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IMPACT OF DICLOFENAC ON INFLAMMATION INDUCED BY CARRAGEENAN ON MICE

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First of all, praise be to Allah who has guided me on the right path throughout my studies and inspired me to take the right steps.

I dedicate this thesis: To the one who has always filled me with her love and these sacrifices, who consented to me and supported me through the most difficult times of my life, to my dearest mom "Khali Karima" that I wear in the apple of my eye and that I cherish from the bottom of my heart. Thanks to her I have learned to be ambitious and courageous.

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ABSTRACT

The inflammation is a natural phenomenon used by the body to eliminate the threat effecting his homeostasis. Although inflammation is a reactive to injurious stimuli and considered as beneficial process in body, it causes some discomfort such as pain. It is a molecule highly toxic and could cause even cancer because the body don't recognize it like a food supplement, we mostly find it in dairy products like yogurts, many health high authorities announce it like bad molecule for the safety of consumer. Eucalyptus is a plant known for its anti-inflammatory activity. Our study is based on how to eliminate the inflammation caused by carrageenan molecule, so we injected some mice with carrageenan and treated it with diclofenac also treated with Eucalyptus the main reason of this study is to see the inflammation then to treat it and to see the difference. Result came spectacular and the desperation of inflammation came in first place after seeing the inflammation on both macroscopic and microscopic analysis. The eucalyptus and diclofenac cured the inflammation caused by carrageenan.

Résumé

L'inflammation est un phénomène naturel utilisé par le corps pour éliminer la menace affectant son homéostasie. Bien que l'inflammation soit une réaction aux stimuli nuisibles et considérée comme un processus bénéfique dans le corps, elle provoque un certain inconfort tel que la douleur. C'est une molécule hautement toxique et pouvant même provoquer des cancers car l'organisme ne la reconnaît pas comme un complément alimentaire, on la retrouve surtout dans les produits laitiers comme les yaourts, de nombreuses hautes autorités de santé l'annoncent comme une mauvaise molécule pour la sécurité du consommateur. L'eucalyptus est une plante connue pour son activité anti-inflammatoire. Notre étude est basée sur la façon d'éliminer l'inflammation causée par la molécule de carraghénane, nous avons donc injecté des souris avec du carraghénane et l'avons traité avec du diclofénac également traité avec de l'eucalyptus la raison principale de cette étude est de voir l'inflammation puis de la traiter et de voir la différence. Le résultat est spectaculaire et le désespoir de l'inflammation est arrivé en premier après avoir vu l'inflammation à la fois sur l'analyse macroscopique et microscopique. L'eucalyptus et le diclofénac ont guéri l'inflammation causée par le carraghénane.

ملخص

الالتهاب ظاهرة طبيعية يستخدمها الجسم للقضاء على التهديد الذي يؤثر على توازنه. على الرغم من أن الالتهاب هو رد فعل للمنبهات الضارة ويعتبر عملية مفيدة للجسم، إلا أنه يسبب بعض الانزعاج مثل الألم. إنه جزيء شديد السمية ويمكن أن يسبب السرطان لأن الجسم لا يتعرف عليه كمكمل غذائي، فهو موجود بشكل خاص في منتجات الألبان مثل الزبادي، وتعلن العديد من السلطات الصحية العالية أنه جزيء سيء لسلامة المستهلك. الأوكالبتوس نبات معروف بنشاطه المضاد للالتهابات. تعتمد دراستنا على كيفية القضاء على الالتهاب الناجم عن جزيء الكاراجينان، لذلك قمنا بحقن الفئران بمادة الكاراجينان وعالجناها بالديكلوفيناك كما عولجت بالأوكالبتوس، والسبب الرئيسي لهذه الدراسة هو رؤية الالتهاب ومن ثم معالجته ومعرفة الفرق. كانت النتيجة مذهلة، وجاء اليأس الالتهابي أولاً بعد رؤية الالتهاب في كل من التحليل المجهري والميكروسكوبي. عالج الأوكالبتوس والديكلوفيناك الالتهاب الناجم عن الكاراجينان..

LIST OF FIGURES

Figure 1: Causes and physiological and pathological outcomes of inflammation..... 4

Figure 2: The acute inflammatory response mediated by the release of pro-inflammatory cytokines..... 5

Figure 3: Mice of experiment M.musculus 21

Figure 4 Plant of experiment eucalypts globulus 21

Figure 5: Extraction of essential oil technique..... 22

Figure 6: Dilution of essential oil..... 23

Figure 7: Injection of carrageenan 24

Figure 8: Measurement with piakolis..... 24

Figure 9: Force-feeding..... 23

Figure 10: Decapitation..... 24

Figure 11: Paraffin cassette with organs iced..... 26

Figure 12: Microtome slices cuter..... 26

Figure 13: Graphs indicating macroscopic analysis..... 31

Figure 14: The cellular characteristics of the liver tissue of mice that have undergone inflammation with carrageenan.....36

Figure 15: The cellular characteristics of the intestine tissue of mice that have undergone inflammation with carrageenan.....39

LIST OF TABLES

Table 1: Dehydration Baths 25
Table 2: Average Table 28
Table 3: Weight Table 45
Table 4: Oedema Measurement Table 46
Table 5: Descriptive Statistical Results Over Different Stages or Time Periods 47
Table 6: Student's T Test Results 48

LIST OF ABBREVIATIONS

CGN: Carrageenan

DAMP: Damage-Associated Molecular Pattern

E. coli: Escherichia Coli

EO: Essential oil

GF-β: Growth Factor

H2O2: Hydrogen Peroxide

HE: Hematoxylin Eosin

HSV-1: Herpes Simplex Virus Type 1

IL1β: Interleukine 1β

IL-6: Interleukine 6

IL-8: Interleukin 8

LD 50: Lethal Dose; 50 denotes the dose likely to cause death in 50% of mice

MDMs: Monocyte-Derived Macrophages

NADPH oxydase: Nicotinamide Adenine Dinucleotide Phosphate Oxidase

NF-κB: Nuclear Factor - kappa B

NOD: (Nucleotide-binding Oligomerization-Domain protein)-like receptors (**NLRs**)

NSAIDs: Non-Steroidal Anti-Inflammatory Drug

PAMPs: Pathogen Associated Molecular Patterns

PG: Prostaglandin

PMN: Polymorphonuclear

PRR: Pattern Recognition Receptor

ROS: Reactive Oxygen Species

TLRs: Toll-like Receptors

TNF: Tumor Necrosis Factor **-α**

TTO: Tea Tree Oil

ι: iota

κ: kappa

λ: lambda

μ: mu

TABLE OF CONTENTS

Abstract V
Introduction 1
Literature Review 3
1. Inflammation 4
1.1. Definition 4
1.2. Acute Inflammatory Response 5
1.2.1. End of Acute Inflammatory Response 6
1.3. Chroic Inflammatory State 6
1.4. The Inflammatory Pathway 7
2. Carrageenan 8
2.1. Definition 8
2.2. Chemical Structure 8
2.3. Properties of Carrageenan 8
2.4. Industrial Food Application 9
2.5. Pharmacokinetics of Carrageenan 10
2.6. Toxicity of Carrageenan 11
2.6.1. Carrageenan and Inflammation 11
2 .6.1. Intistinal Inflammation 11
2.6.2. Anti-Tumor and Immunomodulatory Activities 12
3. Diclofenac 13
3.1. Definition 13
3.2. Pharmacokinetic Properties 13
3.2.1. Absorption 13
3.2.1. Elimination 14
4. Essential Oil 15
4.1. Definition 15
4.2. Eucalyptus 15
4.2.1. Eucalyptus Speceis Chemical Composition 16
4.2.2. Pharmacological Activities of Eucalyptus Speceis 16
4.2.2.1. Antibacterial Activity 16
4.2.2.2. Antifungeal Activity 17
4.2.2.3. Antiviral Activity 17
4.2.2.4. Cytotoxic And Antitumor Activity 18
4.2.2.5. Anti-Inflammatory Activity 18
4.2.2.6. Antioxidant Activity 18
4.2.2.7. Antidiabetic Activity (Antihyperglycemic) 18
4.2.3. Inhalation Pharmacokinetics 19
Method and Materials 20
Result and Discussion 27

Conclusion 42
Appendices 43
References 49

Introduction

Introduction

The introduction hereby lays the foundation for this thesis. The current section is dedicated to presenting carrageenan, its characteristics, and its correlation with inflammation, besides to mentioning certain previous studies related to this research. This thesis has two parts, the first part is a bibliographic collection looking at diclofenac and inflammation and carrageenan then the relationship between the three. The second part is the experimental part, it includes the material and methods chapter which consists in the in vivo evaluation of the anti-inflammatory activity of EO of Eucalyptus extracts. The results obtained are summarized and discussed in the results chapter, and discussion.

This study addresses the following questions: Does carrageenan induce inflammation? Can diclofenac treat inflammation induced by carrageenan? Does the essential oil of eucalyptus has an anti-inflammatory effect? Can the essential oil of eucalyptus treat inflammation induced by carrageenan? The main concern of this research is to investigate the effects of the essential oil of eucalyptus.

Carrageenan is a highly sulfated hydrophilic colloid extracted from red algae and is divided into various subtypes such as λ(lambda), κ(kappa), ι(iota), and μ(mu**) (Liu et al., 2015)**, which are divided mainly on the number and location of sulfate groups and the content of 3,6-endo-D-galactopyranoside **(Thomson et al., 1981).** Carrageenan has wide application in the food industry for its good gelation and thickening properties, often used as an emulsifier, thickener and Stabilizer **(Watt and Marcus, 1981).**

However, recent studies have suggested that carrageenan has biological toxicity and is prone to causing damage to the intestinal mucosal immune function of the host, leading to inflammation of the intestine. Currently, most research on carrageenan focuses on tissue and cellular levels **(Kitsukawa et al., 1992).**

Inflammation is an essential defense mechanism for the integrity of the body. However, it is involved in a very large number of human pathologies. On the other hand, oxidative stress is also the cause of several humans' diseases ranging from inflammation to cancer to cardiovascular disease and rheumatoid arthritis disease. Therefore, the search for original plant substances with anti-inflammatory and / or antioxidant activities is very useful for improving human health while avoiding the undesirable effects of synthesis **(Meziti, 2008).**

This process could be defined as the immediate defensive response by vertebrate tissue against harmful stimulus, such as injury and infection which are caused by chemical and physical agents. It is shown that a series of biochemical mechanisms which are managed by

pro-inflammatory factors could initiate and mature the inflammatory response **(Larsson, 2001).**

In view of the large number of conflicting research results, further investigation into carrageenan consumption safety is warranted. To elucidate the mechanism of the effects of CGN on inflammation, we have investigated the interaction between CGN and two molecules (EO eucalyptus and diclofenac).

Statement of the Problem:

Although it is used in food industry, carrageenan has biological toxicity and is prone to causing damage to the intestinal mucosal immune function of the host, leading to inflammation of the intestine.

Significance of the Study:

The possibility of using the essential oil of eucalyptus to treat inflammation naturally without using chemical substances.

Research Questions:

Does carrageenan induce inflammation?

Can diclofenac treat inflammation induced by carrageenan?

Does the essential oil of eucalyptus has an anti-inflammatory effect?

Can the essential oil of eucalyptus treat inflammation induced by carrageenan?

Research Objectives:

Investigating the effects of the essential oil of eucalyptus.

Comparing the anti-inflammatory effect of the essential oil with anti-inflammatory activity of diclofenac.

Literature Review

1. Inflammation

1.1. Definition

Inflammation is an adaptive response that is triggered by noxious stimuli and conditions, such as infection and tissue injury. It is generally thought that a controlled inflammatory response is beneficial (in providing protection against infection) (**Majno and Joris, 2004).** The inflammatory response is associated with the immune system, which can be divided into two interconnected branches. Innate immunity is the oldest. She is present at any multicellular organism. The cells of the innate immune system have PRRs (pattern recognition receptors) and highly conserved signaling pathways to detect and respond to infection or injury. Detection of these original exogenous signals microbial, PAMPs (Pathogen Associated Molecular Patterns), or endogenous alarmins **(Bianchi, 2007),** will lead to initiation of the inflammatory cascade and activation of an acquired immune response or adaptive **(Barton, 2008; Medzhitov, 2008)**. The answer takes place in four stages: recognition of danger signals, recruitment of cells at the site of infection, elimination of the pathogen and resolution of inflammation leading to a return to homeostasis and wound healing. In the absence of a resolution, a chronic inflammation **(Barton, 2008).**

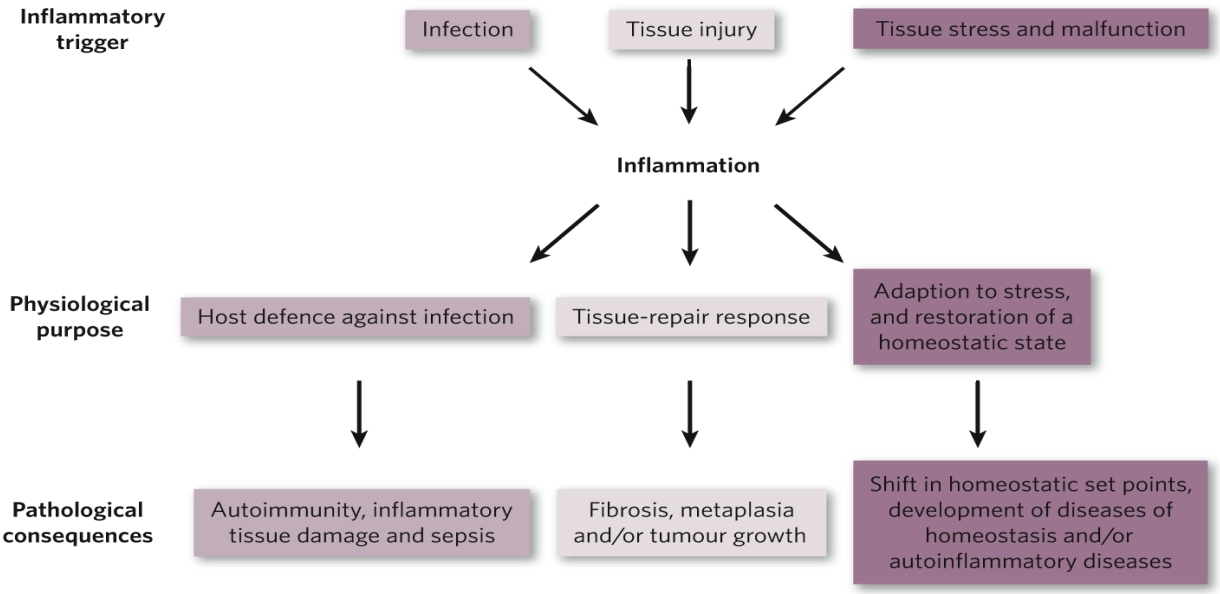


Figure 1. Causes, and physiological and pathological outcomes of inflammation. Depending on the trigger, the inflammatory response has a different physiological purposes and pathological consequences. Of the three possible initiating stimuli, only infection-induced inflammation is coupled with the induction of an immune response. **(Ruslan Medzhitov, 2008)**

1.2. Acute Inflammatory Response

At a basic level, the acute inflammatory response triggered by infection or tissue injury involves the coordinated delivery of blood components (plasma and leukocytes) to the site of infection or injury **(Kumar and Cotran, 2003).** This response has been characterized best for microbial infections (particularly bacterial infections) in which it is triggered by receptors of the innate immune system such as TLRs (Toll-like Receptors) and NOD (nucleotide-binding oligomerization-domain protein)-like receptors (NLRs) **(Barton, 2008).** This initial recognition of infection is mediated by tissue resident macrophages and mast cells, leading to the production of a variety of inflammatory mediators, including chemokines, cytokines, vasoactive amines, eicosanoids and products of proteolytic cascades.

The activated endothelium of the blood vessels allows selective extravasation of neutrophils while preventing the exit of erythrocytes. This selectivity is afforded by the inducible ligation of endothelial-cell selectins with integrins and chemokine receptors on leukocytes, which occurs at the endothelial surface, as well as in the extravascular spaces (where newly deposited plasma proteins form a provisional matrix for the binding of leukocyte integrins) **(Pober and Sessa, 2007).** When they reach the afflicted tissue site, neutrophils become activated, either by direct contact with pathogens or through the actions of cytokines secreted by tissue-resident cells. The neutrophils attempt to kill the invading agents by releasing the toxic contents of their granules, which include ROS (Reactive Oxygen Species) and NOS (Reactive Nitrogen Species), proteinase 3, cathepsin G and elastase **(Nathan, 2006).**

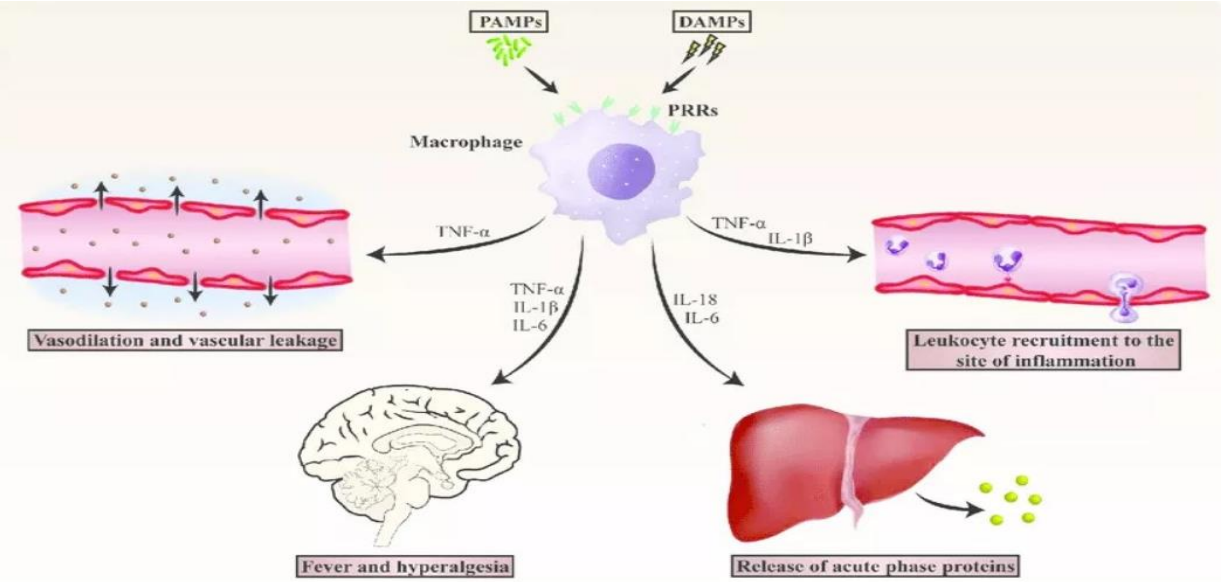


Figure 2. The acute inflammatory response mediated by the release of pro-inflammatory cytokines. **(Netea et al., 2000)**

Following PAMP or DAMP recognition, PRRs trigger proinflammatory and antimicrobial responses by inducing the release of a broad range of cytokines. The archetypical pro-inflammatory cytokines TNF-α, IL1β, and IL-6 are rapidly released upon PRR activation, and they all act as endogenous pyrogens by increasing the hypothalamic thermoregulatory set-point **(Netea et al., 2000)**. In addition, TNF-α and IL-1β orchestrate the release of chemokines and expression of leukocyte adhesion molecules on vascular endothelium, promoting the rapid and efficient recruitment of leukocytes towards inflammatory foci **(Bradley et al., 2008)**. TNF-α is also responsible for multiple hallmark signs of inflammation by inducing local vasodilation (rubor, calor) and vascular leakage (causing swelling) **(Bollenberg et al., 1991) (Hofmann, 2002).** Furthermore, IL-1β evokes inflammatory hyperalgesia and is well known for its induction of IL-6. IL-6, in turn, is a major inducer of acute-phase protein production by hepatocytes. PAMP, pathogen-associated molecular pattern; DAMP, damage-associated molecular pattern; PRR, pattern recognition receptor

1.2.1. End of Acute Inflammatory Response:

A successful acute inflammatory response results in the elimination of the infectious agents followed by a resolution and repair phase, which is mediated mainly by tissue-resident and recruited macrophages **(Serhan, 2005).** The switch in lipid mediators from pro-inflammatory prostaglandins to lipoxins, which are anti-inflammatory, is crucial for the transition from inflammation to resolution. Lipoxins inhibit the recruitment of neutrophils and, instead, promote the recruitment of monocytes, which remove dead cells and initiate tissue remodeling **(Serhan, 2005).** Resolving and protections, which constitute another class of lipid mediator, as well as transforming growth factor-β and growth factors produced by macro phages, also have a crucial role in the resolution of inflammation, including the initiation of tissue repair **(Serhan, 2007).**

1.3. Chronic Inflammatory State

If the acute inflammatory response fails to eliminate the pathogen, the inflammatory process persists and acquires new characteristics. The neutrophil infiltrate is replaced with macrophages, and in the case of infection also with T cells. If the combined effect of these cells is still insufficient, a chronic inflammatory state ensues, involving the formation of granulomas and tertiary lymphoid tissues **(Drayton et al., 2006)**.

