Ministry of Higher Education and Scientific Research

University of Basrah

Pharmacy College

Department of Clinical Laboratory Sciences

In-Vitro Anti-Inflammatory Activity of Some Medicine

A graduation project Prepared by

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Declaration

We hereby declare that this project has been composed by ourselves and has not been submitted in any previous application for a Bachelor's degree. All work presented has been done by us unless otherwise stated. All sources of information have been acknowledged appropriately by means of references.

Acknowledgments

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Abstract

The In-Vitro Anti-Inflammatory Activity of Some Medicines refers to the evaluation of the ability of selected medicines to reduce inflammation using in-vitro experiments. To detect evaluate anti-inflammatory effect of Famotidine, diltiazem, sertraline, and Nifedipine in compere to Ibuprofen. The method (HRBC) was selected to evaluate anti-inflammatory effect and estimate the Percentage of protection. drugs had different levels of protection of Ibuprofen 55% while other drug was Famotidine 79.80%, sertraline 62.69%, Nifedipine 64.038%, diltiazem 50%. The results of these experiments provide important insights into the anti-inflammatory properties of these medicines and could pave the way for the development of new treatments for inflammatory conditions.

Contents

Introduction

The inflammatory response is a crucial aspect of the tissues' responses to deleterious iflammogens. This complex response involves leukocytes cells such as macrophages, neutrophils, and lymphocytes, also known as inflammatory cells [1]. In response to the inflammatory process, these cells release specialized substances which include vasoactive amines and peptides, eicosanoids, pro inflammatory cytokines, and acute-phase proteins, which mediate the inflammatory process by preventing further tissue damage and ultimately resulting in healing and restoration of tissue function. This review discusses the role of the inflammatory cells as well as their by-products in the mediation of inflammatory process [2]. A brief insight into the role of natural anti-inflammatory agents is also discussed. [3]

Inflammation is an essential response provided by the immune systems that ensures the survival during infection and tissue injury. Inflammatory responses are essential for the maintenance of normal tissue homeostasis. [4] The molecular mechanism of inflammation is quite a complicated process which is initiated by the recognition of specific molecular patterns associated with either infection or tissue injury. The entire process of the inflammatory response is mediated by several key regulators involved in the selective expression of pro inflammatory molecules causing a variety of inflammatory diseases and pathophysiological conditions. [5]

Inflammation can be either acute or chronic.

Acute Inflammation

Tissue injury due to trauma, microbial invasion, or deleterious compounds can prompt acute inflammation. It begins quickly, turns severe in a brief time and symptoms may persist for a few days for instance cellulitis or acute pneumonia [6]

Subacute inflammation is the time interval between acute and chronic inflammation and might carry on two to six weeks.

Chronic Inflammation

Chronic inflammation is usually described as slow, long-term inflammation which might continue for prolonged periods of several months to many years. basically, the length and effects of chronic inflammation differ with the cause of the damage and the capability of the body to restore and overwhelm the damage[7]

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a drug category FDA approved for use as antipyretic, anti-inflammatory, and analgesic agents [8] These effects made NSAIDs beneficial for treating muscle pain, dysmenorrhea, arthritic disorders, pyrexia, gout, migraines, and used as opioid-sparing agents in certain acute traumatic conditions [9, 10, 11]. NSAIDs are basically sort out into groups depend on their chemical structure and selectivity: acetylated salicylates (aspirin), non-acetylated salicylates (diflunisal , Salicylate), propionic acids (naproxen, ibuprofen, acetic acids (diclofenac, indomethacin), enolic acids (meloxicam, piroxicam) anthranilic acids (meclofenamate, mefenamic acid), naphthylalanine (nabumetone), and selective COX-2 inhibitors (celecoxib, etoricoxib).Topical NSAIDs (diclofenac gel) are also obtainable for use in acute

tenosynovitis, ankle sprains, and soft injuries.[12, 13, 14, 15]

