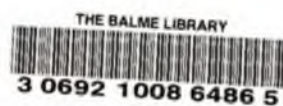




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**ANTI-INFLAMMATORY MEDICINAL PLANTS AS
ANTI-OXIDANTS AND INHIBITORS OF PRO-
INFLAMMATORY EICOSANOID BIOSYNTHESIS**

A THESIS SUBMITTED TO

THE DEPARTMENT OF BIOCHEMISTRY

UNIVERSITY OF GHANA

The crest of the University of Ghana is a shield-shaped emblem. It features a blue shield with a white border. Inside the shield, there are three white triangles pointing downwards at the top. Below these triangles is a white cross with four curved ends. In the center of the cross, the word "BY" is written in a serif font. The shield is set against a light blue background.

BY

KWABENA AMPONSAH-MANAGER

IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE AWARD OF

A MASTER OF PHILOSOPHY DEGREE IN BIOCHEMISTRY

MAY, 2001

Grace is inexplicable

You understand it only by experiencing it

Those who have found it need no explanation

They already know what it is

Those who have not found it, or at least not yet

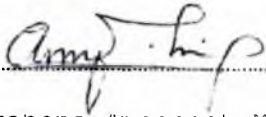
Will never understand it, no matter how often you explain

Kwabena Amponsah-Manager



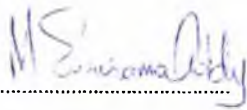
DECLARATION

I carried out the experimental work contained in this thesis in the laboratories of the Department of Biochemistry and the Noguchi Memorial Institute for Medical Research, both of the University of Ghana, under the supervision of Prof. Marian Ewurama Addy and Dr. Alexander Kwadwo Nyarko.



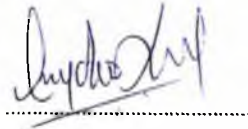
Kwabena Amponsah-Manager

(Student)



Prof. M. E. Addy

(Supervisor)



Dr. A.K. Nyarko

(Co-supervisor)

DEDICATION

***TO MY MOTHER,
FOR OBVIOUS REASONS***



ACKNOWLEDGEMENT

I wish to put on record my deep appreciation of the support and encouragement that were given to me by my supervisors, Prof. Marian Ewurama Addy and Dr. Alexander Kwadwo Nyarko. I cannot thank them enough for their sacrifices throughout this research.

I am highly indebted to Dr. Phyllis Addo, the Head of the Experimental Animals Unit of the Noguchi Memorial Inst. for Medical Research (NMIMR), for offering her laboratory and making some timely suggestions and criticisms. I also thank Messrs Mark Ofosuhene and Kwesi Teye of the Clinical Pathology Unit and the Technical Staff of the Department of Biochemistry, University of Ghana for technical assistance.

I acknowledge the role various friends and colleagues played in this work. I am grateful to them all, especially my best friend Mr. Kojo Aikins who gave so much to get this work done, Ps and Mrs. Dokosi for all the materials they provided and Mr. John Dadzie-Mensah who made some fine suggestions during the initial stages of this work.

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TABLE OF CONTENTS

CONTENTS	Page
CITATION	i
DECLARATION	ii
DEDICATION	iii
ACKNOWLEDGEMENT	iv
TABLE OF CONTENTS	v
LIST OF FIGURE	viii
LIST OF TABLES	ix
ABSTRACT	x

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

General introduction	1
Research objectives	7
Significance of the study	8
Literature review	11
Inflammation	11
Pro- and anti-inflammatory eicosanoids	13
<i>Leukotrienes</i>	14
<i>Thromboxanes</i>	17
Estimation of eicosanoids	21
Anti-oxidants	22
Asthma as inflammatory disorder	24
Anti-inflammatory medicinal plants	28

CHAPTER TWO: MATERIALS AND METHODS

Material	33
Methods	34
Preparation of plant extracts	34
Pre-treatment of animals	35
Preparation of isolated organ	36
Sampling of lung effluent	37
Preparation of microsomes	38
Protein determination	39
In vitro synthesis of pro-inflammatory eicosanoids	39
Quantitative estimation of eicosanoids	40
Anti-oxidant properties	42
Effect on hydroxyl radical generation	42
Quantitative determination of Total phenol	43
Water soluble phenols	44
Statistical analysis	44

CHAPTER THREE: RESULTS

Release of Eicosanoids	45
<i>Effects of 'Tina A'</i>	45
Effect of <i>T. sanguinea</i> on the release of thromboxane B ₂	52
Synthesis of eicosanoids	52
Thromboxane synthesis	52

<i>Cysteinyl leukotrienes</i>	58
Effect on hydroxyl radical generation	59
Total and soluble phenols	61
CHAPTER FOUR: DISCUSSION AND CONCLUSION	63
Reference:	77
Appendix	93

LIST OF FIGURES

Figure	Title	Page
Fig 1.1:	The lipoxygenase pathway	15
Fig 1.2:	The cyclooxygenase pathway	20
Fig 3.1:	Effect of 'Tina A' on the release of TXB ₂	46
Fig 3.2:	Effect on the release of cysteinyl leukotrienes	49
Fig 3.3:	Effects of extracts on synthesis of thromboxanes	54
Fig 3.4:	Effect of 'Tina A' on TX synthesis	56
Fig 3.5:	Effect on hydroxyl radical generation	60

LIST OF TABLES

Table	Title	Page
Table 1.1:	Effect of 'Tina A' on the release of TXB ₂ and Cysteinyl LT under different conditions of treatment.	51
Table 3.2:	Effects of <i>T. sanguinea</i> and <i>L. multiflora</i> on TXB ₂ synthesis	57
Table 3.3:	Effect of 'Tina A' and <i>T. sanguinea</i> on LT synthesis	58
Table 3.4:	Phenolic content of extracts	61
Table 3.5:	Total phenolic content and inhibition hydroxyl radical generation	62

ABSTRACT

Arachidonic acid (AA) metabolism that leads to the production of both anti- and pro-inflammatory eicosanoids is a standard assay used to investigate the basis for the therapeutic action of anti-inflammatory medicinal plants. Earlier investigations have established the efficacy of some herbal preparations in terms of their ability to increase the amounts of anti-inflammatory eicosanoids. *Desmodium adscendens* and *Parkitina sp.* ('Tina A') increased PGE₂ and PGI₂ synthesis and inhibited phospholipase A₂ activity while *Thonningia sanguinea* decreased the release of histamine and slow reacting substances of anaphylaxis (SRA-S). So far none of these extracts has shown any significant effect on thromboxane (TX) synthesis. The present study was conducted to evaluate the efficacy of four plant extracts, *D. adscendens*, Tina A, *T. sanguinea* and *L. multiflora* in decreasing the amounts of the pro-inflammatory eicosanoids, TXB₂ and cysteinyl leukotrienes (cyst.LT), and their antioxidant properties to act as anti-oxidants. Isolated guinea pig lungs perfused via the trachea were used as a model to study the release and inhibition of the release of the TXB₂ and cyst.LTs by the extracts. The organs were perfused with Kreb's solution with and without the plant extracts and TXB₂ and cyst.LT released were estimated by an

ELISA. There were significant decreases in the amounts of TXB₂ and cyst.LT released from lungs perfused with the extract of 'Tina A' or *T. sanguinea* and lungs from animals treated with 'Tina A'. Generally, the treatment of the animals with the extract decreased the release of TX more than LTs, while perfusion of the isolated lungs (i.e. short term treatment) had more effect on cyst.LT than TX. The effects of *D. adscendens*, 'Tina A', *L. multiflora* and *T. sanguinea* on the *in vitro* synthesis of TXB₂ using blood platelet microsomes were investigated. Microsomes for this study were prepared from blood platelets. Except *L. multiflora*, for which higher concentration showed minimum effects on TXB₂ synthesis, there was a concentration-dependent inhibition of TXB₂ by all the extracts. The effect of *T. sanguinea* became significant at a relatively higher concentration compared to that of *D. adscendens* or 'Tina A'. At the highest concentration of 100µg/ml, *D. adscendens*, 'Tina A' and *T. sanguinea* caused 81%, 81.6% and 87.5% decreases respectively in the amounts of TXB₂ synthesized. The effects of the extracts on hydroxyl radical generation and total and water-soluble phenolic content were determined. There was a concentration-dependent inhibition of hydroxyl generation by all the extracts. Large amounts of phenolic compounds were identified in all the extracts. For 'Tina A', 74% of the total phenols was water soluble while *T. sanguinea* which

gave the highest amount of total phenols had 31% being water soluble. There was a positive correlation between total phenolic content and inhibition of hydroxyl radical generation. *T. sanguinea* and *D. adscendens* which had the highest and lowest amounts of total phenols respectively, showed the highest and lowest inhibition of hydroxyl radical generations respectively at all concentrations studied. These findings suggest that the inhibition of both synthesis/release of pro-inflammatory eicosanoids and generation of reactive oxygen species by the plant extracts studied validates their use in folk medicine in the management of asthma and other inflammatory disorders.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

GENERAL INTRODUCTION

Medicinal plants are a major source of healthcare management for a large number of the world's population (Farnsworth *et al.*, 1985). It is estimated that about eighty per cent of the world's population depend solely on local plants as medicine (Lewington, 1990). Today, due to science and technology and improvement in synthetic and phyto-chemistry, the use of non-plant drugs is gradually increasing, thereby changing this percentage. However, due to increasing costs of orthodox drugs, the majority of the poor in the world, especially those in developing countries, continue to use plant medicine for their healthcare management.

In Ghana medicinal plants play a vital role in the Health Delivery System (Anokbonggo, 1992). In many local communities, plant-based drugs are used for the treatment or management of cancer, ulcer, reproductive problems and hypertension. Others are used as contraceptives, laxatives, and for managing a host of inflammatory disorders. For example, *Desmodium adscendens*, *Indigofera erecta*, *Trema occidentalis*, *Capparis erythrocarpus* and *Thonningia sanguinea* are currently used for the treatment of asthma, diabetes

mellitus, hepatitis, arthritis and asthma respectively at the Center for Scientific Research into Plant Medicine (CSRPM) at Mampong in Ghana (Gyamfi *et al.*, 1999; Nyarko and Addy, 1994).

In most communities, it is believed that the knowledge of the curative value of herbs are given to individuals by God, through dreams, psychic powers or some extra sensory perception. As a result such preparations are expected to be administered in a certain form or should follow a certain procedure even when such procedures are unacceptable to a large number of the people. At the moment western medicine is preferred to these local plant extracts by the majority of the people even though they are more expensive. The reasons for this include proper quality control, standardized dosage, documented pharmacological effects and how to handle those effects, safety, and attractive forms of presentation. It is important that the African medicinal herbal preparations are improved upon to make them more acceptable to the majority of the people including those from other continents. Putting science into the practice of herbal medicine will go a long way to address the above concerns.

Currently, a number of plant extracts, the majority in their crude forms, are being used to manage inflammatory diseases.

In spite of their usefulness, detailed scientific studies have not been carried out on these preparations to validate their use in therapy. Such studies are necessary considering the fact that most plant preparations may elicit both therapeutic and toxic effects by interacting with enzymes and/or metabolites of the pathways that those enzymes are involved in. An example is the extract of fever few plant, *Tanacetum parthenium* which has been found to inhibit normal aggregatory and secretory response of blood platelets (Heptinstall, 1987). The fever few plant has been in use since ancient times to treat fever but its effect on platelets became known only recently. Phytochemical studies of some of these plant preparations have shown that they contain alkaloids, some of which can be toxic (Mclean, 1970).

Herbal preparations can be misused, especially by the rural and urban folk who cannot afford orthodox medicine. Lately, on buses and at the market places diverse medicinal plant products are advertised and sold. Some of these materials originate from charlatans and may not have any therapeutic action against the ailment they are claimed to heal. The use of most of these plant preparations is not supported by any scientific data with respect to their efficacy, dosage, shelf life, etc. In order to improve the quality of herbal medicine, the Center for Scientific Research into Plant

Medicine (CSRPM) at Mampong-Akwapim in Ghana was set up to carry out scientific investigation on herbal preparation with the view to addressing these concerns indicated.

The Center stocks medicinal plants, some of which are useful in the management of several diseases including asthma. Some of the plants used to manage asthmatic attacks are *Thonningia sanguinea* and *Desmodium adscendes* (Addy and Nyarko, 1985). The anti-asthmatic effects of these plants have been studied in the Department of Biochemistry and at the Noguchi Memorial Institute for Medical Research, both of the University of Ghana. The early investigations used anaphylaxis in the guinea pig as a model to study the anti-asthmatic effects of the plant preparations (Addy and Dzandu, 1986; Addy and Nyarko, 1985; Addy and Awumey, 1984; Addy and Gbewonyo, 1980). As a result, the effects of the extracts on the biosynthesis of prostanoids/eicosanoids are now known (Addy and Schwartzman, 1995, 1992; Addy and Burka, 1987).