1.4. The Inflammatory Pathway

The inflammatory response is coordinated by a large range of mediators that form complex regulatory networks. To dissect these complex networks, it is helpful to place these signals into functional categories and to distinguish between inducers and mediators of inflammation. Inducers are the signals that initiate the inflammatory response who can be exogenous or endogenous but the mediators that can be vasoactive amines, vasoactive peptides, fragments of complement components, lipid mediators, cytokines, chemokines and proteolytic enzymes. (**medzhitov et all 1997)**

2. Carrageenan

2.1. Definition

Carrageenan is a family of natural linear sulfated polysaccharides that are extracted from red edible seaweeds. It is derived from a number of seaweeds of the class of Rhodophyceae. Carrageenan has no nutritional value and is used in food preparation for its gelling, thickening, and emulsifying properties **(Van de Velde et al., 2002)**. In pharmaceutical applications and experimental medicine, this substance is often used for the testing of anti-inflammatory agents **(Zacharopoulos and Phillips, 1997)**. Carrageenan is widely used in the food industry for their gelling, thickening, and stabilizing properties. Their main application is in dairy and meat products due to their strong binding to food proteins.

2.2. Chemical Structure

Carrageenan is a sulfated polygalactan with 15 to 40% of ester-sulfate content and an average relative molecular mass well above 100 kDa. It is formed by alternate units of d-galactose and 3,6-anhydro-galactose (3,6-AG) joined by α-1,3 and β-1,4-glycosidic linkage. Carrageenan is classified into various types such as *alpha, kappa, iota, epsilon, mu.* all containing 22 to 35% sulphate groups. The primary differences which influence the properties of carrageenan type are the number and position of ester sulfate groups as well as the content of 3.6-AG. These names do not reflect definitive chemical structures but only general differences in the composition and degree of sulfation at specific locations in the polymer. Higher levels of ester sulfate mean lower solubility temperature and lower gel strength. Kappa type carrageenan has an ester sulfate content of about 25 to 30% and a 3,6-AG content of about 28 to 35%. Iota type carrageenan has an ester sulfate content of about 28 to 30% and a 3,6-AG content of about 25 to 30%. Lambda type carrageenan has an ester sulfate content of about 32 to 39% and no content of 3,6-AG **(Barbeyron et al., 2000).**

2.3. Properties of Carrageenan

The chemical reactivity of carrageenan is primarily due to their half-ester sulfate groups which are strongly anionic, being comparable to inorganic sulfate in this respect. The free acid is unstable, and commercial carrageenan are available as stable sodium potassium and calcium salts or, most commonly, as à mixture of these. The associated cations, together with the conformation of the sugar units in the polymer chain, determine the physical properties of

carrageenan. Kappa- and iota-carrageenan form gels in the presence of potassium or calcium ions whereas lambda-carrageenan does not **(Michel et al., 1997)**.

Carrageenan, as linear, water-soluble polymers typically form highly viscous aqueous solutions. Viscosity depends on concentration, temperature, the presence of other solutes, and the type of carrageenan and its molecular weight **(Lai et al., 2000)**.

Viscosity increases nearly exponentially with concentration and decreases with temperature. Carrageenan is susceptible to depolymerization through acid-catalyzed hydrolysis. At high temperatures and low PH this may rapidly lead to complete loss of functionality **(Stanley, 2011)**.

2.4. Industrial Food Applications

Industrial vinegar production is a biochemical process which utilizes bacteria **(Osuga et al., 1984)** described the use of a bubble-mixed reactor and κ-carrageenan gel beads as carriers for the continuous production of acetic acid. An improvement was attempted through the use of an air-lift reactor **(Mori, 1993)**, using the culture of *Acetobacter* species K1024 isolated from a commercial vinegar broth.

More recently, a successful continuous production of vinegar was reported using a bubble mixed tabletop bioreactor with κ-carrageenan-immobilized *Acetobacter suboxydans* cells **(Tosa and Shibatani., 1995)**. Fermented milk products can be obtained by simultaneous acidification and inoculation of skimmed milk by immobilized mixed cultures.

Three different strains of *Lactococcus lactis* and one strain of *Leuconostoc mesenteroides* were separately immobilized in κ-carrageenan/locust bean gum gel (2.75% and 0.25% w/w, respectively) and used in a 2-L stirred reactor **(Sodini et al., 1997)**.

a group of researchers described the immobilization system of yeast cells (*Saccharomyces* sp.) for beer production using κ-carrageenan beads continuously produced by a static mixer process **(Mensour et al., 1996, 1997)**. Ethanol production from glucose using cells of *Zymomonas mobilis* immobilized in κ-carrageenan was investigated in a fluidized bed fermenter. This research was extended to study the production of ethanol from starch **(Krishnan 1999)** using the bacteria co-immobilized with an industrial glucoamylase in carrageenan gel beads and used in a glass column fermenter. The carrageenan gel matrix was reported to provide protection of immobilized *Saccharomyces cerevisiae* cells **(Norton et al., 1995)** and continuous ethanol production from pineapple cannery waste was attempted using these yeast cells immobilized in κ-carrageenan **(Nigam, 2000)**.

2.5. Pharmacokinetics of Carrageenan

Absorption, distribution, metabolism and excretion

Many pharmacokinetics studies concerning the oral administration of carrageenan have been conducted on rats **(Carey, 1958; Hawkins and Yaphe, 1965; Dewar and Maddy, 1970; Grasso et al., 1973; Tomarelli et al., 1974; Coulston et al., 1975; Pittman et al., 1976; Chen et al., 1981; Nicklin and Miller, 1984; Arakawa et al., 1988; Nicklin et al., 1988)**. In groups of five rats that received 0.5% native carrageenan *(iota)* or 5% degraded carrageenan for 10 days, faecal excretion and weight gain were similar between the two polymers **(Dewar and Maddy, 1970)**, and native carrageenan *(kappa/lambda)*, untreated or heat-sterilized in milk, was quantitatively excreted in the faeces of rats **(Tomarelli et al., 1974)**. No carrageenan was found in the livers of rats fed 25% native carrageenan *(kappa/lambda)* in the diet for one month **(Chen et al., 1981)**, of rats fed diets containing 1% or 5% carrageenan *(kappa, iota)* **(Coulston et al., 1975)**, or of rats fed diets containing 5% *Chondrus crispus* carrageenan (*kappa*/*lambda)* for 13 weeks **(Pittman et al., 1976)**. No carrageenan was detected in the small or large intestine of rats fed 5% native carrageenan *(iota)* **(Grasso et al., 1973)**.

Nicklin and Miller **(1984)** reported that orally administered carrageenan of high relative molecular mass could penetrate the mucosal barrier of adult animals via transport by macrophages in Peyer's patches. Carrageenan did not affect the number or distribution of these cells; however, when antigen was administered systematically to carrageenan-fed rats, the antigen-specific antibody response was suppressed.

Analysis of liver samples from rats fed 25% native carrageenan *(kappa/lambda)* in the diet for one month showed that only the second was stored in the liver in two animals, as determined by the presence of *gamma* metachromatic reaction sites in the Kupffer cells **(Chen et al., 1981)**.

Two additional studies concerning the absorption of carrageenan have been reported **(Arakawa et al., 1988; Nicklin et al., 1988).** In the first study, rats quantitatively excreted the carrageenan (*kappa*) in the faeces, and it had the same gel filtration distribution pattern as that of the administered material. In the latter study, in male PVG strain rats given radiolabeled carrageenan (*iota*), there appeared to have been some uptake into the intestinal wall, Peyer's patches, mesenteric and caecal lymph nodes, and serum; however, the method used to radiolabel the carrageenan with tritium is questionable **(International Food Additives**

Council, 1997). Feeding of guinea-pigs with native carrageenan (*iota)* at 5% in the diet for 21–45 days resulted in the accumulation of 36–400 pg/g of caecal or colonic tissue. The carrageenan was contained in macrophages **(Grasso et al., 1973).** Food-grade carrageenan (*kappa, lambda, iota)* administered to guinea-pigs as a 1% solution in drinking-water for two weeks was not retained in the caecum **(Engster and Abraham, 1976),** it was reported that carrageenan was present in the liver, stomach, and small intestine of new-born rabbits given 40 mg native carrageenan orally. Carrageenan was not detected in the cardiac or portal blood 4 h after treatment **(Udall et al., 1981).**

2.6. Toxicity of Carrageenan

Carrageenan and Inflammation

There have been a significant number of in vitro studies in which CGN (Carrageenan) has been reported to activate inflammatory signaling pathways resulting in an induction of proinflammatory cytokines. These studies reported that CGN Induced IL-8, as well as other proinflammatory cytokines, in all of These cell types **(Borthakur et al., 2012; Bhattacharyya et al., 2011)**. There are two ways can induce inflammation by carrageenan:

1) direct binding and activation of the Toll-Like Receptor 4 (TLR4)-Bcl10 pathway

2) induction of oxidative Stress via an increase in H2O2.

Studies have shown that intestinal inflammation is accompanied by the increase of oxidative stress **(Tsuji et al., 2003)**. ROS are the natural Products of aerobic metabolism of animal and human cells The TLR4-NF-κB and MAPK/ERK1/2 pathway, the changes of cytokines, downregulated proportion of Tregs, up-regulated the expression of IL-8 and oxidative stress might be all involved in this enhancement effect. Determined that supplementing carrageenan in drinking water could Cause colitis by reducing certain anti-inflammatory bacteria in the intestine of mice Exposure to carrageenan could activate proinflammatory genes NF-κB and EGR-1 in epithelial cells.

2.6.2. Intestinal Inflammation

Carrageenan in drinking water causes ulcerative lesions associated with clinical and pathological changes that in some respects closely resemble ulcerative colitis in humans.

Clinically, there is weight loss, loose stools, and occult or visible blood in the stool. Pathologically, ulcerations have been found in all parts of the large intestine, with extensive lesions seen in the rectum. Microscopically, focal mucosal hemorrhages and cellular

infiltrates, edema, crypt abscesses, irregular dilation of crypts with loss of mucin-secreting cells and degeneration of the lining epithelium, ulceration involving mainly the mucosa, as well as ulcerations in various stages of progression and healing were present. The ulcerative lesions, however, appear to begin in the cecum and extend distally to the rectum **(Watt and Marcus, 1971)**

2.6.3. Anti-tumor and Immunomodulatory Activities

carrageenan shows antiproliferative activity on cancer cell lines in vitro, as well as tumor growth inhibitory activity in mice **(Yuan et al., 2004, 2006; Zhou et al., 2004, 2006; Yuan and Song, 2005; Rocha et al., 2007).** In addition, they have antimetastatic activity by blocking interactions between cancer cells and the basement membrane, inhibit tumor cell proliferation and tumor cell adhesion to various substrates, but their exact mechanisms of action are not yet fully understood. **Yamamoto et al., (1986)** reported that oral administration of several algae can significantly decrease the incidence of carcinogenesis in vivo. Histopathological examination did not show any enhancement of carcinogenesis by carrageenan, carrageenan has no promoter activity at the highest dietary level of 5.0% for colorectal carcinogenesis under the present experimental conditions **(Hagiwara et al., 2001).**

3. Diclofenac

3.1. Definition

Diclofenac is a non-steroidal anti-inflammatory drug with analgesic and antipyretic activity, and in common with other aspirin-like anti-inflammatory drugs, it is a potent inhibitor of prostaglandin (PG) synthesis. It is a phenylacetic acid derivative, and its chemical structure was designed based on information gained about the structure-activity relationships of other anti-inflammatory drugs **(Sallmann, 1986)**. Diclofenac is extensively metabolized, but none of its metabolites possess significant pharmacological activity compared with the parent drug as assessed from *in vitro* ability to inhibit prostaglandin synthesis and from *in vivo* anti-inflammatory or analgesic activity in animal models **(Maier et al., 1979; Menasse et al., 1978)**

3.2. Pharmacokinetic Properties

3.2.1. Absorption

Bioavailability Studies with single doses of oral and intravenous 14C-Iabelled Diclofenac Indicate that the orally administered drug is almost totally absorbed **(John, 1979; Kendall et al., 1979; Riess et al., 1978)**. However, diclofenac undergoes 'first-pass' metabolism, with about 60% of the drug reaching systemic circulation in an unchanged form **(John, 1979)**. Single doses of commercially available preparations are equally well absorbed whether administered orally as solution or enteric-coated tablets or rectally as suppositories **(John, 1979; Morimoto et al., 1985; Riess et al., 1978; Terhaag et al., 1985).**

Diclofenac is also absorbed percutaneously and reaches systemic circulation when administered as an emulsified gel formulation **(Riess et al., 1986).** Absorption is rapid after administration of the drug as an oral solution, rectally or intramuscularly, with peak plasma concentrations occurring in about 10 to 30 minutes. With enteric-coated tablets peak concentrations are delayed until about 1.5 to 2.5 hours after ingestion in fasting subjects **(Geiger et al., 1975; Willis et al., 1981a).**

3.2.2. Elimination

Diclofenac is eliminated principally by metabolism and subsequent urinary and biliary excretion of glucuronide and sulphate conjugates of the metabolites **(Stierlin et al., 1979)**. The principal metabolite in humans is 4'-hydroxydiclofenac; the amount excreted in urine accounts for 20 to 30% of the dose and that in bile for 10 to 20% of the dose **(Riess et al., 1978; Stierlin & Faigle, 1979).** Three other metabolites each account for 10 to 20% of the dose excreted in urine and small amounts of the dose excreted in bile. Conjugates of unchanged diclofenac account for 5 to 10% of the dose recovered in urine and less than 5% of that excreted in bile.

4. Essential oil

4.1. Definition

Essential oils are generally hydrophobic liquids and soluble in alcohol, nonpolar or weakly polar solvents, waxes, and oils. They are slightly soluble in water and are usually colorless or pale yellow. **(Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M.2008).**
From a chemical standpoint, they are typically composed of hydrocarbons and oxygenated monoterpenes, sesquiterpenes and diterpenes, aromatic compounds (C6-C3 and C6-C1 compounds), and low molecular weight aliphatic compounds. There are more than 3000 EOs described in the existing literature, with approximately 300 in commercial use, including those from various Eucalyptus species. EOs are oily aromatic liquids extracted from plant material by expression, fermentation, enfleurage, extraction or distillation **(Van de Braak & Leijten, 1999)**. The extraction method most often used is distillation **(Guenther, 1948).** Their function in plants is likely antibacterial, antifungal, insecticidal and antiviral defense, and their strong flavor makes plants less palatable for herbivores **(Guenther, 1948).** The biological effects of EOs have been reviewed **(Bakkali et al., 2008).** Although there are over 3000 EOs known, approximately 300 are currently in use, chiefly in fragrances **(Van de Braak & Leijten, 1999).** EOs have been distilled for more than 2000 years, but their widespread use dates back to the 16^{th} century. Over the past 200 years their medicinal purposes have become secondary to their commercial uses, such as flavoring in foods and as fragrances in cosmetics and perfumes **(Guenther, 1948).** Whole EOs tend to vary in their exact composition due to factors such as seasonal variation, climate, subspecies and even the oil-extraction method **(Santoyo et al., 2006).**

4.2. Eucalyptus

A special attention has been drawn worldwide to eucalyptus plants in various fields of industry, among them perfumery, pharmaceuticals, nutraceuticals and furniture. Hence, they represent a fast-growing source of wood as well as a source of oil used for several purposes **(Vecchio, Loganes, & Minto, 2016)**. Conceptually, the name Eucalyptus comes from the prefix “Eu” which means true and “calyptus” (kalypto) which means to cover; describing the flower bud formed by united calyx and corolla parts, which seal the flower till it blooms **(Kantvilas, 1996; Naithani, 2014).** Specifically addressing its phytopharmacological potential, there has been is a growing number of studies assessing in an in-depth manner its biological potentialities. For instance, the oil from leaves, fruits, buds and bark possesses a

wide spectrum of antibacterial, antiseptic, antioxidant, anti-inflammatory, and anticancer effects, and for this reason is used traditionally in the treatment of respiratory diseases, common cold, influenza, and sinus congestion **(Sebei, Sakouhi, Herchi, Khouja, & Boukhchina, 2015; Sharifi-Rad, Sureda, et al., 2017).** In fact, the medicinal value of Eucalyptus oil has been owing, in a great extent, to its main constituent 1,8-cineole (cineole or eucalyptol**) (Mohamed et al., 2015; Sebei et al., 2015).**

4.2.1. Eucalyptus species chemical composition

There are more than 700 Eucalyptus species, mostly originating from the Australian continent, with a very small number found on the neighboring islands of Papua New Guinea, Indonesia, and Philippines **(Yang et al., 2017).** One of the widely studied Eucalyptus species is G. globulus.

