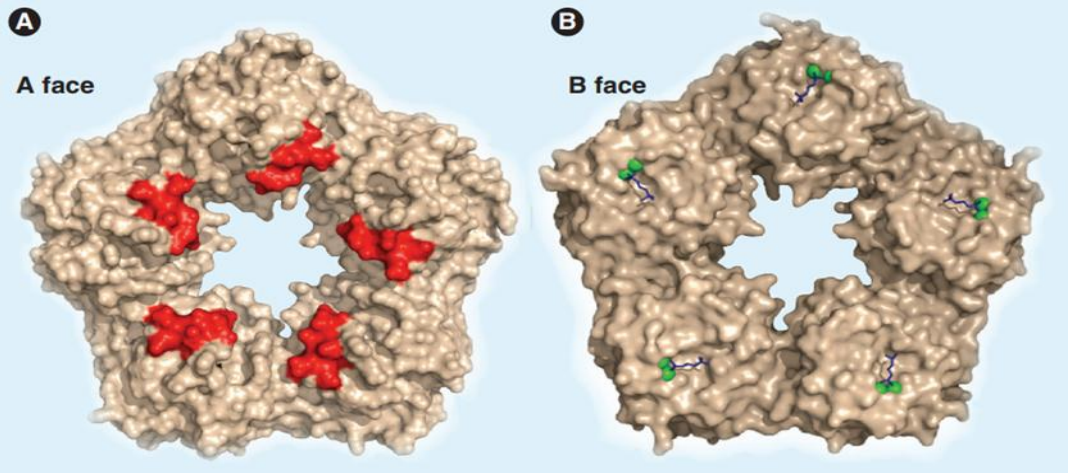


Figure 08: pentameric structure of C-reactive protein (CRP). (A) Space - filling model of the A face (receptor and C1q binding) of the CRP pentamer with the ridge helix in red. (B) Spacefilling model of the CRP molecule showing a phosphocholine molecule (blue) with the two calcium molecules (green) located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

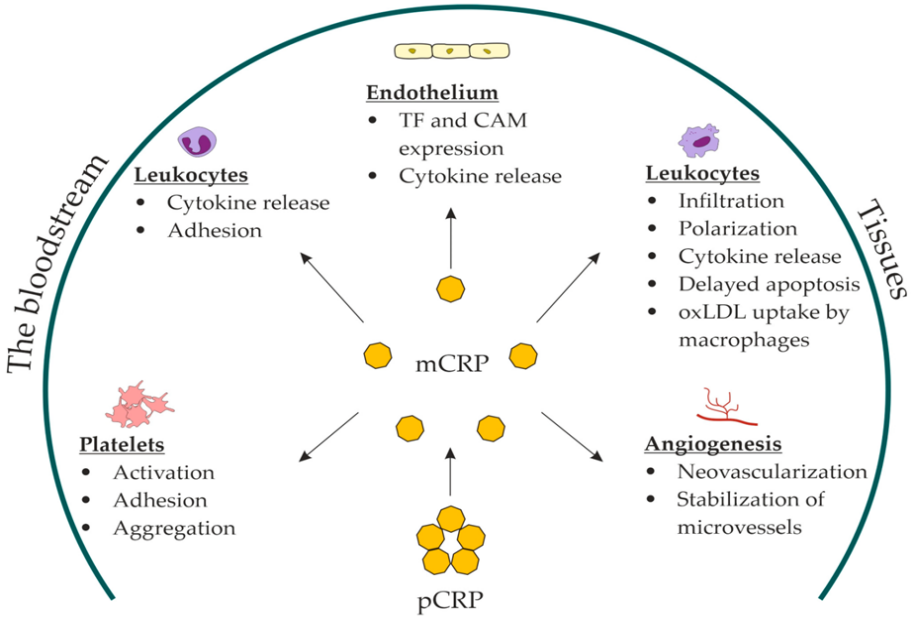


Figure 09: proposed roles for mCRP in atherosclerosis. mCRP, monomeric C-reactive protein; pCRP: pentameric C-reactive protein. TF: tissue factor. CAM: cell-adhesion molecules. oxLDL: oxidized low-density lipoproteins **(Melnikovet al., 2023)**.

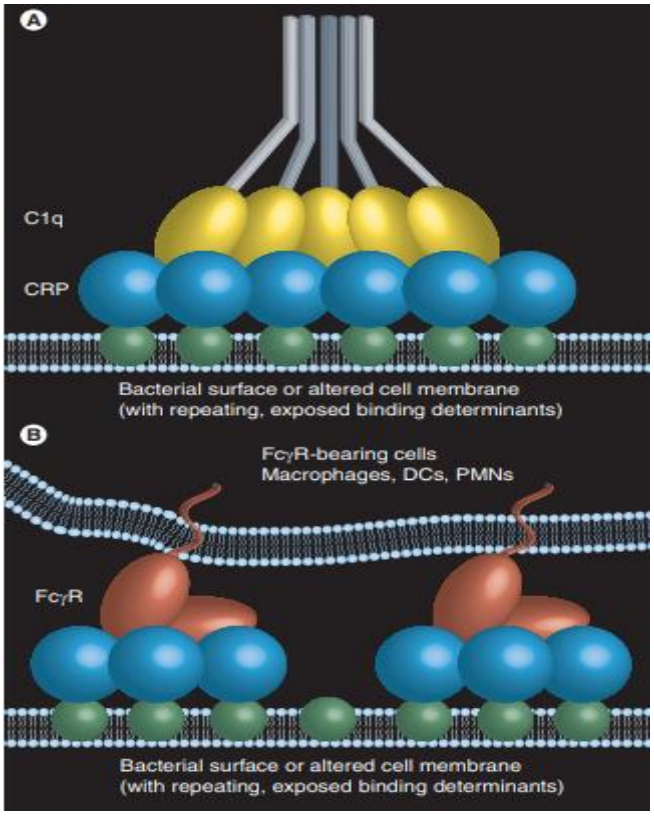


Figure 10: CRP bound tobacterial or altered cell surfaces. CRP binds to a surface on its B-face phosphocholine-binding site, leaving the A face exposed. This allows each pentamer to bind either (A) one of the six globular heads of C1q leading to the activation of the classical complement cascade or;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

Chapter II

Sugar and intestinal inflammation

II. Sugar and intestinal inflammation

II. 1. Intestine

II.1.1 Small intestine:

The small intestine is a crucial component of the digestive system that allows for the breakdown and absorption of important nutrients that permits the body to function at its peak performance **(Lopez et al., 2022).** the small intestine accomplishes this via a complex network of blood vessels, nerves, and muscles that work together to achieve this task **(Chaudhry et al., 2022).** it is a massive organ that has an average length of 3 to 5 meters **(Kahai et al., 2022).**

II.1.1.1 Anatomy of the small intestine

The small intestine extends from the distal end of the pyloric canal to the ileocaecal junction and consists of the duodenum, jejunum, and ileum (Figure 11) **(Susan et al .,2019)**.

II.1.1.1.A Duodenum

The duodenum is the shortest section, on average measuring from 20 cm to 25 cm in length.Its proximal end is connected to the antrum of the stomach, separated by the pylorus, and the distal end blends into the beginning of the jejunum. The duodenum surrounds the pancreas, in the shape of a "C" and receives chyme from the stomach, pancreatic enzymes, and bile from the liver; this is the only part of the small intestines where Brunner's glands are present on histology (Figure 12)

II.1.1.1.B Jejunum

The jejunum is roughly 2.5 meters in length, contains plicae circulares (muscular flaps), and villi to absorb the products of digestion.

II.1.1.1.C. Ileum

The ileum, or terminal section, is about 3 meters long and ends at the ileocaecal valve, which controls the flow of material from the ileum to the caecum, the first part of the large intestine, and prevents regurgitation **(Waugh et al., 2010).**

II.1.1.2 Histology of small intestine

The histological structure of the small intestine is similar to the other organs in the digestive tract. there are four main layers: **(Turiccki., 2023)** (Figure13)

- **Mucosa**: contains the epithelium, lamina propria and muscularis mucosae.
- **Submucosa**: connective tissue layer, which contains blood vessels, lymphatics and the submucosal plexus.
- **Muscularis externa** : consists of two smooth muscle layers; the outer longitudinal layer and inner circular layer. The myenteric plexus lies between them.
- **Adventitia:** comprised of loosely arranged fibroblasts and collagen, with the vessels and nerves passing through it. The majority of the small intestine adventitia is covered by mesothelium and is commonly called the serosa.

Cells of the Epithelium

The epithelium of the small intestine lines the luminal surface. There are a number of components to the epithelium **(Turiccki., 2023) :**

- **Enterocytes** : tall columnar cells, which have an absorptive function. They contain brush border enzymes on the surface which have an important digestive function.
- **Goblet cells** : exocrine glands which secrete mucin.
- **Crypts of Lieberkuhn** : the Crypts of Lieberkuhn are glands found in the epithelial lining. They contain numerous cells such as stem cells to produce new cells to replenish the cells lost due to abrasion, as well as **entero endocrine** cells to synthesis and secrete hormones in response to various stimuli. There are four main classes of entero endocrine cell, each with a different secretory product. These are **I cells, S cells, K cells and enterochromaffin cells. (Turiccki., 2023).**
- To protect from pathogens, there are **paneth cells** which secrete protective agents (such as defensins and lyzozymes) and **peyer's patches** which are only found in the ileum. Peyer's patches contain mucosal-associated lymphatic tissue (MALT) which house white blood cells and lymphocytes. These cells can produce antibodies to further protect the small intestine from infection **(Turiccki., 2023).**

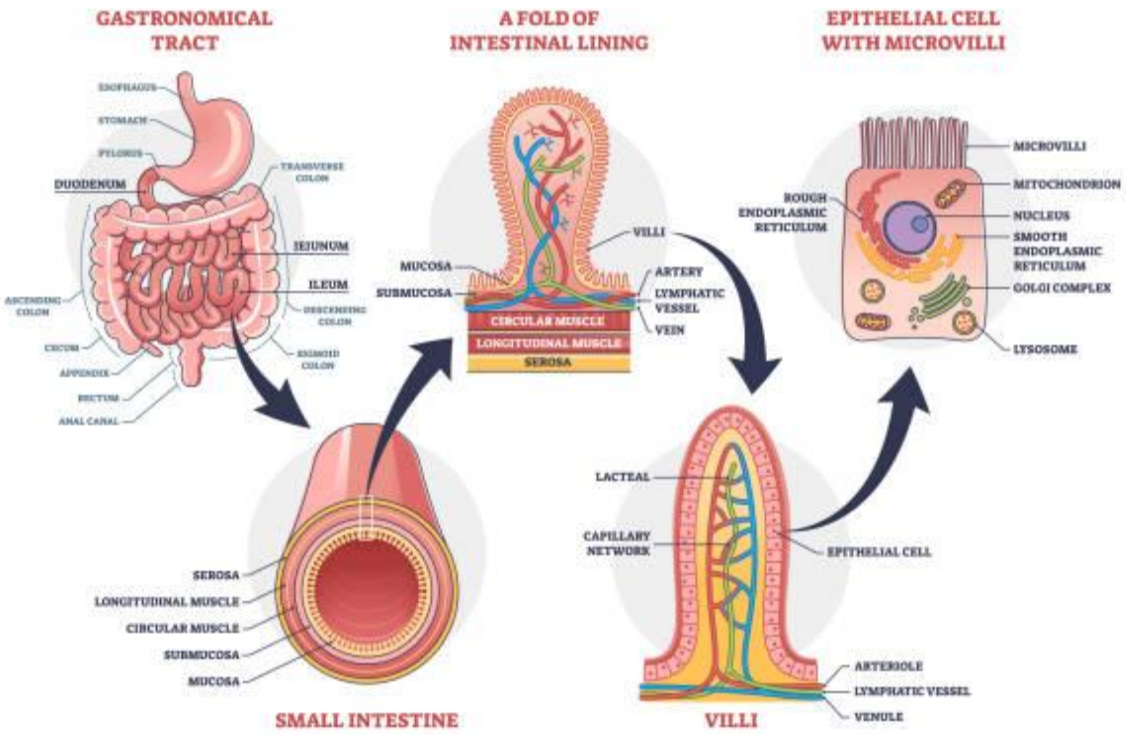


Figure 11: anatomy of the small intestine (7)