A good anti-inflammatory plant product will modulate the metabolism of AA and hence synthesis of eicosanoids such that more anti- and less pro-inflammatory products will be synthesized in response to the administration of the plant material. Inhibition of the cyclooxygenase enzyme protects against inflammation. The non-steroidal anti-inflammatory

drugs (NSAIDs) which have aromatic carboxylic acid moiety, such as acetylsalicylic acid (aspirin) and aspirin-like drugs such as indomethacin, inhibit the cyclooxygenase enzyme. These drugs consequently reduce the amounts of pro-inflammatory eicosanoids such as thromboxane A_2 and the prostaglandin of the F series. The disadvantage in the mode of inhibition described above is that it leads to a reduction of anti-inflammatory eicosanoids, which protect against inflammations. Therefore, a mechanism-based assay that involves the inhibition of the key enzymes, and the consequent reduction in the amounts of pro-inflammatory eicosanoids can be established for use to evaluate potential anti-inflammatory drugs. In this regard, the selective inhibition of AA metabolism will be a precise assay to use in order to evaluate the mode of action of putative anti-inflammatory materials. Previous studies have established the efficacy of some anti-asthmatic herbal preparations. One of these, extracts of *D. adscendens* has been shown to increase the anti-inflammatory prostanoid PGE_2 (Addy and Schwartzman, 1995). This extract however did not decrease the amounts of pro-inflammatory prostanoid $PGF_{2\alpha}$. In another study the extracts of *D. adscendens* greatly increased the synthesis of prostacyclin, PGI_2 , another anti-inflammatory prostanoid, but did not affect the synthesis of thromboxane A_2 (Dadzie-Mensah, 2000), a pro-inflammatory eicosanoid whose

biosynthesis is related to that of PGI₂. The reason for this lack of effect on pro-inflammatory eicosanoid is not apparent but it is likely that the conditions of the assays used were not optimal for their synthesis. For example, for the *in vitro* studies in some of the previous investigations, microsomes used as the source of the cyclooxygenase enzyme were prepared from either the lung or the kidney. These organs, even though rich in the cyclooxygenase enzymes, may not be good sources of the enzyme system for TX biosynthesis. The major product of AA metabolism in platelets is thromboxanes (Ali and Mohammed, 1986). In view of the fact that thrombocytes are a rich source of the enzyme system for TX synthesis (Moore *et al*, 1991), it was important to use microsomes from blood platelets in order to evaluate the effect of these plant extracts on production of pro-inflammatory eicosanoids such as TXs and cysteinyl-leukotrienes.

In vivo measurement of TX has so far not been significantly successful over the years. Measurement of TXB₂, the dominating compound in circulation after injection of AA indicates that, there is great artifactual formation of the compound during sampling (Samuelsson, 1978). Consequently "peripheral plasma levels" of TXB₂ is no doubt very high and certainly does not reflect the true endogenous circulating

levels of the compound. Another problem has to do with the post release metabolism of the compound in the whole animal. This possibility can be minimized by employing an isolated whole organ, which, unlike tissue homogenates, adequately retains vital physiological characteristics, but at the same time has the advantage of excluding some of the complexities associated with whole animal experimentation.

Research Objectives

The overall objective of the research was to investigate the potential of certain plant extracts that are used by herbalist to treat various forms of inflammatory diseases by assaying for their ability to decrease the synthesis of pro-inflammatory eicosanoids, such as TXA₂ and the cysteinyl-leukotriene, and to act as anti-oxidants.

The specific objectives included:

- Establishing of an assay for the biosynthesis of the pro-inflammatory eicosanoids, TX and cysteinyl-leukotrienes, using isolated guinea pig lung.
- Establishing an assay for the synthesis of these eicosanoids *in vitro* using microsomes from platelets as a source of enzyme.

- Using the established assays to evaluate the effects of these plant extracts purported to be used to manage inflammatory diseases.
- Evaluating the anti-oxidant effects of the plant extracts by measurement of hydroxyl radical levels as well as total and water soluble phenols.

It is reported that mediators that contribute to immediate and late asthmatic responses include reactive oxygen species (ROS) produced by epithelial cells and inflammatory cells recruited to the sites of antigen challenge (Comhair *et al.*, 2000). The effects of the ROS produced depend on local anti-oxidant defenses available within the airway epithelial lining fluid. This study therefore proceeded to investigate the anti-oxidant properties of the herbal plant extracts. The results include the effects of the extracts on the hydroxyl radical generation, total and water-soluble phenols.

Significance of the Study

Aspirin, a non-steroidal anti-inflammatory drug (NSAID), has remained the primary drug of choice for the treatment of inflammatory diseases and the majority of articular and musculoskeletal disorders for the past century (Payan and Shearn, 1992). It is the standard against which all anti-inflammatory agents are evaluated. This may be due to its

low cost and minimum side effects. Other NSAIDs, such as indomethacin and phenylbutazone, like aspirin, block prostaglandin production by irreversible inhibition of the cyclooxygenase enzyme (Payan *et al.*, 1992). The steroidal anti-inflammatory drugs (SAIDs) act by inhibiting phospholipase A₂ and consequently interfere with the mobilization of AA and hence the production of all eicosanoids. These two groups of drugs have significant undesirable side effects. For example, phenylbutazone therapy may cause plastic anemia (Glew, 1992). Some of the NSAIDs and SAIDs are very expensive and beyond the means of the majority poor. Our forests however have in abundance trees and herbaceous plants that are used to manage various inflammatory diseases.

Screening putative plants for their anti-inflammatory effects will provide data that could

- be employed as a protocol to standardize herbal preparations that are being used to treat inflammatory diseases
- lead to a more discriminate use of anti-inflammatory herbal products and also increase their acceptance and patronage

- stimulate young and emerging scientists to enter into scientific research into plant medicine thereby providing another avenue for employment
- contribute to our growing economy by cutting down government expenditure on drug importation
- lead to the production of drugs for local as well as foreign markets and hence earn some foreign exchange for Ghana.

LITERATURE REVIEW

Inflammation

Inflammation is the basic cause of a host of diseases and signifies cell injury. It is the body's reaction to invasion by an infectious agent, an antigen or even just a physical damage (Payan and Shearn, 1992). Inflammation occurs when immunologically competent cells are activated in response to foreign organisms or antigenic proteins thereby directing elements of the immune system to sites of infection or injury. The outcome of this response is generally beneficial, as when it causes invading organisms to be neutralized. It could however be deleterious as in the case of arthritis, when it leads to the destruction of bone and cartilage resulting in the limitation of joint function (Roitt *et al.*, 1993).

The manifestations of inflammation are heat, pain, redness and swelling. Redness or rubor is usually the first thing to be noted when inflammation sets in. This is due to the dilation of arteries supplying blood to the area with the result that capillaries, which were previously empty or only partially distended, become rapidly filled with blood. The body controls this condition called hyperemia both neurogenically and chemically via the release of mediator

substances such as histamine. The pain, or dolor, may be the result of change in local pH or in the concentration of certain ions or release of certain chemicals that stimulate nerve endings. Heat, or calor, is the result of more blood being conducted from the inside of the body to the surface, which is normally cooler than 37°C. For inflamed areas deep within the body, local warmth is not a problem since such tissues will already be at the core temperature, and local hyperemia would make no difference. Swelling is the most striking aspect of acute inflammation. It results from the transfer of fluid and cells from the bloodstream to the interstitial tissues. These manifestations are mediated by pro-inflammatory compounds. Some of these pro-inflammatory compounds are eicosanoids that are produced from the metabolism of arachidonic acid (AA) catalyzed by cyclooxygenase, lipoxygenase and monooxygenase enzymes. The release of AA from membrane phospholipids is the rate-limiting step. Once AA is released, it is subsequently metabolized to various eicosanoids (Bhagavan, 1992; Smith, 1992). The excessive production of the pro-inflammatory metabolites is the result of a derangement in the signal transduction mechanism that involves the metabolism of AA.

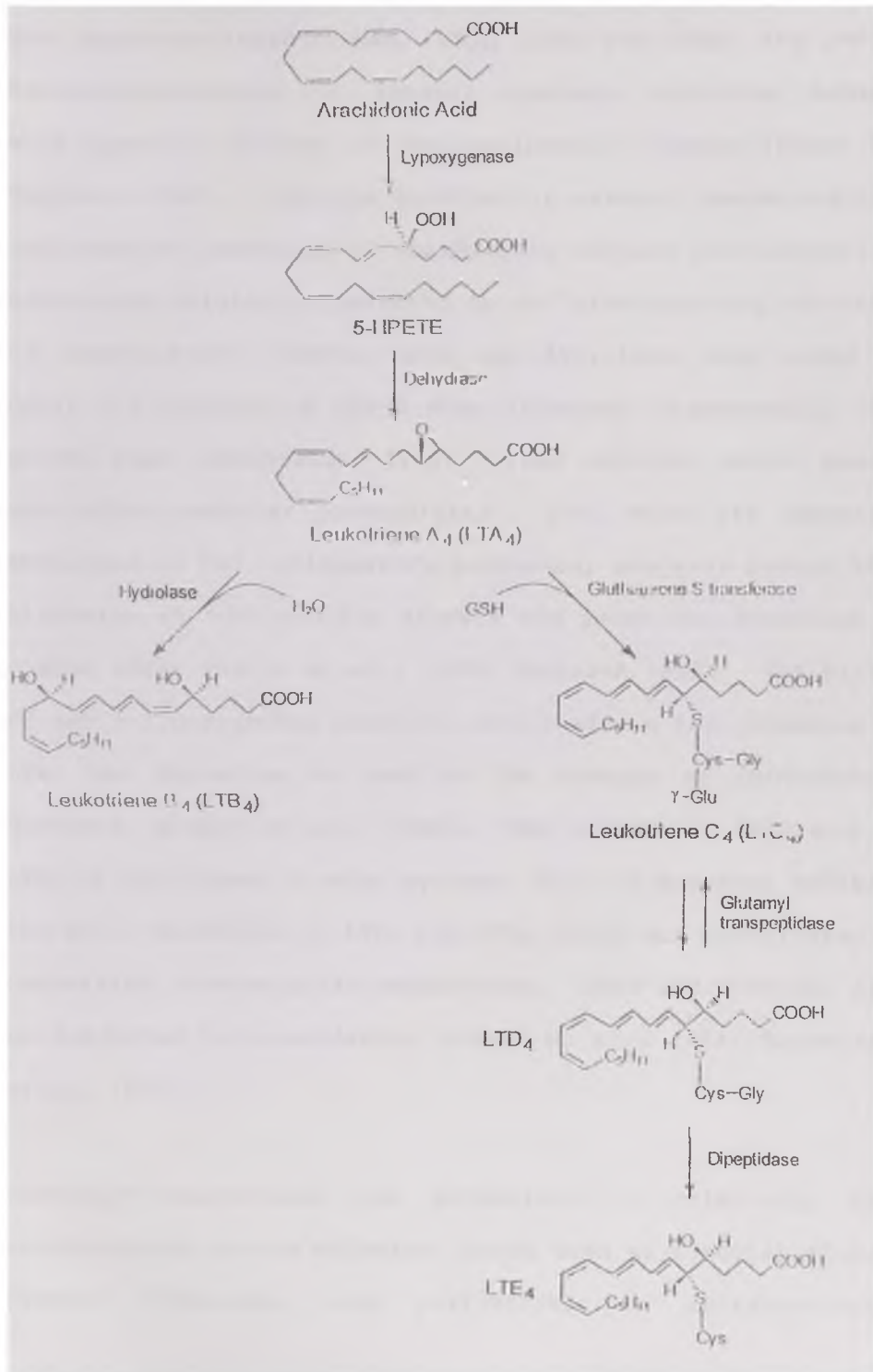
Pro-and anti-inflammatory Eicosanoids

AA metabolism via different pathways gives rise to various C-20 fatty acid (FA) oxygenation products, the eicosanoids. Three of the major classes of eicosanoids namely prostaglandin, prostacyclin and thromboxane, are produced from the cyclooxygenase-mediated metabolism of AA. The lipoxygenase reaction leads to the production of leukotrienes and lipoxins (Fig. 1.1). A third pathway of AA metabolism is mediated by an NADPH-dependent monooxygenase enzyme, a flavoprotein reductase present in the endoplasmic reticulum.

Even though the eicosanoids are produced from the metabolism of a common substrate, some of them are anti-inflammatory while others are pro-inflammatory. Generally, prostaglandin E₂ and prostaglandin I₂ also known as prostacyclin, are anti-inflammatory while thromboxane A₂, prostaglandin F_{2α} and leukotrienes are pro-inflammatory (Whaley and MacSween, 1992). Many anti-inflammatory drugs act by modulating the formation of these eicosanoids or by blocking their action on the target cells. In this study cysteinyl-leukotrienes and thromboxanes are the pro-inflammatory eicosanoids that were measured in response to treatment with the plant extracts.

Leukotrienes

The LTs, which mediate acute inflammatory reactions are formed from AA in leukocytes (Hardie, 1991; Samuelsson et al., 1978). Their biosynthesis has subsequently been demonstrated in other bone marrow-derived cells that express the 5-lipoxygenase including eosinophils, mast cells, and macrophages. Leukotrienes are commonly found in vascular tissues of the lung, heart and platelets. They are synthesized via a lipoxygenase, which catalyze direct oxidation at the 5-carbon of AA to form a hydroxyl peroxy fatty acid intermediate (Fig. 1.1). In the synthesis of LTs, the first product formed is 5-hydroperoxyeicosatetraenoic acid (5-HPETE). 5-HPETE is converted to a monohydroxyeicosatetraenoic acid (5-HETE) by a peroxidase or to the 5,6-epoxide also known as leukotriene A₄ (LTA₄) by a dehydratase. Aspirin and other NSAIDs inhibit the formation of 5-HETE. LTA₄ is transformed into 5,12-dihydroxyeicosatetraenoic acid (LTB₄) by a hydrolase or into a glutathione adduct with the formation of a thioether linkage at carbon-6 by a glutathione-S-transferase to give LTC₄ as the product. LTD₄ results from the removal of the glutamyl residue from LTC₄, while LTE₄ results from the removal of glyceryl residue from LTD₄. The addition of a glutamyl residue to LTE₄ results in the final product LTF₄ (Bhagavan 1992).

Fig. 1.1: Lipoxygenase pathway of arachidonic acid metabolism for leukotriene synthesis

DHIs and HETEs other than the 5 derivatives are also produced (not shown)

The cysteinyl-leukotrienes (LTC₄, LTD₄ and LTE₄) are potent bronchoconstrictors in several species, including humans, with specific effects on the peripheral airways (Piper and Tippins, 1982). They are involved in several immune-mediated inflammatory reactions of anaphylaxis and are constituents of substances originally referred to as "slow-reacting substance of anaphylaxis" (SRS-A). LTC₄ and LTD₄ have been shown to mimic the effects of SRS-A when injected intradermally into guinea pigs (Samuelsson, 1983). They contract smooth muscle and affect vascular permeability. LTs, which are important mediators of the inflammatory processes, are more potent than histamine in constricting airways and promoting formation of tissue edema (Bogic *et al.*, 1998; Bhagavan 1992). Inhibition of the 5-lipoxygenase reaction that leads to the formation of LTs, can therefore be used in the therapy of inflammatory diseases (Higgs *et al.*, 1984). The biological activity of LTE₄ is much lower in most systems, but its presence reflects the prior existence of LTC₄ and LTD₄, which are inactivated by conversion to more polar metabolites. LTC₄ and LTD₄ can also be subjected to ω -oxidation (Higgs *et al.*, 1984; Samuelsson *et al.*, 1978).

