# ASSESSMENT OF ANTI-INFLAMMATORY EFFECT OF CALCIUM CARBONATE AND DILTIAZEM AND THEIR COMBINATION WITH ASPIRIN IN ALBINO RATS

Thesis

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# ABSTRACT

In the present study, we aimed to assess the anti-inflammatory effect of calcium carbonate, diltiazem and their combination with aspirin as well as the possible underlying mechanism.

Calcium carbonate (10 and 50 mg/kg), diltiazem (4mg/kg) and aspirin (54 and 200 mg/kg) were administered orally in different groups of rats to study their effect on acute inflammation induced by yeast.

Two animal models of acute inflammation were used; rat paw edema and rat air pouch model. The effect of the aforementioned drugs on paw volume, total leucocytic count, and intereukin-6 (IL-6) level in air pouch exudates were studied.

In the present study both calcium carbonate (50mg/kg) and diltiazem (4mg/kg) produced significant reduction in paw edema volume by 65.2% and 50 % respectively. In the air pouch exudates, calcium carbonate (50mg/kg) and diltiazem (4mg/kg) significantly reduced the total leucocytic count (by 52% & 53.4% respectively) and IL 6 level (by 37.6% & 35.2% respectively).

We found that neither calcium carbonate in the dose of 10 mg/kg nor aspirin (54mg/kg) has any anti-inflammatory effect. However, when combined with each other they produced significant reduction in paw edema volume by 73.1%. In the air pouch exudates, the same combination significantly reduced the total leucocytic count (by 50.7%) and IL 6 (by 55.6%). It is to be noted that the anti-inflammatory effect of this combination was comparable to that of aspirin (200 mg/kg) in the reduction of paw edema volume and IL 6 level.

Similarly, diltiazem (4mg/kg) potentiated the anti-inflammatory activity of aspirin (54mg/kg), as their combination produced significant reduction in paw edema volume by 61.1%, total leucocytic count (by 58.3%) and IL 6 level (by 49.3%). It is to be noted that the anti-inflammatory effect of this combination was comparable to that of aspirin (200 mg/kg).

The mechanism of anti-inflammatory action of the tested drugs was explored by observing their effect on oxidative stress as measured by level of malondialdehyde (MDA) in the air pouch exudates.

Both calcium carbonate (10 and 50 mg/kg) and diltiazem (4 mg/kg) produced significant reduction in MDA level by 17.8%, 38.1% and 29.4% respectively. When each of calcium carbonate (10 mg/kg) and diltiazem (4 mg/kg) was combined with aspirin (54mg/kg), they produced a more significant reduction in MDA level by 52.3% and 41.6% respectively.

We concluded that both calcium carbonate and diltiazem possess anti-inflammatory action that may be mediated via their ability to modulate oxidative stress. Also both of them can potentiate aspirin in its sub-antiinflammatory dose.

**Key words:** Anti-inflammatory, aspirin, calcium salts, calcium channel blockers.

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## *List of abbreviations*

| | |
|---|---|
| **PAMPs** | Pathogen-associated molecular patterns |
| **APCs** | Antigen-presenting cells |
| **ASA** | Acetylsalicylic acid |
| **$CaCO_3$** | Calcium Carbonate |
| **CCBs** | Calcium channel blockers |
| **CGRP** | Calcitonin-gene related polypeptide |
| **COX** | Cyclo-oxygenase |
| **CRH** | Corticotropin-releasing hormone |
| **CSIF** | Cytokine synthesis inhibitory factor |
| **DCs** | Dendritic cells |
| **ECF** | Extracellular fluid |
| **ELAM-1** | Endothelial leukocyte- adhesion molecule-1 |
| **eNOS** | Endothelial nitric oxide synthase |
| **G-CSF** | Granulocyte- colony stimulating factor |
| **GM-CSF** | Granulocyte-macrophage colony stimulating factor |
| **H2O2** | Hydrogen peroxide |
| **HETE** | Hydroxyeicosatetraeinoate |
| **ICAM** | Intercellular adhesion molecule |
| **IFN** | Interferon |
| **IL** | Interleukin |
| **iNOS** | Inducible nitric oxide synthase |
| **LDL** | Low density lipoprotein |
| **LP** | Lipid peroxidation |
| **LPS** | Lipopolysaccharide |
| **LTB4** | Leukotriene-B4 |

| | |
|---|---|
| **MCP-1** | Monocyte chemotactic protein-1 |
| **MDA** | Malondialdehide |
| **MDATBA** | Malondialdehide-thiobarbituric acid assay |
| **MHC** | Major histocompatibility complex |
| **NADPH** | Nicotinamide adenine dinucleotide phosphate |
| **NF-kB** | nuclear factor kappa-light-chain-enhancer of activated B cells |
| **NK** | Natural killer cells |
| **NO** | Nitric oxide |
| **NSAIDs** | Non-steroidal anti-inflammatory drugs |
| **PAF** | Platelet activating factor |
| **PGs** | Prostaglandins |
| **PMS** | Premenstrual syndrome |
| **PNLs** | Polymorph nuclear leucocytes |
| **ROS** | Reactive oxygen species |
| **STAT-1** | Signal Transducer and Activator of Transcription |
| **TGF** | Transforming growth factor |
| **TLRs** | Toll- like receptors |
| **TNF** | Tumor necrosis factor |
| **TxA2** | Thromboxane A2 |
| **VCAM-1** | Vascular cell adhesion molecule-1 |

