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Diamine Oxidase use as an anti-inflammatory dietary supplement

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May they find in this work the testimony of our sincere gratitude and deep respect

Last but not least, we cannot thank enough our parents; without them none of this would indeed be possible.

DEDICATION

To

My loving parents and their endless support

My brothers

My grandparents

My aunts and cousins

Dolf, a loyal friend

Azedine KACI

DEDICATION

to my beloved family
my brothers

Lynda ARIB

ABSTRACT

Histamine intolerance (HIT) has been the subject of many scientific studies and is thought to be caused by a reduced intestinal degradation of this amine. The enzyme diamine oxidase (DAO) is considered for the gastrointestinal degradation of histamine. Histamine is an essential mediator of the pathophysiology of many allergic diseases. The inflammatory response is one of the effects exerted by histamine through its different receptors (H1, H2, H3, H4). The aim of this bibliographic review was to assess the efficacy of a new approach by implementing DAO enzyme as food supplementation in a preventive treatment for patients diagnosed with histamine intolerance. First we focused on reviewing the main sources of DAO (bacterial, vegetal and animal), discussing the process of extraction from known sources such as *L. sativus* and Porcine Kidney, and purification of plant and animal based DAO. We then reviewed and discussed the anti-inflammatory properties of the enzyme, which showed a significant reduction of every HIT-related symptom and its intensity due to DAO oral supplements.

Key words: DAO, histamine, amine oxidases, inflammatory response, histamine intolerance, oral supplements.

RÉSUMÉ

L'intolérance à l'histamine (HIT) a fait l'objet de nombreuses études scientifiques et serait causée par une dégradation intestinale réduite de cette amine. L'enzyme diamine oxydase (DAO) est considérée comme responsable de la dégradation gastro-intestinale de l'histamine. L'histamine est un médiateur essentiel de la pathophysiologie de nombreuses maladies allergiques. La réponse inflammatoire est l'un des effets exercés par l'histamine à travers ses différents récepteurs (H1, H2, H3, H4). L'objectif de cette revue bibliographique était d'évaluer l'efficacité d'une nouvelle approche par la mise à disposition de l'enzyme DAO en tant que supplémentation alimentaire dans un traitement préventif pour patients diagnostiqués avec une intolérance à l'histamine. Nous nous sommes focalisé en premier lieu à passer en revue les principales sources de DAO (bactériennes, végétales et animales), et discuter du processus d'extraction à partir de sources connues telles que *L. sativus* et le rein de porc, et de la purification du DAO d'origine végétale et animale. Nous avons ensuite examiné et discuté les propriétés anti-inflammatoires de l'enzyme, qui a démontré une réduction importante de chaque symptôme lié à la HIT et de son intensité grâce à la supplémentation orale de la DAO.

Mots clés : DAO, histamine, amine oxydases, réponse inflammatoire, intolérance à l’histamine, suppléments oraux

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LIST OF ABBREVIATIONS

AO: Amine oxidases

APC: Antigen-presenting cells

BA: Biogenic amines

CD: Crohn Disease

CMS: Carboxymethyl starch

CSF: colony-stimulating factor

CuAO: Copper-containing amine oxidases

DAG: Diacylglycerol

DAO: Diamine oxidase

ECL: Enterochromaffin-like cells

ELISA: Enzyme-linked ummunosorbent assay

FAD: Flavin adenine dinucleotide

GI: Gastrointestinal

GM: Granulocyte-macrophage

HDC: Histidine decarboxylase

HIT: Histamine intolerance

HMT: Histamine N-methyltransferase

HPLC: High High performance liquid chromatography

HR: Histamine receptor

IBD: Inflammatory bowel disease

IBS: Irritable Bowel Syndrome

IgE: Immunoglobulin E

IL: Interleukin

ImAA: Imidazole acetic acid

IP3: Inositol triphosphate

MAO: Mono-amines oxidase

MAOI: Mono-amines inhibitors

NCBI: National Center for Biotechnology Information

NEC: Necrotizing Enterocolitis

PKC: Proteinkinase C

PLC: PhospholipaseC

REA: Radio extraction essay

SIBO: Small intestinal bacterial overgrowth

SSAO: Semi carbazide-sensitive amine oxidase

TJ: Tight junction

TPQ: Trihydroxyphenylalanine quinone

UC: Ulcerative colitis

INTRODUCTION

Varying opinions exist on the significance of biogenic amines in non-immunologically-mediated reactions to foods that may be termed "food intolerance". Food intolerance, which is not immunologically mediated, is usually triggered by small molecular weight chemical substances and biologically active components of foods, not by food proteins **(Bischoff and Manns 1998).** Histamine is an important example of a biologically active component that is present in many foods and beverages**.**

Biogenic amines including histamine are produced by bacterial decarboxylation of aminoacids in food **(Doeun et al., 2017).** Histamine is an important mediator of the symptoms of allergy. Allergy is a Type I hypersensitivity reaction, involving the production of allergen-specific IgE, which leads to the release of pre-formed inflammatory mediators that are stored within cytoplasmic granules in tissue mast cells, and to a lesser extent from similar granules in blood basophils. Histamine is the most important of these mediators, and is responsible for the symptoms that are typical of an immediate-onset allergic reaction. Defects in histamine catabolism may contribute to the severity of the reaction. When the endogenous histamine is released in the process of allergic degranulation is not broken down efficiently, excess will accumulate, theoretically leading to a situation that might critically enhance the adverse effects of the amine. The contribution of defects in histamine pharmacodynamics to anaphylaxis has been assessed in very few studies, but where this has been considered, the results seem to indicate that it might represent an important factor in a condition which can be life-threatening **(Hershko et al 2001)**.

There is an imprecise clinical overlap between irritable bowel syndrome (IBS) and other IBS-like disorders. The most commonly reported symptom-oriented conditions are IBS and small intestinal bacterial overgrowth (SIBO). All of these present with various functional, nonspecific, non-allergic gastrointestinal symptoms, predominantly abdominal pain, bloating and diarrhea **(Borghini et al. 2017)**. These GI symptoms, including multiple combinations of these, are also reported by patients with HIT **(Schnedl, Schenk, et al. 2019)**. Generally, there is a lack of specificity of symptoms, therefore symptoms alone or symptom complexes are hardly, if ever, diagnosed **(Verdu, Armstrong, and Murray 2009)**. It is suspected that various pathogenetic mechanisms may be responsible for IBS **(Chey 2016).** However, 80% of IBS patients identified food, containing histamine, as a possible trigger for their symptoms **(Böhn et al. 2013).**

It is thought that the cause of histamine intolerance is most likely a defect in catabolism of histamine **(Lessof et al 1990)**. In humans, enzymatic inactivation of histamine occurs through the operation of two types of enzymes, diamine oxidase (DAO) and histamine N-methyltransferase (HMT). The contribution of each of the enzymes systems (DAO and HMT) to histamine breakdown seems to vary between tissues. Diamine oxidase activity seems to predominate in the intestine, whereas, N-methyltransferase activity predominates in the brain.

The need of a new main strategy to avoid the symptoms of histamine intolerance other than to follow a low-histamine diet, has led to considering supplementation with exogenous DAO. It has been recently postulated as a complementary treatment to enhance dietary histamine degradation in intolerant individuals who have a deficiency of this enzyme in the intestine**.** In this regard we attempted to showcase the efficiency of implementing DAO in daily meals as a supplement.

This bibliographic review consists of five chapters, in which we will take a closer look at intestinal inflammatory diseases and their close link with histamine, an overview on histamine synthesis, regulation and function. Followed by the main topic: Amine oxidases, Diamine oxidases and their use as dietary supplements for their anti-inflammatory properties.

CHAPTER I: INTESTINAL INFLAMMATIONS

1.1 Generalities

The intestine is the important location where absorption of dietary nutrition and waste excretion take place **(Chen et al., 2021)**. The intestinal barrier is critical to health and is one of the most metabolically dynamic systems in the body. The intestines must constantly balance allowing molecules in (e.g., water, electrolytes, nutrients) while keeping inflammatory environmental antigens out **(Bischoff et al., 2014)**. Additionally, the intestinal barrier must manage the prevention of invading and translocating luminal bacteria, but also not become hyper reactive to these commensal or symbiotic microorganisms **(Cho, Berger, Nold-Petry, & Nold, 2016)**. The intestinal barrier is composed of both an external physical and biochemical barrier and a complementary inner immunological barrier **(Bischoff et al., 2014)**.

However, many intestinal diseases can impair intestinal epithelium and flora. Generally, almost all the intestinal ailments of different pathogenic mechanisms like intestinal mucosal atrophy due to deficiency of food stimulation in intensive care patients with total parental nutrition, irritable bowel syndrome (IBS), colitis, ulcerative colitis (UC), Crohn's disease (CD), and abuse of antibiotics can sabotage the integrity of intestinal epithelium and the balance of intestinal flora **(Chen et al., 2021).**

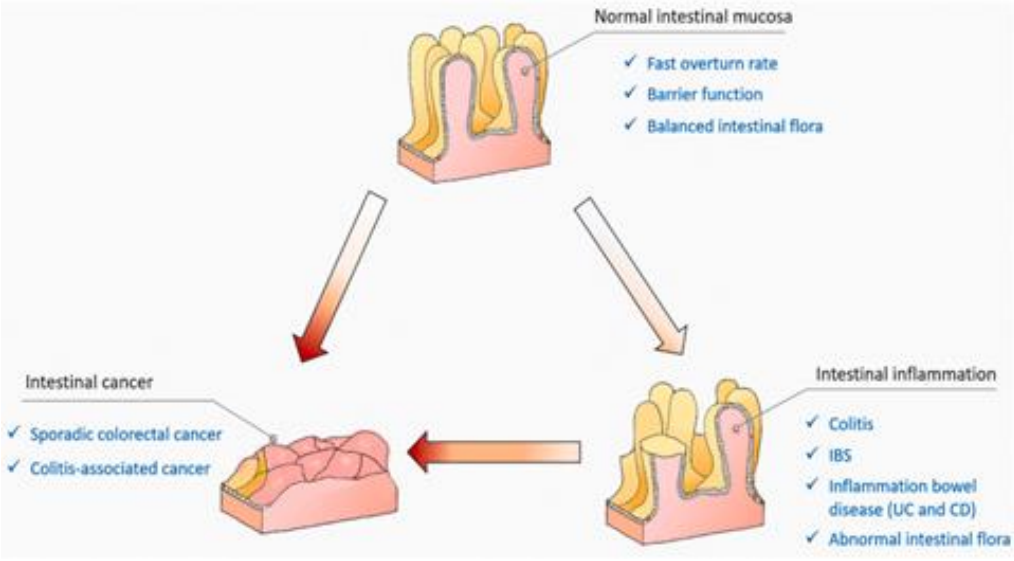

Figure 1: Global relationship between dietary foods and intestinal ailments. *Normal intestinal mucosa is long and in compact arrangement with a high overturn rate. In the inflammation-related intestinal ailments like IBS and IBD, the mucosa is impaired and the barrier function is also disrupted. Both normal and inflammation inflicted intestinal cells can be transformed into colorectal cancer cells under certain circumstance* ***(Chen et al., 2021).***

The incidence of intestinal inflammatory diseases, such as necrotizing enterocolitis (NEC), Crohn's disease (CD), and ulcerative colitis (UC), is increasing worldwide. CD and UC, together referred to as inflammatory bowel disease (IBD), are chronic, relapsing inflammatory diseases with no cure and significant morbidity, most often affecting young adults **(Dias et al., 2018)**. Much like NEC, the etiology of IBD is, as yet, unexplained, but is thought to involve an overstimulation and excessive response of the intestinal mucosal immune system to resident luminal microorganisms **(Lu, Li, Liu, Zhang, & Zhang, 2018).**

In Crohn's disease, inflammation is discontinuous and manifests as distinct granulomas, with inflammation often permeating transmurally and even affecting adjacent lymph nodes **(Pallone & Monteleone, 2001)**. In contrast, ulcerative colitis, occasionally a milder condition, is characterized by continuous mucosal inflammation localized to the colon. Both CD and UC result in extensive epithelial damage.