Cysteinyl-leukotrienes can accumulate to relatively high concentration in the effusion fluids such as synovial fluids, pleural effusions, and pericardial or intraventricular

aspirates that are associated with inflammation. Since LT metabolism is incomplete in these circumstances, substantial amounts of LTC₄, LTD₄, and LTE₄ may be present. For example, bronchoalveolar lavage fluid from asthmatic subjects may contain 700-1000pg/mol cysteinyl-leukotrienes comprising mainly LTC₄ and LTD₄ (Westcott et al., 1990). Consequently, analysis of such fluids could be a useful assay for the evaluation of putative anti-inflammatory plant materials.

Thromboxanes

The cyclooxygenase enzyme complex catalyses oxidative reactions leading to the formation of prostaglandin H₂ (PG H₂), a cyclic endoperoxide with the characteristic 5-membered ring structure of the PGs (Hardie, 1991).

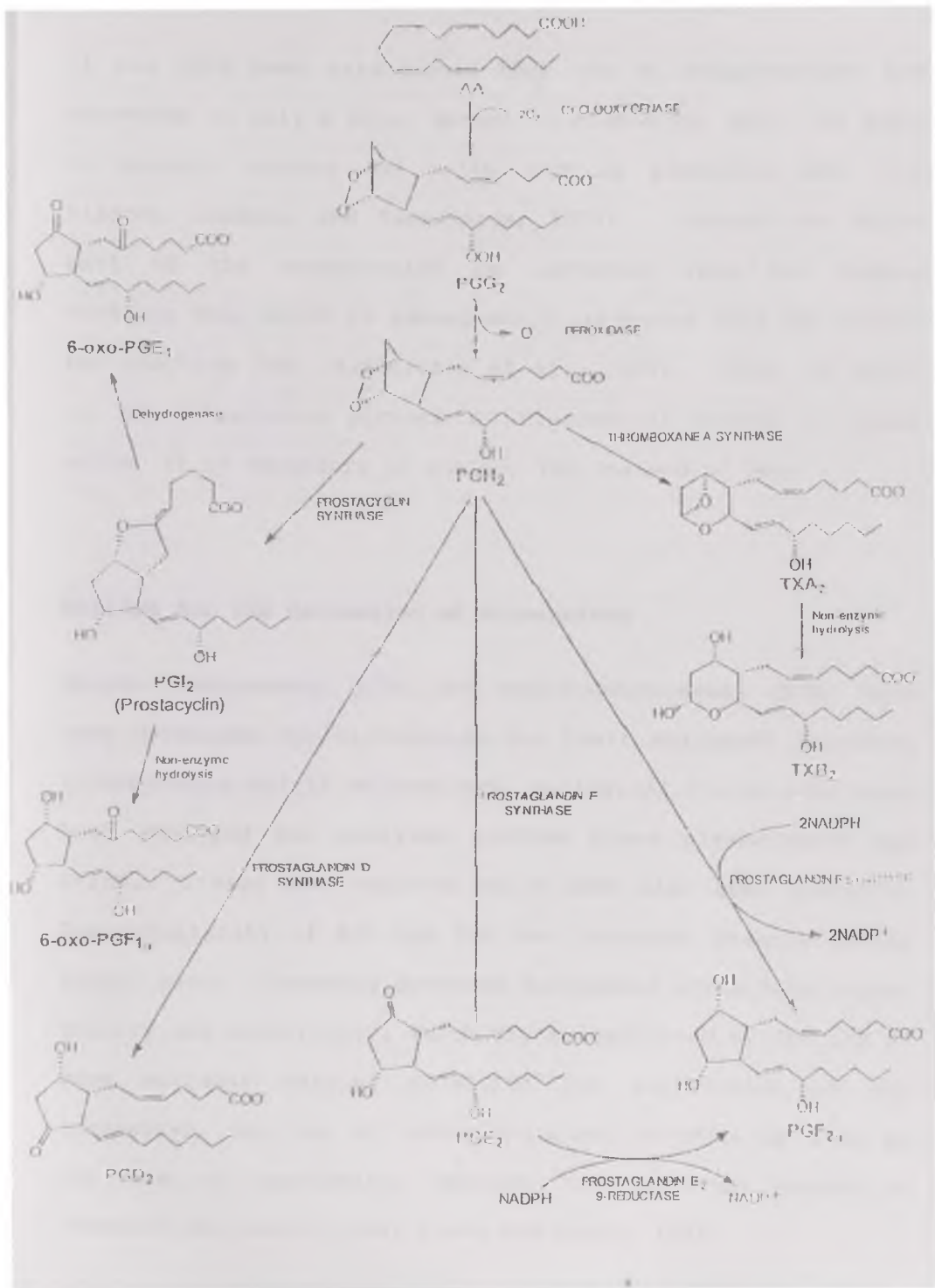
Thromboxanes (TXs) are synthesized by microsomal thromboxane synthases from prostaglandin H₂ via isomerization reaction (Smith 1992; Hardie, 1991). Thromboxanes were first isolated from human and equine thrombocytes (platelets), which have been shown to be the major source of TX (Moore et al., 1991). Thromboxane A₂ has a very short half life (T_{1/2} =30s at 37°C and pH 7.5). It undergoes rapid non-enzymatic hydrolysis to produce the inactive TXB₂ (Bhagavan, 1992). The cyclooxygenase pathway for eicosanoid biosynthesis is shown in Fig. 1.2.

Thromboxane A₂ has an oxane:oxetane structure. It derives its name from both its structure and origin, thrombocytes. It is a potent inducer of platelet aggregation, platelet release reaction (Ali and Mohammed, 1986; Hamberg *et al.*, 1974) and smooth muscle contraction (Samuelsson *et al.*, 1978). In fact, rabbit aorta contracting substance (RCS), which was originally believed to be a PG endoperoxide has been shown to consist of mainly TXA₂ and rather low levels of PGH₂, the intermediate (Heptinstall *et al.*, 1987; Hamberg *et al.*, 1974).

Thromboxane A synthase, present in the endoplasmic reticulum is abundant in the lung and platelets (Glew, 1992; Moore *et al.*, 1991). TX production has been demonstrated in platelets, lungs, spleen, polymorphonuclear leukocytes, brain and inflammatory granuloma (Sameulsson *et al.*, 1978). Cultured vascular endothelial cells (Ingerman-Wojenski *et al.*, 1981) as well as intact blood vessels (Salzma *et al.*, 1980) are also capable of synthesizing TX. Vascular synthesis of TXs may thus contribute to platelet aggregation and vasoconstriction. In an experiment involving the formation of rabbit aorta contracting substance and platelet aggregation factors, Needleman *et al.* (1977) reported that although PGH₁, PGH₂ and PGH₃ were formed by sheep vesicular glands, only PGH₂ and PGH₃ were converted to TX by platelet microsomes.

Thromboxanes have been implicated in the alteration of microvascular permeability from acute lung injury (Sprangue *et al.*, 1992). Recently, Sprangue *et al.* (1992) reported that the TX synthase inhibitor, OKY-046 prevents pulmonary edema induced by phorbol myristate acetate (PMA). TXs are important in alcoholic liver injury and inhibition of their production is accompanied by amelioration of liver injury. Nanji (1993) has demonstrated that compared to feeding with saturated fat and ethanol, liver nonparenchymal cell production of TXB₂ and leukotriene B₄ was higher in rats fed corn oil and ethanol. Feeding rats with corn oil and ethanol caused liver injury and the pathologic changes correlated with plasma levels of TXB₂.

Thromboxanes may also be important in synovial inflammation and certain destructive events in arthritis (Hayes *et al.*, 1994). This stems from the observation that the synthesis of the key metabolites in these conditions, 1,25-dihydroxyvitamin D₃ is increased by thromboxanes, leukotrienes and interferon gamma - a macrophage activating factor secreted by activated T-lymphocytes (Hayes *et al.*, 1994).

Fig. 1.2: Cyclooxygenase pathway for prostanoid biosynthesis

It has long been established that the PG endoperoxides are converted to only a minor extent to stable PG, PGE₂, and PGF_{2a} in certain tissues and cells such as platelets and lung tissues (Hamberg and Samuelsson, 1974). Instead the major part of the endoperoxide is converted into the highly unstable TXA₂, which is subsequently converted into the stable but inactive TXB₂ (Samuelsson et al., 1978). Thus, in order to get a reliable picture of biochemical events in these cells, it is necessary to monitor TXs instead of PGs.

Methods for the Estimation of Eicosanoids

Enzyme immunoassay (EIA) and radio-immuno-assay (RIA) have been developed for eicosanoids and their analogues including thromboxanes and TX derivatives. Biological fluids that have been employed for analyses include blood plasma/serum and urine. Tissue and isolated cells have also been analyzed. The sensitivity of EIA and RIA has increased greatly during recent years. Recently produced antibodies often have higher avidity and specificity, which may be explained by the use of more suitable carrier molecules for preparation of the conjugates, the use of better coupling methods, as well as the use of appropriate species for antibody production (Hubbard and Gould, 1988; Blake and Gould, 1984).

RIA and EIA assays have been developed for the determination of TXB₂ in various minute samples and is thus suitable for detailed kinetic studies such as the events occurring during platelet aggregation. TX measurements are conveniently carried out *in vitro*, either in platelet experiments or in perfusion studies of various organs (Samuelsson *et al.*, 1978). The difficulty of measuring TXB₂ *in vivo* stem from artifactual formation of the compound of interest during sampling, which does not allow 'peripheral plasma levels' of TXA₂ to reflect the true endogenous circulating amounts of the compound. In the case of PGs, this problem is solved by monitoring the 15-keto-13, 14-dihydro metabolites instead. These are not formed artifactually during sampling and reliably reflect endogenous levels. In the case of *in vivo* measurements of TXs, no major circulating metabolites of the compound seem to exist (Samuelsson *et al.*, 1978).

Anti-oxidants

Reactive oxygen species (ROS) are involved in the pathogenesis of a number of diseases including rheumatoid arthritis, arteriosclerosis, skin aging, nephritis, diabetes mellitus and asthma (Florence, 1995; Cerutti, 1994; Stadman and Oliver, 1991; Stadman, 1990; Steingberg *et al.*, 1989).

ROS include superoxide ion, singlet oxygen, and hydroxyl radical, which are generated from exogenous factor or as byproducts of biological reactions (Cerutti, 1991). These free radicals characteristically possess one or more unpaired electrons. Superoxide radical is formed in small amounts as oxyhemoglobin is converted to methemoglobin, and a small percentage of the electrons passing down the mitochondrial electron transport chain leak directly into oxygen (O_2), thereby producing $O_2^{\cdot-}$. Also, during the inflammatory process, inflammatory cells such as mast cells, macrophages, eosinophils and neutrophils become activated by a number of stimuli and produce superoxide radicals during the respiratory burst. This potentially dangerous free radical is acted on by superoxide dismutase and converted to hydrogen peroxide, another ROS, which is removed by catalase and glutathione peroxidase. Hydrogen peroxide can also react with free iron or copper to form hydroxyl radicals. Iron is normally tightly bound to ferritin and transferrin and copper is usually bound to ceruloplasmin. Under conditions of chronic inflammation and tissue damage on the airways, iron is liberated and can catalyze the production of highly reactive hydroxyl radicals, further exacerbating the inflammation and airways tissue damage (Chabot *et al.*, 1998; Barnes, 1990; Heffner and Repine, 1989). Aerobic bacteria produce ROS, and those that are catalase negative can be

another source of chronic exogenous hydrogen peroxide in infected airways (Gabridge *et al.*, 1985)

There are systems, both enzymatic and non-enzymatic, which are available within the airway epithelial lining fluid as local anti-oxidant defenses. These include Cu Zn, superoxide dismutase and reduced glutathione (Maynard *et al.*, 1992). The effect of the ROS on the airways depends on the availability of these local anti-oxidant defenses.

It is known that the respiratory tract anti-oxidant capacity is altered in mild stable asthma (Kelly *at al.*, 1999). Therefore a search for natural anti-oxidants and other preparation of plant origin is highly commendable.

Asthma as an Inflammatory Disorder.

Asthma is a clinically defined condition marked by recurrent, discrete episodes of reversible bronchial narrowing, separated by periods in which ventilation approaches normality (Green, 1999). It is an inflammatory disease that involves mast cells, airway epithelium, eosinophils, and neutrophils. These cells produce the broad array of mediators and cytokines that cause bronchoconstriction, mucosal edema, mucus secretion and bronchial hyper-responsiveness that characterize asthma (Lazarus, 1998).

Current guidelines for asthma therapy recommend that all patients whose asthma is more severe than mild intermittent receive chronic treatment with drugs that interrupt the inflammatory cascade mentioned above.

There is established and growing evidence that asthmatic attacks are also inflammatory responses. Most pharmacological agents used in the management of asthma, for a example, theophylline (a bronchodilator in acute and chronic asthma) and corticosteroids, have anti-inflammatory properties (Minoguchi *et al.*, 1998). Again, asthmatic airways are infiltrated with inflammatory cells that release mediators and cytokines into the microenvironment. It is believed that this inflammatory cell density in peripheral airways in severe asthma may relate to the peripheral airway obstruction that is characteristic of this condition (Maclean *et al.*, 1999; Haley *et al.*, 1998).