The main mechanism of action of NSAIDs is the inhibition of the enzyme cyclooxygenase (COX). Cyclooxygenase is needed to turn arachidonic acid into thromboxanes, prostaglandins, and prostacyclins [16] The therapeutic outcomes of NSAIDs are associated to the absence of these eicosanoids. particularly, thromboxanes take part in platelet adhesion, prostaglandins result in vasodilation, rising the temperature set-point in the hypothalamus, and take part in anti-nociception [17]

There are two cyclooxygenase isoenzymes, COX-1 and COX-2. COX-1 gets constitutively interpreted in the body, and it take part in coordinate gastrointestinal mucosa lining, kidney function, and platelet aggregation. COX-2 is not constitutively interpreted in the body; and rather, it inducibly interpreted throughout an inflammatory response. Almost all the NSAIDs are nonselective and inhibit both COX-1 and COX-2. although, COX-2 selective NSAIDs (e.g celecoxib) only aim COX-2 and consequently have a variety of side effect profile. Crucially, because COX-1 is the chief mediator for confirming gastric mucosal integrity and COX-2 is basically included in inflammation, COX-2 selective NSAIDs must provide anti-inflammatory alleviation without weaken the gastric mucosa. [18]

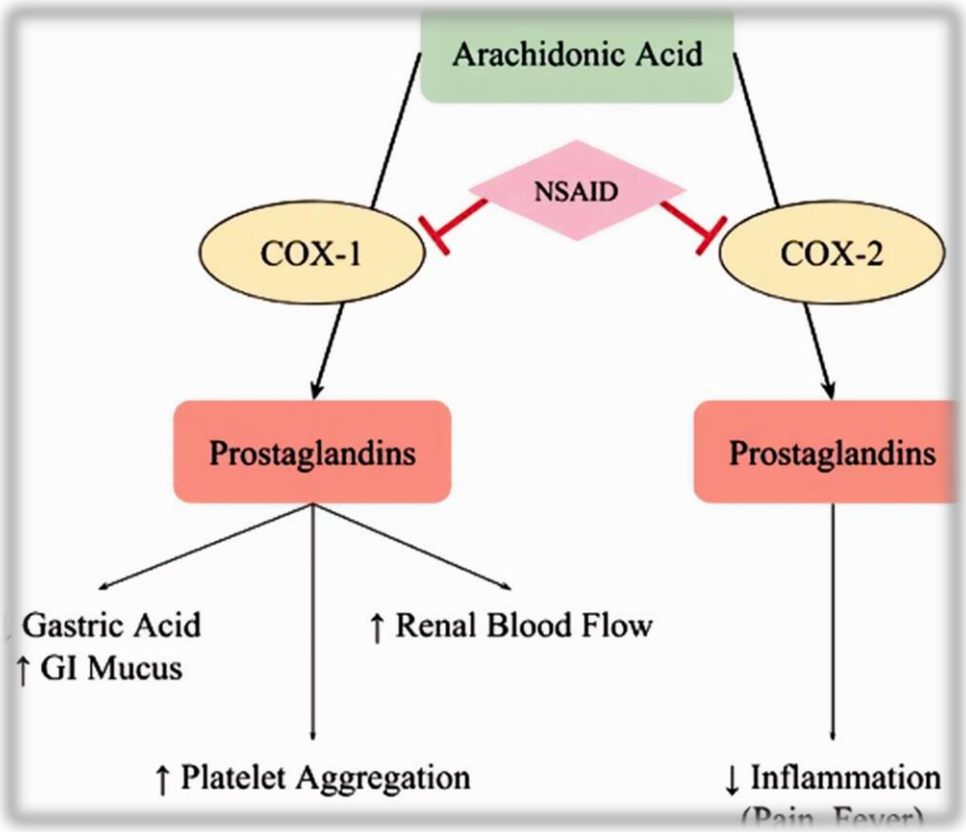
Nonsteroidal anti-inflammatory drugs (NSAIDs) provide their therapeutic benefits through inhibition of cyclooxygenase (COX), the enzyme that produce prostaglandins (PGs). They have, to a larger or lesser degree, the same side effects, take into account, gastric and renal toxicity [19]. Current research has manifest that at least there are two COX isoenzymes. COX-1 is constitutive and produce PGs that afford protection to the stomach and kidney from injury. COX-