E.g., E. globulus: 1,8-cineole, spathulenol, α-terpineol, α-pinene, limonene, tricyclene, camphene, globulol, o-cimene, cisocymene, α-terpinyl acetate, ρ-cymene, β-myrcene, solanone, β-pinene. The main products obtained from Eucalyptus are essential oils **(Small, 2013),** mainly used for its antimicrobial **(Bachir & Benali, 2012; Mulyaningsih, Sporer, Reichling, & Wink, 2011),** antifungal **(Gakuubi, Maina, & Wagacha, 2017),** antiseptic, astringent **(Marzoug et al., 2011),** anti-inflammatory **(J. Silva et al., 2003),** wound healing, disinfectant **(Valeriano et al., 2012)** and expectorant properties **(Horváth & Ács, 2015).** Other products obtained from eucalyptus are gum, cellulose and wood, while the essential oil extracted from the leaves is widely employed for medicinal, cosmetic and food industries **(S. M. Silva et al., 2011).**

4.2.2. Pharmacological Activities of Eucalyptus Species

4.2.2.1. Antibacterial Activity

The antibacterial activity of the essential oils from E. globulus leaves was investigated against E. coli and S. aureus. The plant was collected from Algeria, the oil was prepared by hydro-distillation, and the activity was measured using agar disc diffusion and dilution broth methods. This study showed both positive antibacterial activity of the oil against Gram-positive and Gram-negative representatives, S. aureus and E. coli, respectively **(Bachir and Benali, 2012).** In addition, the antibacterial activity of essential oils from leaves of two Eucalyptus species (E. globulus and E. camaldulensis) was determined against S. aureus and

E. coli bacteria. The inhibiting activity was evaluated by three different assays: aromatogramme, microatmosphere and germs in suspension. The results demonstrated that the two species oils had an inhibitory effect on both bacteria, but with a less degree on E. coli **(Ghalem & Mohamed, 2008)**.

4.2.2.2. Antifungal Activity

The fungicidal activity against rice blast fungi (e.g., Setosphaeria turcica, Magnaporthe grisea, Botrytis cinerea, Fusarium graminearum and others) was performed on leaves essential oil from E. grandis x E. urophylla, a fastgrowing hybrid clone between E. grandis and E. urophylla, which is an economically important pulp tree, widely grown in many provinces of south China. The oil, with a final concentration of 2.5 mg/mL, showed a significant inhibitory activity in mycelium growth. The most interesting findings were variations in colony morphology and abnormal hypha growth, according to the scanning electron microscopy (SEM) observations. These effects may be due to interferences with genes expression involved in cellulose synthesis, respiration process and nucleicacid synthesis **(Yuan et al., 2016).**

4.2.2.3. Antiviral Activity

The antiviral activity of Eucalyptus oil was tested against HSV-1 (Herpes Simplex Virus type 1) strain (KOS). In a dose-response assay (plaque reduction assay), the inhibition of the viral replication was measured. The oil has succeeded to suppress the viral multiplication and infectivity by more than 96%, while its major constituent 1,8-cineole was less active. It was shown that the inhibitory mechanism of HSV was much more prominent before the viral adsorption, indicating the binding of essential oil components to viral proteins involved in host cell adsorption and penetration **(Astani, Reichling, and Schnitzler, 2010).** Thus, given the current insights, the development of an effective and less toxic an anti-influenza virus drug is urgently needed due to continual appearance of drug-resistant mutants. The essential oil from E. globulus have shown a significant virucidal activity against influenza virus following exposures to oil vapors over only 10 min. In addition, this activity was observed without measurable adverse effect on the epithelial cell monolayers **(Vimalanathan & Hudson, 2014).**

4.2.2.4. Cytotoxic and Antitumor Activity

Several reports have confirmed the cytotoxic and antitumor activity of a wide variety of Eucalyptus species extracts. The activity was most likely mediated through induction of apoptotic pathways and cell death against several cancerous cell linese. **(Döll-Boscardin et al., 2012).**

4.2.2.5. Anti-inflammatory activity

E. globulus oil dose-dependently stimulated phagocytosis (according to several parameters) by human monocyte-derived macrophages (MDMs) *in vitro*, without producing pro inflammatory effects. Phagocytic activity was not increased when the MDMs were exposed to tea tree oil (TTO) or lavender oil. Eucalyptus oil's phagocytic ability was halted when EO-stimulated cells were treated with a microtubule-destabilizing chemical, suggesting EO's phagocytic ability is dependent on the microtubule network. **(Serafino et al., 2008).**

4.2.2.6. Antioxidant Activity

EO can react biologically as an antioxidant. The free radical scavenging capability of *E. tereticornis* oil from fresh or decaying leaves and separate oil constituents was studied against superoxide anion and hydroxyl radical. Both oils showed strong antioxidant ability either comparable or exceeding the standard antioxidants ascorbic acid and t-butylhydroxytoluene, respectively. Interestingly, the sum of the major individual constituents of the oil did not match the exceptional results of the whole oil, suggesting a synergistic effect when in combination. Singh HP, Mittal **(Kaur, et al., 2009).**

4.2.2.7. Antidiabetic Activity (Antihyperglycemic)

Leaf essential oil of *E. globulus* has antidiabetic and antioxidant properties, reduces oxidative stress through dropping the plasma glucose level in diabetic rats, thereby checking the unnecessary production of free radicals through glycation of the proteins. *Eucalyptus citriodora* showed momentous antidiabetic activity which is analogous with the standard drug Glibenclamide. **(Dey B, Mitra A.2013).**

4.2.3. Inhalation Pharmacokinetics

Study of the pharmacokinetics of inhaled 1,8-cineole in humans show peak plasma concentration after 18 minutes. Elimination from blood is biphasic, with mean distribution of 6.7 minutes and an elimination half-life of 104.6 minutes. **(Jager W, Nasel B, Nasel C, et al.1996)**. No studies were found showing the inhaled pharmacokinetics of whole EO. Several studies show the pharmacokinetics of skin permeation by EO.

Methods and Materials

1. Biological Model

Our study involved rodent white mice. The mice were out-landed from Group of Saidal in Medea, department of quality control, service of pet shop. The animals considered in this study are 20 adult mice with an intermediate weight of 20 g. (appendices table 3)

1.1. Taxonomy:

Kingdom: *Animalia*
Phylum: *Chordata*
Class: *Mammalia*
Order: *Rodentia*
Family: *Muridae*
Genus: *Mus*
Species: *M. Musculus*



Figure 3. mice of experiment M.musculus

2. Plant material

Eucalyptus globulus **(blue gum)**

2.1. Classification

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Eudicot
Clade: Rosids
Order: Myrtales
Familly : Myrtaceae
Genus: Eucalyptus
Species: E. globulus



Figure 4. plant of experiment: eucalypts globulus

3. Preparation of Essential Oil "Extraction"

Eucalyptus globulus was outland from trees of eucalyptus from Berrouagia, after that the leaves were washed very carefully and wiped and grinned with crusher, we putted 50 g of eucalyptus and 300 ml of distillated water in to hydrodistilator with 150 C° in 2 h, 600 g of eucalyptus leaves ware used but the performance of oil extracted was not enough (The extraction was performed in the technological hall of technology field at Ain Dhab), so we bought an essential oil from the pharmacy which is measured that its 100% pure.

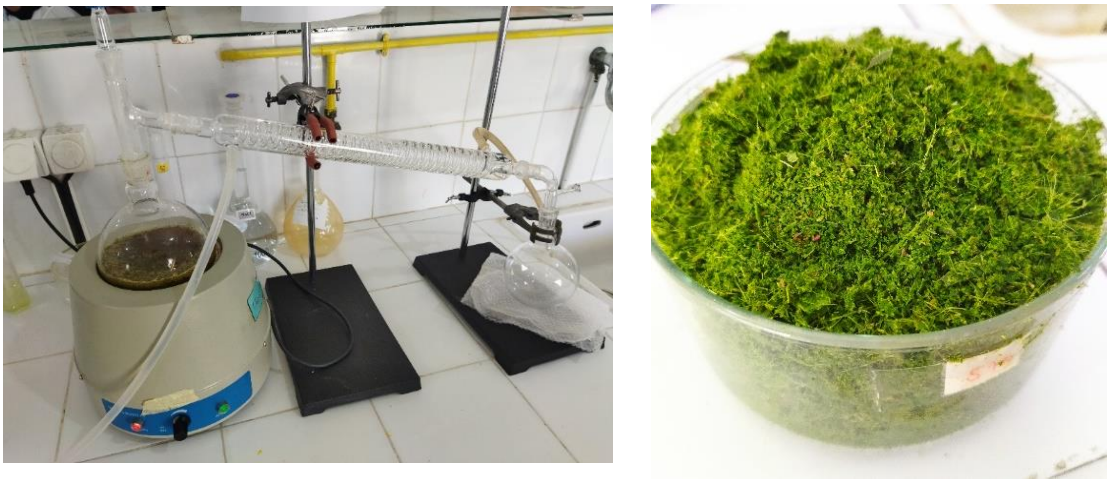


Figure 5. Extraction of Essential Oil Technique

3.1. Determination of the median lethal dose

There was a study experimented on rates to determine the lethal dose of essential oil of eucalyptus, according to the method of Finney [11] for determination of the median lethal dose (LD50), exploratory trials were performed in five groups each of two rats, eucalyptus were administered orally at doses of 500, 1000, 1,500, 2,000, and 2,500 mg/b.w. correspondingly in five groups to find the smallest toxic dose to start with.

Nine groups of rats were used (10 of each), 1st, 2nd, 3rd, and 4th groups were given 1000, 1,500, 2,250, and 3,375 mg/kg b.w. of eucalyptus oil. The 9th group was kept as a control. Mortality of rats was recorded after 24 hr.

The toxicity index of each oil was determined according to Sun **(SUN Y.P. 1950).**

Obtained data concerning the median lethal dose (LD50) of active principle of eucalyptus essential oils revealed that the doses of the LD50 were 2,334.4 mg/kg b.w., respectively, for rats under environmental conditions.
Obtained data revealed that eucalyptus essential oil is moderately hazardous (LD50 2,334.4 mg/kg b.w) according to the WHO classification **(WHO. 2005.)**

3.2. preparation of essential oil

1 ml of essential oil of eucalyptus added to 0,1 ml of tween “80” in 100 ml of distillated water from this dilution we took 8 ml and add to 2 ml of distillated water to obtain 10 ml of solution, the mice were starved 18h Before experiment to make sure that we found a good result.



Figure 6. Dilution of essential oil

4. Method of Study

Animals were placed in individual cages and maintained at a temperature of 22 ±2 °C, a photoperiod of 12h light / 12h dark with free access of nutrition which is water and grains.
The animals were separated in 4 and minting in stander photoperiod (12h L / 12h D) and temperate of 24 ± 2 °C. After two weeks of adaptation, the animals were separated into four lots each lot has 5 mice.

- Lot 1 n=5 injected only with carrageenan.
- Lot 2 n=5 injected with carrageenan and diclofenac.
- Lot 3 n=5 injected with carrageenan and force-feeding of essential oil of eucalyptus.
- Witness lot n=5 without adding anything.

Stimulation of inflammation with injection of 0.2 ml of carrageenan solution in paw in lots 1 and 2 and 3, INRA muscular Injecting 0.06ml of diclofenac in lot 2 after 15 of injection of carrageenan, force-feeding of 0.5ml of eucalyptus essential oil after 30 min of injection of carrageenan, after injection and force-feeding we toke measure of paw oedema each 15 min with professional electronic piakolis.



Figure 7. injection of carrageenan



Figure 8. measurement with piakolis



Figure 9. Steps of force-feeding





Figure 10. Step of decapitation and dissection

5. Statistical Analysis of Pow Oedema

After taking measure of pow oedema, we realized a statistical test to verify that the result are significant

Issue:

Is there a difference between the effect of treatment with diclofenac and that with eucalyptus essential oil during periods of 15 minutes after dosing with carrageenan?

ie the difference between Lot2 and Lot3?

H0: there is no difference in effect between Lot2 and Lot3

H1: there is a difference in effect between Lot2 and Lot3

Conditions for applying the student test: normality and homogeneity

6. Histological study

The manipulations took place in natural photoperiod in accordance with the spring season. The samples were taken during the morning between 10:00 and 12:00 in the laboratory of the faculty of life science at Dr. Yahia Fares Medea University. The animals were sacrificed by decapitacion. The harvested organs (liver and intestine) are fixed by immersion in formalin 10% for 1 week. The purpose of the fixation is to preserve the structures and to harden the parts.

After fixation, the specimens are carefully washed with running water for one to two hours to remove all traces of the fixative liquid until the rinse water clears. They are then dehydrated with ethyl alcohol in increasing degrees of alcohol (Table 1)

Table 1. dehydration baths

Alcohol	70°	96°	100°	Butanol
organs	2 baths: 10mn	2 baths: 10mn	2 baths: 15mn	2 baths:8h

7. The inclusion

The inclusion is done in special molds called "cassette molds ". The mold is filled with filtered paraffin, which is immiscible with water and is carried out in an oven at 56°C, the part is gently immersed in it to avoid any air bubbles and is oriented with a heated clamp to avoid a sudden solidification of the paraffin around the part. A recognition label containing the indications concerning the piece is fixed.



Figure 12. Paraffin cassette with organs

8. Sections, staining and observations

Once the cassette was cooled and unmolded, cross-sections of 5-7 µm thickness were made with microtome (Leica), then spread on slides with 0.4% gelatinized water.

After dewaxing and rehydration, the sections were stained using topographic stains, Groat-Eosin Hematoxylin. The histology slides were examined and photographed with a 'euromex' photomicroscope on which a "premiere" digital camera is adap.



Figure 13. Microtome slices cuter

Results and Discussion

Results and Discussion

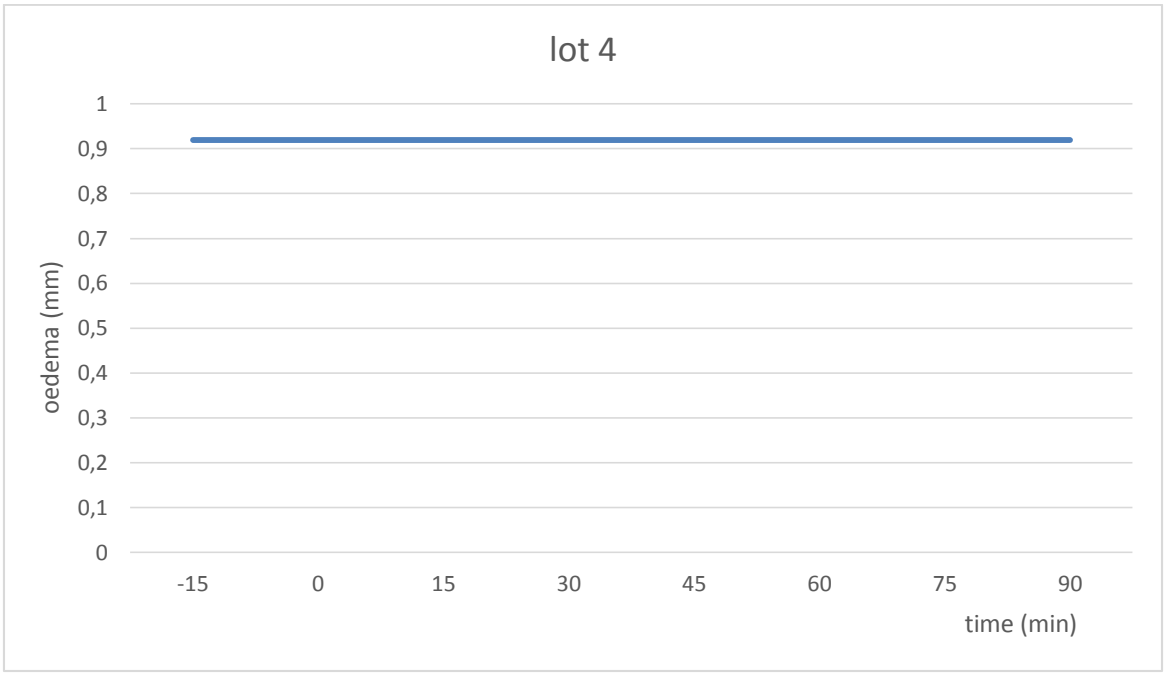
1. Macroscopic Analysis

We have measured the oedema in the four lots of mice with piakolis before and after the injection of carrageenan, and after the treatment of diclofenac and the essential oil of eucalyptus. The time span between each measurement with piakolis of oedema is 15 minutes (the measures are stated in table 3). We have found that the lots injected with carrageenan have the biggest rate of oedema (in the first three lots) while the last lot 4, which is the witness, has no oedema at all.

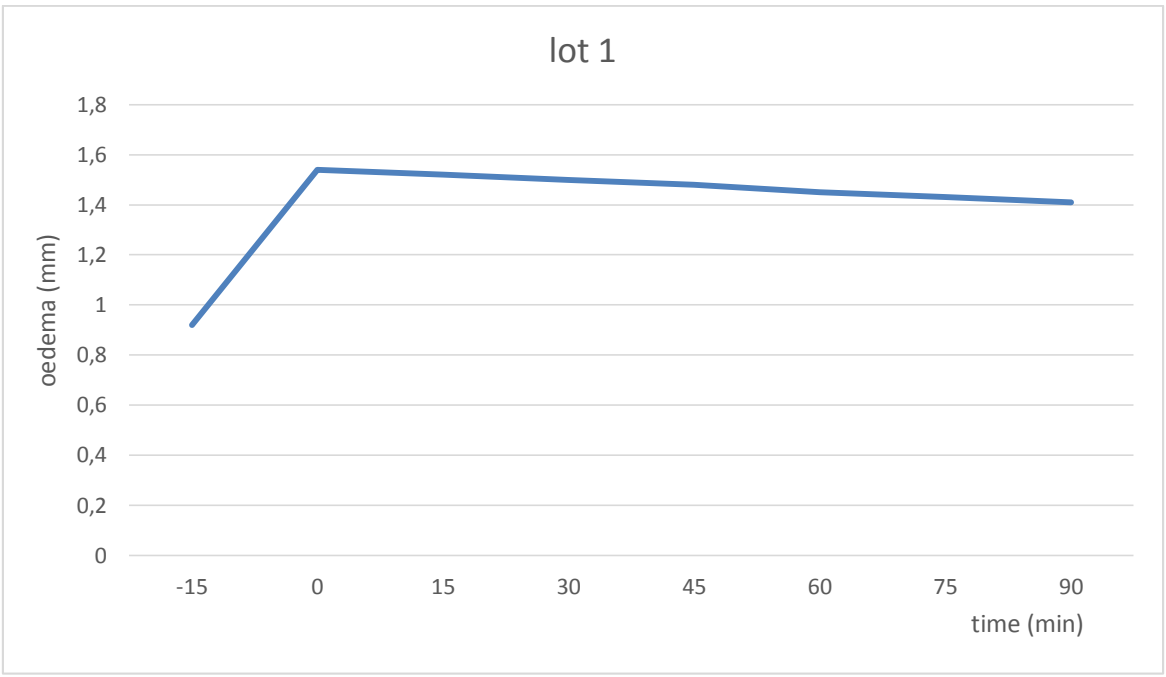
After that, the oedema in lot 1 increased after injection, remained high, and then it slightly decreased. However, the oedema in lot 2, which was treated with diclofenac, decreased faster than the lot 1. Moreover, the oedema in lot 3 decreased rapidly but not as fast as lot 2. The lot 4 remained constant with no oedema registered at all.