In fact Takami and Tsukada, (1998) reported that a marked and sustained bronchoconstriction occurs after antigen challenge in actively sensitized guinea pigs, and the bronchoconstriction observed correlated with increased TXA₂ levels in both the plasma and bronchoalveolar lavage fluid (Takami and Tsukada, 1998). , An inhibitor of TX synthase

(DP-1904) was shown to attenuate TXA₂ levels and the bronchoconstriction in a dose-dependent manner.

In platelet-depleted animals, antigen-induced bronchoconstriction and TXA₂ release in the plasma were significantly reduced compared to those of non-platelet - depleted animals, a further indication that platelets are a major source of TXA₂ production.

Leukotrienes, which are lipoxygenase products of AA metabolism, play an important role in inflammatory reactions (Bogic et al., 1998) and this has been well studied in bronchial asthma. Sulfidopeptide leukotrienes (LTC₄, LTD₄ and LTE₄) are potent bronchoconstrictors that produce mucous secretions and alter vascular permeability (Bogic et al., 1998; Drazen, 1998). They also participate in the inflammation process as well as in early and late asthmatic responses and the administration of specific leukotriene receptor antagonist or leukotriene synthesis inhibitors ameliorates the symptoms and signs of bronchial asthma (Bogic et al., 1998).

The foregoing indicates that asthmatic attacks have similar pathogenesis as other inflammatory disorders. It is in this light that inflammation is emphasized in the management of persistent asthma (Szeffler and Nelson, 1998), with anti-

inflammatory agents recommended as medications for primary long-term control of the disease (Szeffler and Nelson, 1998). One such long-term preferred medication is inhaled corticosteroids (Jatakanon et al., 1998). However recent report indicates that asthmatic patients who rely on long-term, high-dose corticosteroid therapy are at increased risk for osteoporosis (Niewoehner and Niewoehner, 1999). Again, available data provides evidence to show that the cost involved in the therapy of asthma is substantial all over the world. In Switzerland, for instance, this amounts to approximately 1,200 million Swiss France (CHF) per year (Szucs et al., 1999).

In Ghana, the cost of drugs for the management of asthma is beyond the means of many asthmatic patients who therefore turn to self-medication usually with plant medicines. At the Center for Scientific Research into Plant Medicine (CSRPM) in Ghana, the extract of various plants, e.g. *Desmodium adscendens*, *Thonningia sanguinea* and *Parquetina* sp., also known as Tina 'A' are used to treat asthmatic patients who visit the out-patient clinic of the Center. In the case of Tina A, it is reported that in some instances this plant extract provides immediate relief from the symptoms of asthma (Robertson, Personal communication). Scientific investigation into this and other local

preparations that are used in the management of asthma and other inflammatory disorders could lead to the discovery of locally affordable, efficacious and safe plant medicines that can substitute the imported and expensive orthodox drugs.

In view of the fact that eicosanoids such as TXs and LTs mediate both asthmatic and other inflammatory disorders, and the fact that anti-inflammatory and anti-asthmatic agents modulate eicosanoid metabolism, assays involving the release and/or synthesis of these eicosanoids can be used to evaluate the efficacy of herbal preparations that are used to manage asthma and other inflammatory disorders.

Medicinal Plant with Anti-inflammatory Effects

Thonningia sanguinea vahl (Balanophoraceae): This is one of the plants in a herbal preparations used prophylactically against bronchial asthma at the Center for Scientific Research into Plant Medicine. The powdered dried roots of *T. sanguinea* are used in combination with the stem or leaves of *Desmodium adscendens* for the management of asthma. The extracts of the plant were found to be anti-anaphylactic when given to guinea pigs orally. These experiments showed the extracts of *T. sanguinea* caused a decrease in the release of histamine and mepyramine-resistant spasmogens, inhibited

anaphylactic contractions in isolated ileal pieces, and reduced the sensitivity of ileal pieces to exogenous histamine (Nyarko and Addy, 1994; Addy and Nyarko, 1985). The extracts of this plant are reported to have a scavenging action for the stable free radical 1,1-diphenyl-2-picrylhydrazyl (Gyamfi et al., 1999).

Desmodium adscendens Sw. DC var. *adscendens* (Papilionaceae):

The extract of *Desmodium adscendens* has been used by herbalist and studied to some extent by some scientists. According to Ampofo (1977), the plant material is useful against poor lactation and dysmenorrhoea. There are reports that the extract of the plant is used to treat ringworm, constipation, convulsion (Ayensu, 1978), urinary problems, abdominal tumors and catarrh (Ayensu 1978). At the CSRPM in Ghana, aqueous decoction from the stem and leaves of the plant is used to manage asthma. Preparations from the roots are used as cough mixtures and also applied on wounds and abscesses.

Extracts of this plant have been found to reduce anaphylactic contractions and interfere with histamine- and antigen-induced contractions of smooth muscle. It also reduces the amounts of histamine released from lung tissue in a dose-dependent manner (Addy and Dzandu, 1986; Addy and Awumey,

1984). It was found to activate the cyclooxygenase enzyme and increase the synthesis of the anti-inflammatory prostanoid, PGE₂ (Addy and Schwartzman, 1995) and also inhibited NADPH-dependent oxygenation of arachidonic acid and AA-induced contractions of smooth muscles (Addy and Schwartzman, 1992). Several chemical constituents have been isolated from aqueous extract of different species of *D. adscendens*. Some of these are N, N-dimethyltyramine, N, N, dimethyltryptamine, salsoline (Asante-Poku *et al.*, 1988), triterpenoid glycosides dehydrosoyasaponin I (DHS-I), soyasaponin I, soyasaponin II and soyasapogenol B and E (McManus *et al.*, 1993).

***Tenacetum parthenium*:** The extract of this plant has been used in the treatment of migraine, an inflammatory disease resulting from vasospasm and dilation of intra-cranial arteries and their branches resulting from the intermittent release of hydroxytryptamine and prostaglandins (Their and Smith, 1981).

Dioscorea sp. (the yam family): Steroidal compounds with anti-inflammatory properties have been identified in many plants of this species. These include cortisone and hydrocortisone, which inhibit phospholipase A₂ and hence block AA mobilization in eicosanoid biosynthesis.

Lippia multiflora Moldenke (Family Verbanaceae); Syn. ***Lippia adoensis*** Hochst.; ***Lippia grandifolia*** Hochst. ExWalp: *L. multiflora*, also known as Power Tea (Noamesi, 1977) is a herbaceous savannah plant widely distributed in West Africa (Chanh *et al.*, 1988¹; Noamesi *et al.*, 1985). It is used on a large scale in the African folk medicine. In Ivory Coast, infusion of *L. multiflora* is used as for the treatment of hypertension (Chanh *et al.*, 1988²; Noamesi *et al.*, 1985). In Ghana some people in villages hang the dried leaves of the plant in bedrooms and the characteristic aromatic scent that emanates from the leaves is said to repel mosquitoes (Noamesi *et al.*, 1985). Leaves of the plant have also been reported to have anti-malarial, anti-viral and anti-fungal effects (Taboubi *et al.*, 1997; Benoit *et al.*, 1996; Valentin, 1995). The total phenolic extract from *L. multiflora* was found to have a dose-related inhibition of the biosynthesis of TXA₂ (Chanh *et al.*, 1988²), suggesting an anti-inflammatory action of the plant. This anti-thromboxane synthase activity may be the major contributor to the hypotensive effect of the plant.

Euphorbia hirta Linn. (Syn *Euphorbia pilulifera* Linn) also known as Australian asthma herb, Queensland asthma weed or Cat's hair. It is referred to as 'Nimakoa' (in the Twi language) in Ghana. It is a common weed in towns and

villages near drains, roadsides and waste places. Chemical compounds that have been isolated from *E. hirta* include diterpenes (phorbol esters), triterpenes, flavonoids, hydrolysable tannins, aromatic acids, alkaloids, coumarins and anthocyanidins. It is widely used as anti-asthmatic and anti-spasmodic and in some communities it is used against bronchitis, dysentry and amoebiosis (Ayiku, 1992).

CHAPTER TWO

MATERIAL AND METHODS

MATERIALS

Guinea pigs

Male guinea pigs weighing between 150 and 350g initially were initially purchased from local breeders and quarantined in large cages for at least 14 days prior to their being used for the study. The animals were maintained on a standard laboratory diet, obtained from the Tema Food Complex Ltd, Tema, Ghana, and daily rations of fresh grass (*Panicum maximum*). Drinking water, which was occasionally supplemented with vitamin C, was freely provided. The animals were kept in two main groups, A and B.

Plant Materials

The crude powdered product of *Parquetina sp.* (Tina A) was obtained from the Center for Scientific Research into Plant Medicine (CSRPM), Mampong-Akuapem, Ghana. Roots of *Cryptolepsis sanguinolenta* and leaves of *Lippia multiflora* were supplied as dried powdered material by Phyto-Riker (GIHOC) Pharmaceuticals, Ghana. Freeze-dried products of *Thonningia sanguinea* and *D. adscendens* were obtained from the NMIMR, Legon.

Chemicals and Reagents

Biorad reagent was obtained from BIORAD Life Sciences Group, CA, U.S.A. Cysteinyl-leukotriene (Cat # 520501) and thromboxane B₂ (cat # 519031) immunoassay kits were obtained from Cayman Chemical Company, Ann Arbor, U.S.A. Reduced glutathione (GSH), Bovine Serum Albumin (BSA) and arachidonic acid (AA) were obtained from Sigma Chemical Company, St. Louis, MO. USA. Other chemicals used were of analytical grade and obtained from Fluka Chemie, Switzerland.

METHODS

Preparation of Plant Extract

a) For pretreatment of guinea pigs

Amounts of plant materials used and volumes administered to the guinea pigs were based on the dosage administered to humans by CSRPM.

Approximately 9.4 g of the powdered product of *Parquetina sp.* (Tina A) was boiled in 150ml of tap water for 2 minutes. The mixture was strained and the solution stored in a refrigerator until needed. The extract which was prepared and stored this way was warmed to room temperature prior to its administration to the animals and used within five days after which the remainder was discarded.

b) For perfusion studies

Four grams of the powdered product of 'Tina A' and *T. sanguinea* were boiled in 20 ml of water for 2 minutes and strained. The extract was always freshly prepared.

c) For the *in vitro* effect on arachidonic acid metabolism

Pre-determined amounts of the plant material (*Tina A'*, *L. multiflora* and *C. sanguinolenta*) were boiled in distilled water such that concentration of the extract was 0.3g/ml. This was kept as the stock solution. For *D. adscendens* and *C. sanguinea* a solution of 100µg/ml was prepared with the freeze-dried material. All extracts were kept at 4°C for not more than three days. Appropriate dilutions were made with potassium phosphate buffer (pH 7.4) at the time of the experiment.

Pretreatment of Animals

Group A): Animals in this group were pre-treated with the extract of 'Tina A' as prepared above. The extract was administered orally using graduated syringes at a dose of 8ml/kg body weight daily. After fifteen days the animals were sensitized by injection with 2ml (1ml intra-peritoneal and 1ml sub-cutaneous) of egg albumin (100mg/ml) as antigen in normal physiological saline. This group of guinea pigs was sub-divided into two. Extract administration was continued in

one group (TT) while it was terminated in the other (TS). Eight days following the first sensitization, the animals were injected with a booster dose of the same antigen (5mg/ml) as described above.

Group B

The guinea pigs (18 for A and B) in this group without pre-treatment with the extract were sensitized with egg albumin, (100mg/ml, 1ml i.p. and 1ml s.c.) at the same time that those in group A were sensitized. Each animal received a booster dose of 5mg/ml egg albumin 8 days following the initial sensitization as described for group A.

Preparation of Isolated Organ

Fourteen days following the second injection with the antigen, each of the guinea pigs (now 300 to 500g-body weight) was anaesthetized with chloroform. The trachea was cannulated following mid-thoracotomy after which the lungs were removed and suspended in a vessel. The lungs were washed of blood and equilibrated by perfusion via the trachea with warm (37°C) Kreb's solution, gassed with 95% O₂, 5% CO₂, at a rate of 2.0 to 2.2ml/min for 10-12 min.

Sampling of Lung Perfusate

After the equilibration period (about 10-12 min.), lungs removed from pre-treated and non-pretreated guinea pigs were infused with 0.6ml egg albumin (100µg/ml) through the Kreb's stream for 3min.at approximately 0.2ml/min. The lung effluent for the first three minutes was discarded. The perfusate collected between the 4th and 5th minutes was pooled for the estimation of pro-inflammatory eicosanoids by enzyme immunoassay. This was based on previous observation by Serios *et al*, (1987) and de Nucci *et al*. (1986) that maximum concentration of eicosanoids under these conditions were reached in the perfusate 4 to 5 minutes following anaphylactic reaction.

Isolated lungs used to investigate the *ex vivo* effects of the extract were treated as follows: 1ml extract of 'Tina A' was infused (~0.2ml/min) into the isolated organs 10 minutes before antigen challenge. During the antigenic challenge, as described above, 0.5ml of the extract was continuously given as infusion into the lung. The lung perfusate collected between the 4th and 5th minutes were pooled for the estimation of pro-inflammatory eicosanoids, TXB₂ and cysteinyl leukotrienes.

After sampling for the 'Tina A', the no pretreatment (NPT) lungs were washed for ten minutes to get rid of excess 'Tina

A' in the organs. The effect of *T. sanguinea* on the release of TX B₂ was evaluated. One set of lungs was again challenged with 0.6ml of the antigen (0.2ml/min for 3 minutes) and the lung perfusate was collected between the 4th and 5th minutes. Another set of lungs was perfused with 1.0 ml of the solution of *T. sanguinea* ten minutes before the antigenic challenge. During the antigen challenge, 0.5ml of the extract was continuously given as infusion into the lungs. The lung perfusate was sampled again at the 4th and 5th minutes for the estimation of TXB₂.