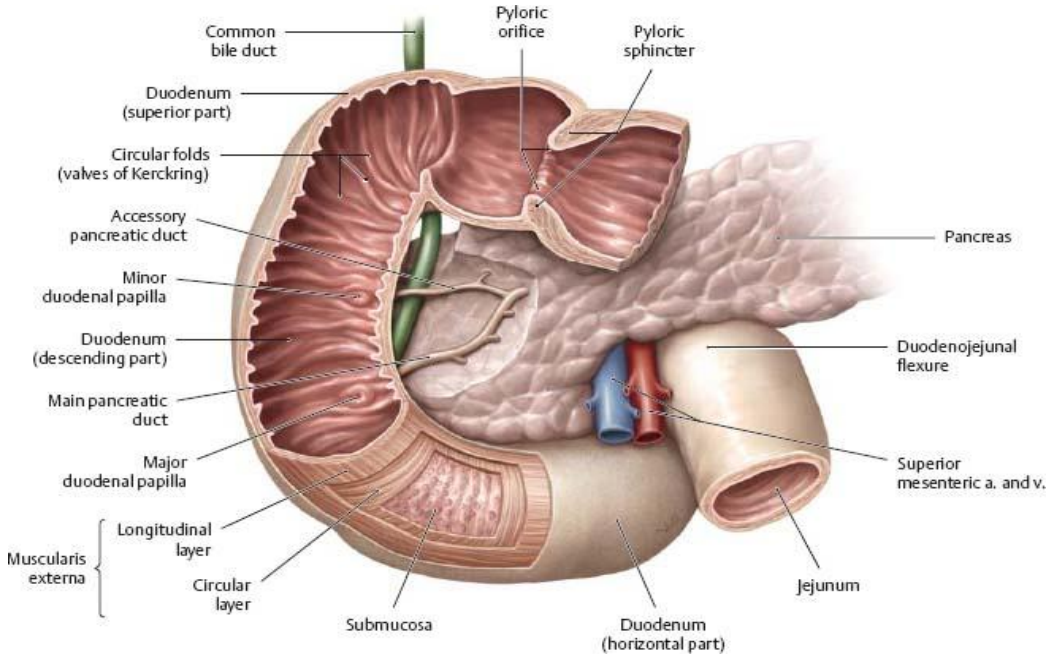


Figure 12: duodenum: Anterior view with the anterior wall opened (8)

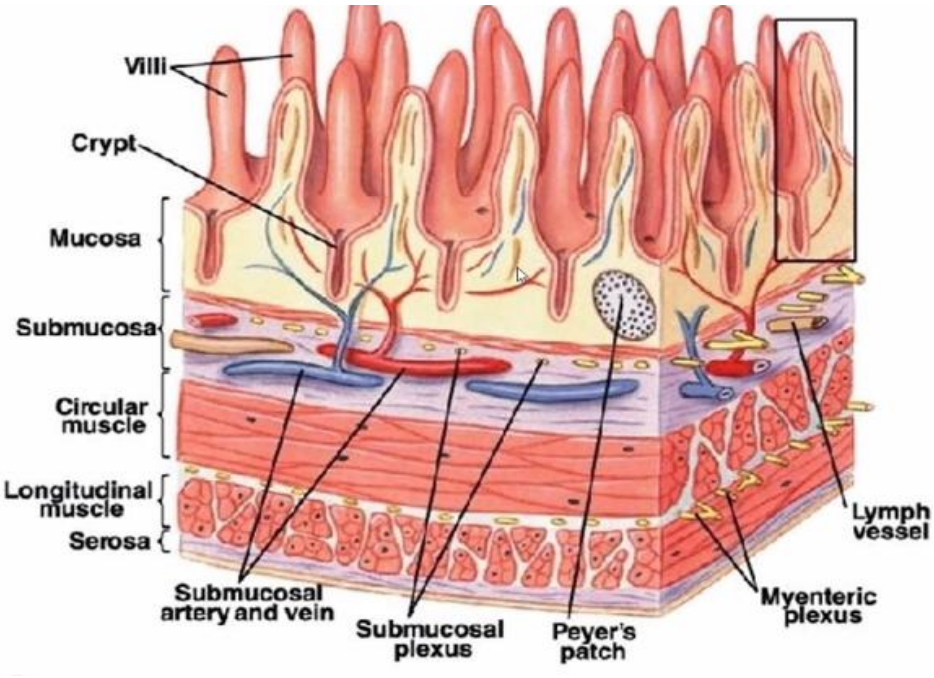


Figure 13: small intestine histology (9)

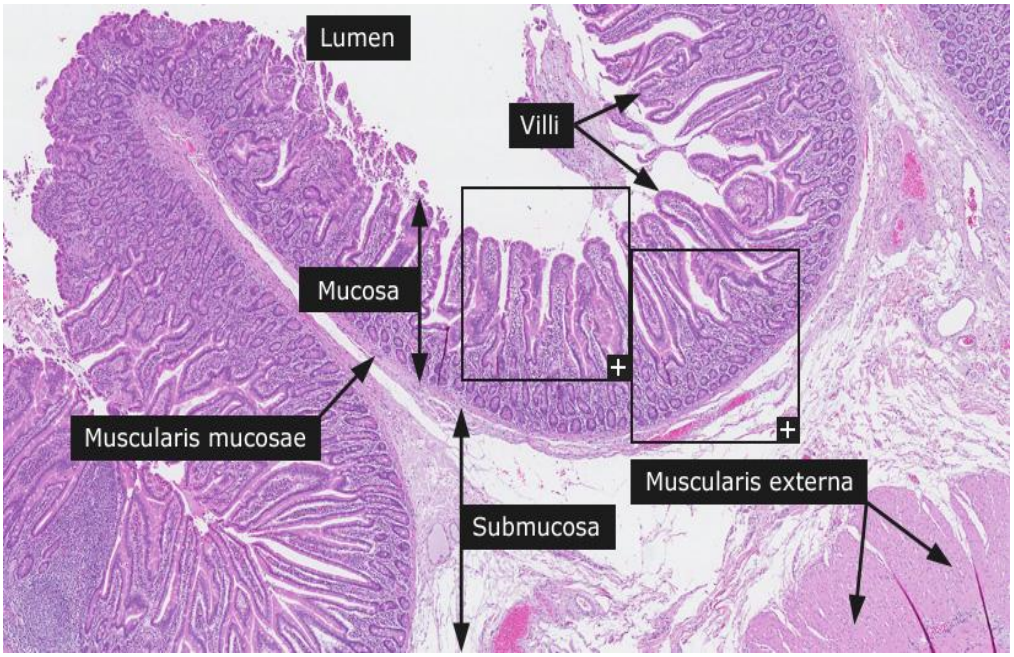


Figure 14: microscope view histology of the small intestine (10)

II.1.2 Large intestine (Colon)

The large intestine is about 1.5 meters long, beginning at the caecum in the right iliac fossa and terminating at the rectum and anal canal deep in the pelvis. Its lumen is about 6.5 cm in diameter, larger than that of the small intestine. It forms an arch round the coiled-up small intestine **(Waugh et al., 2010).**

II.1.2.1 Anatomy of the large intestine

For descriptive purposes the large intestine is divided into the caecum, colon, sigmoid colon, rectum and anal canal (Figure 15) **(Waugh et al., 2010).**

II.1.2.1.A Caecum

This is the first part of the large intestine. It is a dilated region which has a blind end inferiorly and is continuous with the ascending colon superiorly. just below the junction of the two the ileocaecal valve opens from the ileum. The vermiform appendix is a fine tube, closed at one end, which leads from the caecum. It is usually about 8 to 9 cm long and has the same structure as the walls of the large intestine but contains more lymphoid tissue. **(Waugh et al., 2010).**(Figure16)

II.1.2.1.B Colon

The colon has four parts which have the same structure and functions.

Ascending colon or right colon

This passes upwards from the caecum to the level of the liver where it curves acutely to the left at the hepatic flexure to become the transverse colon. **(Waugh et al., 2010).**

Transverse colon

This is a loop of colon that extends across the abdominal cavity in front of the duodenum and the stomach to the area of the spleen where it forms the splenic flexure and curves acutely downwards to become the descending colon **(Waugh et al., 2010)**.

Left colon or descending colon

Runs along the left side of the abdomen for 15–20 cm proximal to the sigmoid **(Poitras et al., 2022).**

Sigmoid colon

The sigmoid is an S-shaped colonic segment, often smaller in size and of variable length (15–50 cm) **(Poitras et al., 2022).**

II.1.2.1.C Rectum

The *rectum* extends 12–15 cm from the anus to the rectosigmoid angle. The posterior rectum, with its mesorectum, rests against the sacrum, while the anterior wall faces the pelvic organs (bladder, uterus). The rectum is located outside the peritoneum, except for its anterior part which is covered by visceral peritoneum up to 5–10 cm from the anus **(Poitras et al., 2022).**

II.1.2.1.D Anal canal

This is a short passage about 3.8 cm long in the adult and leads from the rectum to the exterior. two sphincter muscles control the anus; the internal sphincter, consisting of smooth muscle, is under the control of the autonomic nervous system and the external sphincter, formed by skeletal muscle, is under voluntary control **(Waugh et al., 2010).**

II.1.2.2 Histology of large intestine

The caecum and colon wall was composed of the four tunica (mucosa, submucosa, muscularis externa and serosa or the adeventitia. **(Ahmed et al., 2018)**.

The large intestine differs from the small intestine in the following important ways: villi are absent in the large intestine; the microvilli of the large intestine epithelial cells are much less abundant; goblet cells are more prominent in the large intestine; endocrine cells are less prom

inent in the large intestine; and crypt to epithelial migration is a much slower process in the large intestine. **(Washabau, 2013).**

the tunica mucosa is the inner layer that lined the cecum and colon lumen and didn't revealed villi. the tunica mucosa of cecum and colon was included three different layers;the lining simple columnar epithelium with large number of goblet cells, the lamina propria composed of loose connective tissue and several crypts of Lieberkuhn and smooth muscle of the muscularis mucosa . **(Ahmed et al., 2018)**

submucosa consists of loose connective tissue, thin smooth muscle bundles, nerve plexuses (Meissner plexus and henle deep submucosal plexus) with ganglion cells, stromal cells, adipose tissue and vasculature. **(Patil et al., 2021)**

The muscularis externa of the large bowel is composed of an inner circular layer and an outer longitudinally running layer of smooth muscle that condenses into three longitudinally running taeniae coli, the mesocolic taenia and two antimesenteric taeniae. the taeniae unite at the base of the vermiform appendix. They flare at the rectum and incorporate into its external muscular layer. The inner and outer layers of muscularis externa are separated by the myenteric plexus of Auerbach. The villi of subserosal fat typically hang from the large bowel to form the epiploic appendices. **(Robert et al., 2009)**

Subserosa and serosa: subserosa is composed of fibroadipose tissue and is covered by the serosa lined by cuboidal mesothelial cells. **(Patil et al., 2021)**

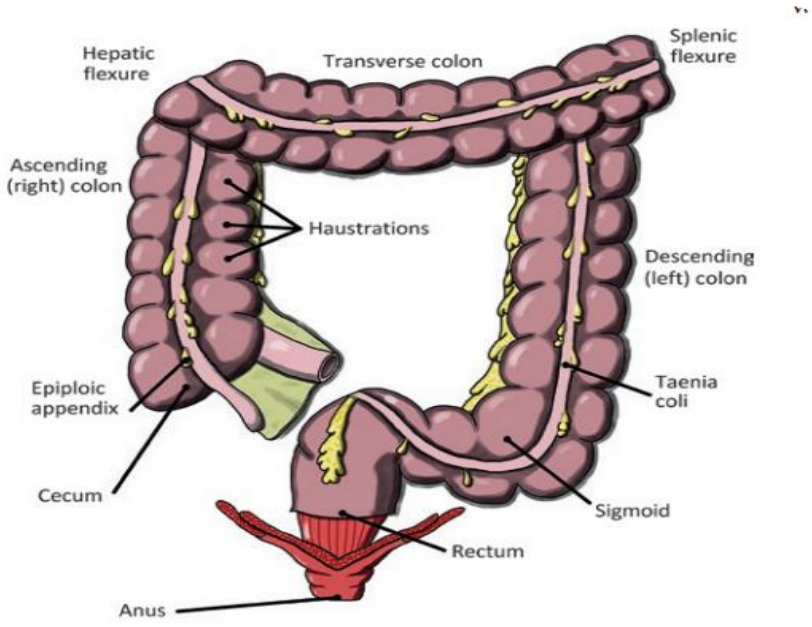


Figure 15: the Anatomy of the human colon (anterior view) **(Poitras et al., 2022)**

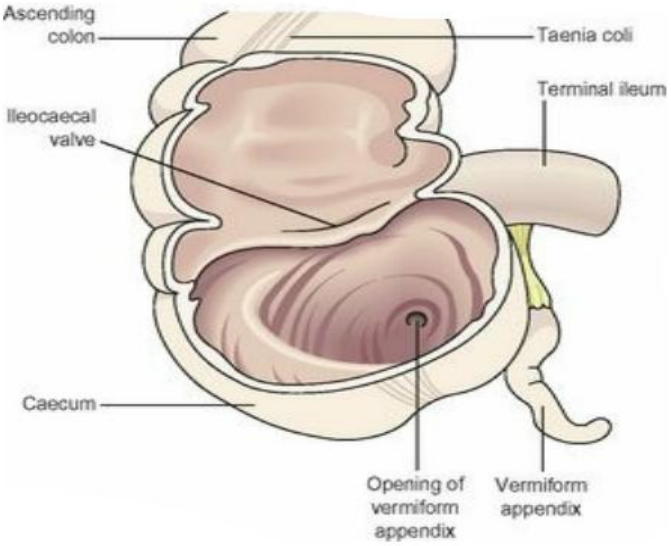


Figure16: interior of the caecum. **(Waugh et al., 2010)**.