Table 2. Average table

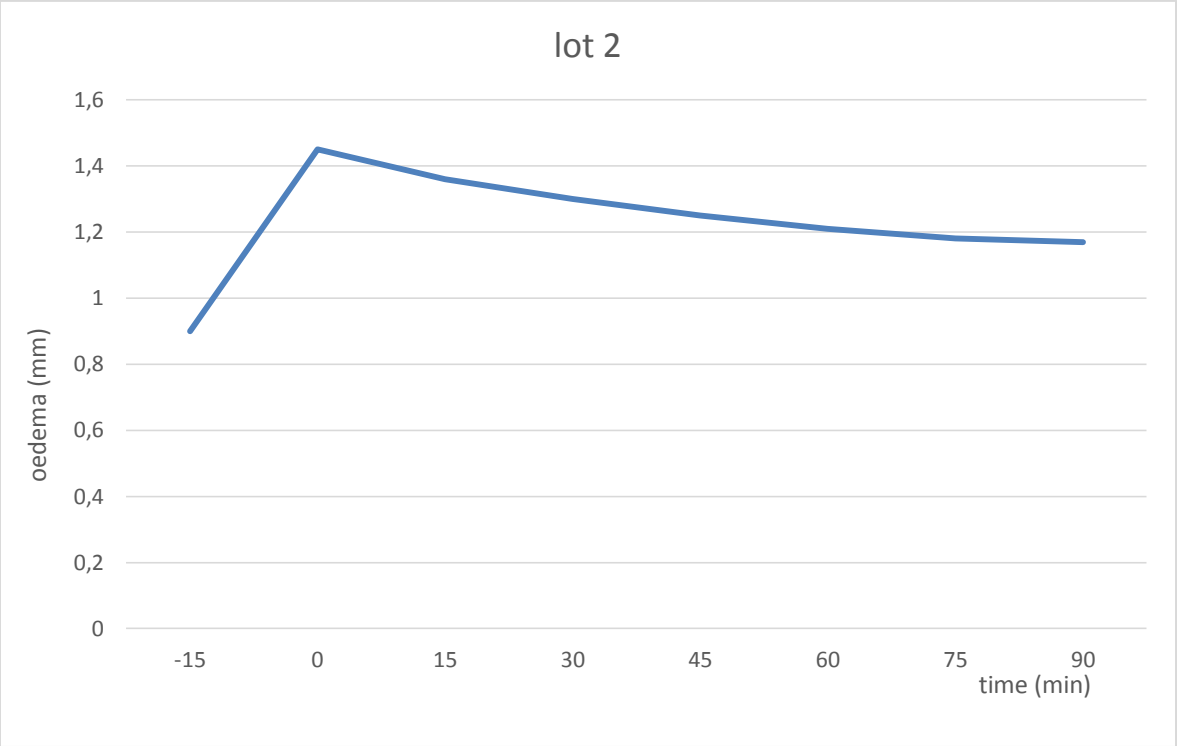
Time / Lot	Befor injection	After ingection	After 15 min	After 30 min	After 45 min	After 60 min	After 75 min	After 90 min
Lot 1	0,92	1,54	1,52	1,5	1,48	1,45	1,43	1,41
Lot 2	0,9	1,45	1,36	1,3	1,25	1,21	1,18	1,17
Lot 3	0,91	1,68	1,51	1,45	1,38	1,31	1,25	1,19
Lot 4	0,92	0,92	0,92	0,92	0,92	0,92	0,92	0,92


lot 4
oedema (mm)
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
-15
0
15
30
45
60
75
90
time (min)

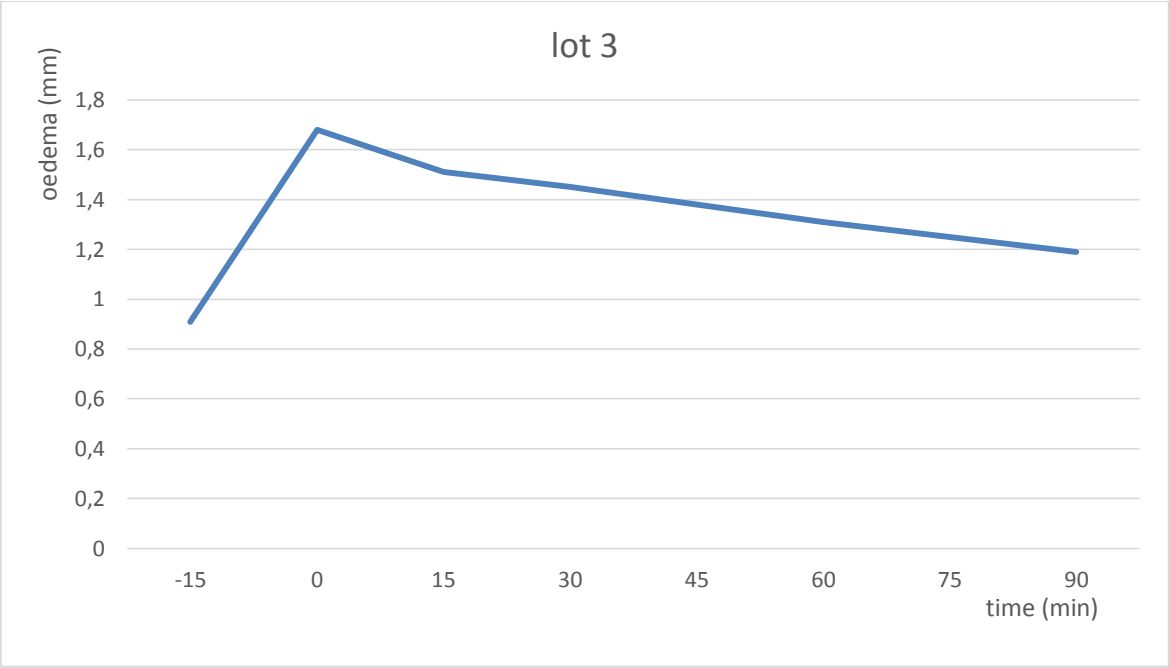
A


lot 1
oedema (mm)
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
-15
0
15
30
45
60
75
90
time (min)

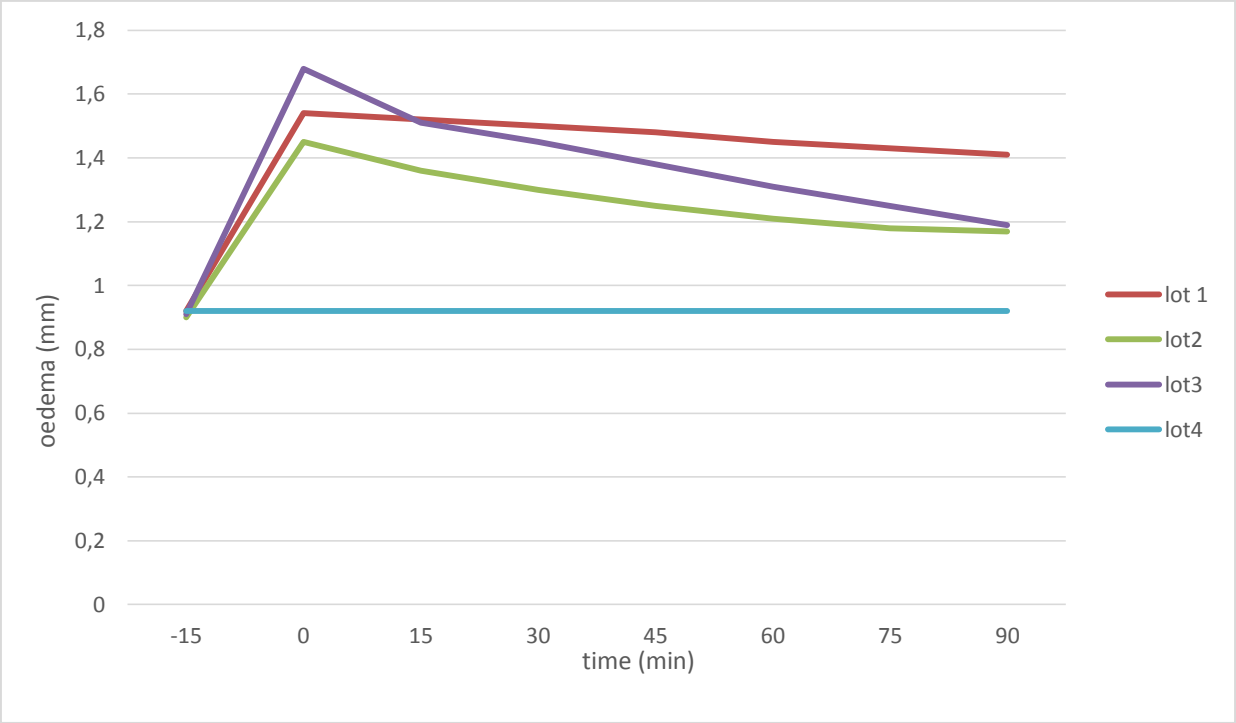
B


lot 2
oedema (mm)
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
-15
0
15
30
45
60
75
90
time (min)

C


lot 3
oedema (mm)
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
-15
0
15
30
45
60
75
90
time (min)

D



E

Figure 14. Graphs indicating macroscopic analysis (paw oedema). A (witness), B (injected with carrageenan), C (injected with carrageenan and diclofenac), D (injected with carrageenan and EO eucalyptus), E (all together)

Homogeneity test: Levene test

If sig sup at 0.05 then the variances are equal and we look at the top line

If Sig inf to 0.05 then the variances are unequal and we look at the bottom line

Student's T test:

If sig is inf 0.05 then there is a difference between the means of the two variables

if sig is sup 0.05 then we accept H0 and there are no differences between the means of the variables

Interpretation in our example

- Before carrageenan injection, Test Levene sig = 0.013 <0.05 then the variances are unequal and we look at the bottom line.
- sig =0,671> 0.05 then we do not reject H0 (there is no difference between the diclofenac effect and eucalyptus) it is logical because the treatment has not yet been

administered (before injection) (the difference between the means observed in the first table 0.9060 and 0.9140 is not significant)

- After carrageenan injection, Test Levene sig = 0.125> 0.05 then the variances are equal and we look at the top line
- sig =0,000 <0.05 then we reject H0 and we accept H1 (there is a difference between the diclofenac and eucalyptus effect in favor of diclofenac) indeed, moy diclofenac = 1.45> moy eucalyptus = 1.6820 that is to say that the diclofenac treatment works faster than that of eucalyptus
- fifteen min Test Levene sig = 0.617> 0.05 then the variances are equal and we look at the top line
- sig =0,000 <0.05 then we reject H0 and we accept H1 (there is a difference between the diclofenac and eucalyptus effect in favor of diclofenac) indeed, moy diclofenac = 1.3640> moy eucalyptus = 1.5140 that is to say that the diclofenac treatment works faster than that of eucalyptus

And so, on

2. Microscopic Analysis

2.1. Study of the Structural Aspect of the Liver of Mice

Histological study and observation under a light microscope allowed us to see the structural differences in the hepatic parenchyma between control and treated mice.

Our observations showed that the liver of control mice contains several hepatic lobules. The center of lobule represents the central vein, each lobule separated from the other by thin connective tissue (septa), which contains portal area. The portal area includes the hepatic artery, portal vain and bile duct. The hepatic lobule contains hepatic cells (hepatocytes) which arranged as cords radically surrounds the central vein and these cords separated from each other by vascular canal (sinusoids). The sinusoids house an important part of livers that represents the defense system composed of fixed macrophage (Kupffer cells) **(Figure 15 A).**

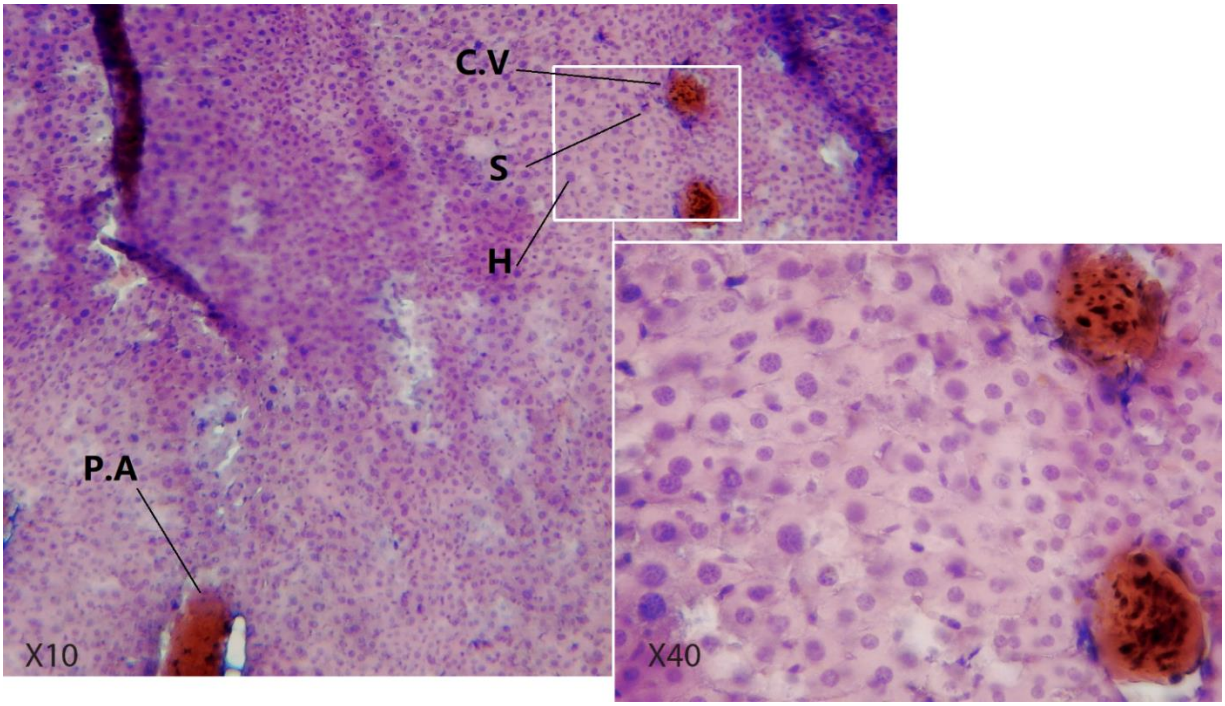
This study compared mice injected with carrageenan to induce inflammation with mice treated with diclofenac and essential oil of eucalyptus and control lot. The individuals injected

only with carrageenan have shown a clot of inflammation and focal degenerative and necrosis in hepatocyte cells with inflammatory cells infiltration, especially near portal area were seen dispersed isolated hepatocytes apoptosis with widening of sinusoids with congestion especially around portal area were observed **(Figure 15 B)**. The individuals treated with diclofenac and essential oil of eucalyptus showed a decrease of the clots of inflammation and reduction of the focal degenerative and necrosis in hepatocyte cells and the number of inflammatory cells **(Figure 15 C, D)**.

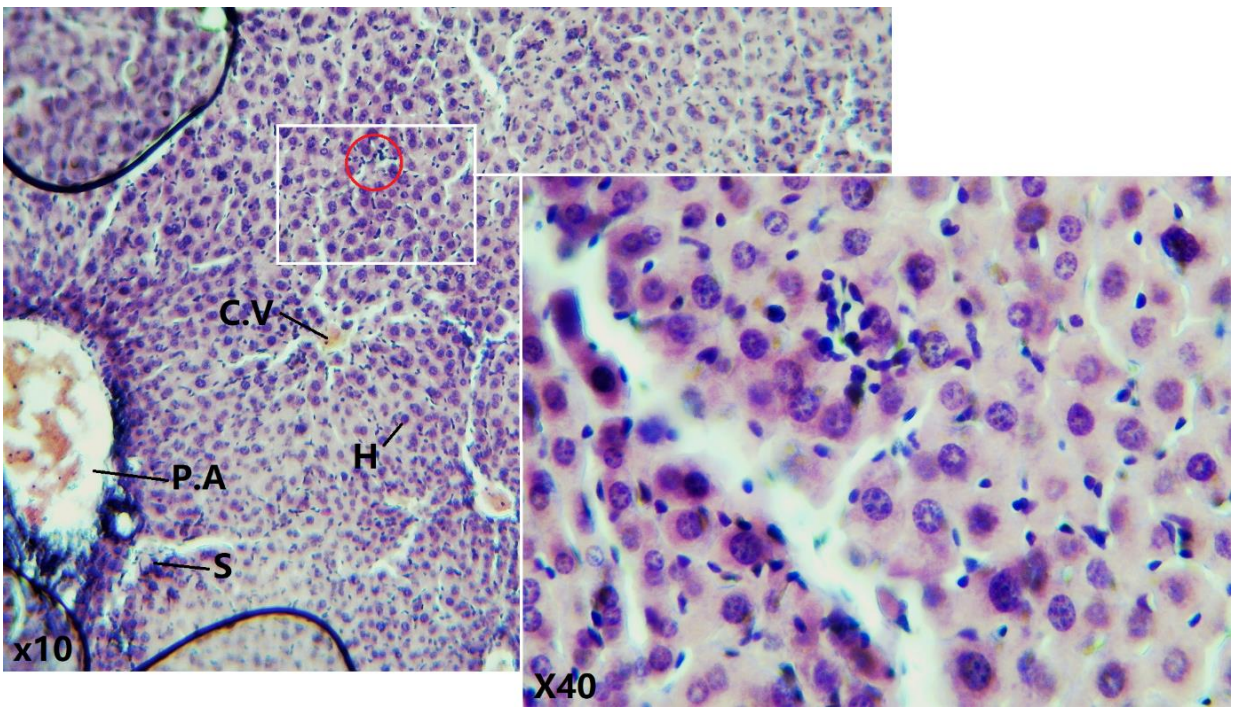
2.2. Study of the Structural Aspect of the Intestine of Mice

The second work is focused on the observation of intestinal cells on photonic microscope allowed us to see the structural differences in intestinal submucosa between control and desynchronized mice.