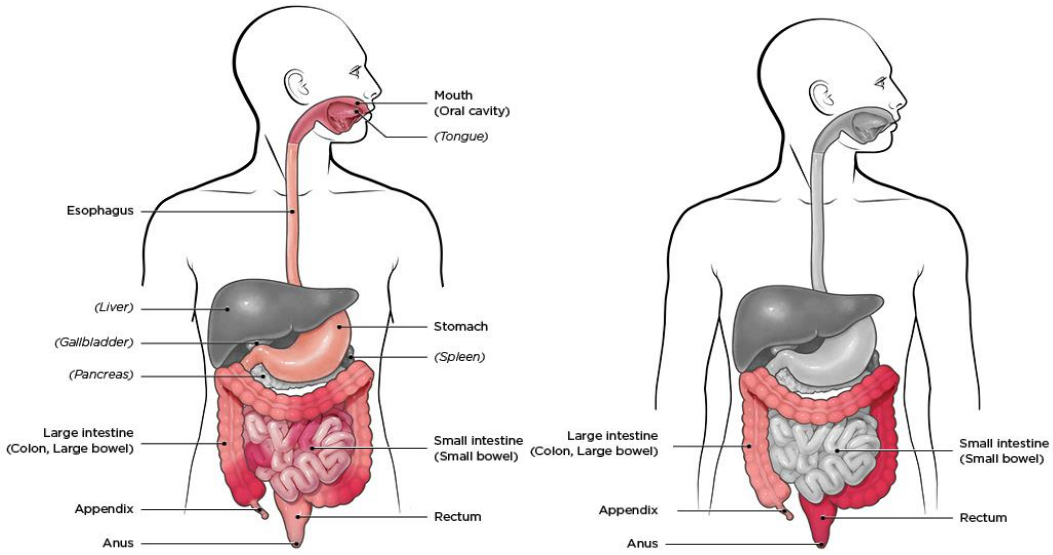

Figure 2: Difference between Crohn's disease (left) and Ulcerative Colitis (right) *Crohn's Disease can affect any part of the GI tract (from the mouth to the anus), most often it affects the portion of the small intestine before the large intestine/colon. Ulcerative Colitis Occurs in the large intestine (colon) and the rectum adapted from* **(Rubin et al., 2017)**.

1.2 Histamine intolerance and intestinal inflammations

The prevalence of patients suffering from gastrointestinal and extra-intestinal afflictions after food ingestion is rising. The spectrum of food intolerances reaches from carbohydrate malabsorption (e.g.lactose, fructose) to immunological IgE or non-IgE-mediated food allergies **(Zopf, Hahn, Raithel, Baenkler, & Silbermann, 2009).**

In addition, histamine intolerance (HIT) is also often considered to be responsible for gastrointestinal symptoms after food intake. Thereby, histamine intolerance is defined as an adverse reaction of ingested histamine that affects different organ systems and results in various intestinal and extra-intestinal symptoms **(Laura Maintz & Natalija Novak, 2007)**.

Ingestion of histamine containing foods and beverages, including fish, cheese or red wine, are supposed to trigger symptoms like flush,pruritus, nausea, vomiting, diarrhea and abdominal pain **(Laura Maintz & Natalija Novak, 2007)**.Other foods like citrus fruits or various drugs further contribute to an elevated histamine concentration through their histamine-liberating effect **(Kovacova-Hanuskova, Buday, Gavliakova, & Plevkova, 2015).**

Recent findings speculated that low serum Diamine Oxidase (DAO) values may be responsible for the symptoms of histamine intolerance HIT **(Schnedl et al., 2019).** which will be discussed further.

However, also other factors are discussed to affect histamine intolerance, for example an alteration of the intestinal bacteria. Various bacteria, which are able to convert histidine from proteins into histamine, naturally occur in the digestive tract as part of the normal intestinal gut flora **(Kohn, 2014).**

Interestingly, some probiotic strains including several lactic acid bacteria, like *Lactobacillus reuteri*, *Lactobacillus casei* and *Lactobacillus delbrueckii subsp. bulgaricus*, possess the enzyme histidine decarboxylase (HDC) and are therefore able to generate biogenic amine **(Deepika Priyadarshani & Rakshit, 2011).** The presence of these bacteria in the human intestine might contribute to increased histamine levels and promote histamine sensitivity in some persons.

CHAPTER II: HISTAMINE

2.1 Generalities

Histamine is one of the earliest identified mediators of allergy. Researchers identified its role in the modulation of allergic reactions as early as 1932. Since then, research has determined histamine to be a mediator of autoimmune conditions, gastric acid secretion, and hematopoiesis. Histamine is present within all bodily tissues; however, its sites of highest concentration include the lungs, basophils, and mast cells. It is also a potent vasoactive agent through its effects on bronchial smooth muscles and nociceptive itch nerves. Histamine regulates a variety of physiological functions by playing a key role in the inflammatory response of the body.It also has a vital role in various pathomechanisms of inflammatory diseases, which have led to the identification of novel histamine receptors over the years and greater recognition of its functions in the immune system **(Patel & Mohiuddin, 2021).**

Histamine is widely distributed in different food categories and in highly variable concentrations, In fresh foods such as fish and meat, and in some derived products, the presence of histamine is due to a lack of freshness or an inadequately hygienic quality of raw materials and/or production processes **(Comas-Basté et al., 2020)**.

Histamine was first purified from ergot fungi in 1910 **(Dale & Laidlaw, 1910)** and from human tissues in 1927 **(Best, Dale, Dudley, & Thorpe, 1927)**. Histamine has pleiotropic effects on skin and the cardiovascular, respiratory, digestive, central nervous, and immune systems **(O'Mahony, Akdis, & Akdis, 2011)**. It is a profound vasodilator that increases blood vessel permeability, allowing blood leukocytes to enter tissues to promote inflammatory responses.

Anaphylaxis is a serious allergic reaction that is rapid in onset and can be life threatening. The clinic manifestations include symptoms that involve the skin, gastrointestinal track, respiratory system, and cardiovascular system. Anaphylaxis can be caused by allergy to foods, insect venoms, medications, and other agents **(Simons, 2010)**.

The incidence of food-induced anaphylaxis has risen dramatically in developed countries during the past several decades **(Sicherer & Sampson, 2010**). The cost of treating food allergy is estimated at about 25 billion dollars annually in the US alone **(Gupta et al., 2013).**

Histamine plays an essential role in IgE-medicated anaphylaxis, the most common type of anaphylaxis **(Kemp & Lockey, 2002).** Relatively large quantities of histamine can cause a rapid decrease in body temperature due to massive leakage of blood plasma into the extravascular space. Rapid release of large amounts of histamine leads to anaphylaxis **(Reber, Hernandez, & Galli, 2017).**

2.2 Definition

Histamine is a low-molecular weight amine found throughout the body**.** It's an essential mediator of the pathophysiology of many allergic diseases. It is synthesized in inflammatory and immunocompetent cells, in gastric parietal cells and in neurons (labile reserve).

It can be liberated in the skin, intestines, liver and bronchi by antigen-antibody interactions as well as by drugs (for example, morphine), venoms, toxins, endogenous agents (for example, kinins), radiation, burns and inflammatory reactions.

It is a potent vasodilator that can increase capillary permeability, induce bronchoconstriction, activate inflammatory cells, stimulate gastric section and, depending on the circumstances, inhibit or stimulate the central and peripheral nervous systems.

This biogenic vasoactive amine, causes symptoms such as allergies and has a pleiotropic effect that is dependent on its interaction with its four histamine receptors.

Histamine is used primarily diagnostically in two test systems: to assess gastric acid secretion and to diagnose pheochromocytoma **(Bylund, 2017).**

Histamine has the ability to regulate other monoamines, such as dopamine and serotonin **(Tochen & Singer, 2016),** and hormone such as adrenaline **(Huang, Li, Liang, & Finkelman, 2018).**

2.3 Synthesis of histamine

Histamine is synthesized primarily by mast cells, basophils, histaminergic neurons in the basal ganglia of the brain and enterochromaffin-like cells (ECL) in the stomach. Synthesis of histamine occurs through decarboxylation of the amino acid histidine by the enzyme L-histidine

decarboxylase (HDC), which is expressed in neurons, parietal cells, gastric mucosal cells, mast cells, and basophils which removes carboxyl group from histidine **(Huang, Li, Liang, & Finkelman, 2018).**

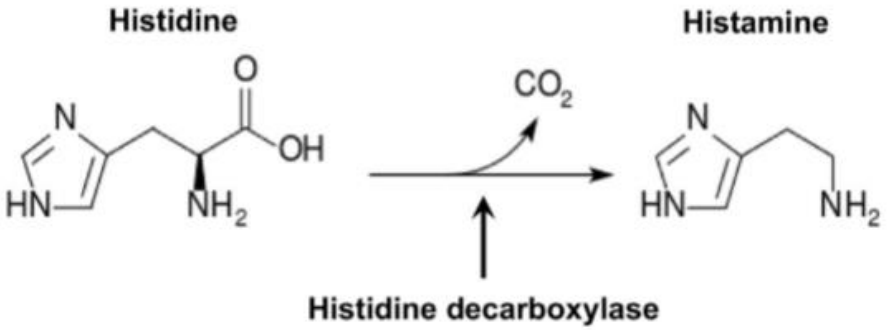

Figure 3: Decarboxylation of the amino acid histidine by the enzyme L-histidine decarboxylase (HDC) into Histamine from **(Huang, Li, Liang, & Finkelman, 2018)**.

Mast cells, basophils, enterochromaffin-like cells of the gastric mucosa, and histaminergic neurons synthesize considerable amounts of histamine and store the mediator in special storage granules inside the cells. Upon appropriate stimulation, these cells can rapidly release relatively large amounts of histamine and thereby efficiently activate suitable effector mechanisms **(Mahdy & Webster, 2017).**

In mast cells, histamine release is IgE dependent; in gastric cells, gastrin and acetylcholine induce release; and in the nervous system, a nerve impulse stimulates release **(Bylund, 2017).**

Apart from these histamine-storing cell types, there have been persistent observations of histamine in other, nonbasophilic, circulating cells; lymphocytes, monocytes, and platelets appear to contain very low levels of histamine (Table 1) they can express HDC and synthesize histamine. However, in these cells, histamine is immediately released and is not stored **(Mahdy & Webster, 2017).**

In experimental animals less than 10 per cent of injected histidine and less than 1 per cent of ingested histidine is converted to tissue histamine. This newly formed intracellular histamine is released slowly from the tissues, with a biological half-life of about 50 days. Since only a

small portion of ingested histidine is converted to histamine, the supply of histidine in the diet has little effect on stores of histamine in tissues **(Beall & Vanarsdel Jr, 1961).**

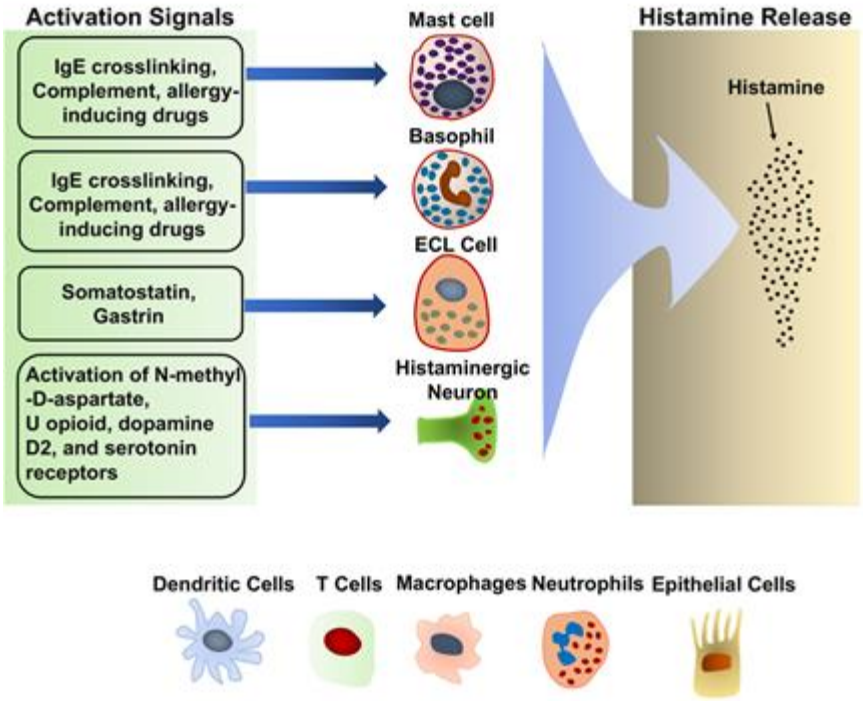

Figure 4: Summary of Major and Minor Histamine-producing Cells and activation signals leading to the release of Histamine from **(Huang et al., 2018).**

Table 1: Low Levels of Histamine Sources from **(MacGlashan, 2003).**

	Relative to basophils
Platelets	1/100,000 (1/1,000 for equivalent cell volume)
Monocytes	1/100
Lymphocytes	1/100–1/1,000

2.4 Gene expression and histamine synthesis

Transcriptional regulation of HDC gene expression in mammals is still poorly understood **(Huang et al., 2018)**. HDC is the rate-limiting enzyme for histamine synthesis. Understanding transcriptional regulation of the HDC gene will advance our knowledge about how this gene

detects extracellular stimuli and increases its transcription, leading to histamine synthesis, replenishment and accumulation that exacerbate allergic inflammation and anaphylaxis.