2 is bringing about by inflammatory stimuli, such as cytokines, and produces PGs that responsible for the pain and swelling of inflammation. Thus, selective COX-2 inhibitors must be anti-inflammatory lacking side effects on the kidney and stomach. Absolutely, selective COX-2 inhibitors may have other side effects and probably other therapeutic prospective. For instance, COX-2 (and not COX-1) is believed to be involved in ovulation and in labor. Adding in, the very known protective action of aspirin on colon cancer might be by an action on COX-2, which is demonstrate in this disease [20]. Furthermore, NSAIDs slow down the progression of Alzheimer's disease. hence, selective COX-2 inhibitors may show new crucial therapeutic benefits as anticancer agents, as well as put a stop to premature labor and perhaps even delay the progression of Alzheimer's disease. [21]

The physiological changes induced by acute inflammation demonstrate significant management challenges for anesthesiologists. Major surgery, trauma, burns and sepsis all have large inflammatory components. Acute inflammation represents by vasodilatation, fluid exudation and neutrophil infiltration. These processes are activated and boost by a series of intracellular and extracellular factors that tightly organize the inflammatory process [22]. The innate immune system reacts rapidly to infection or injury. Macrophages, natural killer cells, CD8 + T-lymphocytes and neutrophils supply an early response to injurious factors in an effort to involve and remove harmful stimuli. The adaptive immune response requires earlier exposure to microbial antigens, is mediated fundamentally by CD4 + T-lymphocytes and perform to further amplify acute inflammation [23]. Although acute inflammation is basically beneficial, severe inflammation can bring about the systemic inflammatory response syndrome. This syndrome is represented by hyper inflammation and can cause organ injury,

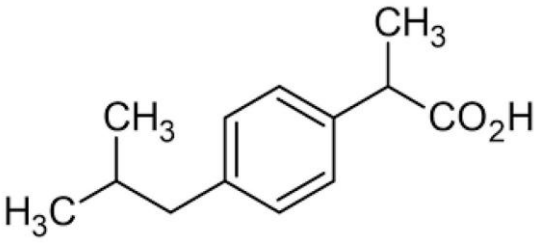
shock and death in its most severe patterns. in general, our knowledge of inflammation has increased significantly during the last 20 years. Anyway, these basic science advances have not yet interpreted into broad benefit for patients experiencing trauma, sepsis and systemic inflammation.

Mechanism of action of a non-steroidal anti-inflammatory



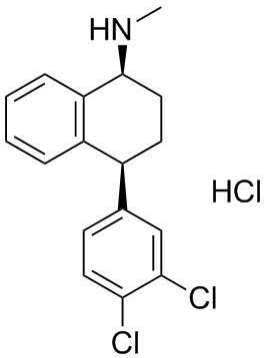
Drugs used in our research

Ibuprofen



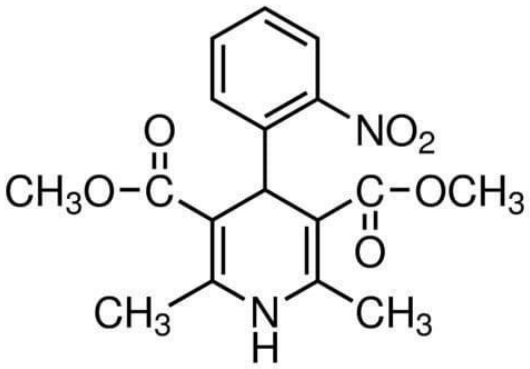
it is a painkiller available over the counter without a prescription?
It's one of a group of painkillers called non-steroidal anti-inflammatory drugs (NSAIDs) and can be used to: to reduce fever and to relieve minor aches and pain from headaches, muscle aches, arthritis, menstrual periods, the common cold, toothaches, and backaches
Ibuprofen contains a carboxyl group
Molecular Formula: $C_{13}H_{18}O_2$