Preparation of Microsomes

The method of Needleman et al. (1976) was used with the following modification. Two liters of whole goat blood was collected in 3.8% w/v trisodium citrate (TSC) to achieve a final concentration of 10% TSC. The mixture was spun at 200g for 10 min. at 4°C to remove red blood cells. The platelet rich plasma (top 2/3 fraction) was separated and centrifuged at 2000g for 15 min. at 4°C. The pellet of platelets was re-suspended in 20ml of Tris-Cl buffer (100mM, pH 7.5). The platelet suspension was subjected to three rounds of freezing and thawing to disrupt the membranes. The disrupted platelets were centrifuged at 5000g for 15 min. at 4°C using a high speed refrigerated centrifuge (Model 20PR-52D, Hitachi Koki Co., Japan) to remove cell debris. The supernatant was

centrifuged at 100,000g using an automatic preparative ultracentrifuge (Model 80P-7, Hitachi Koki Co. Ltd., Japan). The resultant pellet was suspended in 2ml of 100mM Tris-Cl, pH 7.5 and homogenized. The pellet was re-suspended in 6ml Tris buffer, and divided into aliquots and stored at -80°C.

Protein Determination:

The Bradford method for protein determination was used (Bradford, 1976): Five serial dilutions of a protein standard BSA were prepared with concentration range between 0 and 1mg/ml. A volume of 100 μ l of each standard solution and sample to be determined (diluted 1 in 50 dilution) was pipetted into clean dry test tubes. Five milliliters of diluted (1:4) dye reagent (Coomassie Brilliant Blue) was added to each tube and vortexed. All samples were incubated at room temperature for eight minutes and the absorbances were read at 595nm. The protein concentration of the sample was estimated from a standard curve generated with the protein standard.

Effect of Plant Extracts on the *in vitro* Synthesis of Pro-inflammatory Eicosanoids:

The total of 1ml reaction mixture used consisted of 0.86mg/ml microsomal protein, 0.5mM GSH (freshly prepared), and 15 μ M AA (freshly prepared) in 0.1M potassium phosphate buffer, pH

7.4. The mixture was kept in a refrigerator prior to use. It was then placed in a water bath maintained at 37°C for at least 2 minutes and the reaction was initiated by the addition of 100uL AA (15uM in 0.1M phosphate buffer, pH 7.4). For the test reaction, the mixture was pre-incubated with the plant extract (0.05 to 100 µg/ml) for 10min before initiating the reaction. The reaction was terminated after 5 minutes by the addition of 100µl of 1M citric acid to bring the pH below 3. The terminated reaction mixture was stored in a refrigerator and the amounts of eicosanoids present were determined within four hours. The pH of the mixture was adjusted to slightly alkaline range by the addition of 100µl concentrated NaOH solution just before use.

The extracts evaluated were 'Tina A', *L. multiflora*, *D. adscendens*, *C. sanguinolenta*, and *T. sanguinea*.

Quantitative Estimation of Eicosanoids

An ELISA was used for the estimation of the eicosanoids. The reaction mixture was composed of the eicosanoid released or synthesized (X) and X-acetylcholinesterase complex (known as the tracer) for a limited number of X-specific rabbit antiserum binding sites. The concentration of the tracer was held constant while the concentration of free X was varied. The complex between the X or tracer and the specific rabbit

antiserum then bind to mouse monoclonal anti-rabbit antibody that had been previously attached to the well.

After washing the wells once with wash buffer, 50µl of tracer and 50µl of antibody were put into each well. Fifty microliters of sample (lung perfusate or *in vitro* reaction mixture) were added to each well. All samples were assayed in duplicates. The plates were covered with a plastic film and incubated for 20 hours at room temperature. The wells were washed five times with wash buffer to remove any unbound reagent. Exactly 200µl of Ellman's reagent containing acetylcholine, the substrate for the acetylcholinesterase, were put into each well and incubated for 90 - 120 minutes at room temperature in the dark. The product of the reaction gave a yellow color, the intensity of which was measured at 405 - 450nm. The intensity determined this way is inversely proportional to the amount of free X contained in the samples analyzed. Concentrations of eicosanoids were calculated using software provided by Cayman Chemical Company, USA.

The eicosanoids estimated were TXB₂ for all plant extracts evaluated and cysteinyl leukotrienes for only 'Tina A' and *T. sanguinea*.

Anti-oxidant Properties of the Herbal Plant Extracts

Effect on hydroxyl radical generation

Hydroxyl radicals were generated according to the method of Suarez et al. (1998). Ascorbic acid is oxidized with Fe^{3+} and hydroxyl radicals generated combine with methyl groups donated by DMSO to form formaldehyde.

Approximately 0.375g of the freeze-dried extract of *Desmodium adscendens* and *Thonningia sanguinea* were dissolved in 10ml of 100mM potassium phosphate (PB) buffer (pH 7.4). Appropriate pre-determined amounts of powdered product of *Parquetina* sp. and *Lippia multiflora* were boiled in the buffer for two minutes such that the concentration of the extracts would be approximately 0.38g/ml. The solutions were strained using filter paper. All solutions of extracts were stored at 4°C until used. For concentration dependent assays, appropriate volumes of the extract prepared as described above were added to the buffer such that the total volume was 300µl. Mannitol was used as a standard inhibitor at a concentration of 3.0 mg/ml reaction mixture. The control was made up of just 300µl of PB buffer. Blanks were prepared for each of the assays, that is, controls, mannitol and each of the concentration of the extracts. Exactly 250µl of Fe^{3+} -EDTA mixture and 50µl DMSO (1.771ml of DMSO diluted to 50ml with PB buffer, pH 7.4) were added to each tube. 250µl of trichloroacetic acid (TCA,

1.75g dissolved in 10ml of distilled water) were added to each of the blanks and vortexed before initiating the reaction. The reaction was started by the addition of 150 μ l of freshly prepared ascorbic acid (1.761mg/ml in PB, pH 7.4). Each tube was incubated at 37°C for 3.5 hours. The reaction was stopped by the addition of 250 μ l of TCA. One ml of freshly prepared Nash (1953) reagent (made up of 15g ammonium acetate, 0.2ml acetylacetone and 0.3ml acetic acid made up to 100ml with distilled water) was added to each of the reaction mixtures and each tube incubated for 45 minutes at 37°C. Absorbance was immediately read at 412nm. Results were expressed as percentage inhibition of formaldehyde formation in the presence of the extract: i.e. $(ABS_{\text{control}} - ABS_{\text{test}})/ABS_{\text{control}} \times 100$.

Quantitative Determination of Total Phenols

Total phenolic content was determined by the method of Lowman and Box (1983) with the following modifications. Concentrations of 0.3g/ml plant extract solutions were made with distilled water. A volume of 83.6 μ l of this solution was made up to 25ml with 50% v/v methanol giving a final concentration of 0.1mg plant extract solution. The solutions were boiled for 3 minutes and made up to 25ml with 50% v/v methanol. Five millilitres of each solution was diluted to 25ml with de-ionized water. Successive addition of 1.50ml of

Na_2CO_3 (200g/l) and 0.50ml 2N Folin-Ciocalteu reagent was made to each mixture and absorbance at 750nm read after one hour. Catechin was used as the standard and the amounts of phenols expressed as mg catechin equivalents per gram plant extract.

Quantitative Determination of Water Soluble Phenols

Plant extracts and catechol (0.4mg/ml) were prepared in 50% v/v methanol and allowed to stand at room temperature for 24 hours. The solutions were centrifuged at 3500rpm for 5 minutes and filtered with a filter paper. A volume of 1.50ml Na_2CO_3 and 0.5ml of 2N Folin-Ciocalteu's reagent were added to each solution and left at room temperature for one hour. Absorbance was read at 750nm. The content of water-soluble phenols were calculated as mg catechin equivalents per gram plant extract.

Statistical Analysis

The student t-test or analysis of variance were used for the statistical analysis in the report. Statistical significance was calculated at $p \leq 0.05$.

CHAPTER THREE

RESULTS

A: Release of Eicosanoids

Effects of 'Tina A'

The effects of *Tina A* on the release of thromboxane B₂ (TXB₂) and cysteinyl leukotrienes (cyst.LT) during anaphylaxis in guinea pig lungs are presented in Figs 3.1 and 3.2.

Fig. 3.1 shows the effects of *Tina A* on the release of TXB₂. In these figures NT denotes the group of animals that were not treated with the extract. TS refers to the group of guinea pigs that were pre-treated with the extract before sensitization, while TT applies to the group that were given the extract before sensitization and throughout the experiment.

The figure shows that the highest amount of TXB₂ was released in the NT group. Within this group the amounts of TXB₂ released from lungs with and without extract infusion were significantly different ($p = 0.05$). Infusion of the extract into the isolated lungs caused 25.4% decrease in the amount of TXB₂ released. Amounts of thromboxanes released from the TS set of guinea pigs were lower than the NT group. Among these animals, infusion of the extract caused a

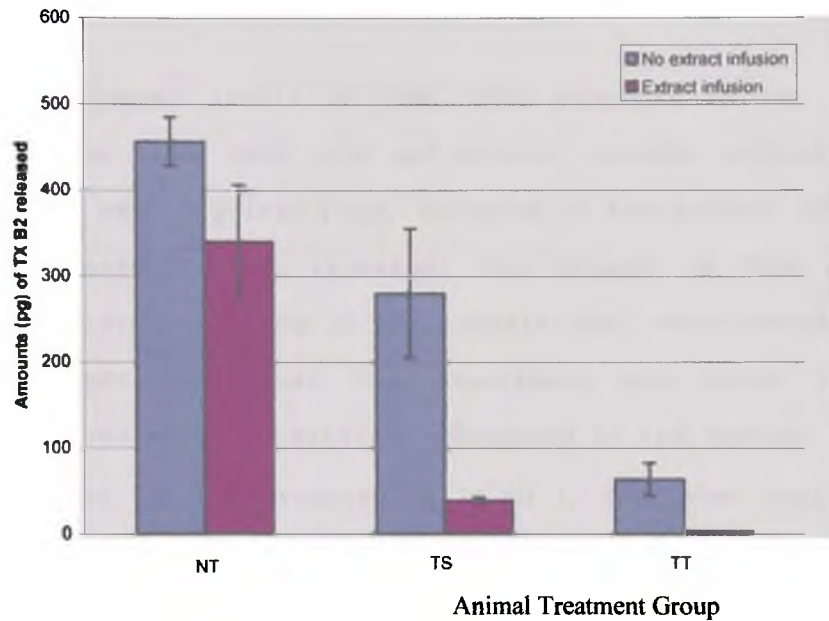


Fig. 3.1: Effect of *Parquetina sp.* on the release of cysteinyl-leukotriene from guinea pigs lungs during anaphylaxis . (The bars represent the mean of 3 experiments and the vertical lines the S.E.M)

NT: Animals in this group were not treated with the extract

TS: Animals pre-treated with the plant extract for two weeks before sensitization

TT: These animals were put on plant extract throughout the experiment.

significant decreased (86.1%) in TXB₂ levels: from 280±75 to 38±4, giving approximately 86.1% decrease in the release of the eicosanoid.

The lowest levels of TXB₂ were recorded in the TT set of guinea pigs, both with and without extract infusion. Within this set of guinea pigs, infusion of the extract caused 95.3% decrease in TXB₂ released. The release of TXB₂ was almost completely blocked in the animals that were treated with the extract throughout the experiment and whose lungs were infused with the extract. Compared to the control value, the amount of TXB₂ produced, 2.33 ±0.3, indicates that there was 99.32% decrease in the amount of TXB₂ released.

The effect of the extract on the release of cysteinyl-leukotriene (cyst.LT) is shown in Fig. 3.2. For the NT group, infusion of the extract caused a highly significant decrease (79.4%) in the amount of cyst.LT released, compared with a decrease of 25.4% in the case of TXB₂. Among the TS group of animals, contrary to the results on the release of TXB₂, and the results obtained for the NT group, there was no significant decrease (42.5%) in the amount of cyst.LT released between the with and without extract infusion. Within the TT group of animals, even though there was a

significant decrease (46.8%) in the amount of cyst.LT during extract infusion, the decrease was not as drastic as it was with the NT group.

It is seen from Fig. 3.1 and Fig. 3.2 that generally there was more TXB₂ released under the conditions of the experiment than cysteinyl leukotrienes, e.g. for the NT group of animals 457pg of TXB₂ and 1443pg of cysteinyl-leukotriene were released in the absence of the extract.

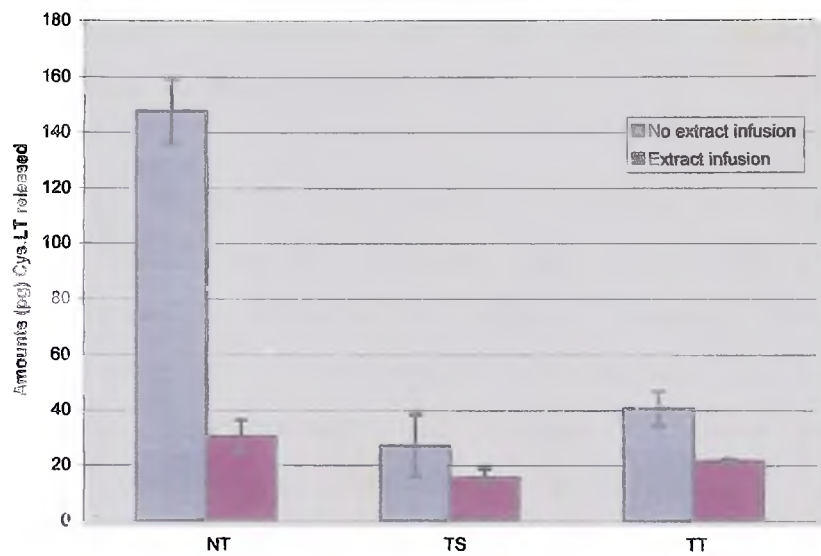


Fig 3.2: Effect of *Parquetina sp.* on the release of cysteinyl-leukotriene from guinea pigs lungs during anaphylaxis . (The bars represent the mean of 3 experiments and the vertical lines the S.E.M).