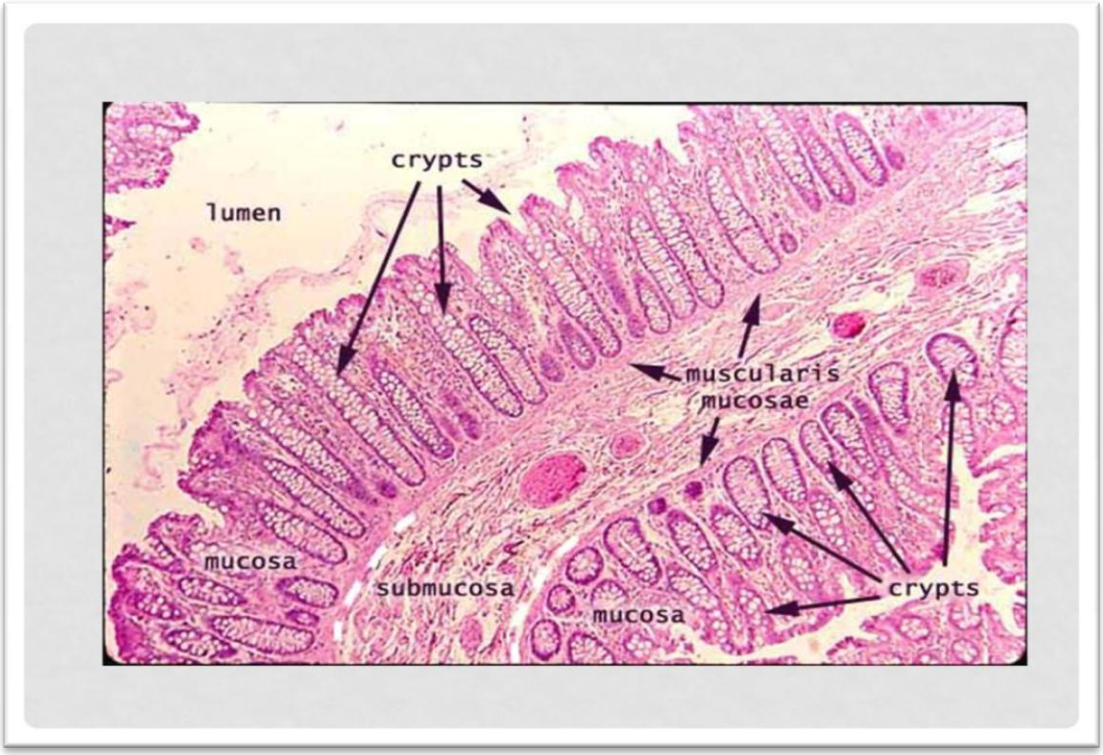


Figure17: histological section of large intestine (11)

II.2. Intestinal inflammation

II.2.1 Definition

Inflammatory bowel disease (IBD), Crohn's disease and ulcerative colitis, are characterized by uncontrolled inflammation in the intestine. While the pathogenesis of Crohn's disease and ulcerative colitis remain incompletely understood, recent advances reveal several key processes that are involved **(Diegelmann et al., 2016).** (Figure18)

II.2.2 Examples for intestinal inflammations

II.2.2.1 crohn's disease

Crohn's disease (CD) is an inflammatory bowel disease (IBD), and it is characterized by different events within time which are in relation to heterogeneity of the disease. This heterogeneity depends on the age of appearance. When the inflammatory process isn't controlled, it can lead to chronic inflammation, thickening of intestinal walls, ulcers, and noticeable symptoms. **(Giuseppe et al., 2015)** (Figure19)

Even though Crohn's disease, it can affect any part of the gastrointestinal (GI) tract from the mouth to the anus. It can also cause symptoms outside the GI tract, including the eyes, skin, and joints.

II.2.2.2 Ulcerative colitis

Ulcerative colitis is a type of inflammatory bowel disease (IBD). It is characterized by continuous and diffuse inflammation that is limited to the colonic mucosa and extends proximally from the rectum. The disease develops most often in the second or third decade of life. The classic symptoms are bloody diarrhea, abdominal pain, fecal urgency, and/or tenesmus **(Danese et al., 2011)** (Figure20)

II.2.3 Cause of intestinal inflammation

The risk of developing IBD has long been recognized to have a genetic contribution. Recent genome-wide association studies (GWAS) have identified more than 160 loci that are linked to IBD susceptibility. while genetic studies indicate the involvement of key regulatory pathways that are associated with risk variants they also reveal that risk alleles, by themselves, are not sufficient to result in inflammation or clinical disease. dysregulation of the innate and adaptive immune systems directed against luminal gut microbiota or their

products, and immune responses to commensal and pathogenic microbiota are the result of altered intestinal permeability and mucosal barrier function (**Diegelmann et al., 2016)**.

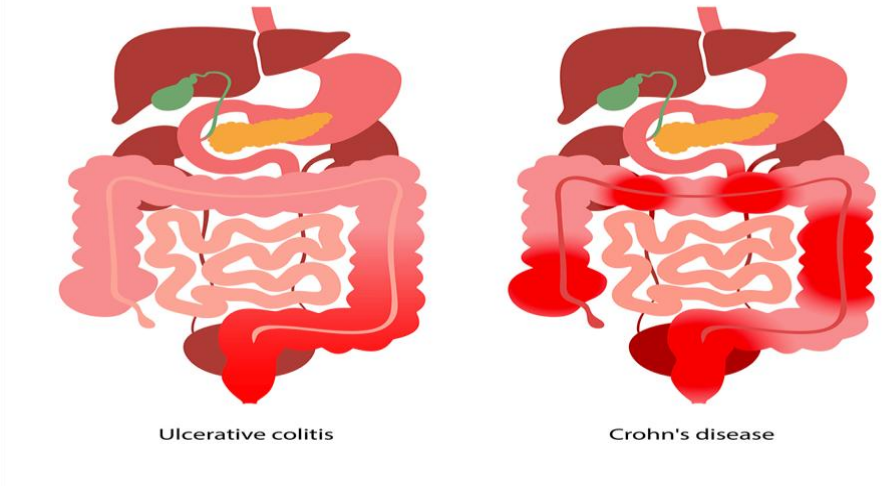


Figure18: inflammatory bowel disease

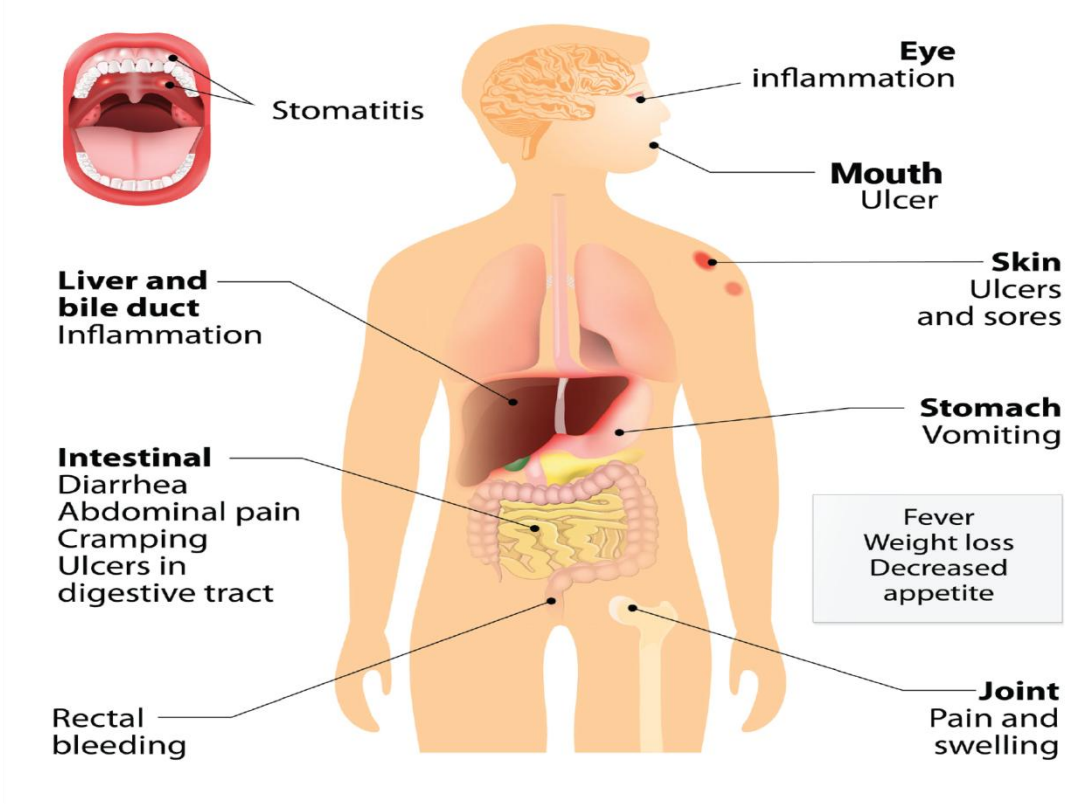


Figure19: signs and symptoms for crohn's disease(12)

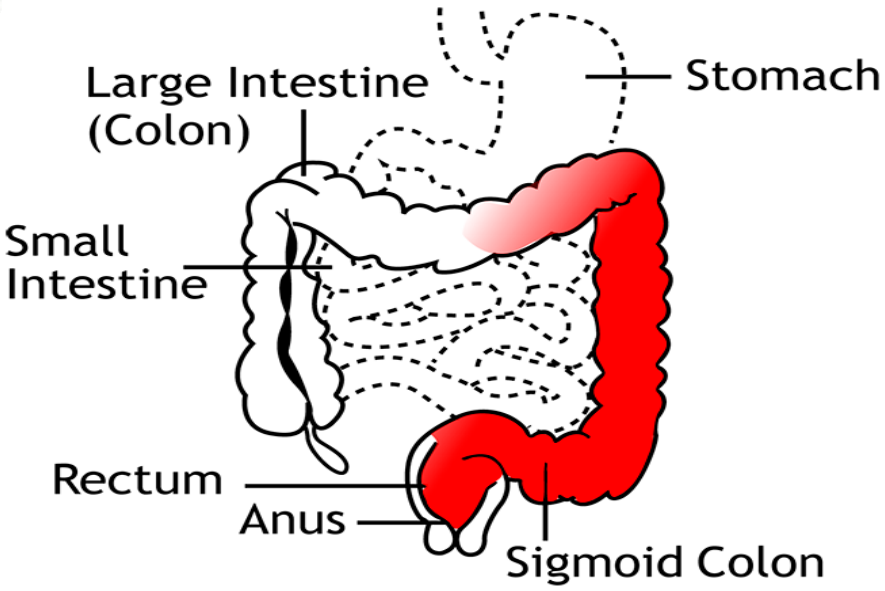


Figure20: site of ulcerative colitis **(Nunes, 2013)** (13)

Chapter III: hot water and traditional yeast

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 **(Sawkaet 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 **(Xiao, 2014)**. 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 18) **(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 19) **(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)**.

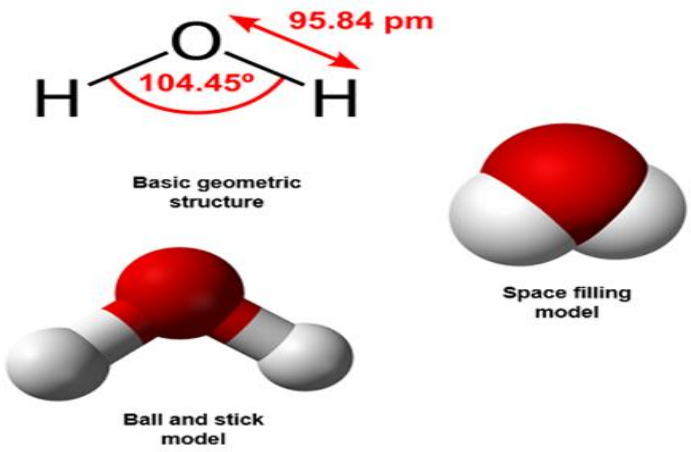


Figure21: water molecules

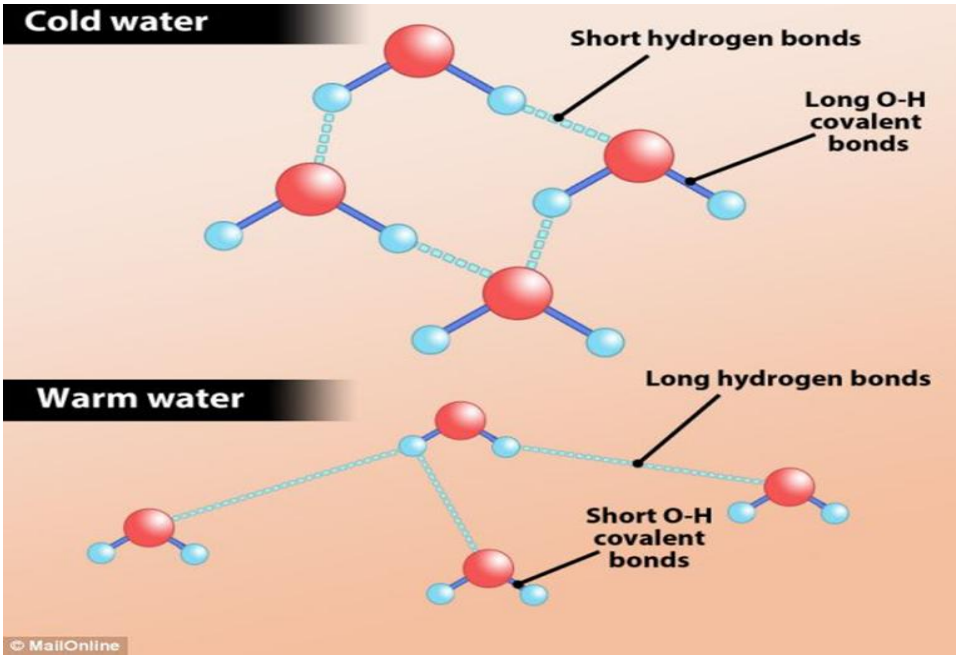


Figure22: cold and warm water structure (14)

III.1.3. Hydrotherapy

III.1.3.1. Definition

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 **(Devcate 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)**.