HDC gene expression and histamine synthesis are regulated both positively and negatively by a range of factors. Notably, crosslinking of FcεRI by antigen binding to FcεRI-associated IgE increases mast cell HDC mRNA expression and histamine synthesis. These mast cell activation-induced increases in HDC mRNA expression and histamine synthesis are also induced by Phorbol 12-myristate 13-acetate. Histamine synthesis also increase as immature mast cells undergo maturation **(Huang et al., 2018).**

However, it is not clear if *in vivo* exposure to IgE promotes maturation and increases HDC mRNA expression. In this regard, **(Katz, Levine, & Austen, 1987)** Demonstrated that chlorotoxin, which induces mast cell maturation by binding to a specific receptor located on the mast cell surface, strongly upregulates HDC gene expression within few hours after the treatment. Chlorotoxin-triggered signals in mast cells then activate transcription factors that directly and rapidly promote HDC gene transcription.

It has been shown that bacteria in the gut of allergic patients can promote HDC mRNA and histamine synthesis by producing substances similar to chlorotoxin.

In line with the notion that factors promoting mast cell maturation also enhance histamine synthesis, cytokines that promote basophil and mast cell maturation, such as IL-3, IL-18, IL-33, GM-CSF, and SCF, have also been reported to increase HDC activity **(Saluja et al., 2015).** Other substances, including chemokines, neuropeptide substance P have also been reported to induce HDC mRNA and histamine synthesis **(Castellani et al., 2008).**

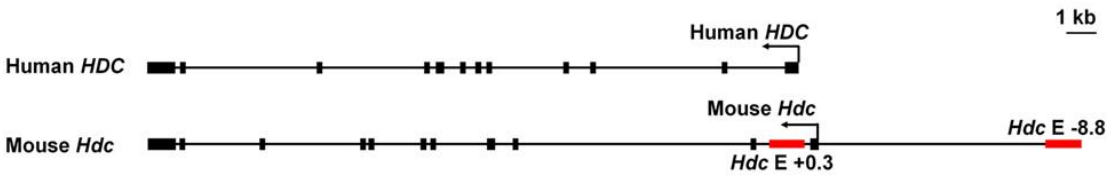

Figure 5: Genomic structures of the human and mouse histidine decarboxylase (HDC) gene. Red bars indicate the enhancers we described: The human HDC gene is located on chromosome 15. It contains 12 exons. HDC mRNA is expressed broadly in many organs, with the highest expression levels found in the gallbladder, stomach, and lung **(Huang et al., 2018).**

2.5 Histamine and histamine receptors

The pleiotropic effects of histamine are mediated by 4 histamine receptors (HRs), H1R, H2R, H3R, and H4R, which are G protein-coupled receptors. The active and inactive conformations of these receptors coexist in equilibrium. Agonists of these receptors stabilize the active conformation, whereas antagonists stabilize the inactive conformation. Curiously, the ageing process impairs expression or activity of HRs, and the enzymes HDC and Diamine oxidase (DAO) may contribute to the progression of allergic reactions and various neurodegenerative disorders **(Branco, Yoshikawa, Pietrobon, & Sato, 2018)**. Chronic itch in the elderly is a common problem that is often multifactorial due to physiological changes in ageing skin, including impaired skin barrier function, and changes in immunological, neurological, and psychological systems associated with age.

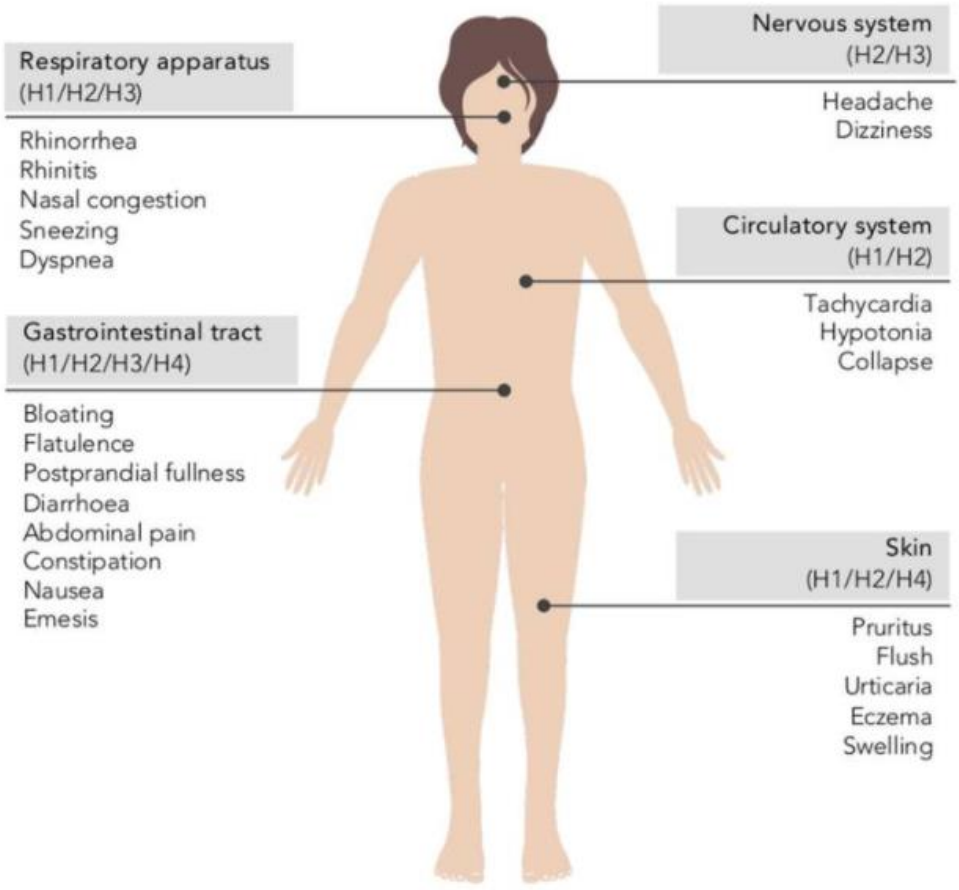

Figure 6: Main symptoms of histamine intolerance and possibly corresponding histamine receptors (Comas-Basté et al., 2020).

- **H1R**

  H1R is expressed in various cell types, such as neurons, endothelial cells, smooth muscle, mast cells, eosinophils (a variety of white blood cells combatting allergic reactions). H1R signaling results in the following: synthesis of prostacyclins; activation of platelet factor; synthesis of nitric oxide, arachidonic acid and its metabolites, and thromboxane; and contraction of smooth muscle cells. In addition, H1R activation leads to increased chemotaxis (movement) of neutrophils at the site of inflammation, higher functional capacity of antigen-presenting cells (APCs), activation of Th1 lymphocytes, and decreased humoral immunity but the promotion of IgE production **(Jutel, Blaser, & Akdis, 2006)**.

  As expected for such biological actions, H1R antagonists, including pyrilamine, fexofenadine, diphenhydramine, and promethazine, are commonly used for the treatment of allergic symptoms. Signaling via H1R leads to the activation of intracellular transcription factors, such as IP3 (inositol triphosphate), PLC (phospholipaseC), PKC (proteinkinase C), DAG (diacylglycerol), and Ca2+ **(Beermann, Bernhardt, Seifert, Buschauer, & Neumann, 2015).**

  Recently, H1R and H4R signaling was implicated in cAMP accumulation, leading to increased pro inflammatory gene expression. In addition, activation of H1R is important for the generation of Th1 lymphocytes responses, whereas H2R regulates Th2 lymphocytes responses. In addition, H1R was demonstrated in an experimental allergy model to play a critical role together with histamine in orchestrating recruitment of Th2 cells to the site of allergic lung inflammation **(Bryce et al., 2006).**

- **H2R**

  H2R is expressed by parietal cells of the gastric mucosa, muscle, epithelial, endothelial, neuronal, hepatocyte, and immune cells. H2R antagonizes some of the effects mediated by H1R and leads to the relaxation of smooth muscle cells, causing vasodilation. H2R activation regulates several of the functions mediated by histamine, including cardiac contraction, gastric acid secretion, cell proliferation, and differentiation **(Schneider, Smoller, & Lamps, 2004).** One recent study demonstrated that histamine acts on H2R and induces inhibition of leukotriene synthesis in human neutrophils through cAMP-dependent protein kinase (PKA) signaling. In a lung inflammation, H2R loss has an effect on T cells, aggravating local inflammation **(Ferstl et al., 2017).**

- **H3R**

  While the activation H1R and H2R mainly accounts for mast cell and basophil-mediated allergic disorders **(Novak et al., 2012)**, H3R functions were identified in the central nervous system, peripheral and presynaptic receptors to control the release of histamine and other neurotransmitters. The asymmetry of histamine via H3R inhibits the acetylcholine released which controls neurogenic inflammation by inhibiting cAMP formation and Ca2+ accumulation **(Dimitriadou et al., 1994)**. H3R regulates insulin resistance as well as a decrease in homeostatic energy: the cellular process for coordinating homeostatic regulation of food intake (energy inflow) and energy expenditure (energy outflow). H3R expression may be associated with bronchoconstriction, pruritus (without involvement of mast cells), increased pro inflammatory activity **(Akdis & Simons, 2006).**

- **H4R**

  H4R is preferentially expressed in the intestine, spleen, thymus, bone marrow and cells of the innate and adaptive immune systems. Activation of this receptor causes chemotaxis in mast cells, leading to an accumulation of inflammatory cells and control of cytokine secretion by Dendritic and T cells. H4R is also involved in increased secretion of IL-31 causing maturation of mast cells **(Gutzmer et al., 2009)**. The binding of histamine to eosinophil H4R induces increased expression of macrophage and adhesion molecules. These events favor the migration of eosinophils from the bloodstream to the site of inflammation. In mast cells, the binding of histamine to this same receptor promotes the intracellular release of calcium and recruitment of mast cells into tissues **(Hofstra, Desai, Thurmond, & Fung-Leung, 2003).**

Table 2: Immunological functions mediated by histamine receptors signaling **(Branco et al., 2018)**.

Receptor	Expression	Intracellular signaling	Immunological activity
H1R	Endothelial cells, nerve cells, epithelial cells, neutrophils, eosinophils, monocytes, macrophages, DCs, and T and B cells	PLC, PIP2, DAG, IP3, Ca^{2+}, and PKC	Allergic reactions and inflammation, histamine release, eosinophil and neutrophil chemotaxis, antigen presentation ability, Th1/IFN-γ activity, and recruitment of Th2 cells; decreases humoral immunity and IgE production
H2R	Endothelial cells, nerve, epithelial, neutrophils, eosinophils, monocytes, macrophages, DCs, and T cells and B	Adenyl cyclase, cAMP, PKA, CREB, and EPAC	Increases IL-10 production and humoral immunity; decreases cellular immunity; inhibits Th2 cells and cytokines, chemotaxis of eosinophils, and neutrophils; suppresses IL-12p70 of MoDCs
H3R	Histaminergic neurons, monocytes, eosinophils	Inhibitor of adenyl cyclase and cAMP; increases levels of Ca^{2+}	Control of neurogenic inflammation, increased proinflammatory activity, and antigen presentation capacity
H4R	Eosinophils, DCs, Langerhans cells, neutrophils, T cells, basophils, and mast cells	Inhibitor of adenyl cyclase and cAMP; increases levels of Ca^{2+}	Affects pDC and mDC functions, Th1/Th2 differentiation, eosinophil and mast cell chemotaxis, IL-6 production, leukotriene B4, and migration of Tγ/δ cells; increases IL-17 secretion by Th17 cells, and regulatory T recruitment; suppresses IL-12p70 of MoDCs

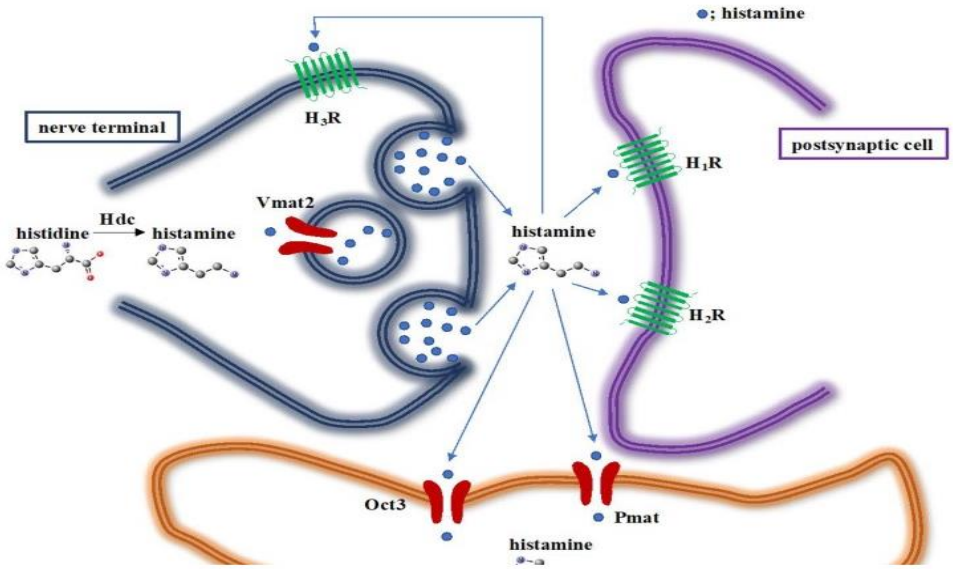

Figure 7: Neurotransmission and termination of histaminergic nervous system. Histidine decarboxylase (Hdc) synthesises histamine from histidine. Histamine is stored in synaptic vesicles via vesicular monoamine transporter 2 (Vmat2). Upon stimulation, histamine is released to extraneuronal spaces. Histamine exerts its effects through interactions with postsynaptic histamine h1 receptor (H1R) and H2R, and presynaptic H3R. Extracellular histamine is transported via organic cation transporter 3 (Oct3) and plasma membrane monoamine transporter (Pmat). Finally, histamine is metabolised by histamine N-methyltransferase (Hnmt) from **(Yoshikawa, Nakamura, & Yanai, 2019).**