Under various conditions of treatment the comparative effect of 'Tina A' on the release of thromboxane B₂ and cysteinyl-leukotriene is shown in Table 3.1. It is seen from the data whenever the animals received treatment prior to sensitization (TS) or throughout the sensitization period, subsequent perfusion with the extract caused a higher decrease in TXB₂ than cyst.LT.

In the absence of perfusion, LT release was more affected than TX in the TS. However, both LT and TX release were significantly affected in animals treated throughout the experiment (TT). The cyst.LT infusion was just as effective as the pre-treatment but further treatment was not as effective, and actually reduced effectiveness. For the NT set of animals, perfusion with the extract effected the release of LT more than the release of TXB₂.

Table 3.1:Effect of *Tina A.* on the release of TXB₂ and Cysteinyl.LT under different conditions of treatment. LT values are shown in parenthesis.

Treatment	% Inhibition of release TXB ₂		
	None (NT)	TS	TT
Between treatment groups without infusion ^a	-	38.7 (81.6)	86.1 (72.5)
Within same treatment group as a result of extract infusion ^b	25.4 (79.4)	86.1 (42.5)	95.3 (46.8)
Between different treatment groups with extract infusion ^c	-	88.6 (48.9)	99.3 (29.0)

Values were computed as follows: $a = \frac{NT(ni) - T_{-}(ni)}{NT(ni)} \times 100$

$$b = \frac{ni - ei}{ni} \times 100$$

$$c = \frac{NT(ei) - T_{-}}{NT(ei)} \times 100$$

where ni denotes no infusion, ei infusion and T₋ = TS or TT

Effect of *T. sanguinea* on the release of thromboxane B₂

TXB₂ levels from the NT group after antigenic challenge of the isolated lungs perfused with the Kreb's solution with and without *T. sanguinea* were 200 ± 11pg and 305 ± 18.5pg respectively. This value indicates approximately 34% decrease in the release of the eicosanoid in response to infusion with *T. sanguinea* compared to 25.4% for 'Tina A'.

B: Synthesis of Eicosanoids from Arachidonic Acid.**Thromboxane synthesis**

The effect of the different concentrations of herbal extracts on the *in vitro* synthesis of thromboxane B₂ from exogenous arachidonic acid is presented in Fig. 3.3. The figure shows a concentration dependent effect of the different preparations on platelet-microsome mediated synthesis of TXB₂

Compared to the control value, *D. adscendens* at 1, 10 and 100µg significantly decreased the amounts of TXB₂ produced. At the lowest concentration of *D. adscendens* (1µg/ml), there was 66% decrease in TXB₂ production compared to the control. The extract at 100µg/ml caused approximately 81% inhibition in TXB₂ synthesis.

Parquetina sp caused an effect similar to that caused by *D. adscendens*. However, unlike *D. adscendens* and 'Tina A', more TXB₂ was produced at 10 and 100µg/ml of *L. multiflora* than at 1µg/ml, the highest concentration having the least effect. For *T. sanguinea*, while up to 10µg/ml did not show a significant decrease in TXB₂ synthesis, 100µg/ml of the extract gave a percentage decrease as much as those of *D. adscendens* and 'Tina A'.

At all the concentrations used, *C. sanguinolenta* had no significant effect on the synthesis of TXB₂. There was only 4.3% inhibition of TXB₂ synthesis at the highest concentration (100µg/ml) of the extract.

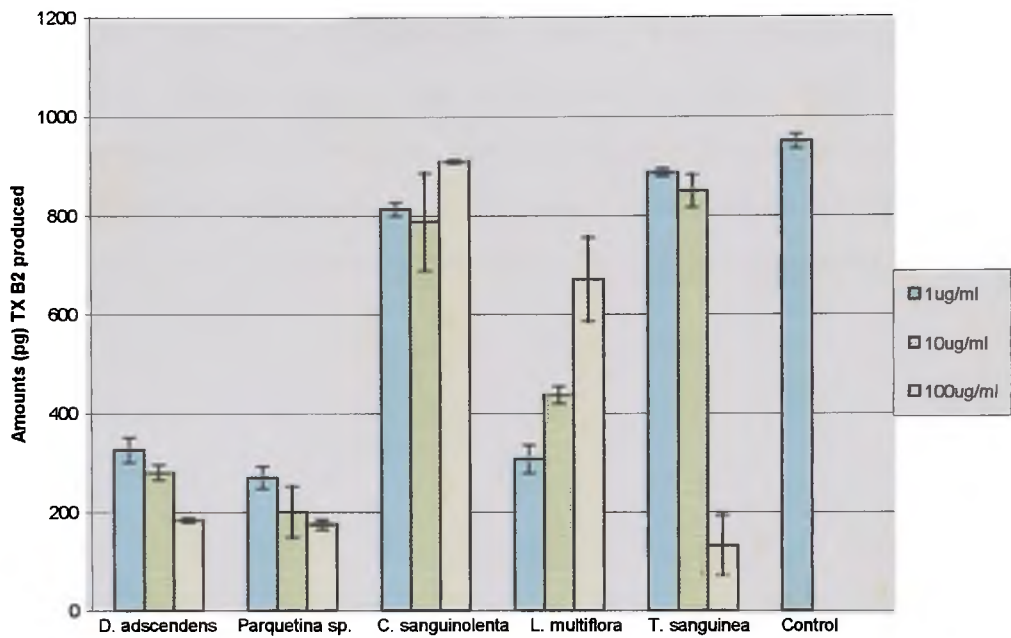


Fig. 3.3: Effect of plant extracts on the *in vitro* synthesis of TX B₂ from arachidonic acid using platelet microsomes. (The bars represent the mean of three experiments and the vertical bars the S. E. M)

Based on the data presented in Fig. 3.3, the experiment was repeated for 'Tina' A, *L. multiflora*, and *T. sanguinea* using a different set of concentration range. The results for this set of experiment are shown in Fig. 3.4 and Table 3.2. With Tina A. (Fig. 3.4), generally there was a concentration dependent inhibition of the synthesis of TXB₂. Even though the levels at 0.1 and 0.3 mg/ml extract concentration were significantly different from the control, no major effect was observed until extract concentration reached 1mg/ml and above.

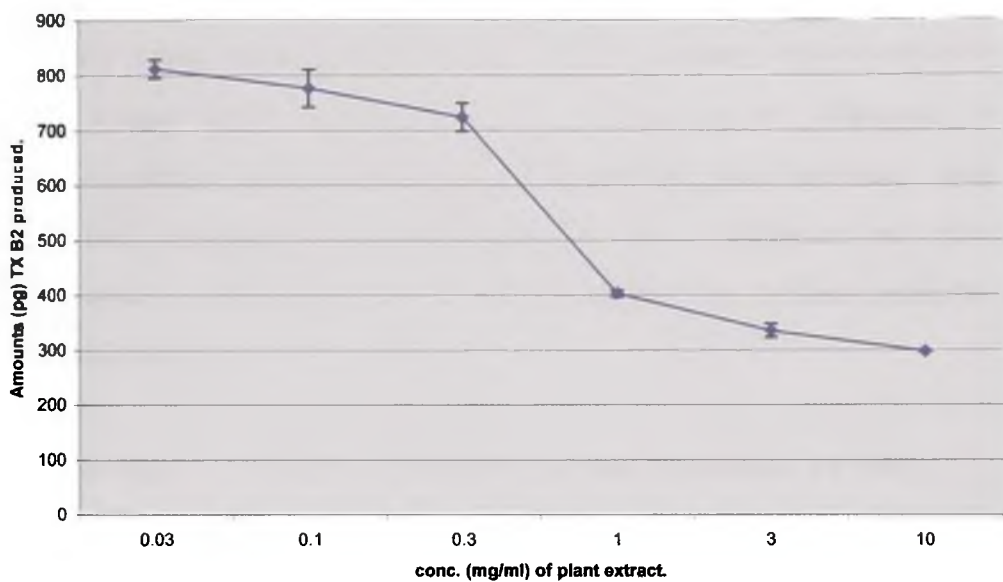


Fig. 3.4: Effect of 'Tina A' on the *in vitro* synthesis of TXB₂ from arachidonic acid using platelet mocosomes. n=3.

For *L. multiflora*, (Table 3.2) at a lower concentration of 0.3µg/ml, there was a significant decrease in the synthesis of TXB₂. Higher concentrations of 10 and 30µg/ml were not as effective. The most effective concentrations are 1 and 3µg/ml confirming previous results of higher concentrations not being more effective. *T. sanguinea* at 30µg/ml, a concentration between 10µg/ml and 100µg/ml that had been used earlier, caused 54.1% decrease in the synthesis of TXB₂ compared to values of 10.6% and 86.2% for the 10 and 100µg/ml respectively.

Table 3.2: Effect of extract on the synthesis of TXB₂.

Extract concentration (µg/ml)	Amount of TXB ₂ produced (pg)	
	<i>T. sanguinea</i>	<i>L. multiflora</i>
0 (Control)	860±19	860±19
0.3	nd	573.7±21 (33.4)
1.0	nd	494.7±6 (42.6)
3.0	752.1±13 (12.6)	486.2±14 (43.5)
10.0	718.0±20 (16.5)	515.3±16 (40.1)
30.0	391.5±8 (54.5)	511.4±12 (40.6)

(Values are means ± SEM for N = 3). nd denotes not determined
Values in parenthesis are percentage decrease compared to control

Cysteinyl leukotrienes

The effects of 'Tina A' and *T. sanguinea* on the synthesis of cysteinyl-leukotrienes from arachidonic acid were studied. The results shown in Table 3.3 indicate that both extracts at concentrations of 10 and 100µg/ml caused significant decrease in the amounts of cyst.LT synthesised. At the lower concentration, 10µg/ml, 'Tina A' caused a higher percentage inhibition (52.4%) than *T. sanguinea* (18.0%). At the higher concentration of 100µg/ml, *T. sanguinea* caused 95.3% inhibition while 'Tina A' caused 91.7 % inhibition.

Table 3.3: The effects of 'Tina' A and *T. sanguinea* on leukotriene synthesis. (Values are means + SEM of three determinations.)

Extract	Concentration (µg/ml)	Amount (pg) of cyst.LT synthesized
Control	0	607± 17.6
'Tina A'	10	289.± 9.4 (52.4)
'Tina A'	100	50± 10.7 (91.8)
<i>T. sanguinea</i>	10	498± 7.1 (18.0)
<i>T. sanguinea</i>	100	29± 5.0 (95.2)

Values are mean + SEM of three determinations

Values in parenthesis are percentage inhibition compared to control

C: Effect of Plant Extracts on Hydroxyl Radical Generation

The effect of the extracts on the generation of hydroxyl ion was investigated (fig. 3.5). The results show a concentration dependent inhibition of hydroxyl radical generation. At all the concentration of extracts used, *T. sanguinea* caused the highest percentage inhibition of hydroxyl radical generation while *D. adscendens* gave the least percentage inhibition. The standard inhibitor, mannitol, at 3mg per ml of reaction mixture caused 20% inhibition of hydroxyl radical formation. At that concentration all the extracts but *D. adscendens* caused a percentage inhibition greater than mannitol.

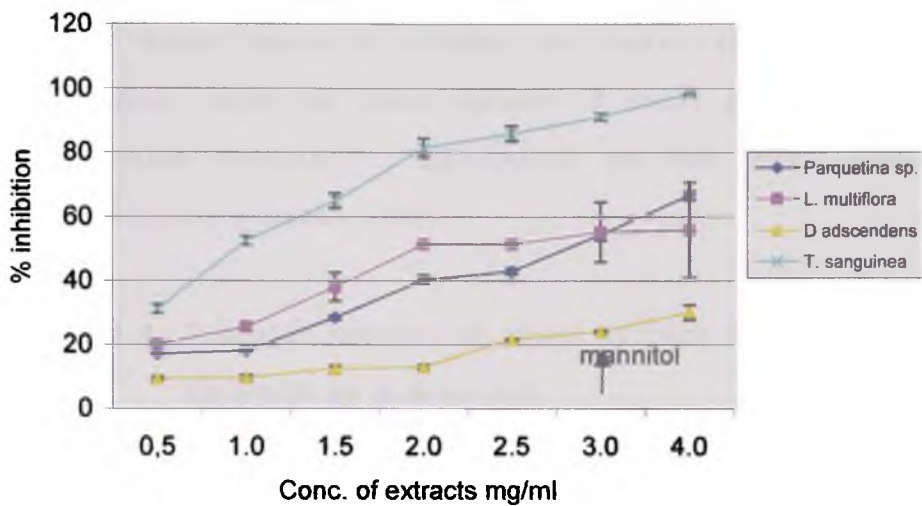


Fig. 3.5: Effects of plant extracts on the generation of hydroxyl radical.
(The arrow in the figure shows mannitol at 3mg/ml)

D: Total and water Soluble Phenols

The total and water-soluble phenolic content of the plants extract (as mg catechin equivalent) are presented in Table 3.4. *T sanguinea* had the greatest amount of total phenols with approximately 31% being water soluble. For 'Tina A', 74% of total phenolic content was water soluble while *D. adscendens*, with the least amount of total phenols, had 51% being water soluble. *L. multiflora* had the least amount of water-soluble phenols (18%).

Table 3.4: Phenolic content of plant extract using (+) - catechin as a standard.

Extract	Total	Water-soluble
<i>D. adscendens</i>	106± 8	53.7±7.5
'Tina A'	211±11	156.4±9.0
<i>L. multiflora</i>	308±9	54.8±7.6
<i>T. sanguinea</i>	390±37	121.1±6.2

Values are expressed as mg catechin equivalent/g plant extract

In Table 3.5, a correlation is made between the total phenolic content and the percentage inhibition of hydroxyl radical generation at extract concentration of 4mg/ml. *T. sanguinea* which has the highest amount of total phenols also shows the highest effect on hydroxyl radical generation, giving approximately 100% inhibition at a concentration of 4mg/ml. *D. adscendens* has the lowest amount of total phenols and the least effect on formation of hydroxyl radicals.