Tabel 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 or sitz bath, steam bath, steam inhalation, and full immersion bath.
Uses Of hydrotherapy	For pain relief, for circulationm 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 0detoxification 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. Yeasts sourdough

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 20) **(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)**.

III.2.3. Formulation yeasts

Sourdough starter can be considered as a mixture of water and flour fermented by yeasts and bacteria (Figure 22° **(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 19) **(De et al., 2021)**.

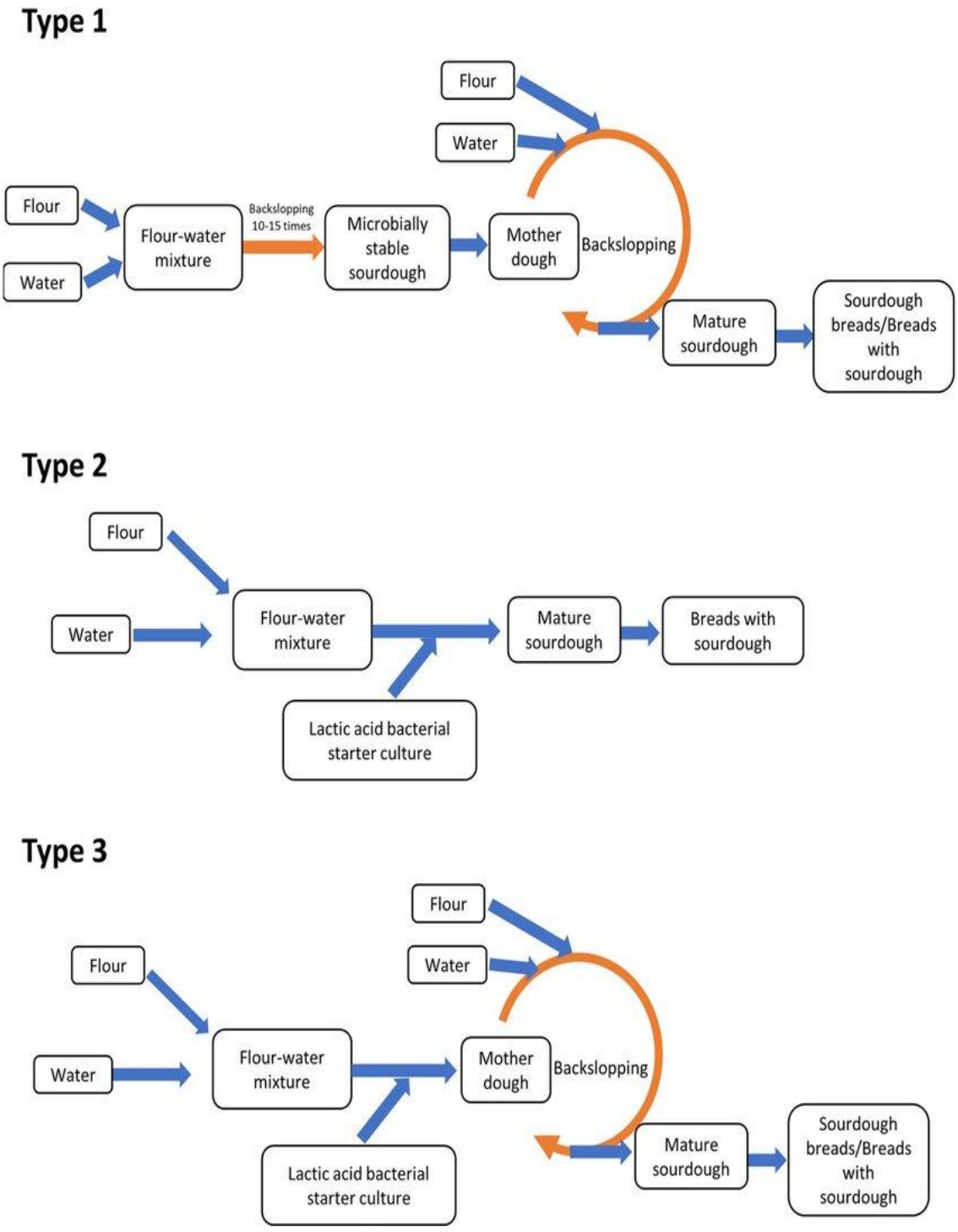


Figure 23: types of sourdough starter: various types of sourdough production processes based on the way of inoculation of the flour-water mixture **(De et al., 2021)**.

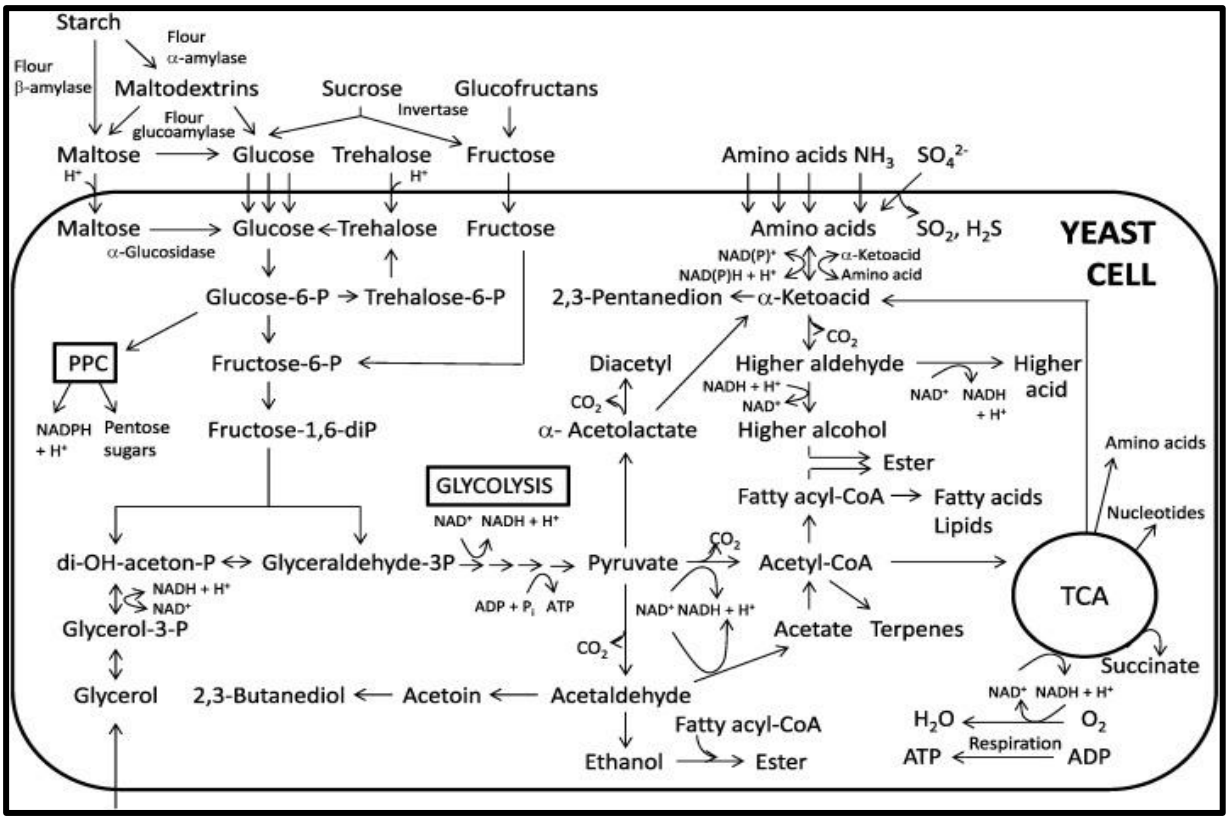


Figure 24: overview of the metabolism of yeasts in a sourdough matrix **(De et al., 2021)**.(15)

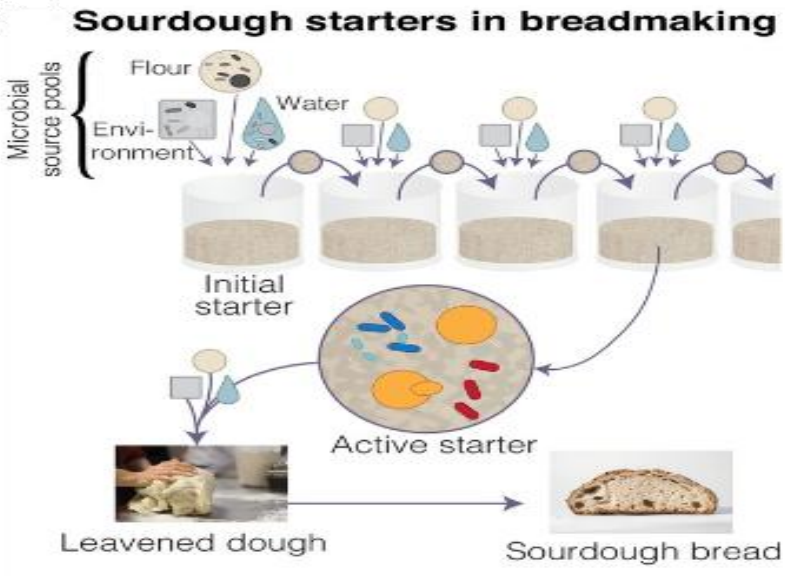


Figure 25: sourdough starters in breadmaking **(Landis et al., 2021)**

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, 2023)**.

Material and Methods

IV.Material and methods

IV.1. Material

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 (25%, 60%, 70%, 95% and 96%), HCl, NaOH, NaCl, butanol, xylene, paraffin and glycerin, acetic acid, heamatoxylin eosin, NaH_2PO_4, Na_2HPO_4, Coomassie Brilliant Blue G-250.

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 (Figure27).

Figure26: materials used during experimental work.

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.

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 abino*Mus Musculus Albino* 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. 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 standad diet and drunk hot water at fifty degrees.

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

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

Group sugar + yeast (SY, G6): group was fed on standard diet rich in sugar with traditional yeast and drinking water at room temperature (Table 4). The diet and weight were measured every day at the same time during 21days of treatment.

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 (Y)	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 (Y+S)	Normal water Standard diet+ Sugar	6	21	125ml / day 120 g / day 200g/65kg/ day

	Yeast			50g/65kg/ day

IV. 2.2 Blood and tissue sampling

After 21days of treatement, blood samples were collected after fasting the animals from the 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 for the experimental scientific research. Then, organs used for histological analysis (the intestine) were quickly removed, rinsed with saline solution (0.9%), and fixed in formalin 10%, the rest of the liver are stored in the freezer without rinsing them with a saline solution at-20°c for the dosage of the antioxidant (GSH).

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 -20°C until the determination of proteins 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 amount of absorption 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 samples is determined from the calibration graph (Figure 27) (Tabel 07).