2.6 Histamine in health and diseases

As discussed above, the pleiotropic effects of histamine are a consequence of the existence of four different receptors that belong to the same family of G-coupled proteins and trigger different signaling cascades; these receptors are differentially distributed across tissues and cells **(Smolinska, Jutel, Crameri, & O'mahony, 2014)**. In addition to its well-known three functions (smooth muscle contraction, increased vascular permeability and stimulation of gastric acid secretion), histamine is recognized as a key player in immune regulation. **(Mahdy & Webster, 2017).**

Elevations in plasma or tissue histamine levels have been noted during anaphylaxis and experimental allergic responses of the skin, nose, and airways. Histamine is only one of many mediators of allergic disease, it plays a primary role in allergic rhinitis, urticaria, anaphylaxis, and to a lesser degree, asthma **(White, 1990).**

Histamine plays various roles in immunomodulation, inflammation, regulation of cell proliferation and differentiation, hematopoiesis, embryonic development, regeneration and wound healing. Moreover, as a neurotransmitter, histamine is involved in the regulation of sleep and wakefulness, cognition, memory, energy and endocrine homeostasis. It also modulates the release of several neurotransmitters through presynaptic receptors located on histaminergic and non-histaminergic neurons of the central and peripheral nervous system. Histamine also plays a pivotal role in the pathogenesis of allergic inflammation. In response to an antigen, reaginic antibodies of the immunoglobulin E type are generated **(Mahdy & Webster, 2017).**

Dietary histamine has also been implicated in the pathogenesis of migraine in susceptible individuals suffering from DAO deficiencies **(L. Maintz & N. Novak, 2007).** Histamine-rich food has been reported by migraine patients to trigger headache which are alleviated by a histamine-free diet **(Wantke, Götz, & Jarisch, 1993).**

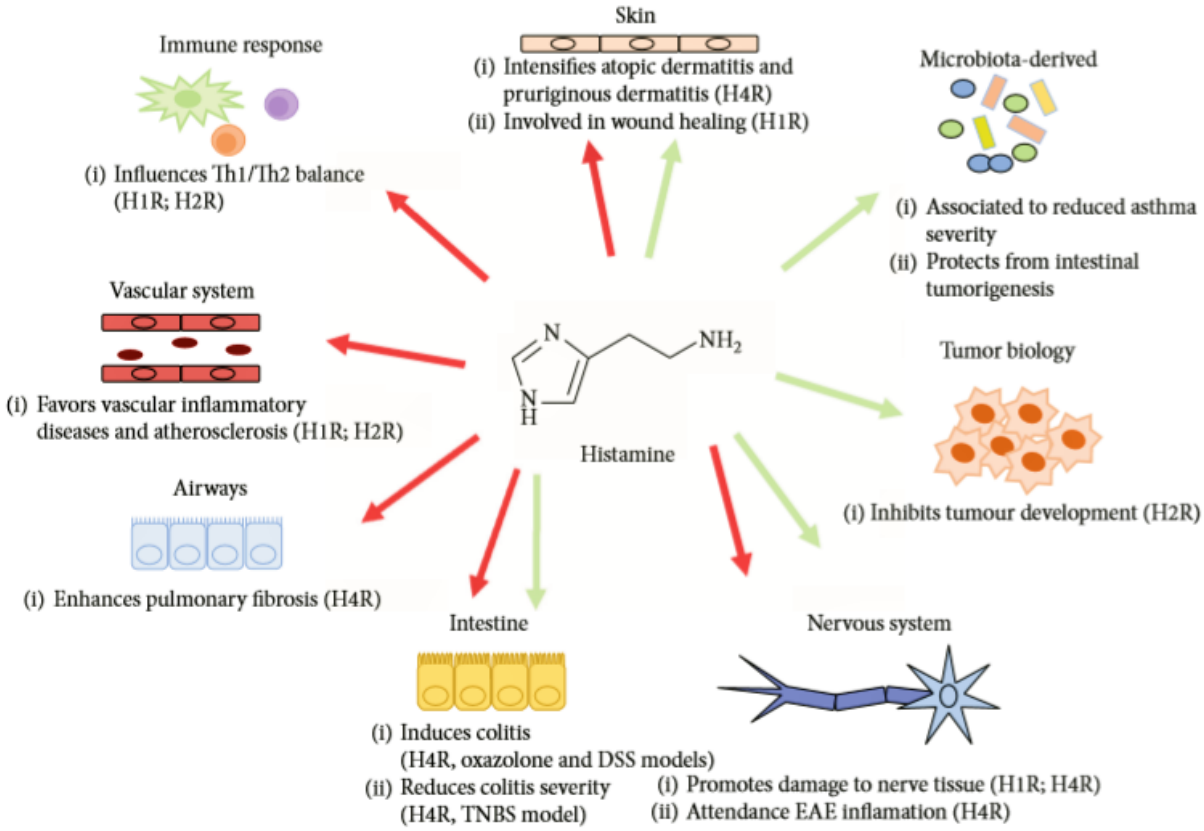

Figure 8: Inflammatory and regulatory functions of histamine on different body sites. Histamine plays dual functions according to the cell type and the receptor. As an inducer of inflammation, histamine can contribute to pulmonary fibrosis, cardiovascular diseases and atherosclerosis, atopic dermatitis, central nervous system damage, and colitis in some experimental models, besides favoring the polarization of the immune response to a Th1 profile. On the other hand, histamine can regulate inflammation in models of experimental autoimmune encephalomyelitis (EAE) and colitis, favor wound healing in skin lesions, and inhibit tumour development. Also, microbiotaderived histamine can regulate the inflammatory picture of asthma. Red arrows indicate proinflammatory action; green arrows indicate regulatory action of histamine from **(Branco, Yoshikawa, Pietrobon, & Sato, 2018).**

2.6.1 Role of histamine in pathogenesis of IBD

Knutson and colleagues (1990) found that the histamine secretion rate was increased in patients with Crohn's disease compared with normal controls, and the secretion of histamine was related to disease activity, indicating strongly that degranulation of mast cells was involved in active Crohn's disease. The highly elevated mucosal histamine levels were also observed in allergic enteropathy and ulcerative colitis. Moreover, enhanced histamine metabolism was found in collagenous colitis and food allergy **(Schwab, Hahn, & Raithel, 2003)**, and increased level of N-methylhistamine, a stable metabolite of the mast cell mediator histamine, was detected in the urine of patients with active Crohn's disease or ulcerative colitis **(Winterkamp**

et al., 2002). Since increased level of N-methylhistamine was significantly correlated to clinical disease activity, the above findings further strongly suggest the active involvement of histamine in the pathogenesis of these diseases. Interestingly, mast cells originated from the resected colon of active Crohn's disease or ulcerative colitis were able to release more histamine than those from normal colon Fox **(Lichtenstein, & Roche, 1993).**

Similarly, cultured samples from patients with IBD secreted more histamine towards neuropeptide substance P alone or substance P with anti-IgE than the samples from normal control subjects under the same stimulation **(Raithel, Schneider, & Hahn, 1999)**. As a proinflammatory mediator, histamine is selectively located in the granules of human mast cells and basophils and released from these cells upon degranulation. To date, a total of three histamine receptors H1, H2 and H3 have been discovered in human gut **(Bertaccini & Coruzzi, 1995)**. It proves that there are some specific targets that histamine can work on in intestinal tract. The finding that H1-receptor antagonist pyrilamine was able to inhibit anti-IgE induced histamine release and ion transport **(Crowe, Luthra, & Perdue, 1997)** suggested further that histamine is a crucial mediator responsible for diarrhea in IBD and food allergy.

2.7 Histamine catabolism

The degradation of endogenous histamine following its release from cellular stores has not been directly studied due to the technical difficulties involved. Instead, most studies have involved the intravenous or subcutaneous injection of trace amounts of histamine. Isotopic labeling of histamine has aided the identification of metabolites **(Beall & Vanarsdel Jr, 1961).**

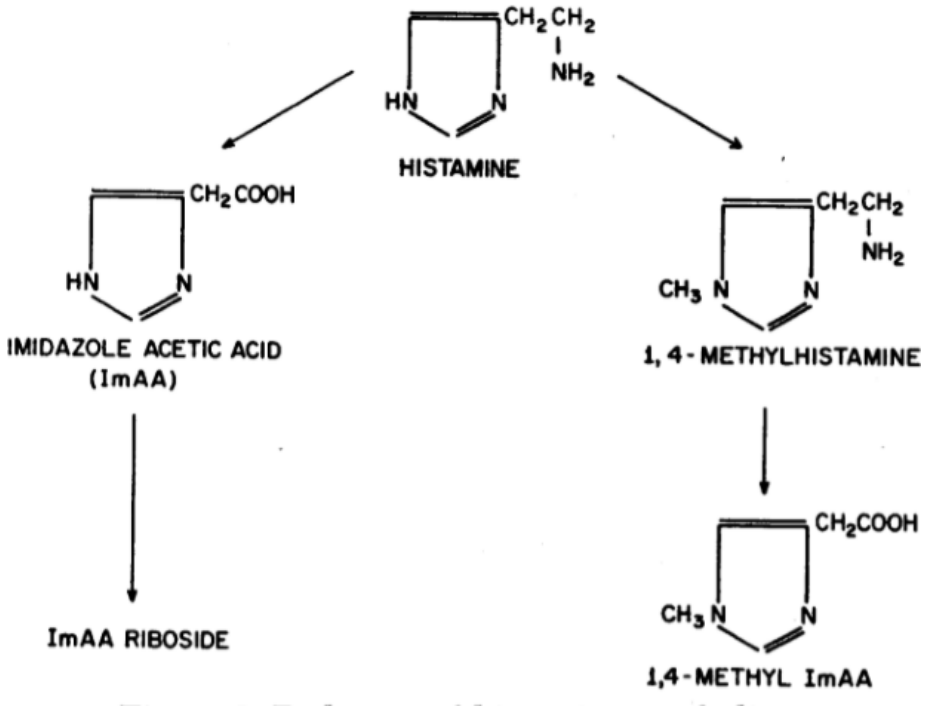

Figure 9: Pathways of Histamine catabolism **(Beall & Vanarsdel Jr, 1961)**.

Beall and Vanarsdel (1961) experimentation showed that the Injected radioactive histamine is rapidly cleared from the plasma and the metabolic products are rapidly excreted in the urine. When small amounts are injected, very little unchanged histamine appears in the urine.

There are two main pathways of intracellular histamine catabolism (Figure 9). First, histamine may be oxidized to imidazole-4(5) -acetic acid (ImAA) through imidazole-4(5) -acetaldehyde. Much of the resulting ImAA is then conjugated with ribose, leading to urinary excretion of both ImAA and ImAA riboside. In the second pathway, histamine is methylated by histamine N-methyltransferase which is a cytosolic enzyme expressed in the liver, colon and kidney, to form 1,4methyl-histamine. This is followed by oxidation of the side chain, presumably through the corresponding aldehyde to 1,4 methylimidazoleactic acid (meImAA) by monoamine oxidase.