Table 3.5: Total phenolic content in mg catechin equivalent/g plant extract compared with the % inhibition at 4mg/ml extract

Extract	Total phenols	% inhibition at 4mg/ml
<i>D. adscendens</i>	106± 8	30.1
'Tina A'	211±11	55.5
<i>L. multiflora</i>	308±9	66.8
<i>T. sanguinea</i>	390±37	99.0

CHAPTER FOUR

DISCUSSION AND CONCLUSION

Several investigations have been carried out in the Department of Biochemistry and the Noguchi Memorial Institute for Medical Research (NMIMR), both of the University of Ghana to investigate the therapeutic basis for the actions of some herbal preparations used to treat various diseases, including inflammatory diseases, particularly asthma. Arachidonic acid metabolism has been used in these studies on the premise that a putative anti-inflammatory plant material will modulate the metabolism of AA such that more anti- and less pro-inflammatory eicosanoids will be synthesized in the presence of the extract. Results from these investigations have shown that some herbal preparations significantly increased anti-inflammatory eicosanoids such as PGE₂ and PGI₂ (Addy and Schwartzman, 1995).

Anti-inflammatory materials could also block the synthesis/release or action of pro-inflammatory eicosanoids (Dadzie-Mensah, 2000). Yet in these studies the extracts did not significantly decrease the pro-inflammatory eicosanoid TXB₂ (Dadzie-Mensah, 2000). The present studies were conducted with the view to optimizing thromboxane synthesis and to use its inhibition as an assay to screen putative anti-inflammatory medicinal plant extracts. The release and

in vitro biosynthesis of pro-inflammatory mediators were investigated and the effects of putative anti-inflammatory medicinal plants evaluated. Two methods were used: the release of two pro-inflammatory eicosanoids TXB₂ and cysteinyl leukotrienes during anaphylaxis in guinea pig lung and their *in vitro* biosynthesis from AA.

Isolated lungs taken from immunologically sensitized guinea pigs perfused via the trachea were used as a model to study the release of eicosanoids during anaphylaxis and the inhibition of the release by medicinal plant extracts. In conditions like asthma or hyaline membrane disease, the cells of the alveolar surface of the lungs play a major role in the development of the pathophysiology (de Nucci et al, 1986). Perfusion via the trachea thus provided a better way of studying these cells, including investigation of the effects of external agents on mediators released by the cells. The results of these experiments showed that antigen delivered via the airways to the isolated sensitized guinea pig lung induced the release of pro-inflammatory eicosanoids such as TXB₂ and cysteinyl leukotrienes.

'Tina A', one of the extracts studied, decreased the release of TXB₂ and cysteinyl-leukotrienes and the inhibition of release was time-dependent. Thromboxane and leukotriene

levels in animals that were not pre-treated with the extract were higher than the amounts from the set of guinea pigs that were pre-treated with the plant extract.

Dadzie-Mensah (2000) reported that among three extracts (*Cassia sieberiana*, *Tina A* and *Desmodium adscendens*) studied, *Tina A* was the most effective best in terms of inhibition of Phospholipase A₂ (PLA₂). Since the release of AA is the rate limiting step in the production of eicosanoids (Loeb and Gross, 1986), the lowering of the amounts of TXB₂ and cysteinyl leukotrienes observed in the present study could be due to inhibition of PLA₂ activity. It could also be due to induction of PLA₂ isoforms with reduced activity, induction of inhibitors of PLA₂ or inhibition of the key enzymes involved in the synthesis of these pro-inflammatory eicosanoids.

On lungs that had been challenged and used to evaluate the effects of 'Tina A', perfusion with *T. sanguinea* caused an even higher percentage decrease in the release of TXB₂. This experimental design was adopted due to limited space and time for another set of experimental animals to evaluate the effects of *T. sanguinea* on the release of TXB₂. The result showed that so far as the Kreb's flow is maintained and the

organs remain viable, a second wave of anaphylactic shock was possible and the release and inhibition of release of inflammatory mediators could be observed. This is consistent with earlier investigation in which, using the Okpako's modification of the Schultz-Dale's reaction (Okpako, 1970), Addy and Nyarko (1994) showed that anaphylactic contractions can be measured in repeatedly challenged sensitized guinea pig ileal tissues.

Both pre-treatment of the animals and perfusion of the isolated lungs with the extracts of either 'Tina A' or *T. sanguinea* caused a decrease in the levels of both TXB₂ and cysteinyl-leukotriene. In the animals that were treated with the extract throughout the experiment, there was a pronounced inhibition of TXB₂ release. With the introduction of the extract *ex vivo*, there was almost a complete inhibition of the release of TXB₂. Pre-treatment of the animals with the extract prior to sensitization is analogous to the use of the extract as a prophylactic. This indicates that the extracts could be used for both prophylactic and curative purposes.

It appears from the results that generally infusion of the extract, that is short-term (or 'acute') administration has more inhibitory effect on Cyst-LT than it has on TXB₂ release while long-term treatment of the animals with the extract has

more inhibitory effect on TX than LT synthesis. There are different pathways involved in stimulus-induced arachidonate mobilization. The two major phospholipid classes utilized are phosphatidylinositol and phosphatidylcholine and the mobilization of these phospholipids involves either diacylglycerol lipase/phospholipase C or phospholipase A₂ pathways. Evidence indicates that the PLA₂ pathway which is quantitatively the most important (Bettazoli *et al.*, 1990; Dennis, 1987) is the major pathway for AA mobilization for thromboxane biosynthesis. Again thromboxanes and leukotrienes are synthesized via the cyclooxygenase and lipoxygenase pathways of AA metabolism respectively. Selective inhibition of either of these pathways at different points in the experiment could account for the different levels of inhibition of TXB₂ and the cysteinyl leukotrienes.

The cysteinyl-leukotrienes are potent mediators of airway narrowing. In lower animals and humans, the cysteinyl-leukotrienes are among the most potent airway contractile substances ever identified (Drazen, 1998). Inhibition of the synthesis of the Cyst-LTs or prevention of their action at the cysteinyl LT receptor is associated with an improvement in the airway dysfunction that occurs in both induced and spontaneous asthma. The reduction in the amounts of the eicosanoids released by the administration of the extracts

validates the use of these extracts as folk remedies for the management of asthma and inflammatory related diseases.

In addition to the release of the two pro-inflammatory eicosanoids, the effects of 'Tina A', *T. sanguinea* and other medicinal plants on their *in vitro* synthesis were also investigated.

In earlier investigations in which microsomes were used as a source of the cyclooxygenase enzyme, the microsomes were prepared from either the lung or the kidney. In all of these investigations, thromboxane synthesis has never been optimized. Knowing that the thrombocytes are the major sites of TX synthesis, microsomes were prepared from blood platelets as a source of the cyclooxygenase enzyme in this study. The results showed that each of 'Tina A', *D. adscendens*, and *T. sanguinea* exhibited a concentration-dependent inhibition of TXB₂ synthesis. Earlier experiments showed that 'Tina A and *D. adscendens* increased the synthesis of the anti-inflammatory prostanoids, PGI₂ and PGE₂ (Dadzie-Mensah, 2000). PGI₂ is anti-thrombogenic while TXA₂ is pro-thrombogenic. Therefore a balance between the amounts of PGI₂ and TXB₂ is necessary for blood vessel smooth muscle tone. It appears that in addition to the use of these extracts to manage asthma, they could be useful against

hypertension and other cardiovascular pathologies including stroke.

For *D. adscendens*, previous work done in which microsomes were incubated with the extract established that the anti-inflammatory effect was due to the activation of the cyclooxygenase enzyme to increase the synthesis of anti-inflammatory PGs such as PGE₂ and PGI₂ (Dadzie-Mensah, 2000; Addy and Schwartzman, 1995). Among three extracts investigated previously, *D. adscendens* was the best anti-inflammatory product with respect to enhancing anti-inflammatory eicosanoid synthesis (Dadzie-Mensah, 2000). However in that study the effect of *D. adscendens* on TXA₂ synthesis was not significant. This led to the conclusion that *D. adscendens* works through a mechanism different from that of aspirin. Aspirin, a known NSAID, acts by both selectively blocking TXB₂ synthesis and inhibiting the prostacyclin endoperoxide synthase (Cairns *et al.*, 1985; Lewis *et al.*, 1983). The result in the present study suggests that the effect of *D. adscendens* may be similar to aspirin in terms of inhibition of TXB₂ synthesis.

At lower concentrations, *T. sanguinea*, did not inhibit the synthesis of TXB₂. However, at 100µg/ml, the highest concentration used, the effect was more pronounced than was

observed for both *D. adscendens* and 'Tina A'. That means that even though *T. sanguinea* is efficacious against the synthesis of TXB₂, a higher concentration is needed to achieve the same effect as that caused by *D. adscendens* or 'Tina A'. Similarly at 100µg/ml the decrease in LT synthesis in the presence of *T. sanguinea* was higher than that observed in the presence of 'Tina A'. *T. sanguinea* has been shown to decrease the release of histamine and slow reacting substances of anaphylaxis (SRS-A) and to decrease exogenous histamine-induced contractions in ileal pieces from sensitized guinea pigs (Nyarko and Addy, 1994; Addy and Nyarko, 1985). These studies used classical pharmacological tools in the evaluations. As a result the identities of the SRS-A released were not determined. Evidence indicates that the SRS-A comprises the cysteinyl leukotrienes (LTC₄, LTD₄ and LTE₄) which are involved in inflammatory disorders (Samuelsson, 1983). Therefore the current result which shows *T. sanguinea*-mediated decrease in LT synthesis/release is consistent with the previous findings.

Lippia multiflora, an anti-hypertensive plant (Chanh et al., 1988) inhibited the synthesis of TXB₂ at all concentrations studied. Increasing concentration of the extract however resulted in decreasing inhibition of synthesis of TXB₂. The reason for this is not clear but it is likely that the crude

extract contains some components, which at higher concentrations, counteract the inhibitory effect or tilt arachidonic acid metabolism in favor of thromboxane synthesis. Crude plant extracts contain not only one but many chemical compounds that are secondary plant metabolites with different and possibly opposing effects. It is for this reason that in certain instances a crude plant preparation may have several pharmacological effects on a particular tissue or organ.

That *L. multiflora* decreases TXB₂ synthesis is not new. This has been shown by Chanh *et al.* (1988). However because that experiment was carried out using only one concentration of the extract, the decreasing effect with increasing concentration of the extract was not reported. The present study cautions that whenever the extract is administered for its anti-inflammatory properties, the right dosage must be prescribed. This is one of the major reasons why scientific study into the therapeutic action of these indigenous herbal preparations is important.

The extract of *C. sanguinolenta* did not show any significant effect on TX synthesis in this study. This plant is used in Ghana for its anti-microbial and anti-malarial properties

(Ayiku, 1992). It therefore served as negative control in the study.

Leukotrienes are involved in several immune-mediated inflammatory reactions of anaphylaxis (Samuelsson, 1984). They cause slowly-evolving but protracted contractions of smooth muscles in the airway and gastro-intestinal tract and enhance capillary permeability (Samuelsson, 1983). Thromboxanes are involved in smooth muscle contraction, platelet aggregation, serotonin release and vasoconstriction. These pathological conditions are associated with inflammation. The ability of these extracts to decrease the amounts of these eicosanoids validates their use in managing inflammatory disorders in Ghana.

It is well established that asthma is an inflammatory disorder of the airways in which the release of mediators from activated mast cells and eosinophils plays a major role (Green, 1999). The inflammatory process results in endogenously generated oxidative stress that contributes to tissue injury and asthma symptomatology. Exogenous oxidants also exacerbate existing asthmatic manifestations (Green, 1999). The effect of the oxidants on airways will depend on the local anti-oxidant defenses available within the airway epithelial lining fluid. Since respiratory tract anti-

oxidant capacity is altered in mild, stable asthma, (Kelly et al., 1997), medicinal plants, which have substantial anti-oxidant properties, could play an important protective role in the management of this condition.

The study has revealed that the extracts have anti-oxidant properties; they inhibited the generation of hydroxyl radicals. There could be diverse mechanism by which the extracts decrease the amounts of hydroxyl radicals generated. This could be via direct inhibition of hydroxyl radical generation, or through the mopping up of generated hydroxyl radicals or both. Further studies would be needed in this direction to identify the mechanisms involved. In a study that was conducted to evaluate the anti-oxidant action of five plant extracts, *T. sanguinea* exhibited the highest scavenging action for the stable free radical 1,1-diphenyl-2-picrylhydrazyl (Gyamfi et al., 1999). In that study the free radical was formed in the reaction mixture so the decrease was an indication of an actual mopping up action. It is therefore reasonable to conclude that in the present study, *T. sanguinea* could be a scavenger for the OH radicals.

Among the four extracts investigated, *T. sanguinea*, which had the highest level of total phenols, gave the highest percentage inhibition of the hydroxyl radical generation. In

the *in vitro* experiment on eicosanoid synthesis, *T. sanguinea* caused the highest percentage inhibition of the synthesis of both TXA₂ and cysteinyl leukotrienes compared to 'Tina A' at the highest concentration of extract used. *D. adscendens*, which showed the least phenolic content, produced the least effect on reactive oxygen species (ROS) generation. The result showed a positive correlation between total phenolic content of the extracts and inhibition of ROS production.

Most of these plants used in the management of asthma are administered as decoctions and infusions. Therefore, the biologically active components are most likely water-soluble. It was for this reason that the water-soluble phenolic content of the extracts was determined. The result obtained does not show any correlation between the content of water-soluble phenols and inhibition of ROS generation.