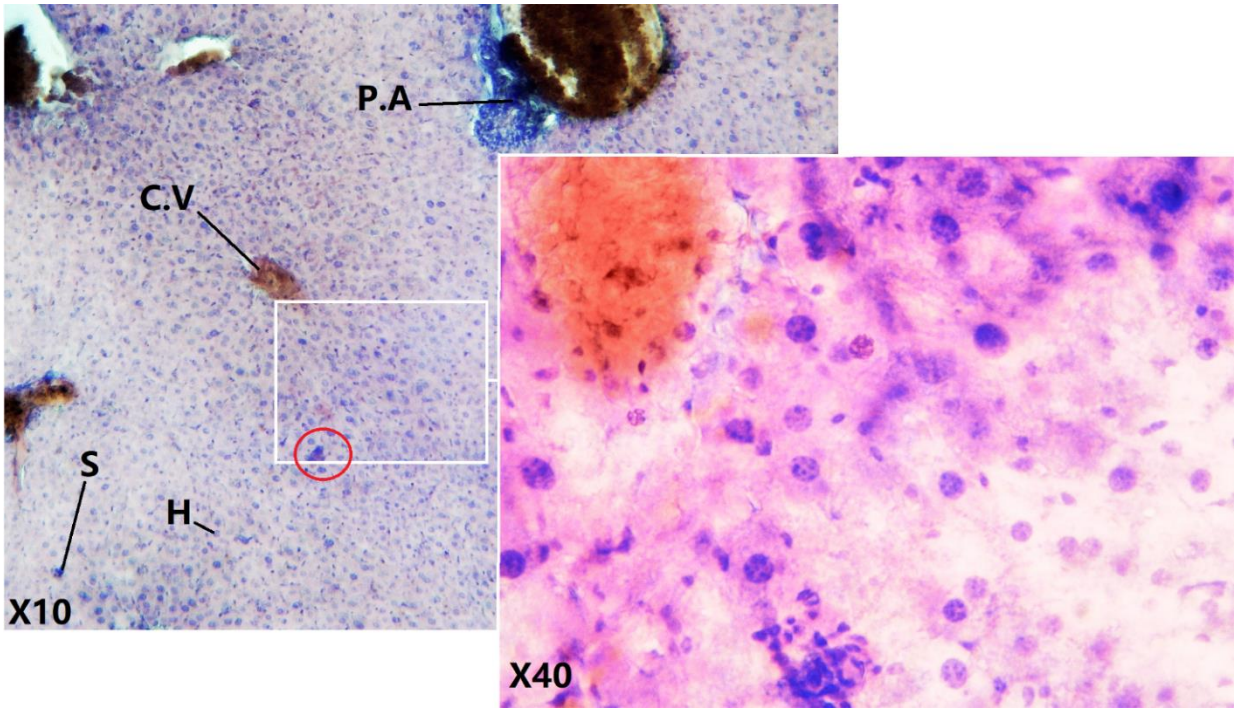
Our observation showed a normal structure of intestine witch contain an intestinal villi and submucosa empty of inflammatory cells and necrotic cells in control individuals, also there is no inflammation resisted in muscular layer **(Figure 16 A)**. In the individuals injected with carrageenan we have noticed inflammatory cells and necrotic cells gathered in submucosa and muscular layer **(Figure 16 B)**. In the individuals treated with diclofenac and eucalyptus showed decrease in inflammatory cells and necrotic intestine cells **(Figure 16 C, D)**



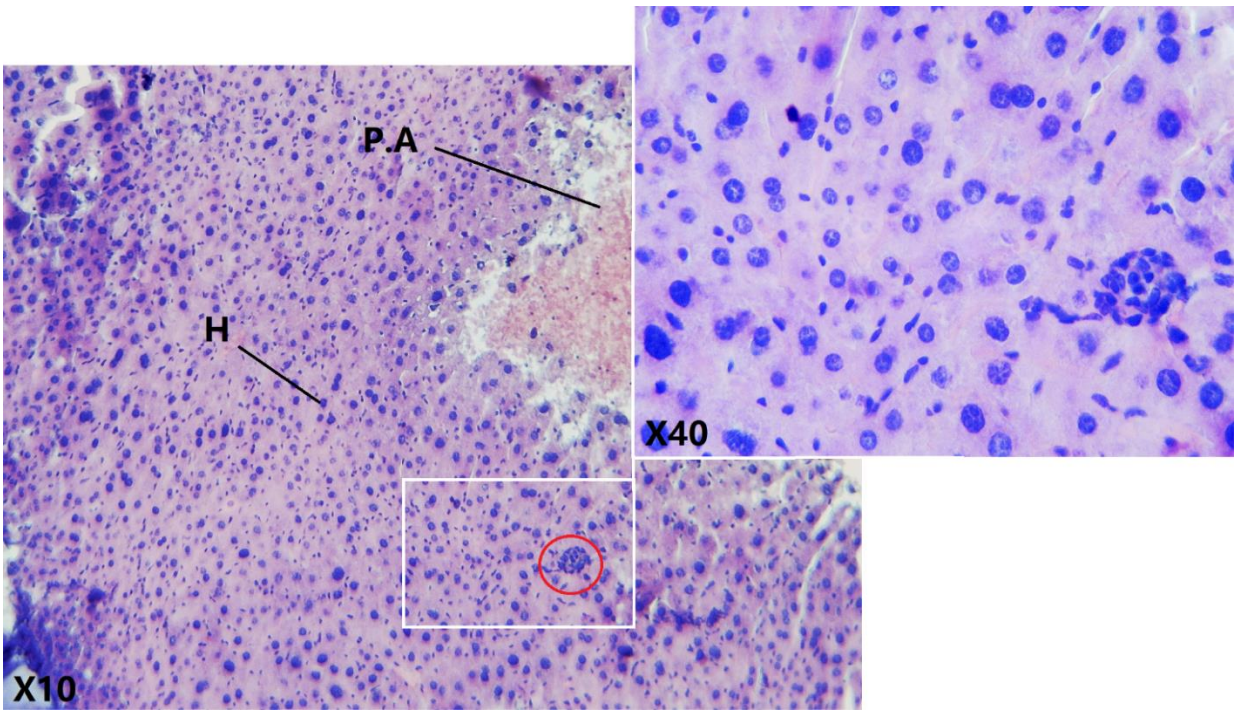
A



B



C



D

Figure 15. the cellular characteristics of the liver tissue of mice that have undergone inflammation with carrageenan.

Section (A): lot 4/ Section (B): lot 1/ Section (C): lot 2/ Section (D): lot 3.

(S): Sinusoid / (H): Hepatocytes / (C.V): Central vein / (P.A): Portal area

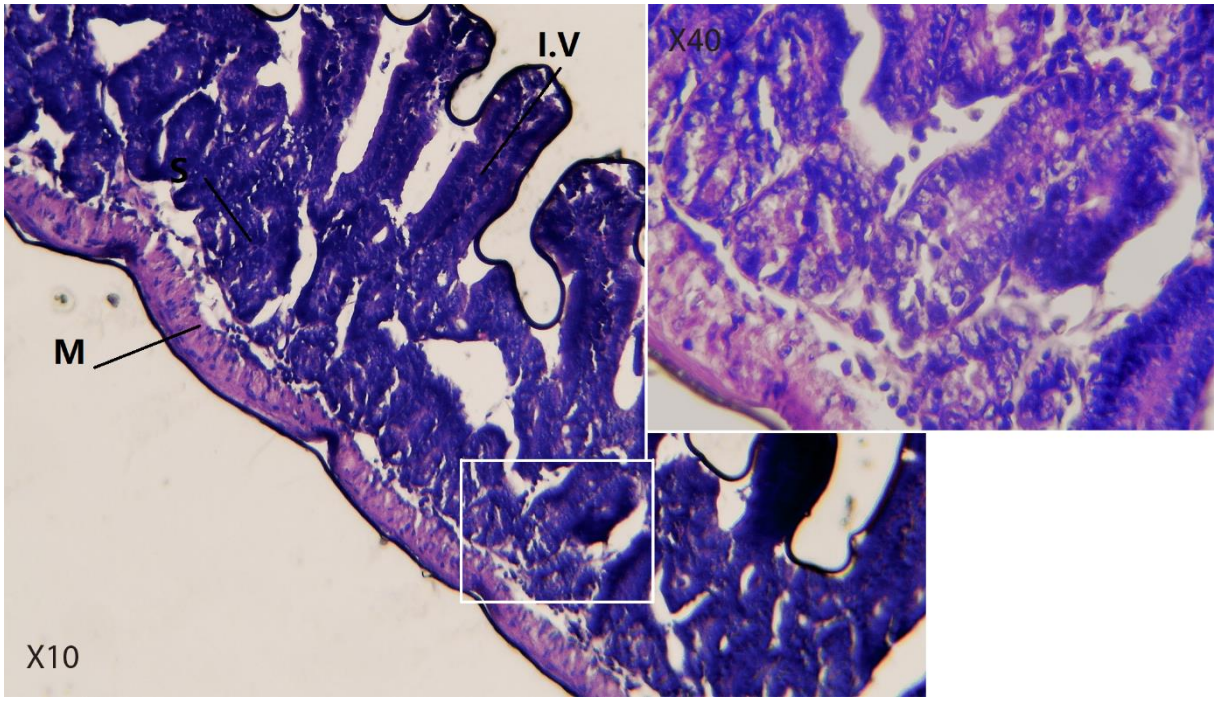
In section (A) there is no inflammation noticed or inflammatory cells (control)

The section (B) injected with carrageenan, we see inflammation and polynuclear cells forming a clot marked by red circle.

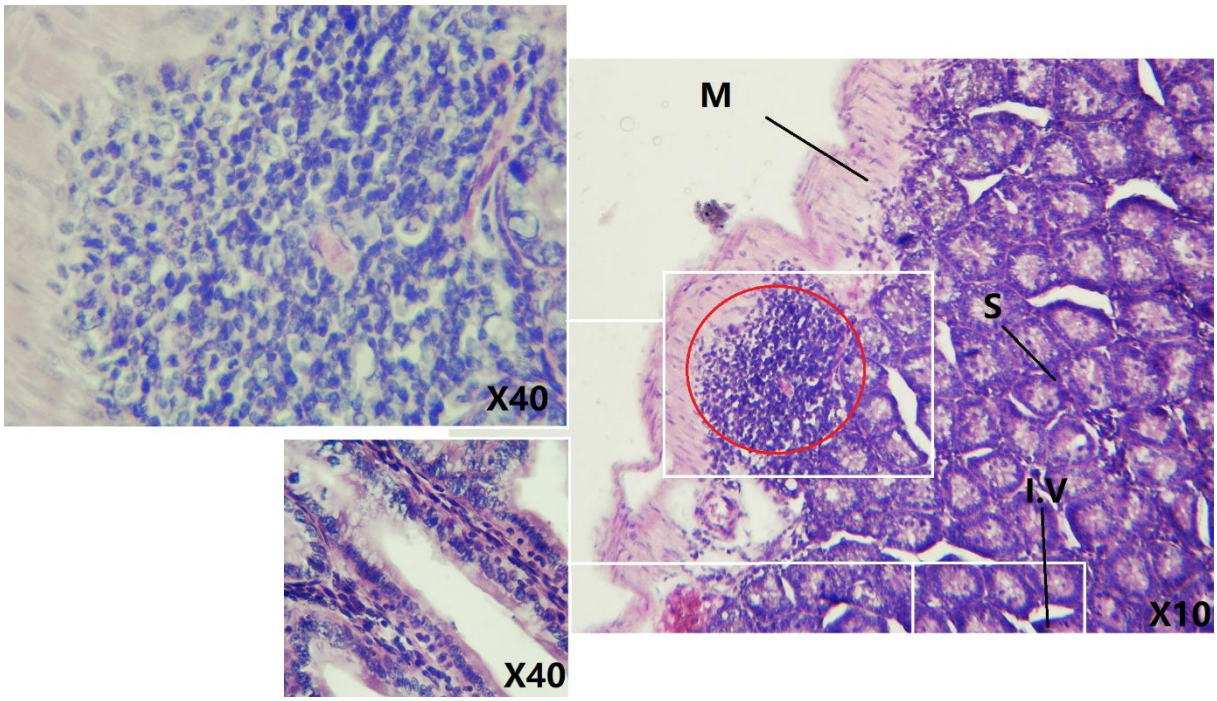
Note also at the level of the section (C) the diminution of inflammation and inflammatory cells around hepatocytes.

We notice in the section (D) a little desperation of inflammation and small clot marked by red circle.

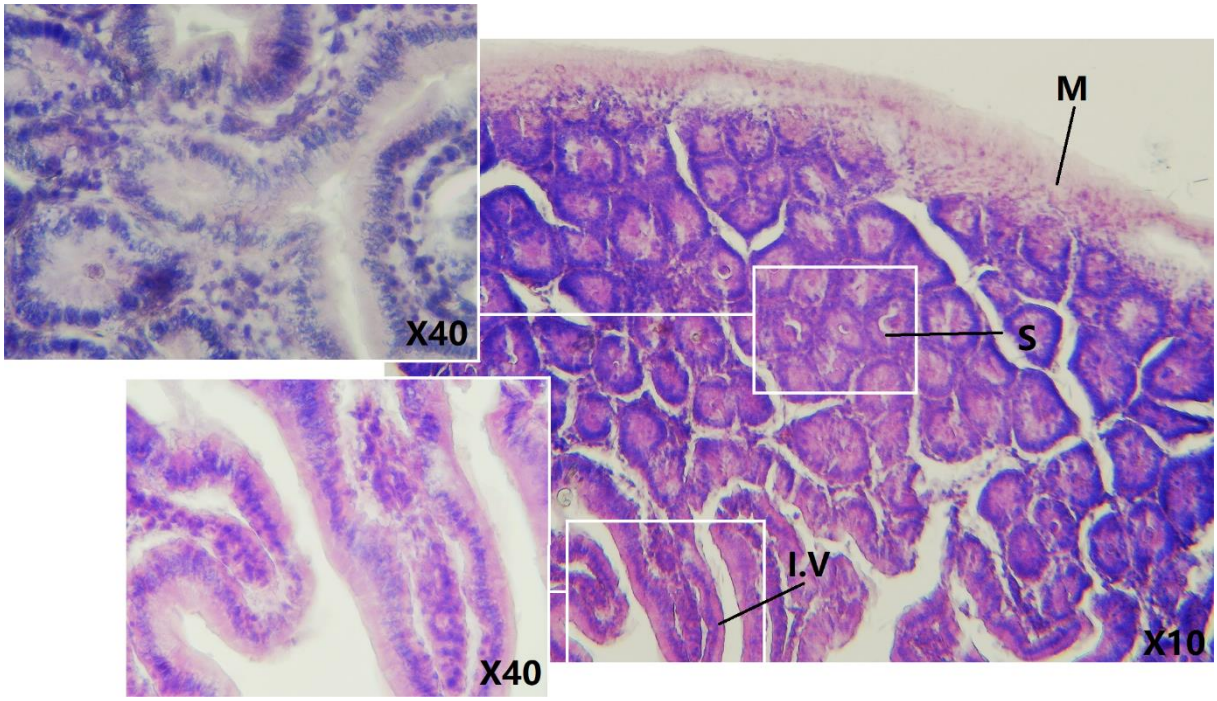
A, B, C and D coloration with HE (hematoxylin, eosin)



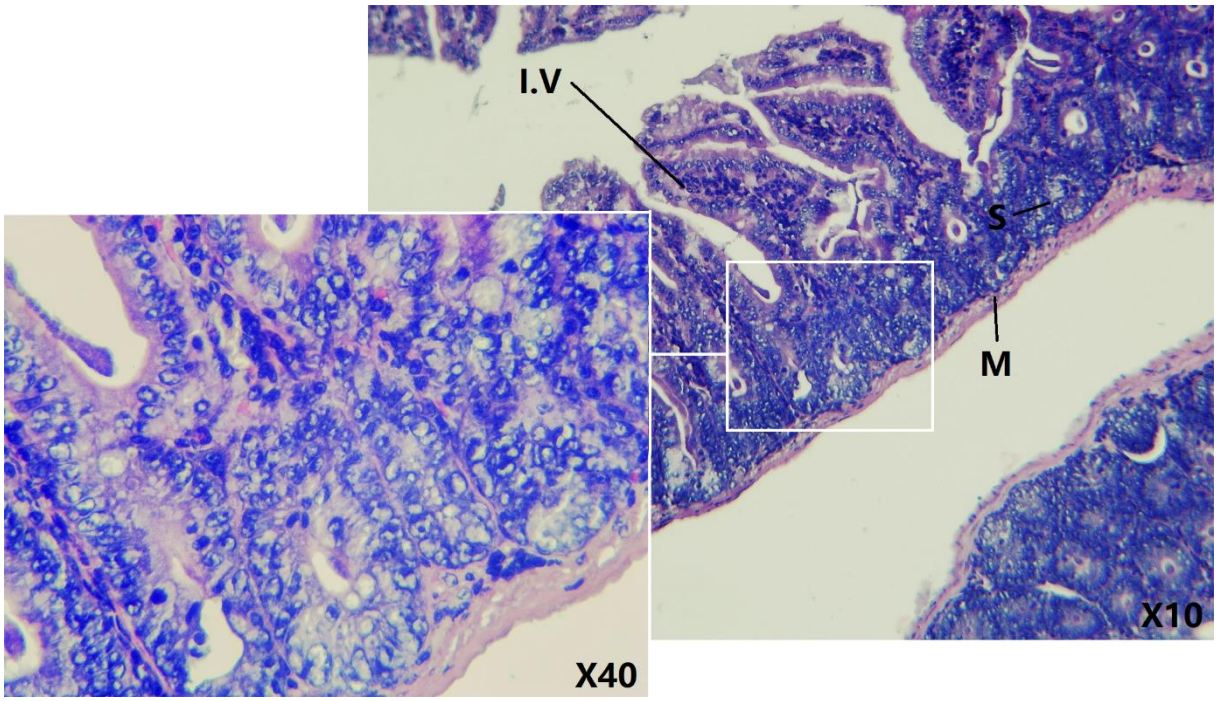
A



B



D



E

Figure 16. the cellular characteristics of the intestinal tissue of mice that have undergone inflammation with carrageenan.

Section (A): lot 4/ Section (B): lot 1/ Section (C): lot 2/ Section (D): lot 3.

(M): Muscular / (S): Submucosa / (I.V): Intestinal villi

In section (A) there is no inflammation noticed.