Alternatively, DAO is responsible for metabolizing extracellular dietary histamine, that enters the body from food or made by bacteria in the gut **(Thompson, 1990)**, to imidazole acetic acid, which can be further conjugated to form imidazole acetic acid ribose, preventing its uptake into the circulation. However, the DAO pathway is not active in the central nervous system **(Mahdy & Webster, 2017).**

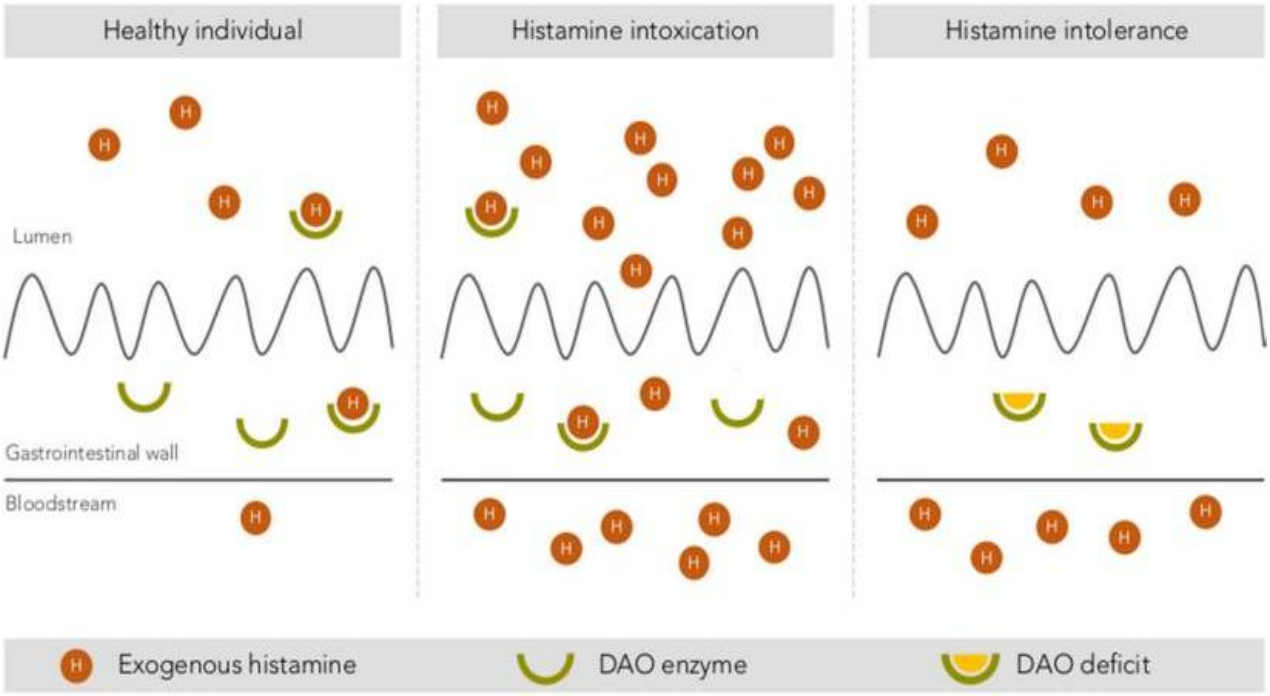

Figure 10: Intestinal degradation of histamine by the DAO enzyme in three different situations: in a healthy individual, with histamine intoxication and with histamine intolerance **(Comas-Basté et al., 2020).**

Although the exact mechanism of the pathogenesis is still unclear, a reduced intestinal diamine oxidase(DAO) activity, which is important for degradation of exogenously supplied histamine, lead to an insufficient degradation of food derived histamine, which passes into the blood stream leading to increased plasma histamine concentrations and evoking the described symptoms by affecting various organ systems (e.g.cardiovascular system, respiratory tract, skin, nervous system, intestine) **(Kovacova-Hanuskova, Buday, Gavliakova, & Plevkova, 2015)** an thus promote histamine sensitivity in some persons.

We should also note that there are several kinds of anti-histaminics that suppress the symptoms originated from an elevated amount of histamine, however DAO remains the only compound that is naturally synthesized and presents no risk upon ingestion.

CHAPTER III: AMINE OXIDASES

3.1 Biogenic amines

Biogenic amines (BAs) are compounds found in different foods and are produced by specific microorganisms (bacteria, yeasts, and molds) as a result of the metabolism of some free amino acid by enzymatic reactions which are mostly of microbial origin.

This enzymatic reaction can be decarboxylation, reductive amination, transamination, and degradation of certain precursor amino compounds and are cause of poisoning.

The main BAs found in foods are cadaverine, putrescine, tyramine, histamine, spermine and spermidine. The number of food poisoning cases associated with BAs in food has increased in the recent years reinforcing the need for early detection to ensure high levels of food quality and safety.

The quantity and variety of BAs formed in foods is influenced by the microbiota growth during food storage and processing, by extrinsic parameters such as, storage time and temperature, and intrinsic food characteristics, including water activity and pH. Decarboxylases enzymes are produced by certain group of bacteria, such as, Enterobacteriaceae, Clostridium, Streptococcus, Lactobacillus, and Pseudomonas **(Özogul et al., 2018).**

3.2 Amine oxidases

Amine oxidases (AOs), a widespread class of enzymes, are present in all living systems **(G. Floris & Finazzi Agrò, 2013)**, distributed among living organisms in plant microorganisms and animal tissues **(Mondovì & Agrò, 1982),** where they control the level of very active compounds such as, mono-, di-, and polyamines.

Two main types of amine oxidases have been described **(Mondovì & Agrò, 1982),** based on the chemical nature of the attached cofactor **(Jalkanen & Salmi, 2001).**

Monoamine oxidases **(**EC 1.4.3.4) that are the flavin containing amine oxidases and copper-containing amine oxidases (EC 1.4.3.6) that contain copper (CuAO) **(Gundidza, 1985),** which are dual-functioning enzymes that catalyzes the biosynthesis of a self-derived coenzyme and subsequent oxidative deamination of primary amines.

(CuAO) are homodimers in which each subunit contains a copper ion and a 2,4,5-trihydroxyphenylalanine quinone (TPQ) cofactor generated by posttranslational autocatalytic modification of an active site tyrosine residue. Analyses of genes and cDNAs encoding copper

amine oxidases revealed that all members of this enzyme family have homologous sequences with several absolutely conserved amino acid residues. The conserved residues appear to be important for the overall protein structure and for the catalytic function and include the tyrosine that is converted to topa quinone, three histidine residues that bind the copper ion, and an aspartic acid residue important for substrate conversion **(Wilflingseder & Schwelberger, 2000).**

These enzymes are critical to both homeostatic and xenobiotic metabolic pathways and are involved in the biotransformation of aminergic neurotransmitters (such as catecholamines, histamine, and serotonin) as well as toxins and carcinogens in foods and the environment

(Chan, 2019).

They catalyze the oxidative deamination of a terminal amino group of primary amines, with the formation of the corresponding aldehyde, hydrogen peroxide and ammonia according to the following equation **(Mondovì & Agrò, 1982).**

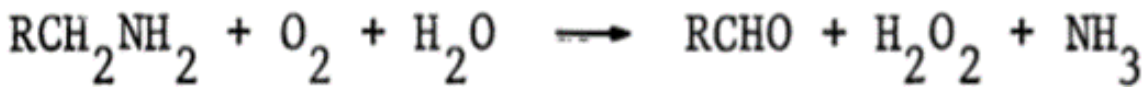

Figure 11: Oxidative deamination catalyzed by amine oxidases. **(Achee, Chervenka, Smith, & Yasunobu, 1968)**

3.3 Flavin containing amine oxidases

Mono-amines oxidases (MAO) are Flavin containing enzymes located in the outer membrane of mitochondria of several cells. Monoamine oxidase were originally named "Tyramine oxidase", since the pressor amine, tyramine, was its first known substrate. Distribution of this enzyme in nature is widespread, being found in both bacteria and animals. *Sarcina lutea* is the most well-known bacterial source **(Voigt & Eitenmiller, 1978).**

Flavin containing monoamine oxidases are made up of two subunits linked by a disulfide bridge, each subunit contains a flavin adenine dinucleotide (FAD) group linked covalently **(De Colibus et al., 2005).**

Monoamine oxidase acts on ordinary monoamines such as adrenaline, tyramine and tryptamine, also on many aliphatic monoamines **(Yamada, Adachi, & Ogata, 1965).**

MAOs oxidize the neurotransmitters, serotonin and dopamine. The activity of MAO in the brain influences behavior by changing neurotransmitter levels. Inhibition of MAO increases the neurotransmitters, alleviating depression and sparing dopamine in Parkinson's disease (**Ramsay**, **2020**).

In 1968, Johnston discovered that MAO exists in two forms, **(Benedetti & Dostert, 1989)**, MAO A and MAO B, on the basis of their amino acid sequence, substrate and inhibitor specificities **(Mondovì & Agrò, 1982).**

3.3.1 Monoamine oxidase function

MAOs function is the oxidative deamination of mono-amines. Their role in different species is to protect the organism from the invasion of exogenous mono-amines by their detoxification to barriers (intestines, lungs, placenta, liver). They also have a role in the catabolism of endogenous mono-amines, MAO-A with a preference for serotonin and MAO-B that metabolizes phenylethylamine. Both types of enzymes metabolize adrenaline, norepinephrine and tyramine **(Glover, Sandler, Owen, & Riley, 1977)**.

The ubiquity of biogenic amines and their central role in neural and cardiovascular function make MAOs highly relevant to clinical anesthesia. The interactions between MAO inhibitors and drugs commonly used in anesthesia have been well described. Although genetic polymorphisms in MAO genes exist and are of great interest in the fields of neurology and psychiatry, to date none have been identified that specifically concern the handling of anesthetic agents **(Chan, 2019).**

3.4 Copper containing amine oxidases

Humans have three functioning genes that encode copper-containing amine oxidases **(A. P. McGrath et al., 2009),** this encoding gives three subclasses:

3.4.1 Lyslys oxidase

(EC 1.4.3.13) is a copper-dependent AO encoded by the ***LOX*** gene, It catalyzes the conversion of lysine molecules into highly reactive aldehydes that form crosslink in extracellular matrix protein **(Hämäläinen et al., 1991)**, and stabilize collagen and elastin fibers, thus being involved in connective tissue formation. LOX uses lysyl tyrosyl quinone as a cofactor, which is required for its catalytic activity in the extracellular matrix **(Collins, 2017).**

3.4.2 Primary-amine oxidase

(EC 1.4.3.21) enzyme is encoded by the AOC2, AOC3 genes, it is also known as semi carbazide-sensitive amine oxidase (SSAO) because Semi carbazide inhibits the enzyme **(Haywood & Large, 1981)**, SSAO is present in various human tissues, plasma **(Matyus et al., 2004)**, smooth muscle of blood vessels and various other tissues. Like (MAO), SSAO can deaminate short-chain primary amines **(Haywood & Large, 1981),** to afford the corresponding aldehydes, ammonia and hydrogen peroxide **(Matyus et al., 2004).**

They are believed to be detoxifying enzymes, primarily involved in the oxidative deamination of endogenous amines, such as methylamine and aminoacetone, together with some xenobiotic amines. However, it appears that the reaction products may have important signalling functions in the regulation of cell development and glucose homeostasis. Furthermore, enzyme, from some sources, behaves as a cellular adhesion protein under inflammatory and it may also be involved in lipid transport **(O'Sullivan et al., 2004).**

3.4.3 Diamine oxidase

DAO (EC 1.4.3.6) a secretory protein also known as histaminase, encoded by *AOC1* gene **(Wolvekamp & de Bruin, 1994)**. It is a copper-containing amine oxidase that exists in plants and mammals and can also be synthesized by some microorganisms

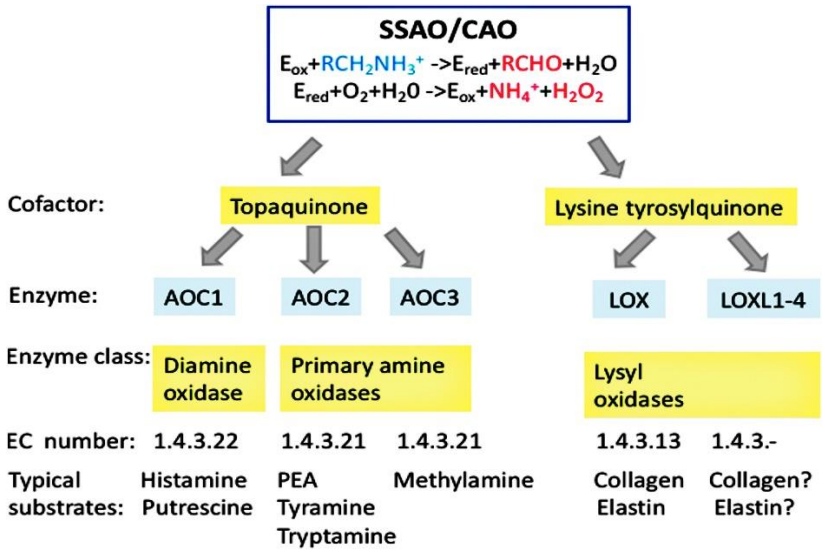

Figure 12: SSAOs/CAOs oxidate primary amines into aldehydes, ammonium and hydrogen peroxide in a two-step reaction. *SSAOs/CAOs can be classified based on their enzymatic properties (cofactor, substrates) or molecular properties (gene sequences). AOC, amine oxidase copper containing; CAOs, copper-dependent amine oxidases; LOX, lysyl oxidase; LOXL, lysyl oxidase like; PEA, phenyl ethylamine; SSAOs, semicarbazide-sensitive amine oxidases* ***(Salmi & Jalkanen, 2019)***.