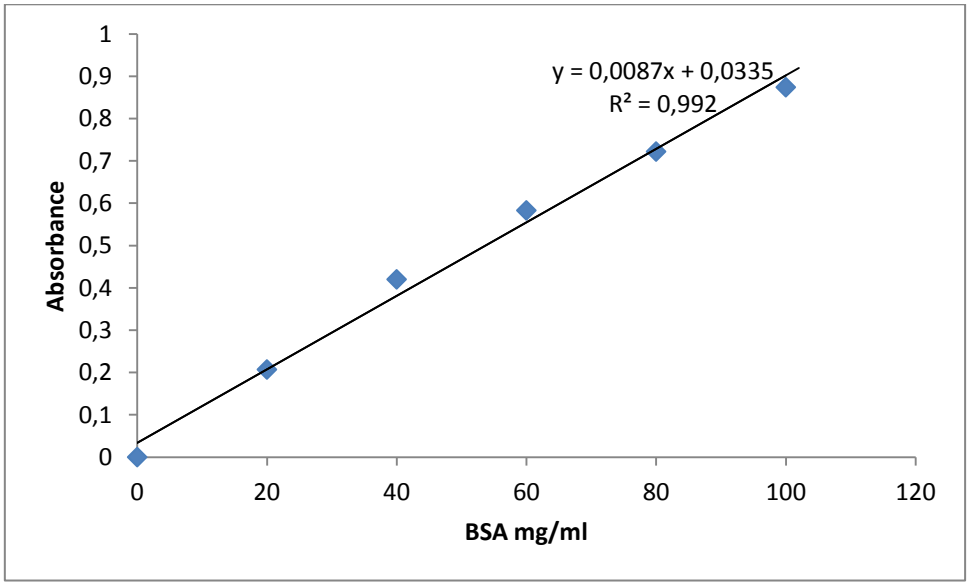


Figure 27: Calibration graph

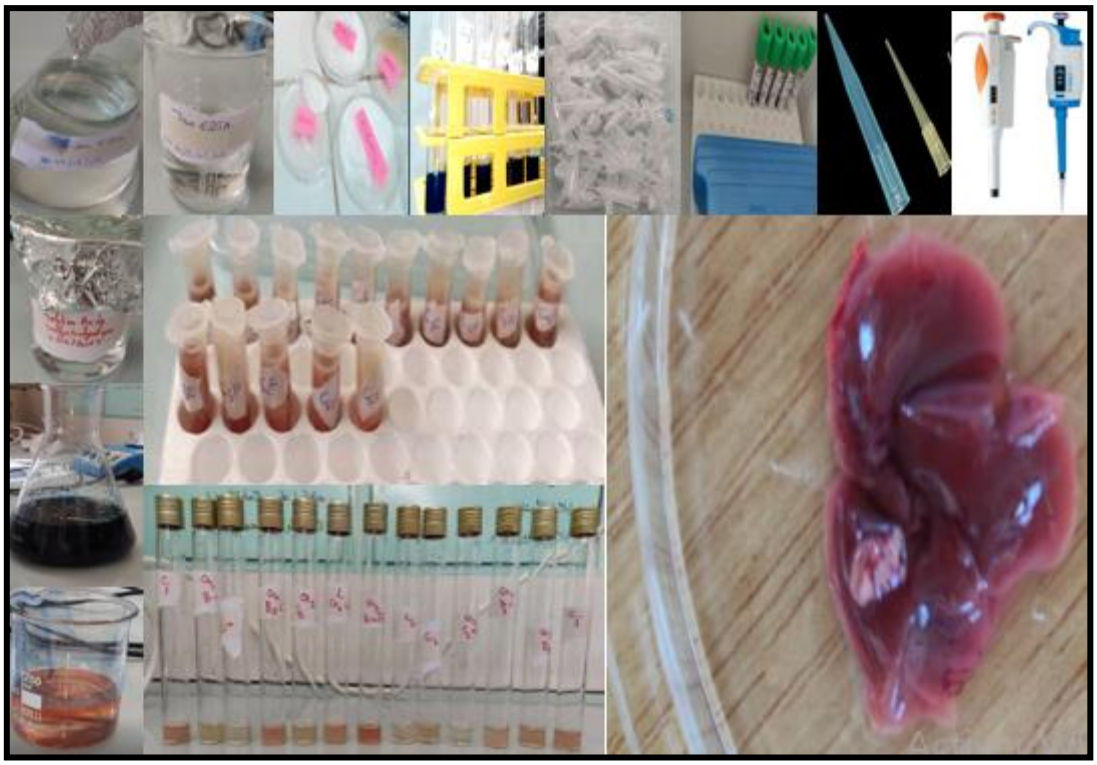


Figure 28: materials and solutions used in protein and reduced glutathione determination

IV.2.4. Preparation of histological sections

Fixation

The intestine 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, samples were placed into paraffin molds and then into tissue cassettes. Then the cut was made with a thickness of 5 µm using a microtome.

Coloring stage

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

The samples immersed in ethanol (96%) for 1 minute, then placed in two xylene baths for 2 minutes each.

Samples are dried in the oven at a temperature of 38 degrees. After this stage, the samples are ready for viewing under a microscope.

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

1.1. First experience (Group C)

The objective of this experiment is to evaluate the effect of normal food on the weight of mice and the consumption of food.

1.1.1. Change in 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 (Figure 29).

1.1.2. Food consumption

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 (Figure 30).

1.2. Second experience (Group S)

The objective of this experiment is to evaluate the effect of food rich in crystallized sugar on mice.

1.2.1. Change in weight

Our results demonstrated that in the group administrated with crystallize sugar in the first and the third week was (35.81g±0.41) (36.07g±1.44) respectively (Figure 29).

1.2.2. Food consumption

Our results demonstrated that in the group administered with crystallize sugar, the food consumption in the first week was (42.57±28.05) and third week was (99.57g±18.54), the food consumed by mice is increased respectively (Figure 30).

1.3. Third experience (Group HW)

The objective of this experiment is to evaluate the effect of hot water in mice fed a normal food.

1.3.1. Change in weight

Our results demonstrated that in the group of mice treated with hot water in the first week the weight was (33.01g±0.71) and in the third week was (33.17g±0.74) (Figure 29).

1.3.2. Food consumption

Our results demonstrated that in the group of mice treated with hot water, in the first week the Food consumption was (63.57g±18.97) and in the third week was (82.71g±29.09) (Figure 30).

1.4. Fourth experience (Group TY)

The objective of this experiment is to evaluate the effect of traditional yeast in mice

1.4.1. Change in weight

Our results demonstrated that in the group administered with traditional yeast, in the first week the weight was (30.50g±1.74) and in the third week was (33.13g±2.32) (Figure 29).

1.4.2. Food consumption

Our results demonstrated that in the group administered with traditional yeast, in the first week the food consumption was (84.14±19.11) and in the third week was (76.41±42.05) (Figure 30).

1.5. Fifth experience (Group S+Hw)

The objective of this experiment is to evaluate the effect of hot water in mice fed a high-crystallized sugar diet.

1.5.1. Change in weight

Our results demonstrated that the weight is increased in the group administered with crystallized sugar and treated with hot water, in the first week was (33.60g±0.99) and in the third week was (34.11g±3.01) (Figure 29).

1.5.2. Food consumption

Our results demonstrated that the food consumed by mice is increased in group administered with crystallized sugar and treated with hot water, in the first week was (80.14g±183.30) and in the third week was (92.42g±28.98) (Figure 30).

1.6. Sixth experience (Group S+TY)

The objective of this experiment is to evaluate the effect of traditional yeast in mice fed

a high-crystallized sugar diet.

1.6.1. Change in weight

Our results demonstrated that the weight is increased in the group administered with crystallized sugar and treated with traditional yeast, in the first week was (29.27g±2.25) and in the third week was (31.60g±1.89) (Figure 29).

1.6.2. Food consumption

Our results demonstrated that the food consumed is decreased in group administered with crystallized sugar and treated with traditional yeast, in the first week was (68.71±29.43) and in the third week was (42.83±41.03) (Figure30).

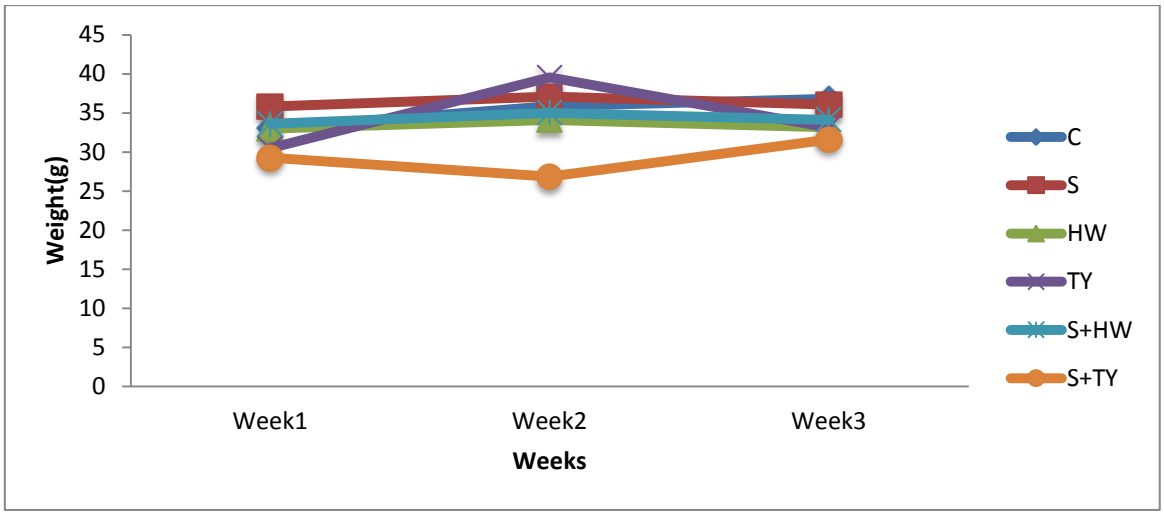


Figure 29: The effect of crystallize sugar, hot water and traditional yeast on the weight in mice during 21 days

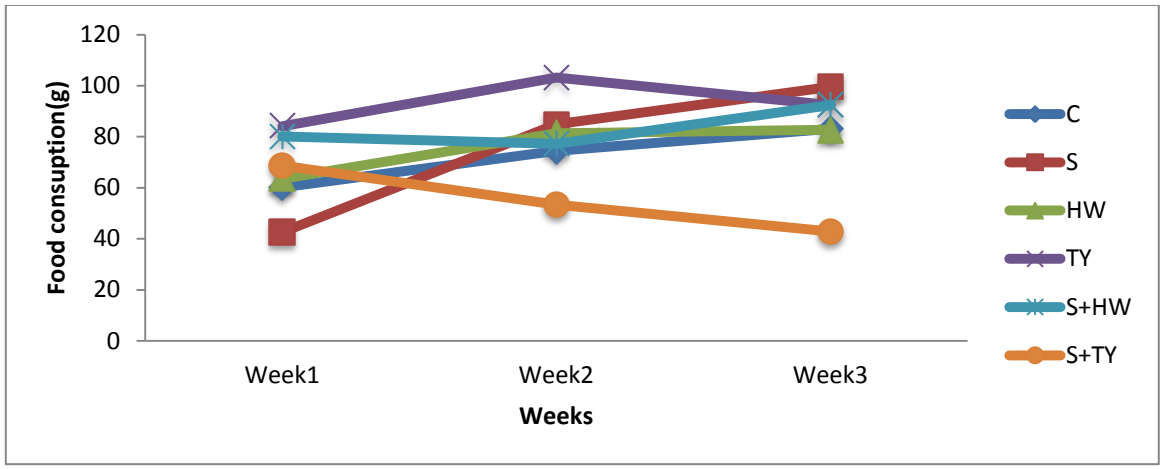


Figure 30: The effect of crystallize sugar , hot water and traditional yeast on the food consumed by mice during 21 days

V. 1. 2. Biochemical analysis

Blood sugar

The (Figure31) 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 increased but not significantly when it is compared to the group of (S).

4. 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 SHW (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 traditional yeast when compared to the control group P>0.05 **(Figure 32).**

Triglyceride

The data showed a difference very highly 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) 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 **(Figure33).**

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 **(Figure34).**

LDL-c

The data showed a difference in the values of LDL-C in groups (C) were (0.06g/l±0.03)and (TY) (0.08g/l±0.06) and (S) (0.18g/l±0.14) and (HW)(0.15g/l±0.07) and (S+HW)(0.16g/l±0.04) and (S+TY) (0.11g/l±0.09)but not significantly P>0.05**(Figure 35).**

CRP

The figure37 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),(SHW) (0.41mg/l±0.34) and (HW) (0.12mg/l±0.05) , (yeast) (0.08mg/l±0.06) and STY (0.12mg/l±0.08) when compared to the (C) (0.74mg/l±0.21) **(Figure 36).**

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 (SHW) and (STY) 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 37).**

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 (Figure40).

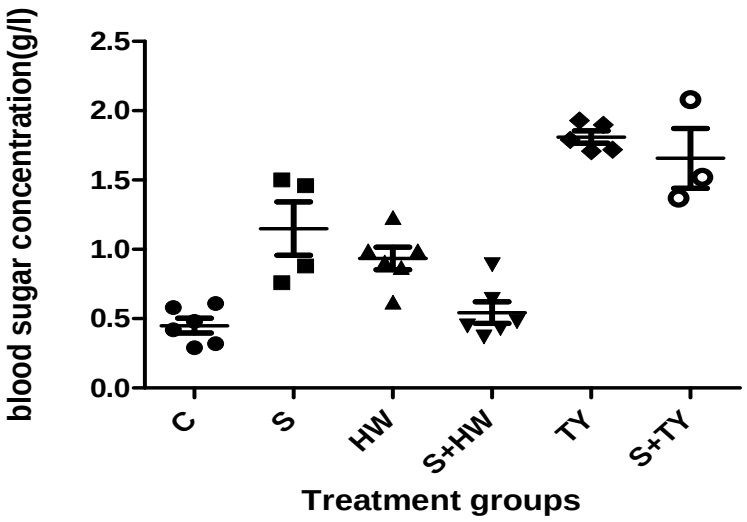


Figure 31: The effect of high consumption of crystallize sugar , hot water and traditional yeast on fasting blood sugar in mice.