Sertraline



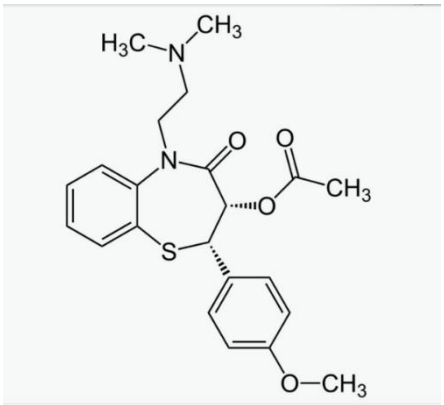
(marketed as Zoloft) it is included in the class of drugs called selective serotonin reuptake inhibitors (SSRIs). This class of drugs is used to treat depression, anxiety, and other mood disorder
Sertraline contains an amine group
Molecular Formula: C17H1C12N

Nifedipine



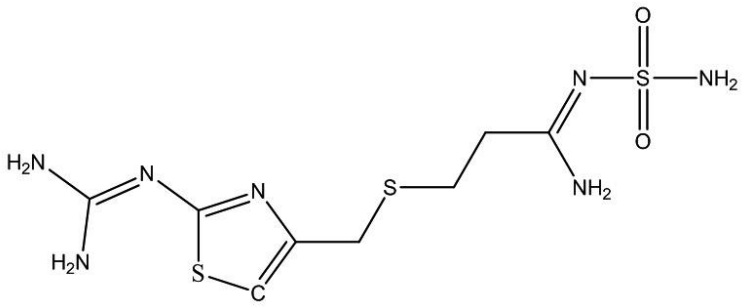
It is in a class of medications called calcium-channel blockers. It lowers blood pressure by relaxing the blood vessels so the heart does not have to pump as hard. It controls chest pain by increasing the supply of blood and oxygen to the heart.
Nifedipine contains an amine group
Molecular formula $C_{17}H_{18}N_2O_6$

Diltiazem



it is a calcium channel blocker. It works by relaxing the muscles of your heart and blood vessels.
used to treat hypertension (high blood pressure), angina (chest pain), and certain heart rhythm disorders
Diltiazem contains an amine group
Molecular formula $C_{22}H_{26}N_2O_4S$

Famotidine



it is in a class of medications called H2 blockers. It works by decreasing the amount of acid made in the stomach.
Famotidine is used to treat ulcers of the stomach and intestines and to prevent intestinal ulcers from coming back after they have healed. This medication is also used to treat certain stomach and throat (esophagus) problems (such as erosive esophagitis, gastroesophageal reflux disease-GERD,
Famotidine contains an amide group
Molecular formula $C_8H_{15}N_7O_2S_3$

Materials and Methods

Chemical

Ethanol, glucose, NaCl, Trisodium citrate, Citric acid, Distilled water, and Phosphate buffer (pH 7.4). All chemical was available in Basra university college laboratory.

Preparation of solution

As an initial step prepare Alsever's solution, Isosaline, and Hypo saline separately according to the concentration in (Table1).

Table 1 . Preparation of Solution

Solvent	material	concentration	Amount of H_2O
Alsever's solution	Glucose	1.025g	50ml
	NaCl	0.205g	
	Trisodium citrate	0.405g	
	Citric acid	0.028g	
Isosaline	NaCl	0.45g	50ml
Hypo saline	NaCl	0.35g	50ml

Preparation of drugs

Preparation of drugs to be use in the research by dissolve each drug sheet in the suitable solvent according to (Table2) then filtrate and finally evaporate the solvent to get the drug without additive. the filtrate solutions were transformed to plate in order to evaporate.