CHAPTER IV: DIAMINE OXYDASES

4.1 Synthesis

DAO mainly an intracellular enzyme named for its substrate preference for diamines particularly histamine **(A. P. McGrath et al., 2009).** Predominantly synthesized in thymus, renal proximal tubular and intestinal epithelial cells of the digestive tract. it is stored in plasma membrane associated vesicular structures **(Blemur et al., 2016).**

The enzyme from porcine kidney was purified by consecutive chromatography on concanavalin A Sepharose, heparin Sepharose and Mono Q. Besides being simpler and faster than previous methods, this new purification scheme results in a homogenous product with a considerably higher yield and allows the rapid purification of large amounts of DAO from mammalian tissues. The availability of sufficient pure protein greatly facilitate studies of the structure and function of the enzyme **(Wilflingseder & Schwelberger, 2000).**

4.2 Structure

The homodimeric structure has the archetypal amine oxidase fold. Two active sites, one in each subunit, are characterized by the presence of a copper ion and possesses a covalently linked active-site cofactor, the topaquinone, derived from the post translational modification of an endogenous tyrosine residue **(Blemur et al., 2016),** which is absolutely required for catalysis **(Agostinelli et al., 2010).**

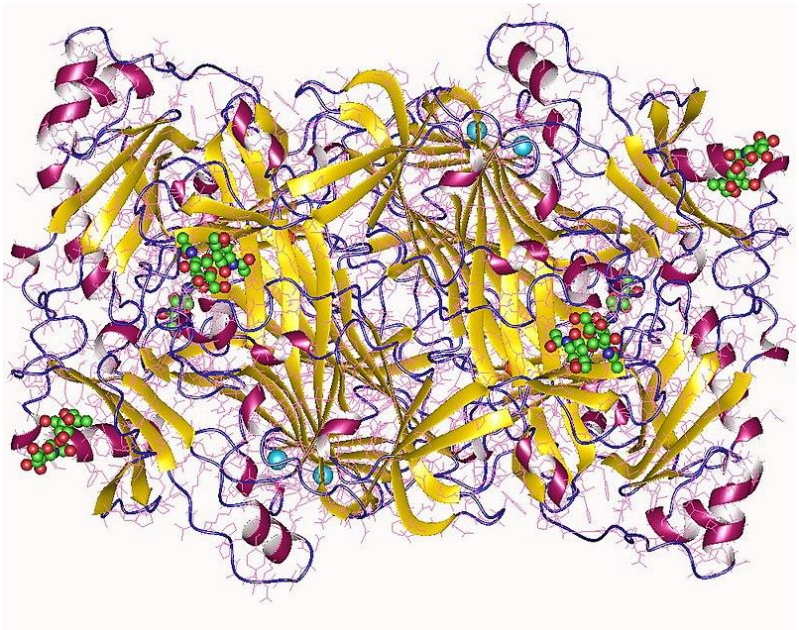

Figure 13: Ribbon Structure of human DAO with copper ions in both active centers**.(Aaron P. McGrath, Hilmer, Collyer, Dooley, & Guss, 2010)**

4.3 Function

DAO is considered the main extracellular enzyme for the intestinal degradation of dietary histamine **(Blemur et al., 2016),** that enters the body from food or made by bacteria in the gut **(Thompson, 1990),** to regulate inflammation and allergic reactions. Also for the degradation of other biogenic amines, it catalyzes the oxidative deamination of the primary amine group of polyamines putrescine, spermine, and spermidine, with the release of corresponding aldehydes, hydrogen peroxide, and ammonia **(Blemur et al., 2016).**

Bacteria and yeasts can utilize amines as nitrogen and carbon sources through the reaction with amine oxidase. Plant DAOs have an important role in cell growth by regulating the intracellular di- and polyamine levels, and the aldehyde products might have a key role in the biosynthesis of some alkaloids **(Giovanni Floris & Agrò, 2004)**.

The function of amine oxidases in mammals is even more diverse and elusive. Amine oxidase activity is found in many tissues, the highest levels being in decidual cells of placenta, in kidney tubular epithelial cells, and in intestinal epithelial cells. These localizations may suggest a general barrier function for this enzyme in preventing the entrance of extracellular diamines and polyamines into circulation **(Giovanni Floris & Agrò, 2004)**.

Furthermore, these enzymes may keep under control the endogenous histamine, which may be responsible for several pathological conditions like allergy, peptic ulcer, and anaphylactic reactions **(Giovanni Floris & Agrò, 2004)**.

Several observations point to a relationship between amine oxidase activity and growth, both in normal and tumoral tissues possibly correlated with cell proliferation and differentiation. DAOs have also been proposed as immune response modulators **(Giovanni Floris & Agrò, 2004)**.

It has been demonstrated that human placental diamine oxidase is identical to the amiloride-binding protein and thus in some way is involved in the regulation of epithelial ion transport **(Giovanni Floris & Agrò, 2004)**.

The molecular mass of the enzyme estimated by Sephadex G-100 gel filtration was 121 kDa. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) displayed a single band at a molecular mass of 52 kDa. The enzyme had optimal activity at 40 °C and retained its activity after being incubated at 30 °C for 30 min. It showed higher activity at pH 6.5 than at other pH values. The enzyme was significantly inhibited by Mg(2+), Cu(2+), Fe(3+),

aminoguanidine, ethylene glycol tetra acetic acid (EGTA), ethylene diamine tetra acetic acid disodium salt (EDTA-Na(2)), L-cysteine and β-mercaptoethanol **(Yang, Chen, Han, & Gu, 2012).**

DAO has been cloned and expressed in insect cells and the structure of the native enzyme determined by X-ray crystallography **(McGrath et al., 2009).**

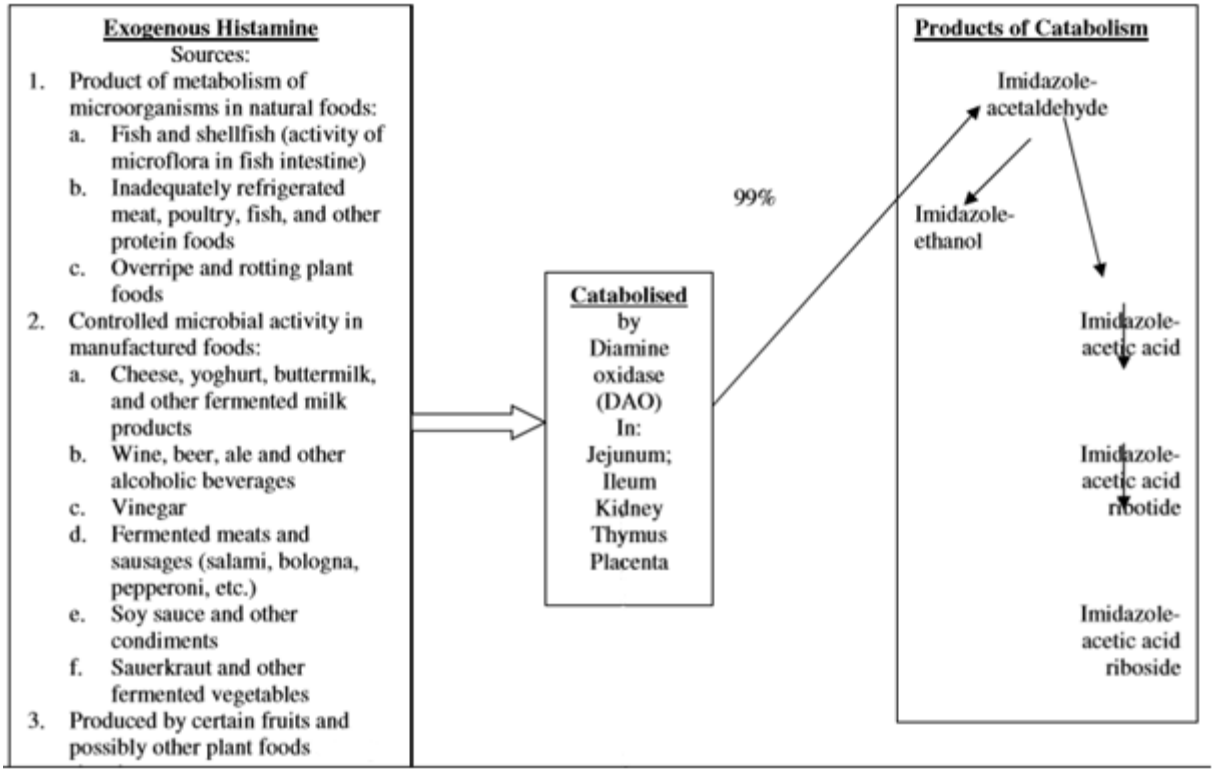

Figure 14: Sources of Exogenous Histamine and the products of its catabolism by Diamine oxidase (DAO) in the gut. **(Joneja & Honorary, 2014)**

4.4 Detection of DAO activity

In need of a simple and sensitive method for detection of diamine oxidase (EC 1.4.3.6) activity in connection with diamine oxidase purification from human placenta, an enhanced chemiluminescence method using putrescine as substrate and horseradish peroxidase and luminol for the detection of the H2O2 produced by diamine oxidase was developed. The method allows direct detection of small amounts of diamine oxidase in serum samples after agarose gel electrophoresis and allows visualization of diamine oxidase activity in tissue sections. Employing this method, diamine oxidase in sera from cow, 44, monkey, rabbit, and pregnant women was detected. On tissue sections from term human placenta diamine oxidase activity

was exclusively localized to the maternal side and was concentrated in vessels and fibrinoid areas **(Bruun & Houen, 1996).**

Diamine oxidase activity is measured by spectrophotometrical methods, monitoring directly the absorbance of formed aldehydes or by subsequent condensation of different compounds.

Other methods are based on radiometric assays, with [1,4-^{14}C] putrescine as substrate, on oximetric or polarographic methods measuring the rate of oxygen consumption in the presence of substrate or on fluorometric determinations, where homovanillic acid is converted into a highly fluorescent compound by the released H_2O_2 in the presence of peroxidase.

All these methods are not giving information on the loss of molecular integrity of DAO (i.e., to acidic or proteolytic hydrolysis).

Thus, supplemental information can be obtained using polyacrylamide gel electrophoresis (PAGE) by monitoring the protein pattern (staining gels with Coomassie Blue) and the enzymatic activity (zymography) **(Calinescu, Federico, Mondovi, & Mateescu, 2010).**

4.5 Diamine Oxidase activity in Inflammatory Bowel Disease

In humans and rodents, DAO is specifically located at the apical end of mature villous cells with high activity and its activity reflects the integrity and maturity of the small intestinal mucosa. Several studies of humans and animals revealed that DAO activity in serum inversely correlates with intestinal permeability of small intestine.

The intestinal barrier is formed by epithelial cells and the junctional complex, including the tight junction (TJ) complex. Alterations of the composition of TJs was reported for both Crohn's disease (CD) and ulcerative colitis (UC) and consisted of an impaired permeability may represent the early onset of inflammatory bowel disease (IBD).

Honzawa et al, conducted a study on human serum samples from patients with active and unactive CD and UC. Serum DAO activity was measured by enzyme-linked immunosorbent assay (ELISA) system according to the methods reported previously in **(Takagi et al., 1994).**

The results showed that Serum DAO activity in patients with CD was significantly lower than that in control subjects (Figure 15.A). In addition, serum DAO activity in patients with UC was significantly lower than that in control subjects (Figure 15.A). There were no significant

differences between patients with active CD and inactive CD or in patients with active UC and inactive UC (Figure 15.B).