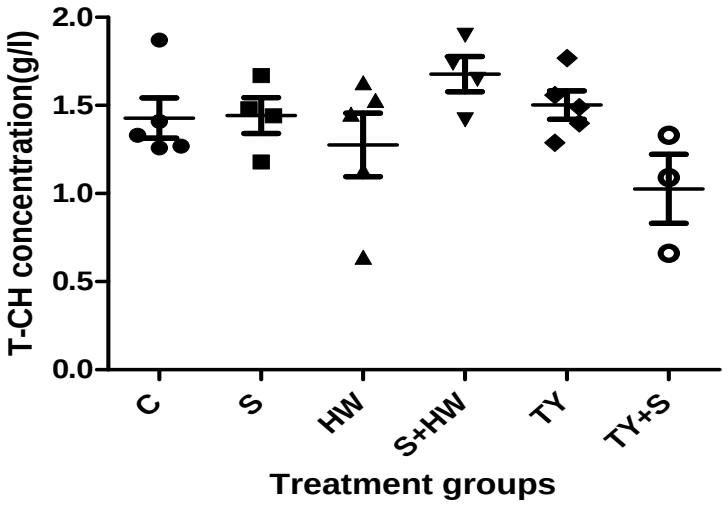


Figure 32: The effect of high consumption of crystallize sugar , hot water and traditional yeast on T- cholesterol in mice

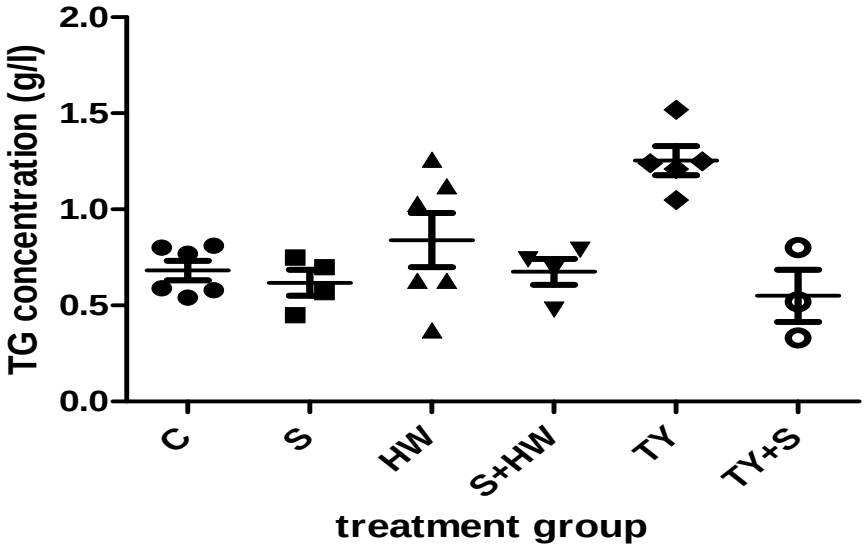


Figure 33: The effect of high consumption of crystallize sugar , hot water and traditional yeast on triglyceride in mice

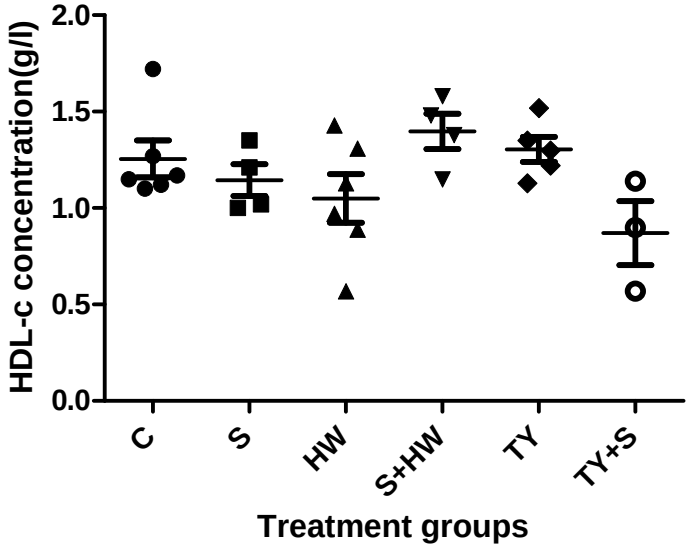


Figure 34: The effect of high consumption of crystallize sugar , hot water and traditional yeast on HDL-c in mice.

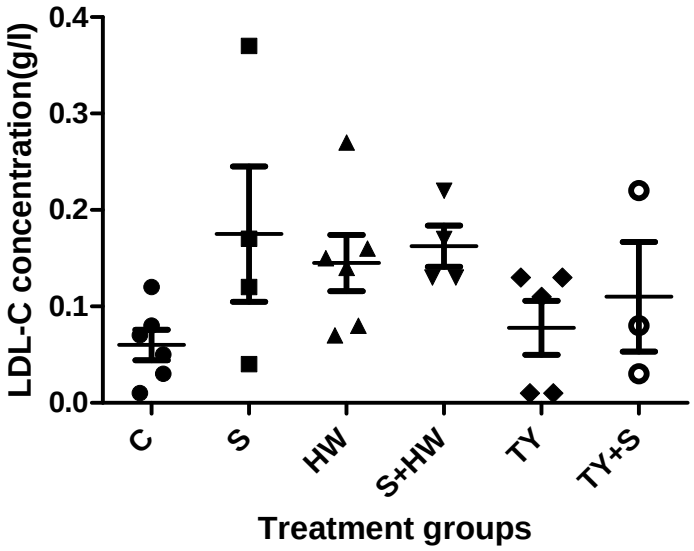


Figure 35: The effect of high consumption of crystallize sugar , hot water and traditional yeast on LDL-c in mice.

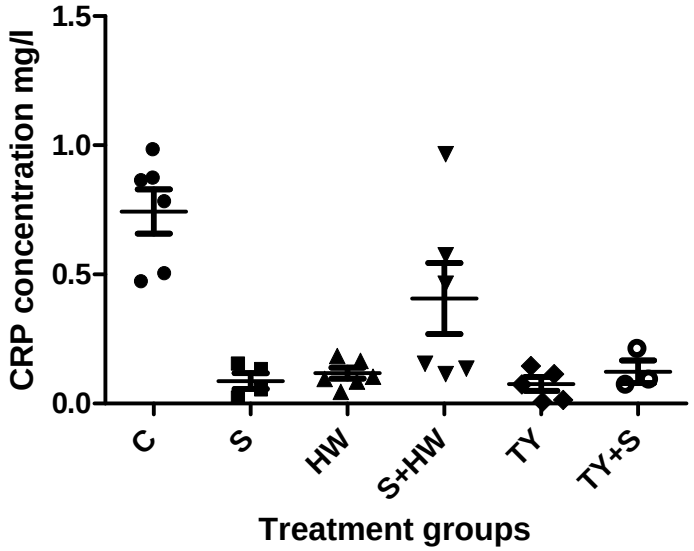


Figure 36: The effect of high consumption of crystallize sugar, hot water and traditional yeast on CRP in mice.

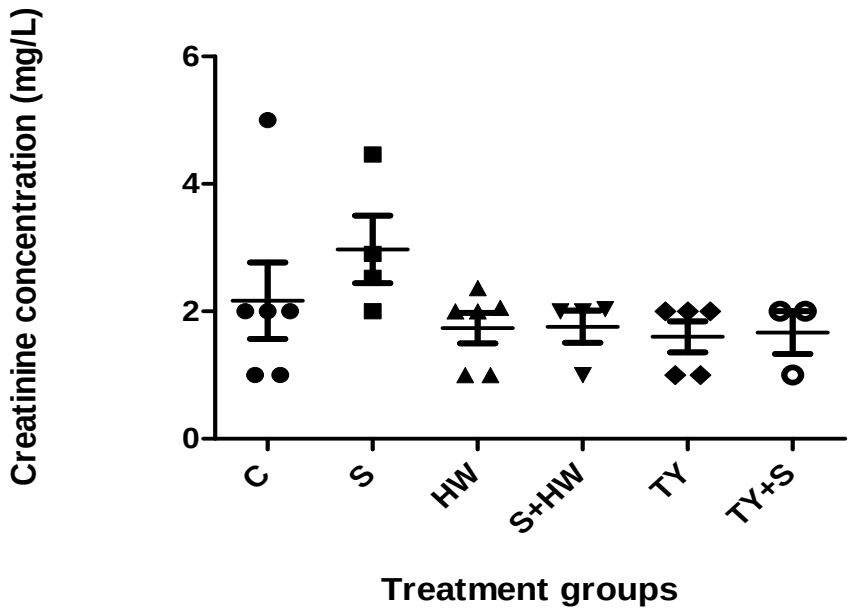


Figure 37: The effect of high consumption of crystallize sugar , hot water and traditional yeast on creatinine in mice.

ASAT and ALAT

The data showed that there is a difference significantly between groups in the level of the 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), (TY+S)(129.18UI/L±11.67)but not significantly $P>0.05$**(Figure38,39)**.

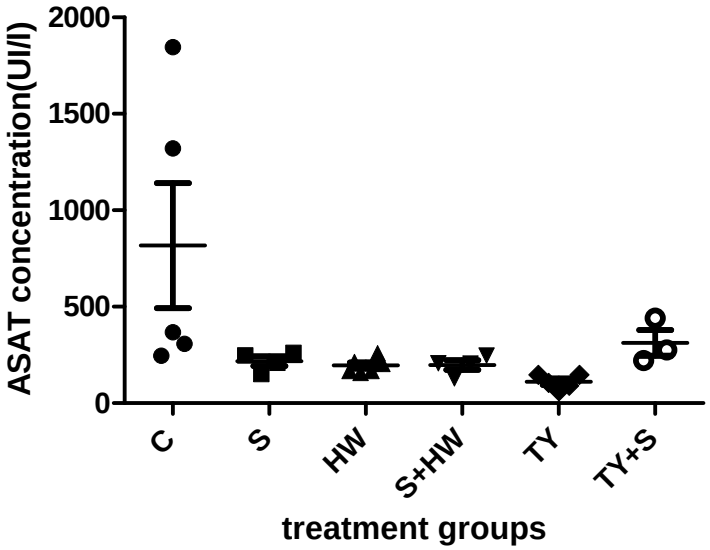


Figure 38: The effect of high consumption of crystallize sugar , hot water and traditional yeast on ASAT in mice.

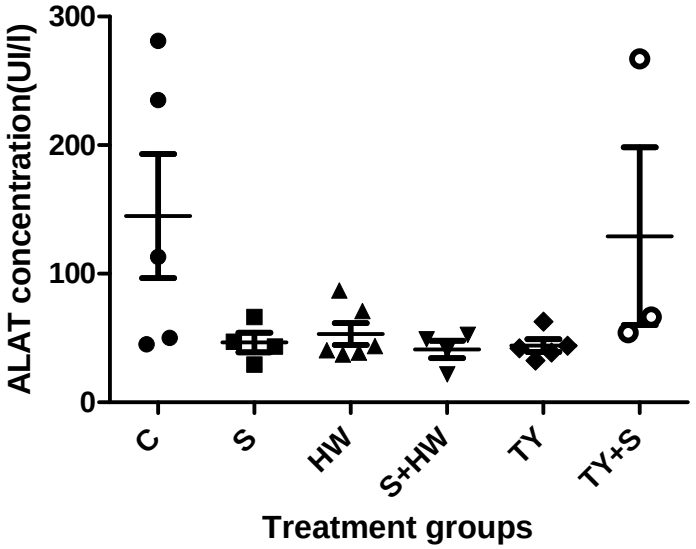


Figure 39: The effect of high consumption of crystallize sugar , hot water and traditional yeast on ALAT in mice.

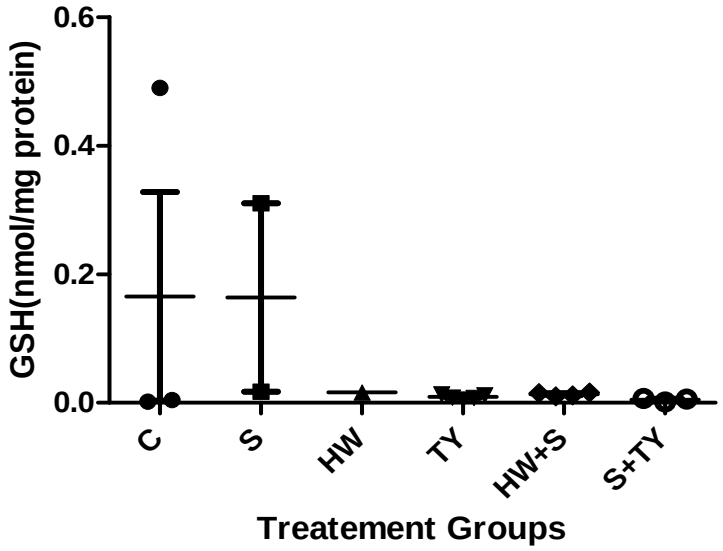


Figure 40: The effect of high consumption of crystallize sugar , hot water and traditional yeast on GSH in mice.

Histological study

9.1 Microscopic observation

Histological investigation showed that the group of mice fed with high crystallize sugar has some alterations in the crypt this was observed by crypt architectural distortion (Figure44, 45). In contrast to the groups, (C) , (HW) treated with hot water and also the group (TY) treated with traditional yeast where we have observed that the colon mucosa is intact and other parts. (Figure 41, 42, 43, 46, 47, 50, 51).