Table 2. Preparation of solution drug				
Drug	**Dose**	**No.of tablet**	**Solvent**	**Amount of solvent**
Ibuprofen (pioneer)	400mg	10 tab.	ethanol	20ml
Diltiazem (Sanofi)	90mg	14 tab.	water	10ml
Sertraline (Tad pharma)	50mg	10 tab.	DMS	20ml
Famotidine (samaraa)	20mg	10 tab.	ethanol	20ml
Nifedipine (Eipico)	20mg	10 film coated tab.	ethanol	40ml

In vitro anti-inflammatory activity

The evaluation of in vitro anti-inflammatory activity of selected drugs done by Human red blood cells (HRBC) membrane standardizing method. A (2.5ml) of Blood was collected from the investigators themselves and mixed with sterilized Alsever's solution (2.5 ml) then take blood tube to be centrifuge at 4000 rpm for 10 min to obtain the serum and separate red blood cell followed by washing with Isosaline solution 3ml two time. The HRBC suspension was prepared by packed

cells, then the volume was complete to 10 ml with Isosaline solution while 50mg of each drug in different tube with 10ml of distilled water. Finally prepare the test samples control (2ml of distilled water), standard (2ml of Ibuprofen), and Drug (2ml of Famotidine, Nifedipine,

sertraline, and diltiazem) were separately mixed with 1 ml of phosphate buffer, 2 ml of hypo saline, and 0.5 ml of HRBC suspension. The assay mixtures were left at room for 30 min. then they were centrifuged at 3000rpm for 10 min. The hemoglobin content in supernatant was picked and with spectrophotometer at 560 nm wavelength absorbance obtained. The percentages of HRBC member stabilization was calculated by the following equation:

$$\text{Percentage of protection} = 100 - \frac{Absorbance\ of\ Sample}{Absorbance\ of\ control} \times 100$$

Results:

After recording the absorbance of drugs we start to estimation Percentage of protection (Table3). (Figure1) Show comparison between Ibuprofen (anti- inflammatory drug as standard) Percentage of protection and (Famotidine, Sertraline, Diltiazem, Nifedipine) Percentage of protection.

1- Ibuprofen (standard)

$$\text{Percentage of protection} = 100 - \frac{0.234}{0.52} \times 100$$

$$= 55\%$$

2- Nifedipine

$$\text{Percentage of protection} = 100 - \frac{0.187}{0.52} \times 100$$

$$= 64.04\ \%$$

3- Diltiazem

$$\text{Percentage of protection} = 100 - \frac{0.26}{0.52} \times 100$$

$$= 50\%$$

4- Sertraline

$$\text{Percentage of protection} = 100 - \frac{0.194}{0.52} \times 100$$

$$= 62.69\%$$

5- Famotidine

$$\text{Percentage of protection} = 100 - \frac{0.105}{0.52} \times 100$$

$$= 79.80\%$$

Table 3. Result- Absorbance and percentage of protection

Sample	concentration	Absorbance	Protection
Ibuprofen	50mg	0.234	55%
Nifedipine	50mg	0.187	64.04%
Diltiazem	50mg	0.26	50%
Sertraline	50mg	0.194	62.69%
Famotidine	50mg	0.105	79.80%