In the section (B) that is injected with carrageenan we see inflammation in the submucosa and polynuclear cells forming a big clot marked red circle.

Moreover, at the level of the section (C), the diminution of inflammation in the submucosa is observed.

We notice in the section (D) a little disappearance of inflammation.

Coloration used in A, B, C and D is with HE (hematoxylin, eosin)

Discussion

In this work we have compared the effect of essential oil of Eucalyptus Globulus and diclofenac in inflammation induced by carrageenan in mice. The main goal is to eliminate the inflammation caused by carrageenan with a non-steroidal drug (diclofenac) and using a natural plant (eucalyptus).

The aim of this study was to investigate the *in vivo* anti-inflammatory effect of Eucalyptus Globulus leaves to explore their benefits against carrageenan inflammation damage in structural level mainly by measuring oedema in all individuals by piakolis. We have noticed a difference in size of oedema in mice's paw in different lots comparing to the control, The table show the oedema size reduction during the different phases of the experiment.

The carrageenan induced paw inflammation is the most used model to test the efficiency of a plant extract against inflammatory response **(Khan et al., 2016).** Inflammation is biphasic: **(Hvattum and Ekeberg., 2003; Ruangsang et al., 2010; Singh et al., 2016)** the first phase (the vascular phase) (2h) involves the increase of chemical mediators such as kinin, histamine and serotonin in the inflammatory area while the second phase (cellular phase), which occurs 2-5h after carrageenan injection, is responsible for the prostaglandins release, neutrophil infiltration and free radicals and lysosome enzymes production **(Cuzzocrea et al., 1998).** This oedema is the systematic consequence of the vasodilatation and vasoconstriction of the first phase **(Ben Khedir et al., 2016).** This phase is sensitive to most of commercial anti-inflammatory drugs **(Kumar and Jain., 2014; Vinegar et al., 1969).**

We have chosen the diclofenac for its great capacity for curing the inflammation with major efficacity, our results proved that with this drug (diclofenac) there is a return to the normal paw size **(table 5).** It is described that inflammation causes disease and the therapy usually targets the inhibition of synthesis or effects of substances that mediate inflammatory reactions. NSAIDs are the most commonly used drugs **(Rao and Knaus., 2008).**

EO eucalyptus is known by his great anti-inflammatory effect, this effect was noticed in our experiment by the reduction of the mice's paw oedema (**table).** Many authors suggest that with the appearance of its side effects and with the ability of the recurrence of the disease after completing the treatment with the synthetic drugs, an impressive interest was focused on natural products **(Upton. 2015).** Indeed, plants have played an important role in human health for ages **(Farombi. 2003)** since they have been used for the treatment of several diseases **(Nair et al., 2005)**.

Anti-inflammatory activity of E. globulus oil dose-dependently stimulated phagocytosis (according to several parameters) by human MDMs (monocyte-derived macrophages) in vitro, without producing pro inflammatory effects. Phagocytic activity was not increased when the MDMs were exposed to TTO (tea tree oil) or lavender oil. Eucalyptus oil's phagocytic ability was halted when EO-stimulated cells were treated with a microtubule-destabilizing chemical, suggesting EO's phagocytic ability is dependent on the microtubule network. **(Serafino A, Sinibaldi Vallebona P, Andreola F, et al., 2008)**. To determine the effect on inflammation, a cytokine profile was performed on MDMs treated with EO only, LPS (lipopolysaccharide) only, and with EO pretreatment, then LPS. EO produced no difference in cytokine profile from the control group. LPS significantly elevated interleukins (IL-4, IL-6) and tumor necrosis factor-alpha (TNF-a). EO reduced the inflammatory effect of LPS. Neither LPS nor EO had an influence on IL-2, IL-10, or interferon-gamma (IFN-g). **(Serafino, Sinibaldi Vallebona, Andreola, et al., 2008)** 1,8-cineole inhibited the stimulated cytokine production from human lymphocytes and monocytes.

The macroscopic analysis proved that essential oil of eucalyptus has anti-inflammatory effect and decreased the oedema due to a special phytochemical involved in the composition of essential oil which promotes the anti-inflammatory effect. The latter decrease the recruitment of inflammatory cells. It is reported that the presence of phytochemicals in plants is one of the reasons which explain the anti-inflammatory power of plants **(Ben Khedir et al., 2016).** Based on the literature, the plant's anti-inflammatory activity is related to its content of these phytochemicals **(Hoensch and Oertel, 2015; De Brum et al., 2016).** These compounds have a great importance because they inhibit the actions of the mediators involved in the oedema formation and inflammation **(Middleton, 1998; Rodrigues et al., 2016).** They also inhibit leukocytes migration and, thus, the metabolism of arachidonic acid will be blocked **(Barar, 2000**).

histopathological observation of liver and intestine tissue showed increase of inflammation in lots injected with carrageenan which means that there is a flow of immune cells as polynuclear neutrophils, but the pre-treatment of carrageenan mice with eucalyptus and diclofenac showed decrease of the number of immune cells in liver and intestine tissue because diclofenac and the essential oil of eucalyptus have an anti-inflammatory effect which impacts the migration and the recruitment of inflammatory cells. Furthermore, eucalyptus helps to reduce inflammation thanks to its eucaluptol constituent but not as fast as diclofenac. That means it has a direct role in leucocyte recruitment. This is what we have noticed in

histological section in mice treated with diclofenac. The same result including the mice treated with eucalyptus essential oil had been obtained.

While NSAIDs (non-steroidal anti-inflammatory drug) are thought to exert their effects mainly by inhibiting prostaglandin synthesis, it has also been postulated that they inhibit a number of leucocyte responses such as lysosomal enzyme release **(Kruze et al. 1976a)** and superoxide production **(Friman et al. 1986).**

Conclusion

Conclusion

The present study allowed us to see the inflammation in individuals (mice) injected with carrageenan and the disappearance of inflammation after treating this individual with diclofenac and EO of Eucalyptus on two levels. On the first level (macroscopic) it was by measuring the oedema and on the second level (microscopic) was by the observation of the individuals' histological sections in both liver and intestine allowing us to describe the two anatomic organs in control and in tested individuals before and after treatment. We noticed that the mice treated have less inflammatory cells and necrotic hepatocytes and intestine cells opposite to those injected only with carrageenan. There is also significant difference between the sections especially in the intestine segment between the submucosa and the muscular layer also in the liver between hepatocyte cell ant the central vein.

The results obtained show that diclofenac and EO extracted from eucalyptus are efficient anti-inflammatory drugs that reduce inflammation through their constituent that reduce the recruitment of inflammatory cell by the secretion of a specific chemokines.

Furthermore, the macroscopic results show an interesting decrease of oedema comparing to mice injected only with carrageenan. This means that the treated individuals responded to the treatment and had a reduction in inflammation that was induced by carrageenan. This denotes that the two molecules above played an important role in reducing inflammation. In the light of this research, further studies are required as:

- The inflammatory process should be studied using transmission electron microscopy to identify the various ultrastructural and morphological alterations that have occurred by carrageenan.
- Blood tests are required to intensively study the plasma enzymes and proteins of inflammation.
- Molecular biology is needed to know better what happened inside the cell like western blot; immunoblotting; PCR…etc.
- Study of NF-kB and transcription factors and their chemokines to help better understand this type of inflammation.
- Choose another type of plant like Mantha Paperita and study its anti-inflammatory effect to find a better molecule to treat this inflammation.

Appendices

Appendices

1. **Table 3.** Weight Table

mice / Lots	S1	S2	S3	S4	S5
Lot 1	20g	25g	20g	18g	20g
Lot 2	22g	20g	20g	24g	18g
Lot 3	25g	21g	24g	19g	23g
Lot 4	21g	18g	19g	23g	18g

2. **Preparation of Carrageenan**

1g of carrageenan ➔ 100 ml of distillated water

3. **Preparation of Diclofenac**

75mg of diclofenac crushed and diluted in 100 ml of distillated water.

4. **Modified Azan Coloration Protocol**

Deparaffinize, hydrate : xylene = 30mn

Alcohol 100°= 2mn

Alcohol 96°= 2mn

Alcohol 70°= 2mn

Rinse with distilled water

Hematoxylin = 1,5mn

Rinse with water (tap water) = 5 mn

Eosin = few secondes

Rinse with water (tap water)

Ethanol at 95° passage

Dehydrate: Alcohol 100°= 2 mn

Xylene = 5 mn

5. **Eosine Preparation Protocol**

Eosine 1g + 100 mL distilled water

Mix well then filter.

6. **Gelatinized Water**

100 ml distillated water with 0.4 mg of gelatin were mixed in hotplate

7. **Table 4.** Oedema Measurement Table

Lots	Time / Mice	Before injection	After injection	After 15 min	After 30 min	After 45 min	After 60 min	After 75 min	After 90 min
Lot 1	**1**	0,97	1,56	1,54	1,51	1,49	1,46	1,44	1,42
	2	0,89	1,49	1,47	1,46	1,44	1,43	1,42	1,40
	3	0,93	1,50	1,49	1,48	1,46	1,43	1,42	1,42
	4	0,95	1,63	1,59	1,57	1,54	1,49	1,45	1,43
	5	0,89	1,55	1,54	1,52	1,49	1,48	1,44	1,42
Lot 2	**1**	0,90	1,45	1,37	1,31	1,27	1,23	1,20	1,18
	2	0,92	1,44	1,36	1,28	1,26	1,21	1,19	1,18
	3	0,89	1,47	1,37	1,33	1,25	1,19	1,18	1,16
	4	0,91	1,43	1,34	1,29	1,24	1,21	1,16	1,16
	5	0,91	1,46	1,38	1,31	1,26	1,22	1,20	1,18
Lot 3	**1**	0,89	1,70	1,53	1,46	1,38	1,31	1,27	1,20
	2	0,86	1,71	1,51	1,45	1,38	1,32	1,25	1,19
	3	0,94	1,69	1,50	1,44	1,38	1,32	1,26	1,21
	4	0,93	1,65	1,51	1,45	1,39	1,30	1,24	1,18
	5	0,95	1,66	1,52	1,48	1,39	1,31	1,25	1,20
Lot 4	**1**	0,95	0,94	0,95	0,96	0,95	0,96	0,96	0,95
	2	0,91	0,92	0,92	0,91	0,90	0,91	0,91	0,92
	3	0,95	0,95	0,94	0,94	0,93	0,93	0,94	0,95
	4	0,90	0,91	0,91	0,90	0,90	0,91	0,91	0,90
	5	0,92	0,91	0,91	0,92	0,92	0,91	0,92	0,92

8. **Table 5.** Descriptive statistical results over different stages or time periods

Group statistic					
	Treatement	**N**	**Average**	**Ecart-type**	**Average standard error**
Before injection	1	5	0,9060	0,01140	0,00510
	2	5	0,9140	0,03782	0,01691
After injection	1	5	1,4500	0,01581	0,00707
	2	5	1,6820	0,02588	0,01158
Fifteen min	1	5	1,3640	0,01517	0,00678
	2	5	1,5140	0,01140	0,00510
Thirty min	1	5	1,3040	0,01949	0,00872
	2	5	1,4560	0,01517	0,00678
Forty-five min	1	5	1,2560	0,01140	0,00510
	2	5	1,3840	0,00548	0,00245
Sixty min	1	5	1,2120	0,01483	0,00663
	2	5	1,3120	0,00837	0,00374
Seventy-five mins	1	5	1,1860	0,01673	0,00748
	2	5	1,2540	0,01140	0,00510
Ninety mins	1	5	1,1720	0,01095	0,00490
	2	5	1,1960	0,01140	0,00510

9. **Table 6.** Student's T test results (significance of the difference in means) during the different stages or time lapse

Independent sample testing										
		Levene's test for equality of variances		T-test for equality of means						
		F	Sig.	T	Ddl	Sig. (Bilateral)	Mean difference	Standard deviation difference	95% confidence interval of the difference	
									Lower	Superior
Before injection	Equal variance assumption	10,182	0,013	-0,453	8	0,663	-0,00800	0,01766	-0,04873	0,03273
	Unequal variance assumption			-0,453	4,721	0,671	-0,00800	0,01766	-0,05422	0,03822
After injection	Equal variance assumption	2,939	0,125	-17,103	8	0,000	-0,23200	0,01356	-0,26328	-0,20072
	Unequal variance assumption			-17,103	6,620	0,000	-0,23200	0,01356	-0,26445	-0,19955
Fifteen min	Equal variance assumption	0,271	0,617	-17,678	8	0,000	-0,15000	0,00849	-0,16957	-0,13043
	Unequal variance assumption			-17,678	7,427	0,000	-0,15000	0,00849	-0,16983	-0,13017
Thirty min	Equal variance assumption	0,487	0,505	-13,761	8	0,000	-0,15200	0,01105	-0,17747	-0,12653
	Unequal variance assumption			-13,761	7,544	0,000	-0,15200	0,01105	-0,17774	-0,12626
Forty-five min	Equal variance assumption	2,326	0,166	-22,627	8	0,000	-0,12800	0,00566	-0,14104	-0,11496
	Unequal variance assumption			-22,627	5,753	0,000	-0,12800	0,00566	-0,14199	-0,11401

Sixty min	Equal variance assumption	0,772	0,405	-13,131	8	0,000	-0,10000	0,00762	-0,11756	-0,08244
	Unequal variance assumption			-13,131	6,311	0,000	-0,10000	0,00762	-0,11841	-0,08159
Seventy-five mins	Equal variance assumption	0,738	0,415	-7,509	8	0,000	-0,06800	0,00906	-0,08888	-0,04712
	Unequal variance assumption			-7,509	7,056	0,000	-0,06800	0,00906	-0,08938	-0,04662
Ninety mins	Equal variance assumption	0,084	0,779	-3,394	8	0,009	-0,02400	0,00707	-0,04031	-0,00769
	Unequal variance assumption			-3,394	7,987	0,009	-0,02400	0,00707	-0,04031	-0,00769

References

REFERENCES

- **Abraham R, Benitz KF, Mankes RF, Rosenblum I, Ringwood N** (1983). Studies on rhesus monkeys (Macaca mulatta) receiving native carrageenan (Chondrus crispus) orally for 7.5 years. Book 1. Unpublished summary of final report from Institute of Experimental Pathology and Toxicology, Albany Medical College. Submitted to WHO by R.J.H. Gray, International Food Additives Council, Atlanta, Georgia, USA and P. Couchoud, Marinalg, Paris, France.
- **Abraham R, Golberg L, Coulston F** (1972). Uptake and storage of degraded carrageenan in lysosomes of reticuloendothelial cells of the rhesus monkey, Macaca mulatta. Experimental and Molecular Pathology 17 ,77–93.
- **Abrams GJ, Solomon L, Meyers OL. A** long-term stud y of diclofenac sodium in the treatment of rheumatoid arthritis and osteo-arthrosis. South African Medical Journal 53 : 442-445, 1978.
- **AI-Sharkawi MS. A multicentre** study of diclofenac sodium slow release (Voltaren Retard) in the treatment of rheumatic disorders in the Kingdom of Saudi Arabia. Journal of International Medical Research 12: 244-249, 1984.
- **AI-Waili NS**. Diclofenac sodium and intractable epilepsy. Acta Neurologies Scandinavica 73: 507,1986.
- **AI-Waili NS**. Diclofenac sodium in the treatment of primary nocturnal enuresis: double-blind crossover study. Clinical and Experimental Pharmacology and Physiology 13: 139-142,1986.
- **Alcalay M, Bussiere JL, Peltier A. Le Voltaren** 100 suppositoire en rhumatologie : bilan-clinique à propos de 16419 nouvelles observations recueillies par 2895 omn ipracticiens en France. Gazette Medicate 87: 2036, 1980.
- **Allion, A.** (2004). Environnement des bactéries et sensibilité aux biocides, thèse de doctorat en sciences alimentaires. Ecole nationale supérieure des industries agricoles et alimentaires, p 22-28.
- **Altman R.** International experiences with diclofena c in osteoarthritis. American Journal of Medicine 80 (Suppl, 4B): 48-52, 1986.
- **Alvarez JLP, de Vallado PF, Ballarin JMP.** A comparative study with tiaprofenic acid *versus* diclofenac in knee osteoarthritis.In Berry & Franchimont (Eds) Tiaprofenic acid, pp, 71-76, Excerpta Medica, Geneva, 1984.

- **Amundsen T, Bleken L, Berkje B, Eidsaunet W, Eidsba P, et al.** Variation in response Ro naproxen and diclofenac in patients with osteoarthritis. Current Therapeutic Research 33: 793-80I,1983.
- **Anderson W, Soman PD** (1966): The absorption of carrageenans. Journal of Pharmacy and Pharmacology 18, 825–827.
- **Appelboom T, Balthazar-Letawe D, Boos P, Claessens J, Famaey J-P.** A'test of lOOmg Voltaren suppos itories in osteoarthritis. Bruxelles-Medicale 56: 273-277, 1976.
- **Araie M, Sawa M, Takase M.** Topical flurbiprofen and diclofenac suppress blood-aqueous barrier breakdown in cataract surgery: a fluorophotometric study. Japanese Journal of Ophthalmology 27: 535-542, 1983.
- **Arakawa S, Ito M, Tejima S** (1988): Promoter function of carrageenan on development of colonic tumours induced by 1,2-dimethylhydrazine in rats. Journal of Nutritional Science and Vitaminology 34, 577–585.
- **Asanza-Teruel ML, Gontier E, Bienaime C, Nava-Saucedo JE, Barbotin JN** (1997). Response surface analysis of chlortetracycline and tetracycline production with κ-carrageenan immobilized *Streptomyces aureofaciens*. Enzyme and Microbial Technology 21, 314–320.
- **Astbury C, Taggart AJ, Zebouni LNP, Bird HA.** Single dose suiphasalazine pharmacokinetics in rheumatoid arthritis with and without concom itan t NSAID therapy. Abstract no. 249. Acta Pharmacologica et Toxicologica 59 (Suppl, 5): 95, 1.986.
- **Avendano-Espinosa O.** Analgesia with diclofenac sodium in anorectal surgery. Prensa Medica Mexicana 42: 347-350, 1977.
- **Averbeck, D., Averbeck, S.,** 1994. Induction of the genes RAD54 and RNR2 by various damaging agents in Saccharomyces cerevisiae.Mutat. Res. 315, 123–138.
- **Averbeck, D., Averbeck, S., Dubertret, L., Young, A.R., Morlie`re, P.,** 1990. Genotoxicity of bergapten and bergamot oil in Saccharomyces cerevisiae. J. Photochem. Photobiol. B 7, 209–229.
- **Aylward M, Maddock J, Lewis PA, Dewland PM.** Mefenamic acid and diclofenac sodium in osteoarthritis of the weight bearing joints: a double-blind comparison. British Journal of Oinical Practice 39: 135-139, 1985.