9.2 The histological sections

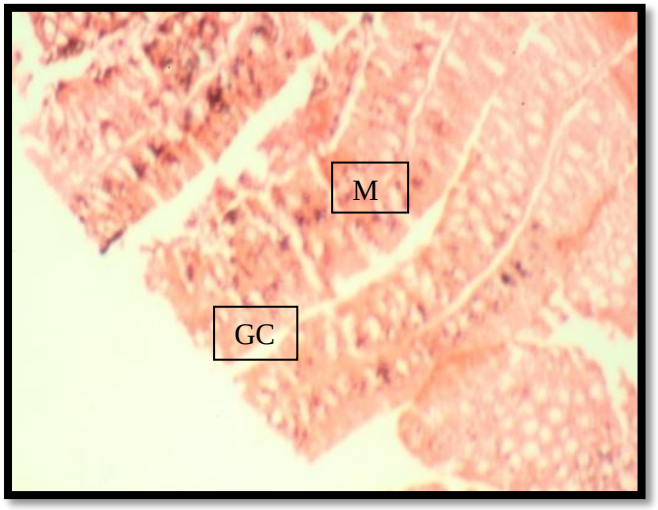


Figure41: Histological section of colon in group control. Heamatoxylin –eosin (x40).

GC: globlet cells, M: mucosa

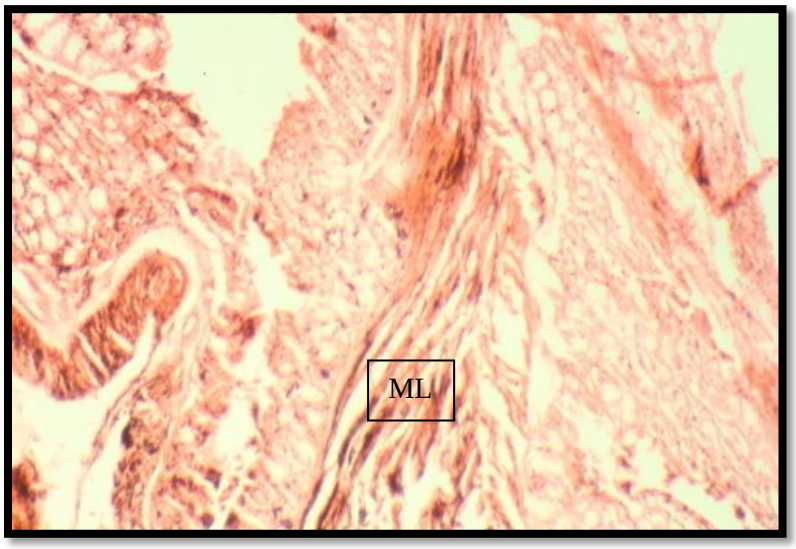


Figure42: Histological section of colon in group control. Heamatoxylin –eosine (x40).

ML: muscularis

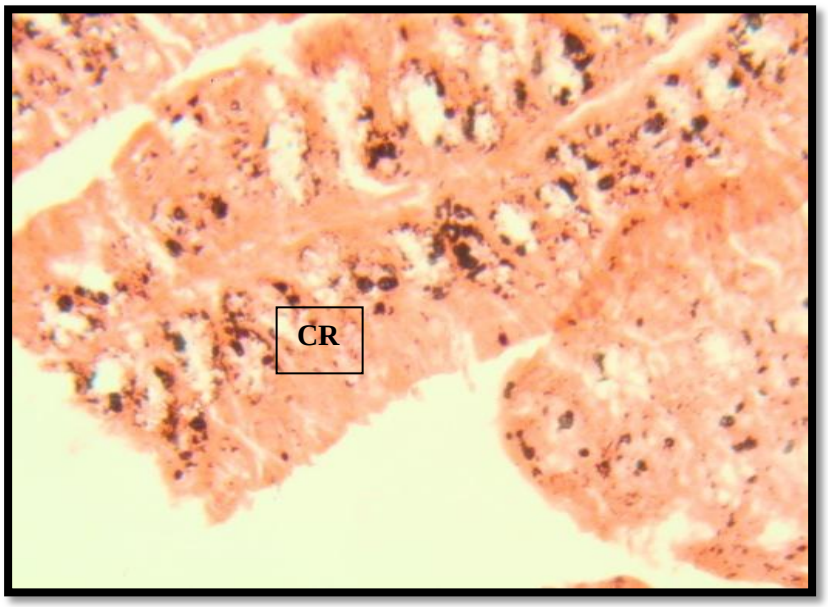


Figure 43: Histological section of colon in group control. Heamatoxylin –eosin (x100).

CR : crypt.

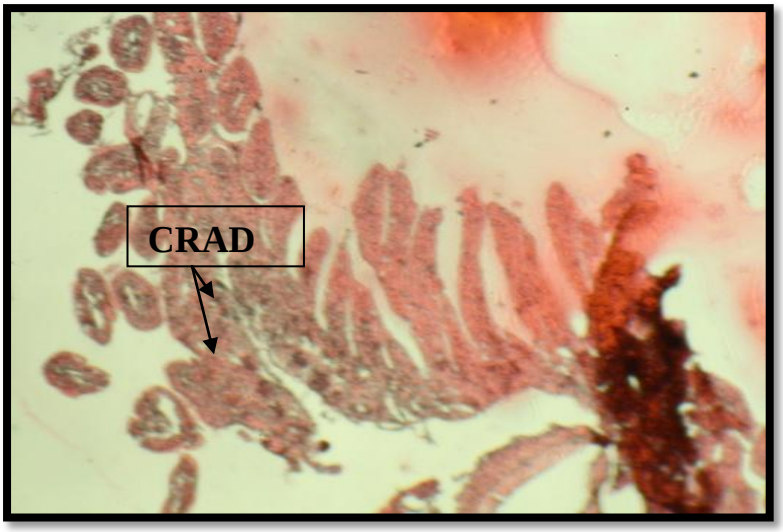


Figure44: Histological section of colon in group fed with sugar . Heamatoxylin –eosin (x40).

CRAD: crypt architectural distortion.

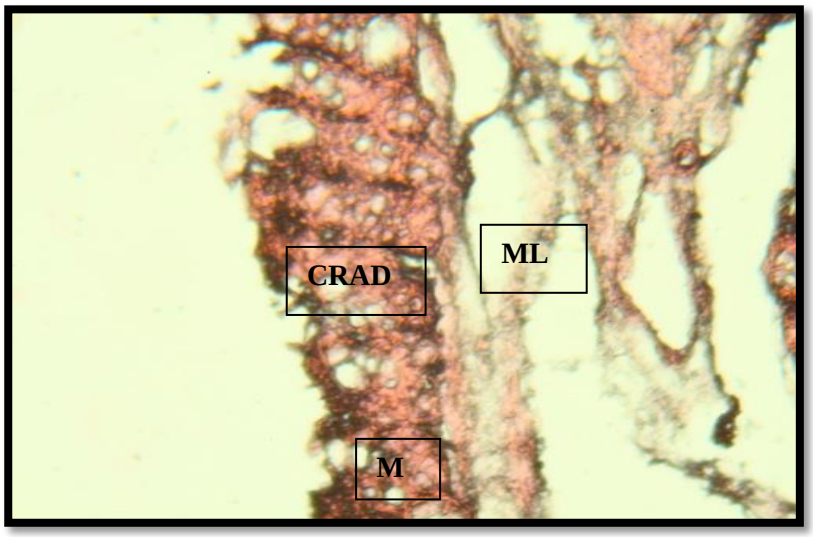


Figure45: .Histological section of colon in group fed with sugar . Heamatoxylin –eosin (x100).

ML: muscularis, M: mucosa, CRAD: crypt architectural distortion.

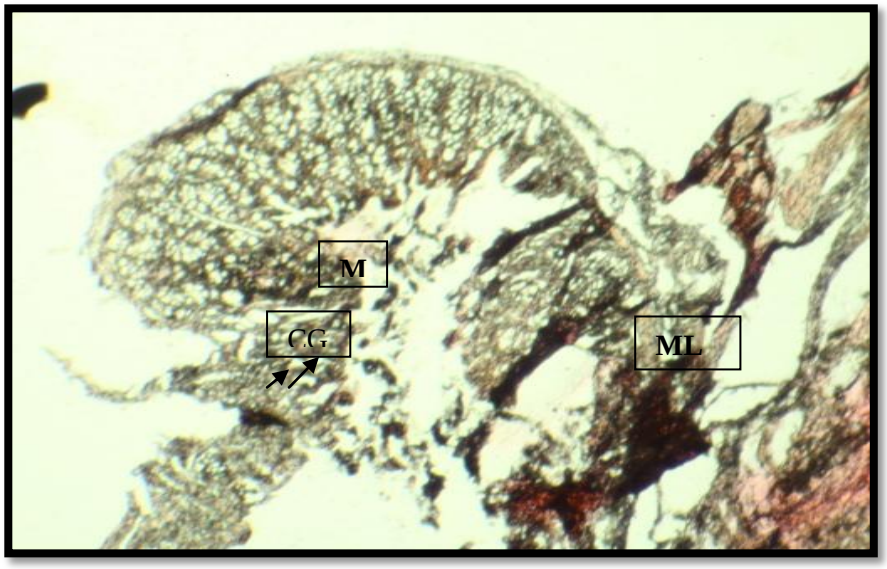


Figure46: Histological section of colon in group treated with hot water . Heamatoxylin – eosin (x40).

ML: muscularis, GC: globlet cells, M: mucosa.

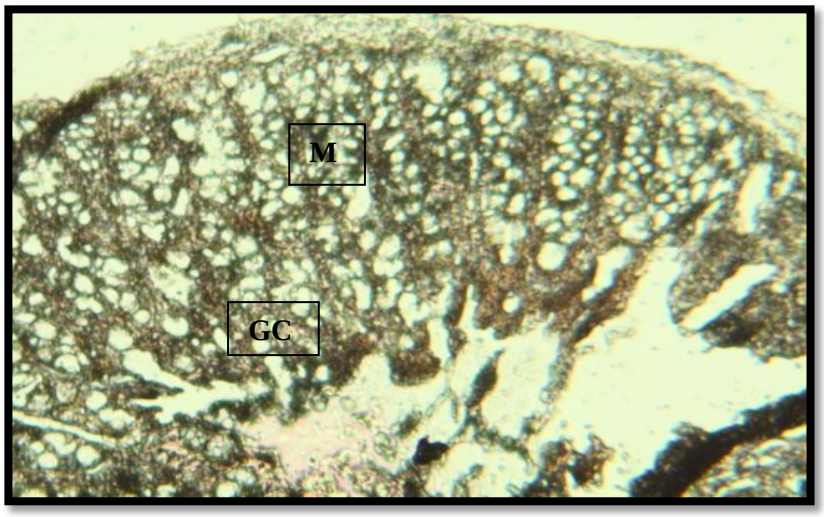


Figure47: .Histological section of colon in group treated with hot water . Heamatoxylin – eosin (x100).

GC: globlet cells, M: mucosa.

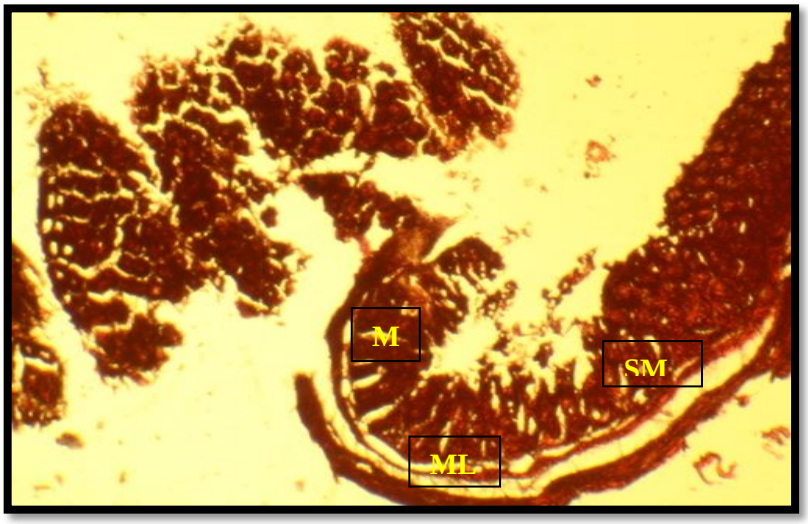


Figure48: Histological section of colon in group fed with sugar and treated with hot water . Heamatoxylin –eosine (x40).

M: mucosa, ML: muscularis, SM: submucosa.