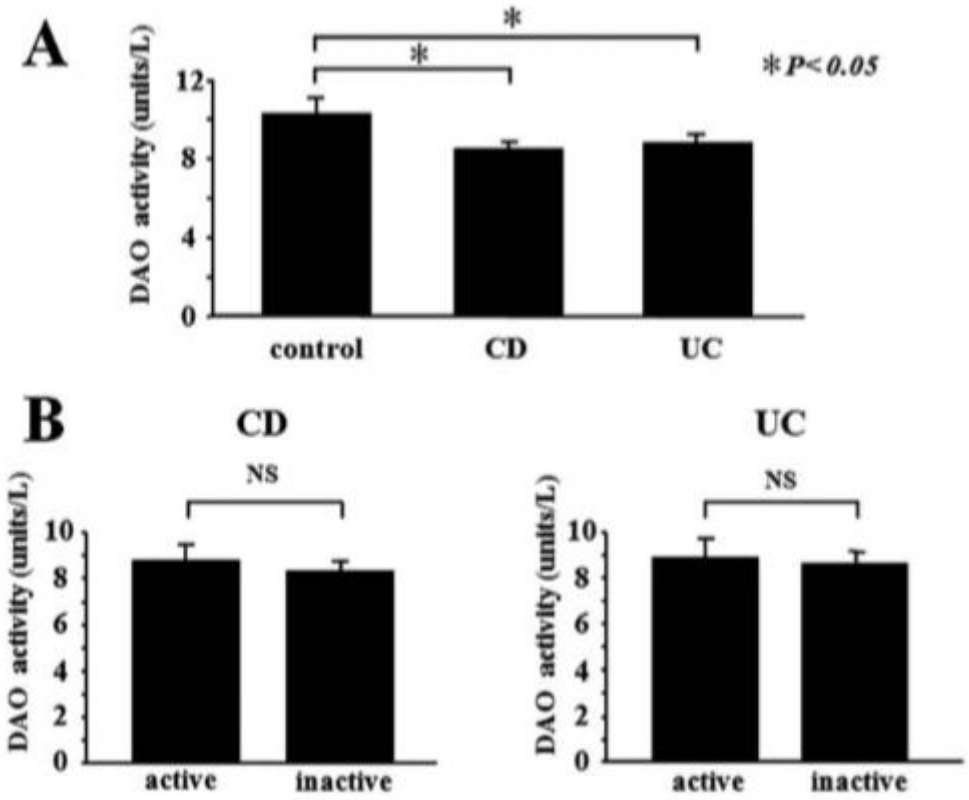

Figure 15: Human serum DAO activity comparison in patients with active, inactive UC and CD (A) Serum DAO activity in patients with IBD. Serum samples were obtained from patients with active CD (n ¼ 13), inactive CD (n ¼ 42), active UC (n ¼ 12), inactive UC (n ¼ 31), and healthy volunteers as normal controls (n ¼ 17). Statistically significant compared to normal controls ($P < 0.05$). NS, not significant. (B) Comparison of serum DAO activity between patients with active and inactive CD or in patients with active and inactive UC. **(Honzawa et al., 2011)**

This study first demonstrates that serum DAO activity is significantly lower in patients with CD and UC regardless of the level of disease activity. Of note, in patients with UC in whom small intestinal inflammation was not involved, serum DAO activity was significantly lower than that in healthy controls. These findings strongly indicate that small intestinal permeability is strongly involved in the pathophysiology of not only CD but also UC.

Another important finding of this study is that serum DAO activity was decreased in both UC and CD patients. In this regard, we must focus on regulating mucosal permeability of the small intestine as a future therapeutic strategy for IBD patients.

Therefore, measurement of serum DAO activity can be an easy and convenient modality for evaluating small intestinal permeability. Reduced serum DAO activity in patients with IBD suggests the importance of mucosal permeability of the small intestine as a pathogenic factor and its measurement together with serology, clinical factors, and genetics might be useful for predicting the disease onset of IBD. Further prospective studies with this modality are required **(Honzawa et al., 2011).**

CHAPTER V: DAO AS A DIETARY SUPPLEMENT

5.1 DAO deficiency and histamine intolerance

Biogenic amines including histamine are produced by bacterial decarboxylation in food **(Doeun et al., 2017)**. If the amount of ingested biogenic amines is high and/or their degradation is inhibited or disturbed in the body, then histamine is thought to cause multiple gastrointestinal (GI) symptoms. These may be accompanied by extra-intestinal symptoms including cardiovascular, respiratory and skin complaints **(Reese et al., 2017)**. Symptoms of a disproportionate amount of histamine are thought to be caused by the reduced activity of the enzyme diamine oxidase (DAO).

The clinical diagnosis of HIT is challenging. Although, patients with low serum DAO values, two or more GI symptoms described for HIT, and a reduction of complaints due to a histamine-reduced diet may be diagnosed with HIT. However, DAO is considered the main extracellular enzyme for the intestinal degradation of histamine and other biogenic amines **(Elmore et al., 2002; Jones and Kearns, 2011).**

DAO deficiency is one of the main causes of histamine intolerance, an alteration in the homestasis of histamine, which results in a reduced intestinal degradation, and its subsequent increase in plasma **(Schwelberger, 2010)**. DAO deficiency may be congenital; resulting from genetic mutations in DAO gene (chromosome 7q36) that code for an altered protein with low enzymatic activity **(Kovacova-Hanuskova et al., 2015)**, or acquired by certain pathologies that limit DAO secretion, especially in inflammatory or degenerative intestinal disorders such as IBD we previously mentioned, or by enzymatic blockade by some commonly used drugs **(Laura Maintz & Natalija Novak, 2007)**. Reduced DAO activity has been described by several clinical studies in patients diagnosed with some pathology such as atopic eczema, chronic urticarial, chronic abdominal pain or inflammatory bowel diseases **(Honzawa, Nakase, Matsuura, & Chiba, 2011)** . A recent study also reported a high prevalence (87%) of DAO deficiency in a group of 137 patients diagnosed with migraine which is one of the major symptoms of HIT.

DAO deficiency is therefore responsible of plasmatic histamine accumulation, thus could be one of the histamine intolerance symptoms triggers.

Intestinal inflammatory diseases, DAO deficiency and histamine intolerance are common but current therapies have limited efficacy **(Fogel & Lewinski, 2006).**

They are treated in different ways, including by supplementing with DAO **(Manzotti, Breda, Di Gioacchino, & Burastero, 2016).**

Low-histamine or histamine-free diet has been proposed as the main strategy for the preventive treatment of histamine intolerance. Conceptually, these diets exclude a number of foods that patients associate with the onset of symptoms, primarily those that may contain high levels of histamine. However, there is no single dietary recommendation of a low-histamine diet **(Comas-Basté et al., 2020)**

A histamine-reduced diet may present a challenge to HIT patients, because the composition of biogenic amines and levels of histamine in food and drinks are frequently unknown.

In general, at least roughly 20 percent of patients with food intolerance/malabsorption cannot comply a comprehensive diet plan. Therefore, a study was conducted in HIT patients and found that oral DAO supplementation helps reduce symptoms. It has been speculated that this may be due to its ability to degrade ingested intestinal histamine**.**

Taking this supplement will not affect the levels of histamine produced internally, as this type of histamine is broken down by N-methyltransferase **(Schnedl et al., 2019).**

5.2 Sources of DAO

The application of diamine oxidase (DAO, EC 1.4.3.6) or bacteria containing this enzyme are emerging approaches to degrade histamine in food. DAO has been isolated from many sources such as the organs of pigs (liver, kidney), human placenta and blood plasma, and from micro-organisms including Microbacterium lacticum and Arthrobacter crystallopoietes KAIT-B-007 **(Naila et *al*., 2012).**

Diamine oxidases (DAOs), are ubiquitous enzymes occurring in microorganisms (fungi and bacteria), plants, and mammals. Some DAOs have been crystallized from bacteria, the yeast *Hansenula polymorpha*, and from pea seedlings. The crystal structure shows that the copper atom is coordinated by three histidine side chains and two water molecules, laying at approximately 6Å distance from TPQ. Plant DAOs from various species have been purified to homogeneity and characterized, the best known and studied being those from lentil (*Lens esculenta*) and pea (*Pisum sativum*) and from latex of the shrub *Euphorbia characias*. In mammals, the best-known enzymes are those from pig kidney and intestine, and from human

placenta. DAOs prefer short aliphatic diamines like putrescine (1,4 diaminobutane) and cadaverine (1,5 diaminopentane) as substrates.

5.2.1 Bacterial DAO

If active diamine oxidase could be supplied to the human digestive tract from a probiotic source, it may be possible to reduce the level of histamine entering the body from the digestive tract. Some preliminary research indicates that certain strains of bacteria, such as species of Lactobacillus, Leuconostoc, Escherichia faecium, Weisella and Sarcina, can produce diamine oxidase **(Dapkevicius et al 2000; Leuschner et al 1998).** It is possible that other micro-organisms able to synthesis the enzyme exist, which could be exploited in the production of a probiotic food able to deliver the deficient enzyme to the human gut.

Search of the NCBI gene catalogue **(http://www.ncbi.nim.nih.gov)** for copper-associated diamine oxidase reveals that the gene sequence has been delineated in a variety of prokaryotes and eukaryotes. Bacteria showing gene homology with the human enzyme include: Enterobacteria: E.coli, E.coli K12, and Klebsiella aerogenes Cyanobacteria: Trichodesmium erythraeum and Nostoc species Gram-positive bacteria: Arthrobacter species and Deinococcus radiodurans.

5.2.2 Vegetal DAO

A higher catalytic capacity of DAO enzymes of plant origin in degrading certain amino substrates has been described by some authors in comparison with those of animal origin**.** Specifically, the germinated sprouts of certain edible legumes have been pointed out as interesting sources of DAO enzyme. The increased presence of DAO enzyme in legume sprouts could be associated with the importance of hydrogen peroxide, a byproduct of the deamination reaction, in the cell wall structuring, lignification and mobilization of seed reserves during germination **(Comas-Basté et al., 2020).**

A histaminase (DAO) of vegetal origin, was proposed for the general treatment of histamine-related pathologic conditions, such as allergic and septic shock, allergic asthma, anaphylaxis, allergic rhinitis and conjunctivitis, urticaria and atopic dermatitis, in which the histamine is the principal chemical mediator **(Calinescu et al., 2010).**

Most of the allergic conditions are chronic and long-term therapy is essential. Therefore, most allergic patients seek better alternatives to synthetic antihistaminic agents. Several plants and their derived natural products have been reported as effective, safe, and cheap antiallergic

agents. Additionally, several plant-derived formulas have reached the clinical trials as safe antiallergic, with similar mechanisms to synthetics drugs**.(Salem, Zayed, & Ezzat, 2021).**

The vegetal DAO was proposed as an antihistaminic agent is orally administered to treat food histaminosis and colon inflammation **(Calinescu et al., 2010)**

Plant histaminase can be obtained from different vegetal sources and can be used as a crude extract or as a purified enzyme **(Calinescu et al., 2010)**

Plants showing gene homology with the human DAO include the Eudicots: Mouse-ear cress (Arabidopsis thaliana), Garbanzo beans (Cicer arietinum), Garden pea (Pisum sativum), Indian mustard (Brassica juncea), Lentils (Lens culinaris), Japanese rice (Oryza sativa) and Soybeans (Glycine max) **(Joneja & Honorary, 2014).**

5.2.2.1 Preparation of vegetal extract from *L. sativus* seedlings and purification of DAO

The vegetal extract and the purified DAO from grass pea *L. sativus* seedlings were prepared as previously described, with minor modifications. Briefly, 500 g of freshly collected shoots of etiolated *L. sativus* seedlings were homogenized in a Waring blender with 1 L of 30 mM NaH_2PO_4 (final pH 4.4), and then filtered. In these conditions, the DAO remains ionically linked to the insoluble fraction. The solid residue, mainly constituted by cell walls and vascular fibers, was washed with the same buffer and the enzyme was finally eluted from the solid residue with 500 mL of 0.1 M sodium phosphate buffer (pH 7) and, then, centrifuged. The supernatant containing the DAO was lyophilized and purified **(Calinescu et al., 2010).**

5.2.3 Animal DAO

In the update of the official list of novel foods in 2017, the European Commission gave the green light to the marketing of a DAO supplement as food supplement or as food for special medical purposes. Specifically, European regulations authorize the formulation of porcine kidney protein extract with an enteric coating to ensure its integrity during its passage through the gastric environment **(Comas-Basté et al., 2020).**

5.2.4 Innovative DAO supply

Several coproducts with high added value are nowdays not valued. Harbaoui & Sadok proposed with the aim of enhancing marine coproduts especially from crustacean. Three species of crustacea, two species of shrimp (*Parapenaeus longirostris* and *Penaeus kerathurus*) as well as *Squilla mantis* were used in order to extract and purify the diamine oxidase enzyme (DAO).

An essay was also done from a vegetarian source using prickly pear racket of *Opuncia officinalis* as specie.

The study was conducted to isolate and characterize the DAO enzyme from different species of crustacean, moreover from vegetarian source, due to the relatively high expression level of DAO in seafood co-products **(Ienistea, 1973).**

The experiment revolved around isolating and obtaining a crude extract of DAO enzyme, first from crustacean coproducts and then from a vegetarian source (*Opuncia officinalis*). The DAO enzyme crude extract was then fractionated with Ammonium sulphate to separate eventual contaminants. Then the enzyme underwent an assay on its activity with spectrophotometric assay like described on a SIGMA ALDRICH data Sheet for the standard of DAO using BioTek Microplates Spectrophotometer. A dosage of protein amount was then realized using the BRADFORD method **(Bradford, 1976),** followed by a gel filtration chromatography to ensure the size separation of proteins and diamine oxidase present in the pellet collected from the first stem and finally, the DAO was subject to a HPLC analysis in order to purify the enzyme.