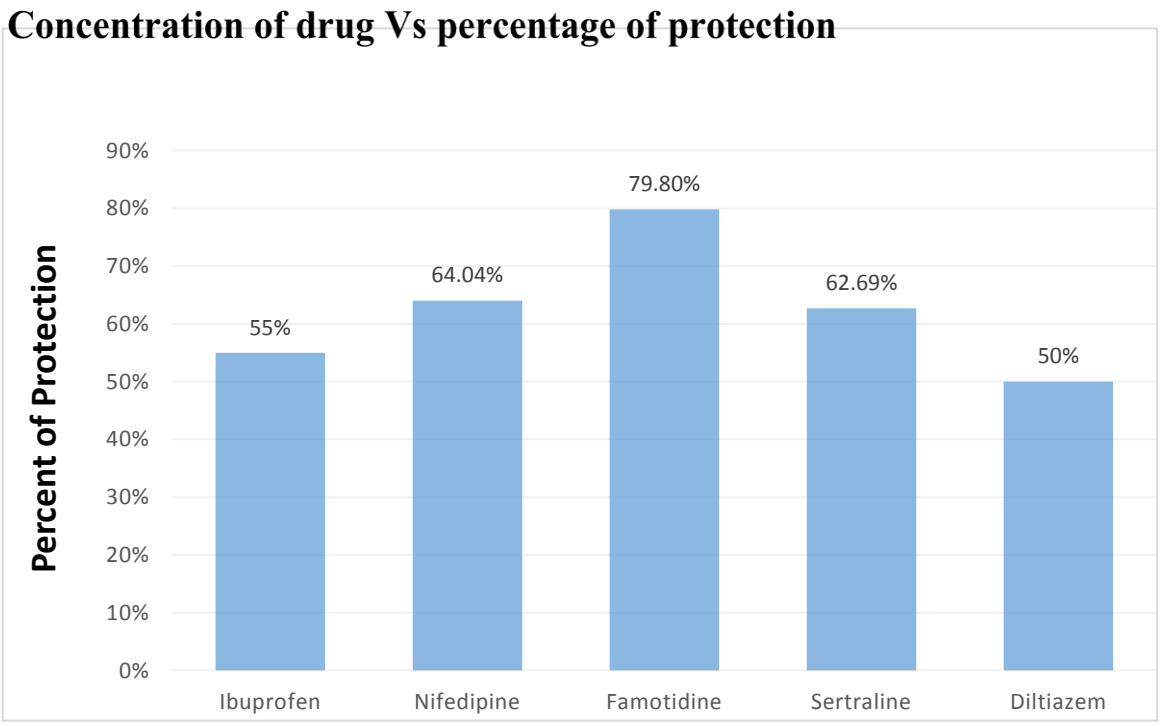


Figure.1 percentage of protection for Ibuprofen, sertraline, Nifedipine, Diltiazem, Famotidine

Discussion

Owing to the efficacy in reducing pain and inflammation, non-steroidal anti-inflammatory drugs (NSAIDs) are amongst the most popularly used medicines confirming their position in the WHO's Model List of Essential Medicines.

Like all medicines, there's a risk of side effects from NSAIDs.

These tend to be more common if you're taking high doses for a long time, or you're elderly or in poor general health so they can't tolerate them without a protective therapy usually proton pump inhibitors are given to minimize GIT upset.

The need arose to find new drugs that have the same efficacy, but with fewer side effects since almost all elderly patient have already other comorbidities that may made them unable to tolerate NSAID drugs.

In our research, we were keen to choose drugs that have

fewer side effects, to increase patient acceptance and protect him as much as possible from the seriousness of side effects of NSAID and reduce the time he needs to recover and possible complications.

Possible side effects of NSAIDs include:

indigestion – including stomach aches, feeling sick and diarrhea •

stomach ulcers – these can cause internal bleeding and anemia; extra • medicine to protect your stomach may be prescribed to help reduce this risk

We choose (Diltiazem, Nifedipine) since both also used as antihypertensive drugs to treat high blood pressure and to control angina (chest pain) so patients suffer from these diseases can take advantage (dual use).

Since Depression is a common mental disorder and More women are affected by depression than men and as many women experience post-menopausal osteoarthritis, arthritis it may be very beneficial to find drug that can act on both depression and joint inflammation.

Therefore, we aimed to select an antidepressant drug to test its efficacy as anti-inflammatory drug as (Sertraline) is

the safest and most popular one among antidepressants.

As we mention previously many patients especially elderly have GIT problems which can limit the use of many drugs. Therefore, we found that it would be great to test a drug that treats stomach diseases and at the same time it can be

Conclusion

As shown in the research study, we predict anti-inflammatory effect of Famotidine, diltiazem, sertraline, and Nifedipine in compere to Ibuprofen, used method (HRBC) was selected to evaluate anti-inflammatory and Percentage of protection to erythrocytes. Our drugs had shown different levels of protection to the red blood cells as: Ibuprofen 55%, Famotidine 79.80%, sertraline 62.69%, Nifedipine 64.038%, diltiazem 50%.

These ratios show promising outcomes for the role of these drugs in the treatment of chronic inflammatory diseases for those who have cautions or extreme contraindications to the use of NSAIDs.

Finally, we can use our research as standard to detect the protection capacity of drugs having same functional group or same chemical properties.

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