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# Introduction

Inflammation is a complex and dynamic condition in which many changes take place at the site of inflammation as well as systemically. It involves a complex array of enzymes activation, release of mediators, extravasation of fluid, migration of cells, tissue breakdown and repair **(Vane and Botting, 1995).**

For many years the pharmaceutical industry attempted to develop non-steroidal anti inflammatory drugs (NSAIDs) which shared the therapeutic action of aspirin but did not cause its main adverse event, namely gastric ulceration. These attempts led to the development of indomethacin, the fenamates, ibuprofen and many others. However, while all these drugs had clinical utility they also eroded the gastric mucosa **(Vane, 1971).**

Interestingly, several other drugs like calcium salts; calcium dobesilate **(Piller, 1990)**, calcium pentosan polysulfate **(Smith et al., 1999**), calcium gluconate and calcium carbonate **(Karnad et al., 2006)** have also been reported to possess anti-inflammatory property**.**

The proposed anti-inflammatory mechanisms of calcium salts may be due to enhanced superoxide anions scavenging through increased activity of superoxide dismutase, peroxidase, glutathione peroxidase and glutathione reductase, which are reported to be increased by calcium glubionate **(Lutnicki et al., 1992).**

It is well known that such antioxidant enzymes suppress inflammation. It is not known whether calcium salts like calcium carbonate and calcium gluconate have a property similar to that of calcium glubionate, which could explain their anti-inflammatory action **(Karnad et al., 2006).**

Calcium channel blockers (CCBs) have also been reported to exert a potent anti-inflammatory action **(Aditya et al., 1997; Gurdal et al., 1997; Kouoh et al., 2006; Suleyman et al., 2006),**

The proposed mechanisms for anti-inflammatory effect of CCBs include inhibition of the synthesis of the produces of cyclooxyenase of lipoxygenase, prevention of aggregation, adhesion and chemotaxis of neutrophils, blockage of the relege of lysosomal enzymes and toxic oxygen radicals and uncoupling of oxidative phosporylation (**Martinez et al., 1999;kouoh et al .,2002; sirmagul et al., 2004).**

# Aim of the work

The aim of this work is to investigate the possible anti-inflammatory effects of calcium carbonate, diltiazem and their combination with aspirin as well as the underlying possible mechanism using two models of acute inflammation in male albino rats.

# Pathophysiology of inflammation

Inflammation is the response of tissue to injury and is characterized by:

(1) In the acute phase by increased blood flow and vascular permeability along with the accumulation of fluid, leukocytes, and inflammatory mediators such as cytokines (innate or non-adaptive response).

(2) The subacute/chronic phase is characterized by the development of specific humoral and cellular immune responses to the pathogen(s) present at the site of tissue injury.

During both acute and chronic inflammatory processes, a variety of soluble factors are involved in leukocyte recruitment through increased expression of cellular adhesion molecules and chemoattraction. The soluble factors that mediate these responses fall into four main categories:

(1) Inflammatory lipid metabolites such as platelet activating factor (PAF) and the numerous derivatives of arachidonic acid (prostaglandins, leukotrienes, lipoxins), which are generated from cellular phospholipids.

(2) Four cascades of soluble proteases/substrates (clotting, fibrinolytic, complement, and kinins), which generate numerous pro-inflammatory peptides.

(3) Nitric oxide, a potent endogenous vasodilator, whose role in the inflammatory process has only recently begun to be explored.

(4) A group of cell-derived polypeptides, known as cytokines, which to a large extent orchestrate the inflammatory response **(Feghali and Wright, 1997).**

Most cytokines are multifunctional and elicit their effects locally or systemically in an autocrine or paracrine manner Thus the role of cytokines is to enable cells to communicate with each other in a local environment **(Wood, 2006).**

## Cytokines involved in acute inflammation (innate response):

-Interleukin-1

-Tumor necrosis factor

-Interleukin-6

-Interleukin-11

-Interleukin-8/chemokines

-Eotaxin

-Interleukin-16

-Interleukin-17

-Colony stimulating factors

## Cytokines involved in chronic inflammation (adaptive response):

- **The humoral inflammatory response:**

-Interleukin-3

-Interleukin-4

-Interleukin-5

-Interleukin-7

-Interleukin-9

-Interleukin-10

-Interleukin-13

-Interleukin-14

-Transforming growth factor-β

- **The cellular inflammatory response:**

-Interleukin-2

-Interleukin-12

-Interleukin-15

-Interferons

-IFN- inducing factor

**(Feghali and Wright, 1997).**

**Figure (1):** Cytokines involved in acute and chronic inflammatory responses **(Feghali and Wright, 1997).**