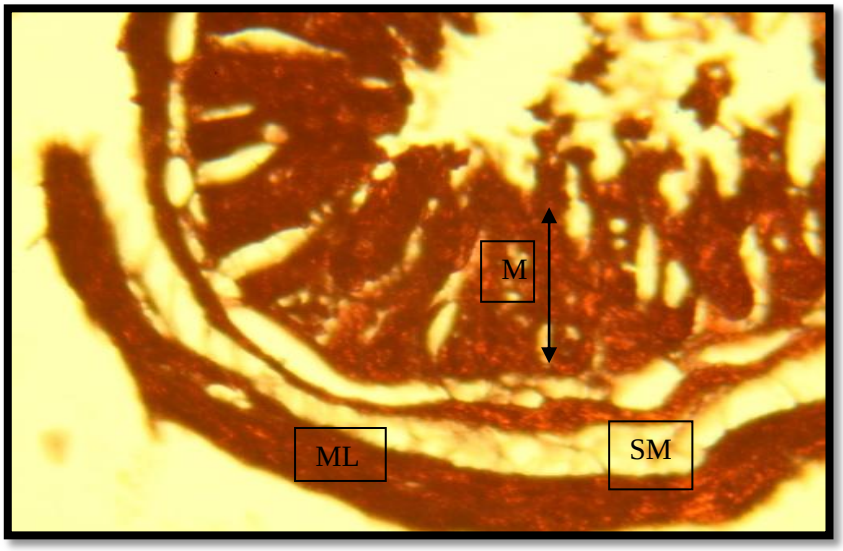


Figure49: Histological section of colon in group fed with sugar and treated with hot water . Heamatoxylin –eosin (x100).

M: mucosa, ML: muscularis, SM: submucosa.

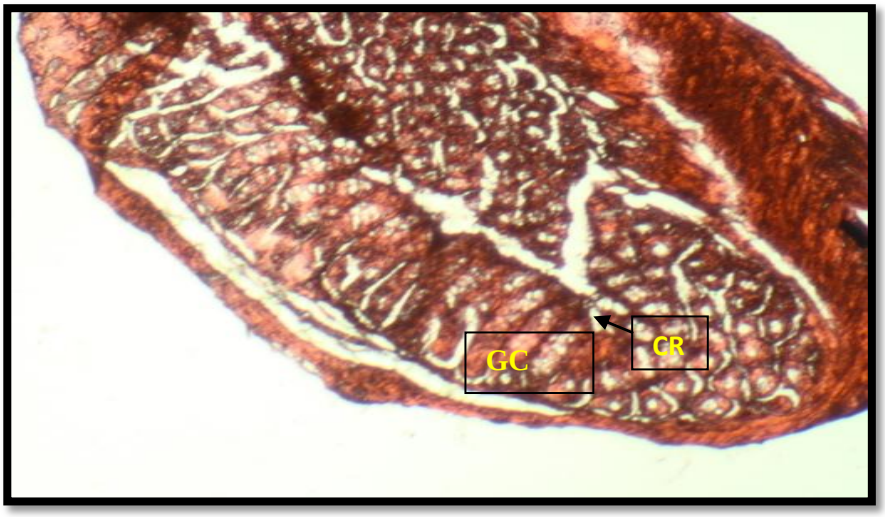


Figure 50: Histological section of colon in group fed with traditional yeast. Heamatoxylin – eosin (x40).

CR: crypt, GC: globlet cells.

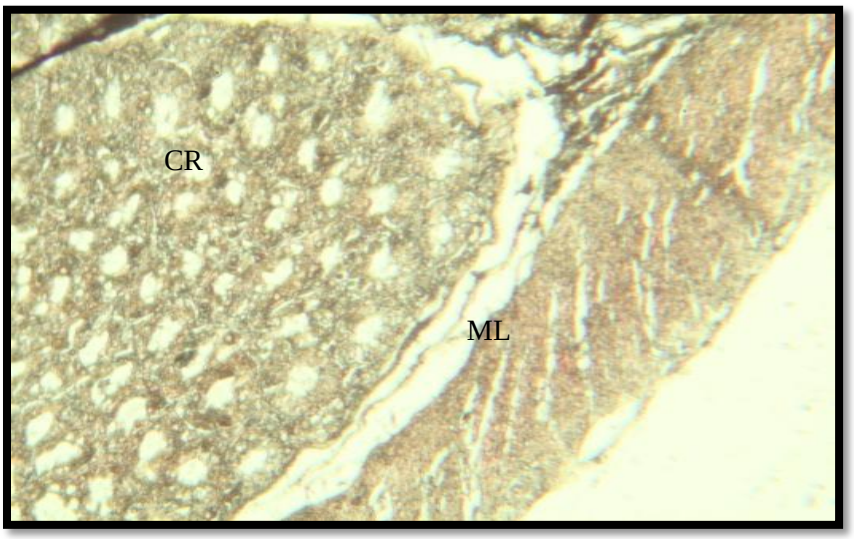


Figure51: Histological section of colon in group fed with traditional yeast. Heamatoxylin – eosin (x100).

CR: crypt, ML: muscularis.

Figure52: Histological section of colon in group fed with sugar and traditional yeast . Heamatoxylin –eosine (x40).

CAD : crypt architectural distortion

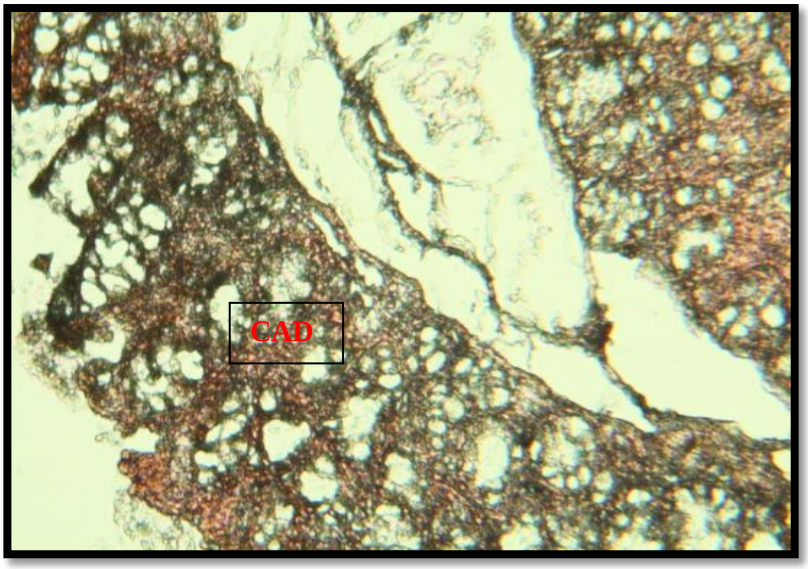


Figure53: Histological section of colon in group fed with sugar and traditional yeast . Heamatoxylin –eosin (x100).

CAD : crypt architectural distortion

V.2.Discussion

Much remains unknown about the role of added sugar in relation to cardiovascular disease (CVD) and the relative contributions of sugar-sweetened beverages (SSB) or artificially sweetened beverages (ASB) to CVD risk **(Yang et al., 2022)**.

Hyperglycemia has become a common disorder that can lead to various complications. High blood sugar is clearly associated with the development of cardiovascular disease, especially in diabetics. The aim of our research is to clarify the effects of a diet high in crystallized sugar on some biochemical parameters (CRP, Total Cholesterol, Triglyceride, HDL-c, LDL-c and glycaemia, creatinine) and on the histological structure of the colon in mice and to examine the therapeutic effect of hot water and traditional yeast on the abnormalities caused by the high-crystallize 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 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 crystallize sugar, treated with hot water, and control group. Same results are obtained by Abderrahmane et al. (2022) who obtained that 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 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 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

The concentration of blood sugar is increased in animals fed with crystallize sugar when compared to the control group. The high intake of sugar can increase the blood pressure, which is a major risk factor for heart disease. People who eat more sugar are more likely to be overweight, and this is a risk factor for heart disease. **(Lennon, 2023)**. Also, a higher percentage of calories from added sugar are associated with significantly increased risk of cardiovascular disease CVD mortality. In addition, regular consumption of sugar-sweetened beverages is associated with elevated CVD mortality **(Yang et al., 2014)**.

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 crystallize sugar and treated with hot water.

We detected in this research, mice administered with 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). Low cholesterol levels and high cholesterol variability were associated with a higher risk of Atrial Fibrillation. 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).

The high density lipoprotein is increased in group TY and decreased in TY+S

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 crytallize sugar and decreased in the groups treated with hot water and traditional yeast.

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 (Xiao et al., 2019).

From our results we found that the liver enzymes (ASAT) are decreased in groups of the experimental study (S+HW, TY and S+TY) and for ALAT in groups (S,HW, TY).

But the level of ALAT was 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 ALAT is 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 oxidized glutathione and the dosage of GSH in the plasma.

V. 2. 3 Histological investigation

Histological investigation showed that the group of mice fed with high crystallize sugar has some alterations in the crypt this was observed by crypt architectural distortion. In contrast to the group fed with standard diet,(C) ,group treated with hot water (HW) and also the group (TY) treated with traditional yeast where we have observed that the colon mucosa is intact and rich with globet cells.

Khelfi et al.(2023), reported that the mice administered with a high dose of methionine during 21 days led to degeneration in the enterocytes membranes cells however the group of mice treated with extarct of fruit *Citrus sinensis L* showed a restoration of enterocyte membrane cell.

Conclusion and future work

Conclusion and future work

The idea 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 intestine.

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 crypt architectural distortion. Meanwhile, a treatment with tratidional yeast and hot water at 50°C was effective in preventing the increase of these metabolic disorders and intestine damages.

Based on the findings of this study our future work 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 intestinal inflammation induced by high crystallize sugar.

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

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Summary

Summary

Sugars family that belong to carbohydrates and closely associated with starch and cellulose are the key energies for humans and animals, its source vary but the healthier one generally found in whole foods like fruits, vegetables and dairy products.

Research found that it's crucial to maintain a balanced sugars intake to protege our bodies from various health problems and mainly intestinal inflammation.

In the present study, we evaluated *in vivo* the interaction of high consumption of crystallize sugar (200mg/kg) , hot water at 50°C and the traditional yeast on the intestine 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 intestine.

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 intestine 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 intestinal inflammation.

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

Résumé

La famille des sucres appartenant aux glucides et étroitement associée à l'amidon et à la cellulose sont les énergies clés pour l'homme et les animaux, sa source varie mais la plus saine se trouve généralement dans les aliments entiers comme les fruits, les légumes et les produits laitiers.

La recherche a révélé qu'il est crucial de maintenir un apport équilibré en sucres pour protéger notre corps de divers problèmes de santé et principalement de l'inflammation intestinale.

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 l'inflammation intestinale 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 des intestins.

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 intestinal 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 intestinal 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 intestinale.

Mot clés: inflammation, intestin, glutathion réduit, profil lipidique, ASAT, ALAT

الملخص

تنتمي السكريات إلى الكربوهيدرات ومرتبطة ارتباطًا وثيقًا بالنشاء والسليلوز، وهي المصدر الرئيسي للطاقة البشرية والحيوانات. يختلف مصدرها، ولكن الأكثر صحة عادة ما يتم العثور عليها في الأطعمة الكاملة مثل الفواكه والخضروات ومنتجات الألبان

أظهرت الأبحاث أنه من الضروري الحفاظ على تناول السكريات المتوازنة لحماية أجسامنا من مشاكل صحية مختلفة وخاصة التهاب الأمعاء

في هذه الدراسة ، قمنا بتقييم أثر استهلاك السكر والماء الساخن عند 50 درجة مئوية مع الخميرة التقليدية على التهابات المعوية 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 للامعاء.

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 المعوي 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 المعوي.

الكلمات المفتاحية :الالتهابات' الامعاء ' الجلوتاثيون المختزل 'الليبيدات' انزيمات كبدية

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 by 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 methanol (96%).

- **Preparation of Bradford**

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

- **Preparation of Sulfosalycilique**

0.25g Sulphosalic acid ⟶ 100ml distilled water.

- **Preparation of Tris EDTA**

6.06g tris + 0.96g EDTA ⟶ 12.5ml distilled water ⟶ pH= 9.6 (modified by HCl.

- **Preparation of Bouin alcohol**

45ml(1g picric acid + ethanol (95%)) + 26 ml formalin (37%) + 7ml acetic acid ⟶ 22ml distilled water.

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

5 mg BSA⟶ 5 ml distilled water

- **Preparation of heamatoxylin**

1 g haematoxylin ⟶ 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 06: composition of water used in the experimental study.

Composition	**Mg/litre**
Calcium	4.6
Magnesium	3.75
Potasuim	1
Soduim	29
Bicarbonates	48.8
Sulfates	10
Chlorides	30
Nitrates	9

Nitrites	0.06
R.S à 105 c°	140
Ph	6.87

Table 07: 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 08: calibration 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	5	5	5	5	5	5
DO	0	0.207	0.420	0.583	0.722	0.874

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THE EFFECT OF HOT WATER AND TRADITIONAL YEAST ON INTESTINAL INFLAMMATION INDUCED BY REFINED CRYSTALLIZE SUGAR

Sugars family that belong to carbohydrates and closely associated with starch and cellulose are the key energies for humans and animals, its source vary but the healthier one generally found in whole foods like fruits, vegetables and dairy products.

Research found that it's crucial to maintain a balanced sugars intake to protege our bodies from various health problems and mainly intestinal inflammation.

In the present study, we evaluated *in vivo* the interaction of high consumption of crystallize sugar (200mg/kg) , hot water at 50°C and the traditional yeast on the intestinal inflammation 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 intestine.

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 intestine 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 intestinal inflammation.

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

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