5.2.5 Purification of porcine kidney diamine oxidase

DAO from porcine kidney was purified according to existing procedures. Briefly, cortexes of 10-12 fresh or frozen porcine kidneys were minced (typically 1.3 kg) and homogenized for 3-5 min in 4 1 of 0.1 M phosphate buffer pH 7.4 using a Waring commercial blender until a smooth suspension was obtained.

Under agitation, the resulting homogenate was adjusted to 66°C in a water bath (80°C). Once the temperature had been reached. the homogenate was immediately removed from the water bath and placed on ice to achieve a temperature of 25°C.

The homogenate was then centrifuged at 14,300 g for 30 min at 10°C. Ammonium sulfate was added to the resulting crude extract up to 35% saturation at 4°C and the pellet was centrifuged at 14,300 g for 10 min at 10°C.

The supematant was adjusted to 60% saturation (at 4°C) and the 35-60% precipitate containing the DAO activity was collected by centrifugation. The pellet was dissolved in 0.1 M phosphate buffer pH 7.4 containing 0.4 M NaCl, dialyzed overnight against the same buffer at 4°C and applied to a Concanavalin A-sepharose column (2.5 cm x 18 cm) equilibrated with the dialyzing buffer.

After washing, 30 ml of 2 M methyl-@-Lrglucopyranoside in the equilibration buffer was pumped on the column and left at 4°C overnight (peak broadening effect is limited with this procedure).

The enzyme was eluted by turning the pump back on. The active fractions were pooled and dialyzed overnight at 4°C against 0.1 M phosphate buffer pH 7.4 containing 0.07 M NaCI. The dialysate was then loaded onto a heparin-agarose column (2.5 cm x 9 cm) equilibrated with the previous buffer.

The column was washed and the enzyme eluted with a linear gradient of NaCl (0.07?0.7 M) in the equilibration buffer. The fractions containing the DAO activity were pooled and dialyzed overnight at 4°C against 0. I M phosphate buffer pH 7.4. The resulting dialysate was applied onto a HA-Ultrogel column (I.5 cm x 18 cm) previously equilibrated with 0.1 M phosphate buffer pH 7.4. After washing, the enzyme was eluted with a linear gradient of ammonium sulfate (O-l M) in the previous buffer.

The active fractions were pooled, concentrated on a YM-30 membrane (Amicon ultrafiltration apparatus), and dialyzed overnight at 4°C against 0.1 M phosphate buffer pH 7.4. The resulting dialysate was concentrated as above, 10% glycerol was added. and the enzyme was stored at -80°C. Under this condition. the enzyme was stable for several months **(Bouvrette, Male, Luong, & Gibbs, 1997).**

5.3 Immobilization of purified DAO on membrane

The immobilization procedure was based on the copolymerization method using glutaraldehyde activation. In this reported procedure, a 1.5 cm x 1.5 cm piece of prewetted Immunodyne TM membrane was stretched on the top of a l-cm diameter hollow plastic chamber and held in place by an O-ring.

Glutaraldehyde was added to the enzyme solution (DA0 12-15 U ml-’ in 0.1 M phosphate pH 7.4) to initiate the cross-linking. This mixture was layered into the stretched membrane and allowed to dry at room temperature for 45 min. The membrane was then removed and washed extensively with phosphate buffer and stored at 4°C in buffer. The immobilized membrane was tested with respect to pH and temperature, Kg (apparent Michaelis-Menten constant), susceptibility to salt, substrate selectivity, and storage stability **(Bouvrette et al., 1997).**

5.4 Minimum DAO enzymatic capacity

For the purpose of this study, we mainly focus on porcine kidney diamine oxidase, in order to understand the minimum enzymatic capacity required for DAO.

A wide variability of the DAO capacity of porcine kidney extracts has been reported (with values ranging from 0.1 to more than 100 mU/mg), depending on the purification grade applied to the matrix and/or the amine compound used as the reaction substrate.

The minimum DAO enzymatic capacity required for the supplement is determined through a radio extraction assay (REA). This technique, based on the radioactive labeling of putrescine and the scintillation counting of its consumption, is advantageous in terms of rapidity and sensitivity, but it was mainly conceived to be applicable to serum samples. a rapid and reliable methodology have been recently developed through ultra-high-performance liquid chromatography and fluorometric detection (UHPLC-FL) for the in vitro determination of DAO activity specifically for the analysis of unpurified complex matrixes, such as porcine kidney extract and DAO supplements. This methodological approach is based in the direct determination of histamine degradation and overcomes certain drawbacks in terms of matrix interferences and handling of radioactive material **(Comas-Basté et al., 2020)**

5.5 DAO sensitivity

When given orally, DAO supplements are sensitive to proteolytic degradation and can be inactivated prior to reach the target site. For this reason, they should be formulated with excipients able to protect them against the proteolytic activities of the intestinal content.

Oral administration forms of diamine oxidase (DAO), formulated as monolithic tablets with the association of carboxymethyl starch (CMS) and alginate is proposed as a novel matrix for the entrapment of bioactive agents in microspheres affording their protection against gastrointestinal degradation. In this case, the enzyme diamine oxidase (DAO) from white pea (Lathyrus sativus) was immobilized by inclusion in microspheres formed by ionotropic gelation of CMS/alginate by complexation with Ca2+ **(Blemur, Le, Marcocci, Pietrangeli, & Mateescu, 2016).**

Microspheres can be given orally as free-flowing suspensions but the effective dose is not precise enough and thus can lead to failure of treatment. To facilitate oral administration, microsphere dosage forms are often filled within hard gelatin capsules **(Blemur et al., 2016)**

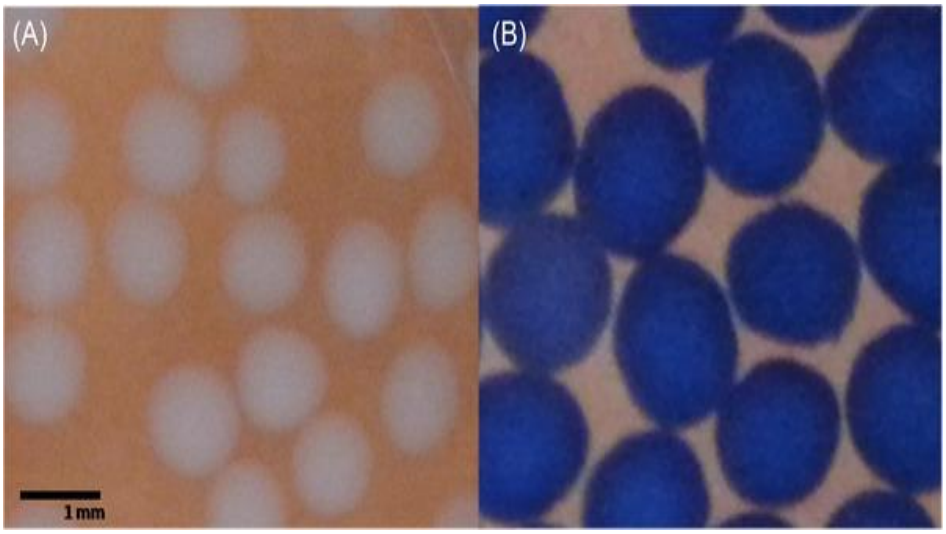

Figure 16: Macroscopic image of CMS/alginate microbeads after Coomassie blue staining: (A) without enzyme and (B) with entrapped enzyme **(Blemur et al., 2016)**

The association of CMS to alginate generated a more compact structure presenting a lesser porosity, thus decreasing the access of gastric fluid inside the microspheres and preventing the loss of entrapped enzyme **(Blemur et al., 2016).**

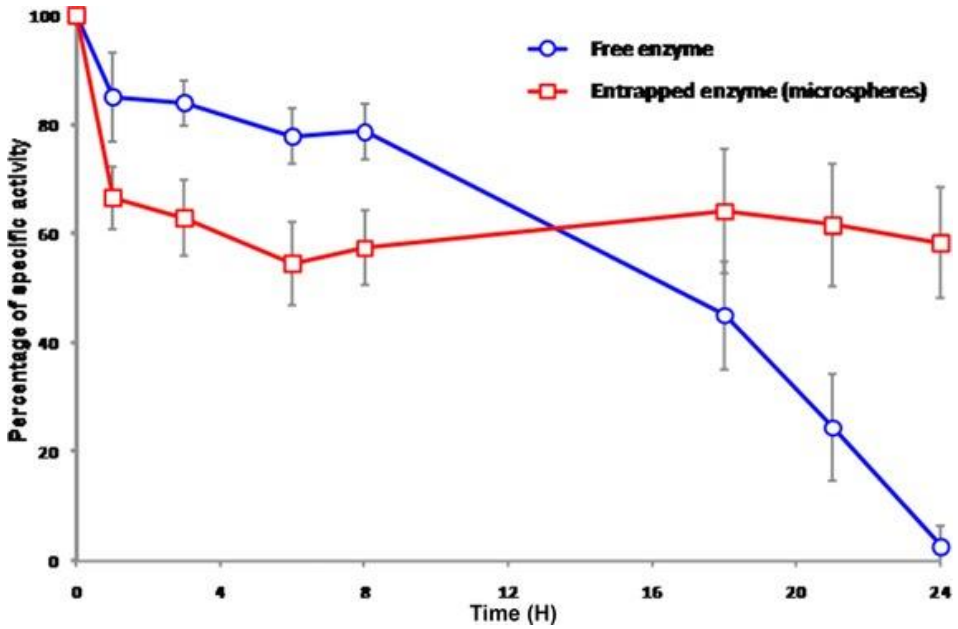

Figure 16: Activity of free and entrapped enzyme at various incubation times**, (Blemur et al., 2016).**

5.6 DAO activity

In 2019, Schnedl & al conducted a study for the purpose of evaluating the activity of DAO; DAOSIN (Diamine oxidase Capsule) was provided by Sciotec Diagnostic Technologies, Tulln in Austria.

28 patients (male/female 7/21, median age 47.5 years, age range 19–72) were included in the evaluation, the patients were instructed not to change their diets or medication throughout the study period of 8 weeks.

Symptoms, compliance of DAO capsule ingestion, and determinations of serum DAO and histamine in plasma were recorded at each visit, in 2-week intervals.

For 4 weeks the patients were instructed to take DAO capsules, each containing 4.2 mg extracted pig kidney proteins with 0.3 mg DAO, before meals, up to three times per day. During the follow-up period of 4 weeks, the patients were instructed not to take DAO capsules.

A standardized questionnaire was then used to assess the symptoms experienced by patients before and during the study period. The questionnaire was based on known symptoms and the four histamine receptors (Schnedl et al., 2019). Twenty-two symptoms were listed in four categories: GI, cardiovascular, respiratory and skin complaints. For each symptom, a severity score, from 0 (no symptoms) to 5 (very intense), was used. The patients were instructed to fill out this questionnaire at each visit. A radio extraction assay DAO Rea 100 (Sciotec Diagnostic Technologies, Tulln, Austria) was used for determination of DAO in the serum. The amount of histamine in the plasma was measured with an enzyme linked immunoassay, Histamin ELISA BA 10-1000 (Diagnostika Nord GmbH & Co. KG, Nordhorn, Germany).

All twenty-two symptoms, including GI, cardiovascular, respiratory and skin complaints improved significantly during the oral supplementation of DAO from visit 1 to visit 3 **(Schnedl et al., 2019).**

Figure 17: DAOSin 30 capsules for Dietetic food for special medical purposes **(Schnedl et al., 2019).**

CONCLUSION

Recent studies and researches showed interest in exploiting the potential of enzymes, specifically amine oxidase, copper-containing for its involvement in the metabolism, oxidation, and inactivation of histamine and other polyamines.

Many trials took place to assess the effects of DAO supplementation in histamine intolerant patients and currently the main answer to prevent or mitigate the symptomatology related to HIT is the dietary management avoiding foods with high contents of histamine or other bioactive amines. However, the wide and variable distribution of histamine in foods together with other additional restrictions makes difficult the diet adherence and increases the risk of nutritional imbalances.

The use of DAO enzyme microencapsulated with a gastro-resistant coating guaranteed its resistance in the Ph of the stomach and subsequently its hydrolysis by pepsin. Although only few works have assayed the clinical efficacy of this preventive treatment, promising results have been obtained so far after only a short treatment period. On the other hand, research is currently underway to identify new sources of DAO enzyme, especially marine coproducts such as crustaceans **(Harbaoui & Sadok, 2015)**

Likewise, it would be of interest to conduct more controlled studies depending on the patients DAO deficiency state and by implementing a histamine free-diet together with DAO supplementation, to assess the benefits of this latter for a longer treatment period, which would make diets less restrictive and improve patient's quality of life.

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