Pharmacological Studies on the Potential Anti-inflammatory Effects of a Natural Product(s)

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(Pharmacology and Toxicology) By

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I dedicate this thesis to my dear family, without their patience, understanding and support; this thesis would not be existed.

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Abstract

Background:

Over the past two centuries, natural products have played an invaluable role in drug discovery contributing enormously to the development of therapeutic agents currently used in modern medicine. *Dietes bicolor*, commonly known as Yellow Wild Iris, Peacock Flower or Butterfly Iris is a rhizomatous perennial herb belonging to family Iridaceae. The phytochemical and pharmacological properties of this genus have never been reported.

Aim: The current study was designed to investigate the anti- inflammatory of *Dietes bicolor* leaf extract. Moreover, phytochemical analysis of the biologically active fraction was carried out.

Methodology: The total leaf extract and its fractions were examined for possible anti-inflammatory activity in two experimental models: the carrageenan-induced rat edema model and the croton oil– induced ear edema model. Collectively, the parameters assessed included paw volume, PGE2 level, cytokines (TNF-α, IL-6, IFN-γ, IL-1α, IL-1β, MCP-1, RANTES and Macrophage Inflammatory Protein (MIP)), ear edema, ear tissue MPO and histopathology.

Results: *Dietes bicolor* extract demonstrated a dose-related reduction in both carrageenan-induced rat paw edema and croton oil-induced ear edema models. A flavone C-glycoside was isolated from the biologically active fraction which may contribute to its anti- inflammatory.

Topical application of *Dietes bicolor* reduced ear edema induced by croton oil in rats. In the same animal model, *Dietes bicolor* reduced neutrophil infiltration, as indicated by decreased myeloperoxidase (MPO) activity. In addition, Dietes bicolor reduced the histopathological changes affected by croton oil application.

The biologically active fraction was subjected to further phytochemical analysis utilizing several chromatographic techniques. One major compound was isolated and its structure was

elucidated using different spectroscopic techniques.

Conclusion: *Dietes bicolor* exhibited promising anti-inflammatory activity *in vivo*. The flavonoid content of *Dietes bicolor* may contribute to the possible biological effects of the extract.

Keywords:

Dietes bicolor; Iridaceae; anti-inflammatory; carrageenan-induced paw edema; croton oil-induced ear edema; Vitexin.

Contents

List of Abbreviations

AA	Arachidonic Acid
COX	Cyclooxygenase
CRP	C Reactive Protein
HOCL	Hypochlorous acid
HPLC-DAD	Chromatography Liquid High-Performance with Diode-Array Detection
ICF	Intracellular Fluids
IL-1 α	Interleukin-1 alpha

IL-1 β	Interleukin-1 beta
IL-6	Interleukin-6
IFN-γ	Interferon gamma
IVF	Intravascular Fluids
MCP-1	Monocyte Chemoattractant Protein-1
MIP	Macrophage Inflammatory Protein
MPO	Myeloperoxidase
M ϕ	Macrophage
NK cells	Natural Killer cells
NO	Nitric Oxide
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
PGE2	Prostaglandin E2
RANTES	T-cell Normal Activation, upon Regulated Expressed and Secreted
ROS	Reactive Oxygen Species
TAC	Total Antioxidant Capacity
TNF-α	Tumor Necrosis Factor-alpha

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Table (1)	The main differences between acute and chronic inflammatory processes.

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Introduction

Inflammation

Inflammation is the immune system's response to infection and injury. It has been implicated in the pathogeneses of arthritis, cancer and stroke, as well as in neurodegenerative and cardiovascular disease. Inflammation is an intrinsically beneficial event that leads to removal of offending factors and restoration of tissue structure and physiological function. Inflammation has been known to humankind for at least a few thousand years, in part because it accompanied two major scourges of the past, wounds and infections, and in part because it is rather conspicuous (Nathan, 2002).

Although references to inflammation can be found in ancient medical texts, apparently the first to define its clinical symptoms was the Roman doctor Cornelius Celsus in the 1st century AD. These symptoms came to be known as the four cardinal signs of inflammation: rubor et tumor cum calore et dolore (redness and swelling with heat and pain). Celsus mentioned these signs in his treatise De medicina, while describing procedures for treating chest pain, where he became an oft- quoted medical celebrity (Majno 1975). The physiological basis of the four cardinal

signs of inflammation were revealed much later by Augustus Waller (1846) and Julius Cohnheim (1867), who discovered leukocyte emigration from the blood vessels and other vascular changes characteristic of an acute inflammatory response. Analyzing living tissues under the microscope, Cohnheim observed vasodilation, leakage of plasma, and migration of leukocytes out of blood vessels and into the surrounding tissue (Majno and Joris, 2004). Once the initiating noxious

stimulus is removed via phagocytosis, the inflammatory reaction can decrease and resolve. During the resolution of inflammation, granulocytes are eliminated, while macrophages and lymphocytes return to normal pre-inflammatory numbers and phenotypes. The usual outcome of the acute inflammatory program is successful resolution and repair of tissue damage, rather than persistence and dysfunction of the inflammatory response, which can lead to scarring and loss of organ function. It may be anticipated, therefore, that failure of acute inflammation to resolve may predispose to auto-immunity, chronic dysplastic inflammation and excessive tissue damage (Nathan 2002).