- **Aylward M, Maddock J; Jones M.** Simultaneous pharmacokinetics of ant irheumatic drugs in plasma and synovial fluids of rheumatoid patients. Abstract presented at the World Congress of Clinical Pharmacology and Therapeutics, London, 1980.
- **Ayrolles D, Fourn ie A.** Study on a new anti-inflammatory nonhormonal drug, diclofenac sodium, in 50 cases. Rhumatologie 28: 53-56, 1976.
- **Baba M, Snoeck R, Pauwels R, de Clercq E** (1988): Sulfated polysaccharides are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, and human immunodeficiency virus. Antimicrobial Agents and Chemotherapy 32, 1742–1745.
- **Babany G, Bernuau J, Danan G, Rueff B, Benhamou J-P.** Hepatite fulminante chez une femme prenant de la g1afenine et du diclofenac. Gastroenterologie Clinique et Biologique 9: 185,1985.
- **Bach GL.** Comparative clinical trials with diclofenac sodium (Voltarol<») and naproxen in rheumatic conditions: investigation of possible changes in diclofenac dose and dose interval. Rheumatology and Rehabilitation (Suppl. 2): 69-71, 1979.
- **Bach GL.** The acute gouty attack: comparative treatment with two non-steroidal antirheumatic drugs. Medizinische Welt 30: 1696, 1979.
- **Badia-Flores J, Garcia-Rubio R, Barradas FM**. Efecto sintomatico y tolerabilidad del diclofenac sodico en la artritis reumatoide. Investigacion Medica International 2 : 194-199, 1975.
- **Bahous I.** Diflunisal - ein neues, lang wirksames steroidfreies Antirheumatikum: klinische Pnifung gegen Diclofenac. Praxis. 68 : 390-393, 1979.
- **Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar**, M. Biological effects of essential oils□A review. Food Chem. Toxicol. 2008, 46 (2),446−475.
- **Barbeyron T, Michel G, Potin P, Henrissat B, Kloareg B** (2000): ι-Carrageenases constitute a novel family of glycoside hydrolases, unrelated to that of κ-carrageenases. Journal of Biological Chemistry 275, 35499–35505.
- **Barnes CG, Berry H, Carter ME, Downie WW, Fowler PD, et al.** Diclofenac sodium (Voltarol) and indomethacin: a multicentre comparative study in rheumatoid arthritis and osteoarthritis. Rheumatology and Rehabilitation (Suppl. 2): 135-143, 1979.
- **Barton, G. M.** (2008). A calculated response: control of inflammation by the innate immune system. *J Clin Invest*. 118: 413-420.

- **Barton, G. M.** A calculated response: control of inflammation by the innate immune system. *J. Clin. Invest.* 118, 413–420 (2008).
- **Baumgartner H.** Comparison of sulindac and diclofenac in the treatment of degenerative joint diseases. Praxis 68: 916-922,1979.
- **Bedi SS, Eberl R, Million R, Silas AM.** Fenclofenac and diclofenac in the treatment of rheumatoid arthritis. British Journal of Rheumatology 23: 214-219,1984.
- **Behrens U, Dumpert HP, Thummler W, Vetter G. A** doubleblind multicenter comparison of isoxicam and diclofenac in the treatment of osteoarthritis. Current Therapeutic Research 37: 739-746, 1985.
- **Bendix T, Schmidt I, Rasmussen K-JE, Ibfelt HH.** Diclofenac (Voltaren<») and ketoprofen (Orudiss'), in rheumatoid arthritis: a randomized double-blind multicentre trial. Current Therapeu~ic Research 33: 192-199, 1983.
- **Benitz KF, Golberg L, Coulston F** (1973): Intestinal effects of carrageenans in the rhesus monkey (Macaca mulatta). Food and Cosmetic Toxicology 11, 565–575.
- **Benson MD, Aldo-Benson M, Brandt KD.** Synovial fluid concentrations of diclofenac in patients with rheumatoid arthritis or osteoarthritis. Seminars in Arthritis and Rheumatism 15 (Suppl, I): 65-67, 1985.
- **Benvenuti C, Guidoni G, Longoni A, Mordini M.** Controlled study of flurbiprofen and diclofenac in the treatment of rheumatic disorders. International Journal of Tissue Reactions 5:61-65,1983.
- **Berry H, Bloom B, Hamilton EBD.** A comparative study of piroxicam (Feldene), diclofenac and placebo in osteoarthritis. Clinical Trials Journal 19: 349-358, 1982.
- **Berry H, Bloom B, Mace BEW, Hamilton EBD.** Comparison of indoprofen (F1osint) and diclofenac in rheumatoid arthritis. Clinical Trials Journal 19:'248-257, 1982.
- **Bethlen L, Dannhorn R.** Results of testing diclofenac sodium in general practice. Therapiewoche 26: 3039-3047, 1976.
- **Bettini R, Grossi E, Rapazzin i R, Giardina G.** Diclofenac sodium versus acetylsalicylic acid: a randomized study in febrile patients. Journal ofInternationai Medical Research 14: 95-100, 1986.
- **Beur GDM, Leunissen KML, Van Breda Vriesman PJC, Van HooffJP, Grave W.** Isolated minimal change nephropathy associated with diclofenac. British Medical Journal 295: 182 183, 1987.

- **Bijlsma A, ten Pas JG.** A study of the anti-inflammatory effect of Voltaren in patients with rheumatoid arthritis. Scandinavian Journal of Rheumatology (Suppl, 22): 46-50, 1978.
- **Bijlsma A.** The long-term efficacy and tolerability of Voltaren (diclofenac sodium) and indomethacin in rheumatoid arthritis. Scandinavian Journal of Rheumatology (Suppl, 22): 74 80, 1978.
- **Bird HA, Hill J, Leatham P, Wright V.** A study to determine the clinical relevance of the pharmacokinetic interaction between aspirin and diclofenac. Agents and Actions 18: 447-449, 1986.
- **Bll1thazar-Letawe D, Cleppe D, Coigne E, Decrans L, Dhondt E, et al.** Single daily dose of diclofenac retard tablets for the treatment of osteo-arthritis: a double-blind comparison with piroxicam. Acta Therapeutica 10: 413-420, 1984.
- **Bodem H, Haringer E, Maier-Lenz H.** Open multicentre study comparing the efficacy and tolerability of diclofenac sodium and piroxicam in degenerative joint diseases. Presented at the 15th International Congress of Rheumatology, Paris, 1981.
- **Bonfiglioli D, Sommariva D, Zanaboni L, Scarpellini P, Bott A, et al.** Influence of two non-steroidal anti-inflammatory drugs on lipolysis and on plasma post-heparin lipoprotein lipase activity in normal man. European Journal of Clinical Pharmacology 20: 263-267, 1981
- **Borghi C, Como C, Palummeri E, Giorgi D.** Pharmacokinetics, efficacy and safety of diclofenac: elderly *vs*non elderly arthritic patients. Abstract no. 270. Acta Pharmacologica et Toxicologica 59 (Suppl, 5): 10I, 1986.
- **Botting RM** (2000): Mechanism of action of acetaminophen: is there a cyclooxygenase 3? Clinical Infectious Diseases 31, 202–210.
- **Breen EG, McNicholl J, Cosgrove E, McCabe J, Stevens FM.** Fa-. tal hepatitis associated with diclofenac. Gut 27: 1390-1393, 1986.
- **Brendl K, Bruhn R, Ganote DP, Lucker PW, Scholz H-J.** Imadyl<»: die gastrische Irritation in Vergleich zu Acetylsalicylsaure, Indometacin, Piroxicam und Diclofenac-Na. Methods and Findings in Experimental and Clinical Pharmacology 5: 579-580, 1983.
- **Brogden RN, Heel RC, Pakes GE, Speight TM, Avery GS.** Diclofenac sodium: a review of its pharmacological properties and therapeutic use in rheumatic diseases and pain of varying origin.

- **Buck ChB, Thompson CD, Roberts JN, Muller M, Lowy DR, Schiller JT** (2006). Carrageenan is a potent inhibitor of papillomavirus infection. PLoS Pathogens 2, 0671–0680.
- **Caceres PJ, Carlucci MJ, Damonte EB, Matsuhiro B, Zuniga EA** (2000). Carrageenans from chilean samples of Stenogramme interrupta (Phyllophoraceae): structural analysis and biological activity. Phytochemistry 53, 81–86.
- **Cannon, W**. Organization for physiological homeostasis. *Physiol. Rev*. 9, 399–431 (1929).
- **Carey PL** (1958). The digestibility of polysaccharides by rats. [Thesis.] Purdue University. Submitted to WHO by R.J.H. Gray, International Food Additives Council, Atlanta, Georgia, USA, and P. Couchoud, Marinalg, Paris, France.
- **Carlucci MJ, Pujol CA, Ciancia M, Noseda MD, Matulewicz MC, Damonte EB, Cerezo AS** (1997). Antiherpetic and anticoagulant properties of carrageenans from the red seaweed Gigartina skottsbergii and their cyclized derivatives: correlation between structure and biological activity. International Journal of Biological Macromolecules 20, 97–105.
- **Carlucci MJ, Scolaro LA, Damonte EB** (1999). Inhibitory action of natural carrageenans on herpes simplex virus infection of mouse astrocytes. Chemotherapy 45, 429–436.
- **Carlucci MJ, Scolaro LA, Noseda MD, Cerezo AS, Damonte EB** (2004). Protective effect of a natural carrageenan on genital herpes simplex virus infection in mice. Antiviral Research 64, 137–141.
- **Carson, C.F., Mee, B.J., Riley, T.V.,** 2002. Mechanism of action of Melaleuca alternifolia (tea tree) oil on Staphylococcus aureus determined by time-kill, lysis, leakage and salt tolerance assays and electron microscopy. Antimicrob. Agents Chemother. 46, 1914–1920.
- **Cassidy MB, Shaw KW, Lee H, Trevors JT** (1997). Enhanced mineralization of pentachlorophenol by kcarrageenan encapsulated Pseudomonas sp. UG30. Applied Microbiology and Biotechnology 47, 108–113.
- **Chao YP, Fu H, Lo TE, Chen PT, Wang JJ** (1999). Onestep production of D-phydroxyphenylglycine by recombinant Escherichia coli strains, Biotechnology Proggress 15, 1039–1045.

- **Chen J, Appleby DW, Weber P, Abraham R** (1981). Detection of a carrageenan in rat liver homogenates after feeding in the diet. Toxicologist 1 (Abstr.), 133.
- **Chorianopoulos, N.; Kalpoutzakis, E.; Aligiannis, N.; Mitaku, S.; Nychas, G.-J.; Haroutounian, A. S.** Essential Oils of Satureja, Origanum, and Thymus Species: Chemical Composition and Antibacterial Activities Against Foodborne Pathogens. J. Agric. Food Chem. 2004, 52 (26), 8261−8267.
- **Coulston F, Abraham R, Benitz KF, Ford W** (1976). Response of the livers of male and female rats (Osborn/ Mendel and Sprague-Dawley) to Alphacel and a carrageenan (HMR) for nine months using two different basal diets. Nine-month progress report from Institute of Comparative and Human Toxicology, Center of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York, USA. Submitted to WHO by R.J.H. Gray, International Food Additives Council, Atlanta, Georgia, USA, and P. Couchoud, Marinalg, Paris, France.
- **Coulston F, Golberg L, Abraham R, Benitz KF, Ford W** (1975). Carrageenans (Hercules Incorporated). Safety evaluation. Nine-month study. Unpublished interim progress report from Institute of Comparative and Human Toxicology, Center of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York, USA. Submitted to WHO by R.J.H. Gray, International Food Additives Council, Atlanta, Georgia, USA, and P. Couchoud, Marinalg, Paris, France.
- **Cuzzocrea S, Sautebin L, De Sarro G, Costantino G, Rombola L, Mazzon E, Lalenti A, De Sarro A, Ciliberto G, Di Rosa M, Caputi AP, Thiemermann CH** (1999): Role of IL-6 in the pleurisy and lung injury caused by carrageenan. Journal of Immunology 163, 5094–5104.
- **De SF-Tischer PC, Talarico LB, Noseda MD, Guimaraes SMPB, Damonte EB, Duarte MER** (2006). Chemical structure and antiviral activity of carrageenans from Meristiella gelidium against herpes simplex and dengue virus. Carbohydrate Polymers 63, 459–465.
- **Dewar ET, Maddy ML** (1970): Faecal excretion of degraded and native carrageenan by the young rat. Journal of Pharmacy and Pharmacology 22, 791–793.
- **Dos Santos VAPM, Bruijnse M, Tramper J, Wijffels RH** (1996a): The magic-bead concept: An integrated approach to nitrogen removal with co-immobilized micro-organisms. Applied Microbiology and Biotechnology 45, 447–453.

- **Dos Santos VAPM, Tramper J, Wijffels RH** (1996b): Dynamic modelling of an integrated nitrogen removal system using Co-immobilized microorganisms Immobilized Cells. Progress in Biotechnology 11, 486– 493.
- **Drayton, D. L., Liao, S., Mounzer, R. H. & Ruddle, N. H**. Lymphoid organ development: from ontogeny to neogenesis. *Nature Immunol.* 7, 344–353 (2006).
- **Eccles R, Meier Ch, Jawad M, Weinmüllner R, Grassauer A, Prieschl-Grassauer E** (2010): Efficacy and safety of an antiviral iota-carrageenan nasal spray: a randomized, double-blind, placebo-controlled exploratory study in volunteers with early symptoms of the common cold. Respiratory Research 11, 1–10.
- **Ekstrom LG** (1985): Molecular-weight-distribution and the behaviour of kappa-carrageenan on hydrolysis. Part II. Carbohydrate Research 135, 283–289.
- **Ekstrom LG, Kuivinen J** (1983): Molecular weight distribution and hydrolysis behaviour of carrageenans. Carbohydrate Research 116, 89–94.
- **Elsenhans B, Blume R, Caspary WF** (1981): Long-term feeding of unavailable carbohydrate gelling agents. Influence of dietary concentration and microbiological degradation on adaptive responses in the rat. American Journal of Clinical Nutrition 34, 1837–1848.
- **Engster M, Abraham R** (1976): Cecal response to different molecular weights and types of carrageenan in the guinea pig. Toxicology and Applied Pharmacology 38, 265–282.
- **Epifanio EC, Veroy RL, Uyenco F, Cajipe GJB, Laserna EC** (1981): Carageenan from Eucheuma spiratum in bacteriological media. Applied and Environmental Microbiology 41, 155–163.
- **Estakhr J, Sanchooli N, Najafi SH, Javdan N** (2011): Anti-inflammatory activity of ethanolic extract of Physalis alkekengi. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2, 421–425.
- **Gardin H, Pauss A** (2001): Kappa-carrageenan/gelatin gel beads for the co-immobilization of aerobic and anaerobic microbial communities degrading 2,4,6-trichlorophenol under air-limited conditions. Applied Microbiology and Biotechnology 56, 517–523.
- **Gibson GR, Macfarlane S, Cummings JH** (1990): The fermentability of polysaccharides by mixed human faecal bacteria in relation to their suitability as bulkforming laxatives. Letters of Applied Microbiology 11, 251–254.

- **Girond S, Crance JM, Van Cuyck-Gandre H, Renaudet J, Deloince R** (1991): Antiviral activity of carrageenan on hepatitis A virus replication in cell culture. Research in Virology 142, 261–270.
- **Gonzalez ME, Alarcon B, Carrasco L** (1987): Polysaccharides as antiviral agents: Antiviral activity of carrageenan. Antimicrobial Agents and Chemotherapy 31, 1388–1393.
- **Grassauer A, Weinmuellner R, Meier Ch, Pretsch A, Prieschl-Grassauer E, Unger H** (2008): Iota-carrageenan is a potent inhibitor of rhinovirus infection. Virology Journal 5, 1–13.
- **Grasso P, Sharratt M, Carpanini FMB, Gangolli SD** (1973): Studies on carrageenan and large-bowel ulceration in mammals. Food and Cosmetics Toxicology 11, 555–564.
- **Guay J, Bateman K, Gordon R, Mancini J, Riendeau D** (2004): Carrageenan-induced paw edema in rat elicits a predominant prostaglandin E2 (PGE2) response in the central nervous system associated with the induction of microsomal PGE2 synthase-1. Journal of Biological Chemistry 279, 24866–24872.
- **Guenther E.** (1948). The essential oils. Vol. 1. New York: D. van Nostrand Company Inc. Bakkali F, Averbeck S, Averbeck D, Idaomar M. (2008). Biological effects of essential oils – a review. Food Chem Toxicol 46:446–75.
- **Hagiwara A, Miyashita K, Nakanishi T, Sano M, Tamano S, Asai I, Nakamura M, Imaida K, Ito N, Shirai T** (2001): Lack of tumor promoting effects of carrageenan on 1,2-dimethylhydrazine-induced colorectal carcinogenesis in male F344 rats. Journal of Toxicology and Pathology 14, 37–43.
- **Handy RLC, Moore PK** (1998): A comparison of the effects of L-NAME, 7-NI and L-NIL on carrageenan induced hind paw oedema and NOS activity. British Journal of Pharmacology 123, 1119–1126.
- **Hawkins WW, Yaphe W** (1965): Carrageenan as a dietary constituent for the rat: Faecal excretion, nitrogen absorption, and growth. Canadian Journal of Biochemistry and Physiology 43, 479–484.
- **Henriques MGMO, Silva PMR, Martins MA, Flores CA, Cunha FQ, Assreuy-Filho J, Cordeiro RSB** (1987): Mouse paw oedema. A new model for inflammation? Brazilian Journal of Medical and Biological Research 20, 243–249.

- **International Food Additives Council** (1997): Unpublished monograph prepared by the International Food Additives Council, Washington DC. Submitted to WHO by **R.J.H. Gray,** International Food Additives Council, Atlanta, Georgia, USA, and **P. Couchoud, Marinalg,** Paris, France.
- **Jain NK, Patil CS, Singh A, Kulkarni SK** (2001): A simple technique to evaluate inflammatory pain along with anti-inflammatory studies in carrageenan-induced paw edema. Indian Journal of Pharmacology 33, 114–115.
- **JECFA** (1974): Toxicological evaluation of certain food additives with a review of general principles and of specifications. Seventeenth report of the Joint FAO/ WHO Expert Committee on Food Additives. FAO Nutrition Meetings Series, No.53. WHO Technical Report Series, No.539 and corrigendum. World Health Organization, Geneva.
- **Kantvilas, G.** (1996). The discovery of tasmanian eucalypts: An historical sketch. Tasmanian Herbarium,
- **Kindness G, Long WF, Williamson FB** (1979): Enhancement of antithrombin III activity by carrageenan. Thrombosis Research 15, 49–60.
- **Kindness G, Long WF, Williamson FB** (1980a): Anticoagulant effects of sulphated polysaccharides in normal and antithrombin III-deficient plasmas. British Journal of Pharmacology 69, 675–677.
- **Kindness G, Williamson FB, Long WF** (1980b): Involvement of antithrombin III in anticoagulant effects of sulphated polysaccharides. Biochemical Society Transactions 8, 82_83.
- **Kobayashi T** (eds.): Marcel Dekker Inc., New York. 291–313.
- **Krishnan MS, Nghiem NP, Davison BH** (1999): Ethanol production from corn starch in a fluidized-bed bioreactor. Applied Biochemistry and Biotechnology 77–79, 359–371.
- **Kumar, V., Cotran, R. S. & Robbins, S. L.** ***Robbins Basic Pathology*** (Saunders, 2003).
- **Lai VMF, Wong PA-L, Lii C-Y** (2000): Effects of cation properties on sol-gel transition and gel properties of κ-carrageenan. Journal of Food Science 65, 1332–1337.
- **Leibbrandt A, Meier Ch, Konig-Schuster M, Weinmullner R, Kalthoff D, Pflugfelder B,Graf P, Frank-Gehrke B, Beer M, Fazekas T, Unger H, Prieschl-**

Grassauer E, Grassauer A (2010): Iota-carrageenan is a potent inhibitor of influenza a virus infection. PLoS ONE 5, 1–12.

- **Lynch G, Low L, Li S, Sloane A, Adams S, Parish C, Kemp B, Cunningham AL** (1994): Sulfated polyanions prevent HIV infection of lymphocytes by disruption of the CD4-gp120 interaction, but do not inhibit monocyte infection. Journal of Leukocyte Biology 56, 266–272.
- **Mallett AK, Rowland IR, Bearne CA, Nicklin S** (1985): Influence of dietary carrageenan on microbial biotransformation activities in the cecum of rodents and on gastrointestinal imine status in the rat. Toxicology and Applied Pharmacology 78, 377–385.
- **Mallett AK, Wise A, Rowland IR** (1984): Hydrocolloid food additives and rat caecal microbial enzyme activities. Food and Chemical Toxicology 22, 415–418.
- **Mankes R, Abraham R** (1975): Lysosomal dysfunction in colonic submucosal macrophages of rhesus monkeys caused by degraded iota carrageenan. Proceedings of the Society for Experimental Biology and Medicine 150, 166–170.
- **Marchetti M, Pisani S, Pietropaolo V, Seganti L, Nicoletti R, Orsi N** (1995): Inhibition of herpes simplex virus infection by negatively charged and neutral carbohydrate polymers. Journal of Chemotherapy 7, 90–96.
- **Mariathasan, S. *et al***. Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* 440, 228–232 (2006).
- **McGill HC Jr, McMahan CA, Wigodsky HS, Sprinz H.** (1977): Carrageenan in formula and infant baboon development. Gastroenterology 73, 512–517.
- **Medzhitov, R. & Janeway, C. A**. Jr Innate immunity: the virtues of a nonclonal system of recognition. *Cell* 91, 295–298 (1997).
- **Mensour NA, Margaritis A, Briens CL, Pilkington H, Russel I** (1996): Application of immobilized yeast cells in the brewing industry. Progress in Biotechnology 11, 661–671.
- **Mensour NA, Margaritis A, Briens CL, Pilkington H, Russell I** (1997): New developments in the brewing industry using immobilised yeast cell bioreactor systems. Journal of the Institute of Brewing 103, 363–370.
- **Michel A-S, Mestdagh MM, Axelos MAV** (1997): Physico-chemical properties of carrageenan gels in presence of various cations. International Journal of Biological Macromolecules 21, 195–200.

- **Morard JC, Fray A, Abadie A, Robert L** (1964): Nature of the renal lesions induced by intravenous injection of carrageenan. Nature 202, 401–402.
- **Mori A** (1993): Industrial Application of Immobilized Biocatalysts. **Tanaka A, Tosa T,**
- **Nagalakshmi V, Pai JS** (1997): Immobilisation of penicillin acylase producing E. coli cells with κ-carrageenan. Indian Journal of Microbiology 37, 17–20.
- **Nantel F, Denis D, Gordon R, Northey A, Cirino M, Metters KM, Chan ChCh** (1999): Distribution and regulation of cyclooxygenase-2 in carrageenan-induced inflammation. British Journal of Pharmacology 28, 853–859.
- **Nathan, C.** (2002). Points of control in inflammation. *Nature*, 420 : p 846-852.
- **Nathan, C.** Neutrophils and immunity: challenges and opportunities. *Nature Rev. Immunol.* 173–182 (2006).
- **Nathan, C.** Points of control in inflammation. *Nature* 420, 846–852 (2002).
- **Naude PJ, Cromarty AD, van Rensburg CE** (2010): Potassium humate inhibits carrageenan-induced paw oedema and a graft-versus-host reaction in rats. Inflammopharmacology, 18, 33–39.
- **Nicklin S, Baker K, Miller K** (1988): Intestinal uptake of carrageenan: Distribution and effects on humoral immune competence. Advances in Experimental Medicine and Biology 237, 813–820.
- **Nicklin S, Miller K** (1984): Effect of orally administered food-grade carrageenan on antibody-mediated and cell-mediated immunity in the inbred rat. Food and Chemical Toxicology 22, 615–621.
- **Nigam JN** (2000): Continuous ethanol production from pineapple cannery waste using immobilized yeast cells. Journal of Biotechnology 80, 189–193.
- **Nilson HW, Schaller JW** (1941): Nutritive value of agar and Irish moss. Food Research 6, 461–469.
- **Nilson HW, Wagner JA** (1959): Feeding test with carrageenin. Food Research 24, 235–239.
- **Norton S, Watson K, D'Amore T** (1995): Ethanol tolerance of immobilized brewers' yeast cells. Applied Microbiology and Biotechnology 43, 18–24.
- **Ochuba GU, von Riesen VL** (1980): Fermentation of polysaccharides by Klebsiellae and other facultative bacteria. Applied and Environmental Microbiology 39, 988–992.

- **Omote K, Hazama K, Kawamata T, Kawamata M, Nakayaka Y, Toriyabe M, Namiki A** (2001): Peripheral nitric oxide in carrageenan-induced inflammation. Brain Research 912, 171–175.
- **Osuga J, Mori A, Kato J** (1984): Acetic acid production by immobilized Acetobacter aceti cells entrapped in a κ carrageenan gel. Journal of Fermentation Technology 62, 139–149.
- **Panlasigui LN, Baello OQ, Dimatangal JM, Dumelod DB** (2003): Blood cholesterol and lipid-lowering effects of carrageenan on human volunteers. Asia Pacific Journal of Clinical Nutrition 12, 209–214.
- **Paschapur MS, Patil MB, Kumar R, Patil SR** (2009): Evaluation of anti-inflammatory activity of ethanolic extract of Borassus flabellifer L. male flowers (inflorescences) in experimental animals. Journal of Medicinal Plants Research 2, 49 54.
- **Petersson M, Wiberg U, Lundeberg T, Uvnas-Moberg K** (2001): Oxytocin decreases carrageenan induced inflammation in rats. Peptides 22, 1479–1484.
- **Pittman KA, Golberg L, Coulston F** (1976): Carrageenan: The effect of molecular weight and polymer type on its uptake, excretion and degradation in animals. Food and Cosmetics Toxicology 14, 85–93.
- **Pober, J. S. & Sessa, W. C.** Evolving functions of endothelial cells in inflammation. *Nature Rev. Immunol.* 7, 803–815 (2007).
- **Porto GG, Vasconcelos BC, Silva-Junior VA, Souza Andrade ES** (2010): The use of carrageenan for limiting the mandibular movement in rats: A preliminary experimental study. Medicina Oral, Patologia Oral y Cirugia Bucal 15, 653–657.
- **Posadas I, Bucci M, Roviezzo F, Rossi A, Parente L, Sautebin L, Cirino G** (2004): Carrageenan-induced mouse paw oedema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression. British Journal of Pharmacology 142, 331–338.
- **Poulsen E** (1973): Short-term peroral toxicity of undegraded carrageenan in pigs. Food and Cosmetics Toxicology 11, 219–227.
- **Poupin P, Mazure N, Truffaut** N (1996): Morpholine degradation by strain Mycobacterium aurum MOI: Improvement of cells growth and morpholine degradation rate by cells immobilization. Progress in Biotechnology 11, 770–776.

- **Radhakrishnan R, Bement MKH, Skyba D, Sluka KA** (2004): Models of muscle pain: carrageenan model and acidic saline model. Current Protocols in Pharmacology 25, 1–28.
- **Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S. & Medzhitov, R.** Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. *Cell* 118, 229–241 (2004).
- **Raut, J. S.; Karuppayil, S. M.** A status review on the medicinal properties of essential oils. Ind. Crops Prod. 2014, 62, 250−264.
- **Reddy BS, Watanabe K, Sheinfil A** (1980): Effect of dietary wheat bran, alfalfa, pectin and carrageenan on plasma cholesterol and fecal bile acid and neutral sterol excretion in rats. Journal of Nutrition 110, 1247–1254.
- **Rizki, T. M. & Rizki, R. M**. Lamellocyte differentiation in *Drosophila* larvae parasitized by *Leptopilina*. *Dev. Comp. Immunol.* 16, 103–110 (1992).
- **Robbins MC** (1997): A 90-day feeding study in the rat with semi-refined carrageenan from two sources, including a recovery phase. Unpublished report of project No. 3160/1/2/97 from BIBRA International, Carshalton, Surrey, United Kingdom. Submitted to WHO by Dr H.J. Bixler, Seaweed Industry Association of the Philippines, Searsport, Maine, USA.
- **Rocha de Souza MC, Marques CT, Dore CMG, da Silva FRF, Rocha HAO, Leite EL** (2007): Antioxidant activities of sulfated polysaccharides from brown and red seaweeds. Journal of Applied Phycology 19, 153–160.
- **Rosen A, Lundeberg T, Bytner B, Nylander I** (2000): Central changes in nociceptin dynorphin B and Met-enkephalin-Arg Phe in different models of nociception. Brain Research 857, 212–218.
- **Rustia M, Shubik P, Patil K** (1980): Lifespan carcinogenicity tests with native carrageenan in rats and hamsters. *Cancer Letters* 11, 1–10.
- **Salvemini D, Wang S-Q, Wyatt PS, Bourdon DM, Marino MH, Manning PT, Currie MG** (1996): Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. British Journal of Pharmacology 118, 829–838.
- **Salyers AA, West SEH, Vercellotti JR, Wilkins TD** (1977): Fermentation of mucins and plant polysaccharides by anaerobic bacteria from the human colon. Applied and Environmental Microbiology 34, 529–533.

- **Santoyo S, Cavero S, Jaime L, et al.** (2006). Supercritical carbon dioxide extraction of compounds with antimicrobial activity from Origanum vulgare L.: determination of optimal extraction parameters. J Food Prot 69:369–75.
- **SCF** (1978): Reports of the Scientific Committee for Food. Seventh series. Commission of the European Communities, Luxembourg.
- **Serhan, C. N. & Savill, J**. Resolution of inflammation: the beginning programs the end. *Nature Immunol.* 6, 1191–1197 (2005).
- **Serhan, C. N.** Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu. Rev. Immunol.* 25, 101–137 (2007).
- **Shanmugam M, Mody KH** (2000): Heparinoid-active sulphated polysaccharides from marine algae as potential blood anticoagulant agents. Current Science 79, 1672–1683.
- **Sharratt M, Grasso P, Carpanini F, Gangolli SD** (1970): Carrageenan ulceration as a model for human ulcerative colitis. Lancet 297, 192–193.
- **Silva FRF, Dore CMPG, Marques CT, Nascimento MS, Benevides NMB, Rocha HAO, Chavante SF, Leite EL** (2010): Anticoagulant activity, paw edema and pleurisy induced carrageenan: Action of major types of commercial carrageenans. Carbohydrate Polymers 79, 26–33.
- **Sini JM, Yaro AH, Ayanwuyi LO, Aiyelero OM, Mallum SM, Gamaniel KS** (2010): Antinociceptive and antiinflammatory activities of the aqueous extract of the root bark of Combretumsericeum in rodents. African Journal of Biotechnology 9, 8872–8876.
- **Sodini I, Boquien CY, Corrieu G, Lacroix C** (1997a): Use of an immobilized cell bioreactor for the continuous inoculation of milk in fresh cheese manufacturing. Journal of Industrial Microbiology and Biotechnology 18, 56–61.
- **Sodini I, Boquien CY, Corrieu G, Lacroix C** (1997b): Microbial dynamics of co- and separately entrapped mixed cultures of mesophilic lactic acid bacteria during the continuous prefermentation of milk. Enzyme and Microbial Technology 20, 381–388.
- **Sokol, C. L., Barton, G. M., Farr, A. G. & Medzhitov, R.** A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nature Immunol.* 9, 310–318 (2008).

- **Stancioff DJ, Renn DW** (1975): Physiological effects of carrageenan. American Cancer Society Symposium Series 15, 1–12.
- **Stanley N** (2011): FAO Corporate document repository. Chapter 3: Production, properties and uses of carrageenan. FMC Corporation, Marine Colloids Division 5 Maple Street, Rockland Maine 04841, USA.
- **Stiles J, Guptill-Yoran L, Moore GE, Pogranichniy RM** (2008): Effects of κ-carrageenan on in vitro replication of feline herpesvirus and on experimentally induced herpetic conjunctivitis in cats. Investigative Ophthalmology and Visual Science 49, 1496–1501.
- **Sugishita E, Amagaya S, Ogihara Y** (1981): Anti-inflammatory testing methods: comparative evaluation of mice and rats. Journal of Pharmacobio-Dynamics 8, 565–575.
- **Swain A, Waterhouse KV, Venables WA, Callely AG, Lowe SE** (1991): Biochemical studies of morpholine catabolism by an environmental mycobacterium. Applied Microbiology and Biotechnology 35(1), 110–114.
- **Takamatsu, S, Tosa T** (1993): Production of L-alanine and D-aspartic acid. Bioprocess Technology 16, 25–35.
- **Tobacman JK** (2001): Review of harmful gastrointestinal effects of carrageenan in animal experiments. Environmental Health Perspectives 109, 983–994.
- **Tobacman JK, Wallace RB, Zimmerman MB** (2001): Consumption of carrageenan and other water-soluble polymers used as food additives and incidence of mammary carcinoma. Medical Hypotheses 58, 589–598.
- **Tomarelli RM, Tucker WD Jr, Bauman LM, Savini S, Weaber JR** (1974): Nutritional qualities of processed milk containing carrageenan. Journal of Agricultural and Food Chemistry 22, 819–824.
- **Tosa T, Shibatani T** (1995): Industrial Application of Immobilized Biocatalysts in Japan. Annals of New York Academy of Sciences 750, 364–375.
- **Udall JN, Harmatz P, Vachino G, Galdabini J, Walker WA** (1981): Intestinal transport and liver uptake of a food additive present in infant formulas (Abstract). Pediatric Research 15, 549.
- **Van de Braak SAAJ, Leijten GCJJ.** (1999). Essential oils and oleoresins: a survey in the Netherlands and other major markets in the European Union. Rotterdam, The Netherlands: CBI, Centre for the Promotion of Imports from Developing Countries.

- **Van de Velde F, Lourenco ND, Pinheiro HM, Bakkerd M** (2002): Carrageenan: a food-grade and biocompatible support for immobilisation techniques. Advanced Synthesis and Catalysis 344, 815–835.
- **Vecchio, M. G., Loganes, C., & Minto, C**. (2016). Beneficial and healthy properties of Eucalyptus plants: A great potential use. The Open Agriculture Journal, 10, 52–57.
- **Wang JL, Li P, Shi H, Qian Y** (1997): Immobilization of microorganisms using carrageenan gels coated with chitosan and application to biodegradation of 4-chlorophenol. Journal of Environmental Sciences 9, 283–287.
- **Watt J, Marcus R** (1969): Ulcerative colitis in the guineapig caused by seaweed extract. Journal of Pharmacy and Pharmacology 21, 187S–188S.
- **Watt J, Marcus R** (1971): Carrageenan-induced ulceration of the large intestine in the guinea pig. Gut 12, 164–171.
- **Weiner ML** (1991): Toxicological properties of carrageenan. Agents Actions 32, 46–51.
- **Wijesekara I, Pangestuti R, Kim SK** (2011): Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. Carbohydrate Polymers 84, 14–21.
- **Witvrouw M, De Clercq E** (1997): Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. General Pharmacology 29, 497–511.
- **Wunderwald P, Schrenk WJ, Port H** (1979): Antithrombin BM from human plasma: an antithrombin binding moderately to heparin. Thrombosis Research 15, 49–60.
- **Yamamoto I, Maruyama H, Takahashi M, Komiyama K** (1986): The effect of dietary or intraperitoneally injected seaweed preparations on the growth of sarcoma-180 cells subcutaneously implanted into mice. Cancer Letters 30, 125–131.
- **Yuan H, Song J** (2005): Preparation, structural characterization and *in vitro* antitumor activity of kappa-carrageenan oligosaccharide fraction from Kappaphycus striatum. Journal of Applied Phycology 17, 7–13.
- **Yuan H, Song J, Li X, Li N, Dai J** (2004): Immunomodulation and antitumor activity of κ-carrageenan Oligosaccharides. Pharmacological Research 50, 47–53.
- **Yuan H, Song J, Li X, Li N, Dai J** (2006): Immunomodulation and antitumor activity of κ carrageenan oligosaccharides. Cancer Letters 243, 228–234.

- **Zacharopoulos VR, Phillips DM** (1997): Vaginal formulations of carrageenan protect mice from herpes simplex virus infection. Clinical and Diagnostic Laboratory Immunology 4, 465 468.
- **Zeitlin L, Whaley KJ, Hegarty TA, Moench TR, Cone RA** (1997): Tests of vaginal microbicides in the mouse genital herpes model. Contraception 56, 329–335.
- **Zhou G, Sheng W, Yao W, Wang Ch** (2006): Effect of low molecular-carrageenan from Chondrus ocellatus on antitumor H-22 activity of 5-Fu. Pharmacological Research 53, 129-134.
- **Zhou G, Sun YP, Xin X, Zhang Y, Li L, Xu Z** (2004): In vivo antitumor and immunomodulation activities of different molecular weight lambda-carrageenan from Chondrus ocellatus. Pharmacological Research 50, 47–53.
- **Zong, W. X. & Thompson, C. B.** Necrotic death as a cell fate. *Genes Dev.* 20, 1–15 (2006). **Huang, H. & Tindall, D. J.** Dynamic FoxO transcription factors. *J. Cell Sci.* 120, 2479–2487.
- **Medzhitov , R. and Janeway , C. A.** (1997) Jr innate immunity: the virtues of a nonclonal system of recognition .