In conclusion, the use of isolated guinea pig lungs and blood platelet microsomes provided good bioassays for evaluating the effects of anti-inflammatory plant extracts on the release or synthesis of TXB₂ and cysteinyl leukotrienes. There was inhibition of the release of TXB₂ and cysteinyl LT when the animals were treated with the extract of 'Tina A' prior to their being used for the study or when isolated

lungs were perfused with the solutions of 'Tina A' and *T. sanguinea*. Generally the treatment of the animals with the extracts decreased the release of TXB₂ more than LTs while perfusion of the isolated lungs decreased the release of LTs more than TXB₂. Extracts of *D. adscendens*, 'Tina A' and *T. sanguinea* inhibited the synthesis of TXB₂ from AA using platelet microsomes in a concentration dependent manner. *L. multifora* inhibited the synthesis of TXB₂ but lower concentrations were more effective than higher concentrations.

All four plant extracts studied inhibited the release of free radicals and had high amounts of phenolic compounds indicating their anti-oxidant properties. There was a positive correlation between total phenolic content and inhibition of free radical generation.

The inhibition of synthesis or release of TXB₂ and cyst.LT and generation of free radicals by these extracts provide the basis for their therapeutic action against inflammatory diseases. It is anticipated that in addition to these laboratory studies clinical investigations will be carried that and will lead to the discovery of herbal preparations

that are therapeutically effective even for diseases in which conventional approaches are ineffective.

Reference :

Addy, M.E. and Awumey, E.M.K. (1984) Effects of the extracts of *Desmodium adscendens* on anaphylaxis. *J. Ethnopharm.* **11**: 283-292.

Addy, M.E. and Burka, J.F. (1988) Effect of *Desmodium adscendens* fractions on antigen- and arachidonic acid-induced contractions of guinea pig airways. *Can. J. Physiol. Pharmacol.* **66**: 820-825.

Addy M.E. and Dzandu W.K. (1986) Dose-dependent effects of *Desmodium adscendens* aqueous extract on histamine response, content and anaphylaxis. *J. Ethnopharm.* **18**:13-20.

Addy, M.E. and Gbewonyo, W.S.K. (1980). Inhibition of anaphylaxis by an extract of the anti-asthmatic plant, *Desmodium adscendens*. *Ghana Med. J.* **5**. (19), 193.

Addy, M.E. and Nyarko, A.K. (1985) Anti-anaphylactic properties of *Thonningia sanguinea*. *Planta Medica.* **5** 360-363.

Addy, M.E. and Schwartzman, M.L. (1995). An Extract of *Desmodium adscendens* activates cyclooxygenase and increases

prostaglandin synthesis by RAM seminal vesicle microsomes. *Phytother Res.* **9**: 298-293.

Addy, M.E. and Schwartzman, M.L. (1992). An extract of *Desmodium adscendens* inhibits NADPH-dependent oxygenation of arachidonic acid by kidney cortical microsomes. *Phytother. Res.* **6**: 245-250.

Ali, M. and Mohammed, S.Y. (1986). Selective suppression of platelet thromboxane formation with sparing of vascular prostacyclin synthesis by aqueous extract of garlic in rabbits. *Prostaglandins, Leukotrienes and Medicine* **25**: 139-146

Ampofo, O. (1977). Traditional medicine: Some examples of its use. *Plants That Heal. WHO Chronicle* **9**: 287-293.

Anokbongo, W.W. (1992). The role of African traditional medicine in health-care delivery alongside modern medicine. In: *Botany 2000: East and Central Africa. NAPRECA Monograph series No. 5* Edwards, S., Asfaw, Z. (eds) NAPRECA, Addis Ababa pp 25-35.

Asante-Poku, S., Sakakibara, J. and Addy, M.E. (1988). Alkaloids of *Desmodium adscendens*. *Ghana Med J.* **22**(4): 111-115.

Ayensu, E.S. (1978). Medicinal plants of West Africa. Reference Publications, Inc., Michigan. USA pp 149-152.

Ayiku, M.N.B. (1992). Ghana Herbal Pharmacopoeia. The Advent Press, Ghana. pp 1-185.

Barnes, P.J. (1990). Reactive oxygen species and airway inflammation. *Free Rad. Biol. Med.* **9**: 235.

Benoit, F., Valentin, A., Pelissier, Y., Diafouka, F., Marion, C., Kone-Damba, D., Kone, M., Mallie, M., Yapo, A. and Bastide, J-M. (1996). *In vitro* anti-malarial activity of extracts used in West African traditional medicine. *Am. J. Trop. Med. Hyg.* **54**: 76-71.

Bettazzoli, L., Zirrolli, J.A., Reidhead, C.T., Shahgholi, M., Petro, M.A. and Robinson, L.T. (1990). Incorporation of arachidonic acid into glycerophospholipids of a murine bone marrow derived mast cells. *Adv. Prostaglandin, Thromboxane and leukotrienes Res.* **20**: 71-78.

Bhagavan, N.V. (1992). *Medicinal Biochemistry*. Jones and Bartlett Publishers Inc. pp 411-419.

Blake, C. and Gould, B.J. (1984). Use of enzymes in immunoassay. *A Review Analyst*. **109**: 533.

Blunting, S., Moncada, S., and Vane J.R. (1983). The prostacyclin-thromboxane A₂ balance - pathophysiological and therapeutic implication. *Br. Med Bull.* **39**: 371.

Bogic, M., Raskovic, S., Peric-Popadic, A., Tomic-Spiric, V., Andrejevic, S., and Jovicic, Z. (1998). Leukotrienes in allergic inflammatory reactions. *Srp. Arch. Celok. Lek.* **126**: (9-10) pp 382-387.

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248-254.

Cairns, J.A., Gent, M., Singer, J., Finnie, K.J., Floggart, G.M., Holder, D.A., Jablonsky, G., Kostuk, W.J., Melendez, L.J., Myers, J.G., Sackett, D.L., Sealey, G.J. and Tanser, P.H. (1985). Aspirin, sulfinpyrazone or both in unstable angina. *New Eng. J. Med.* **313**: 1369-137.

Cerutti, P.A. (1994). Oxy-radical and cancer. *Lancet* **344**: 862-863.

Cerutti, P.A. (1991). Oxidant stress and carcinogenesis. *Eur. J. Clin. Invest.* **21**: 1-11.

Chabot, F., Mitchel, J.A., Gutteridge, J.M.C. et al (1998). Reactive oxygen-species in acute lung injury. *Eur. Resp. J.* **11**: 745.

Chanh, H.C., Koffi, Y. and Chanh, A.P.U. (1988). Comparative hypotensive effects of compounds extracted from *Lippia multiflora* leaves. *Planta Med.* 294-296.

Chanh, H.C., Koffi, Y. and Chanh, A.P.U. (1988²). Comparative effects on TXA₂ biosynthesis of products extracted from *Lippia multiflora* Moldenke leaves. *Prostaglandins, Leukotrienes and Essential Fatty Acids.* **34**: 83-88.

Comhair, S.A.A., Bhathena, P.R., Dweik, R.A., Kavuru, M. and Erzurum (2000). Rapid loss of superoxide dismutase activity during antigen-induced asthmatic response. *The Lancet.* **365**: 624.

Dadzie-Mensah, J. (2000). Modulation of eicosanoid biosynthesis *in vivo* as mechanism-based evaluation of putative anti-inflammatory plant extracts. M. Phil. Dissertation. Department of Biochemistry, University of Ghana.

Dennis, E.A. (1987). Regulation of eicosanoid production: role of phospholipases and inhibitors. *Biotechnology* **5**: 1294-1300.

De Nucci, G., Salmon JA., Moncada, S. (1986). The synthesis of eicosanoids induced by anaphylaxis in guinea pig isolated lungs perfused via the trachea. *Eur. J. Pharmacol.* **130** (3) 249-56.

Drazen, J.M. (1998). Leukotrienes as Mediators of Airway obstruction. *Am. J. Respir. Crit. Care Med.* 158 (3PT3).

Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D. and Guo, Z. (1985). Medicinal plants in therapy. *Bull World Health Org.* **63**: 965-981.

Florence, T.M. (1995). The role of free radicals in disease. *AUST-NZJ Ophthalmol* (Sydney) **23**: 3-7

Gabridge, J.M, Chandler, D.K.F., and Daniels, M.J (1985). Pathogenicity factors in mycoplasmas and spiroplasmas. In: The mycoplasmas (Razin, S., Barile, MF. Eds) Academic Press, Orlando FL. **4**: 313.

Glew, R.H. (1992). Lipid metabolism II: Pathways of special lipids. In 'Textbook of Biochemistry. 3rd Ed. (Devlin, T.M., ed.), Wiley-lics. pp 461-470.

Green, S.L. (1999). Asthma, Oxidant Stress, and Diet Nutrition **15**: Nos 11/12 5-20.

Gyamfi, M.A., Yonamine, M. and Aniya, Y. (1999). Free-radical scavenging action of medicinal herbs from Ghana *Thonningia sanguinea* on experimentally-induced liver injuries. *Gen. Pharmacol.* **32**: 661-667.

Haley, K.J., Sunday, M.E., Wiggs, B.R., Kozakewick, H.P., Rreilly, J.J., Mentzer, S.J, Sugarbaker, D.J., Doerschuk, C.M., and Drazen, J.M. (1998). Inflammatory cell distribution within and along asthmatic airways. *Am. J. Respir. Crit. Care Med.* **158** (2): 565-72.

Hamberg, M. and Samuelsson, B. (1974). Prostaglandin endoperoxides: a new concept concerning the mode of action and release of prostaglandins. *Proc. Natl. Acad. Sci. USA* **71**: 3824-3828.

Hamberg, M., Svensson, J., Wakabayashi, T. and Samuelsson, B. (1974). Isolation and Structure of Two prostaglandin Endoperoxides That cause Platelet aggregation. *Proc. Natl. Acad. Sci. USA*. **71**, 345-349.

Hardie, D, G. (1991). Biochemical Messengers: Hormones, neuro-transmitters, and growth factors. Chapman and Hall. pp 39, 51-55.

Hayes, M.E., Yuan, J. Y., freemont, A, J. and Mawer, E.B. (1994). Interferon gamma and eicosanoid regulation of 1, 25-dihydroxyvitamin D.

Heffner, J.E. and Repine, J.E. (1989). Pulmonary strategies of anti-oxidant defense. *Am. Rev. Resp. Dis.* **140**: 531.

Heptinstall, S., and Groenewegen, W.A., Knight D.W., Sprangenberg, P and Losche, W. (1987). Studies on feverfew:

and its mode of action. In 'Advances in Headache Research (Rose, F.C., ed.) London, Paris; John Libbey. pp 129-134.

Higgs, G.A., Mugridge, K.C., Moncada, C., Vane, J.R. (1984). Inhibition of tissue damage by the arachidonate lipoxygenase inhibitor BW 755C. *Proc. Natl. Acad. Sci. (U.S.A.)* **81**, 2890-2892.

Hubbard, R. and Gould, B.J. (1988). Enzyme immunoassay. In 'Principles of Clinical Biochemistry 2nd Ed. (Williams, D.L., Marks, V., eds.) Heineman Medical Books. pp 508-522.

Ingerman-Wojenski, C., Silver, M.J., Smith, J.B. and Macarak, E. (1981). Bovine endothelial cells in culture produce thromboxanes as well as prostaglandin. *J. Clin. Invest.* **67**: 1292.

Jatakanon, A., Lim, S. Chung, K.F and Barnes, P.J. (1998). Inhaled steroid improves markers of airways of inflammation in patients with mild asthma. *Eur. Resp. J.* **12**(5): 1084-1088.

Kelly, F.J., Mudway, I., Blomer, A., Flew, A., and Sanrom, T. (1999). Altered lung anti-oxidant status in patients with mild asthma. *Lancet* **354**: 482-83.

Lazarus, S. C. (1998). Inflammation, inflammatory mediators and mediator antagonist in asthma. *J. Clin. Pharmacol.* **38**(7) 577-82.

Lewington, A. (1990). Plants for the people. The Natural History Museum, Cromwell Road. London. pp 135-167.

Lewis, H.D., Jr, Davis, J.W., Archibald, D.G., Steinke, W.E., Smitherman, T.C., Doherty, J.E., Schnaper, H.W., LeWinter, M.M., Linare, E., Pouget, J.M., Sabharwal, S.C., Chesler, E. and DeMots, H. (1983). Positive effects of aspirin against acute myocardial infarction and death in men with unstable angina: Results of a Veterans Administration cooperative study. *New Eng. J. Med.* **309**: 396-403.

Loeb, L.A. and Gross, W.R. (1986). Identification and purification of sheep platelets phospholipase A₂ isoforms. *J. Biol. Chem.* **86** 10467-10470.

Lowman, M.D. and Box, J.D. (1983). Variation of leaf toughness and phenolic content among five species of Australian rain forest trees. *Aus. J. Ecol.* **8**: 17-25.

Maclean, J.A., Sauty, A. Luster, A.D., Drazen, J.M. and De Sanctis G.T (1999). Antigen-induced airway hyper-

responsiveness, pulmonary eosinophilia, chemokine expression in B-cell-defined mice. *Am. J. Respir. Cell Mol. Biol.* **20**(3) 379-87.

Maynard, P.M., Graupner, W.G., and Bottje, W.G (1992). Effect of glutathione depletion on tissue and plasma prostacycline and thromboxane in rats. *Biochem. Pharmacol.* **43**, (5): 1043-1051.

Mclean, E.K. (1970). The toxic actions of pyrrolizidine. *Alkaloids Pharmacol. Rev.* **22**: 429.

McManus, O.B., Harris G.N., Giangiacomo, K.M, Feigenbaum, P., Reubin, JP, Addy, M.E., Burka K.F., Kaczorowski, G.J and Garcia, M.L (1993). An activator of Calcium-dependent potassium channels isolated from a medicinal herb. *Biochem.* **32**: 6128-6133.

Minoguchi, K., Kohno, Y., Oda, N., Wada, K., et al (1998). Effects of theopilline withdrawal on airway inflammation in asthma. *Clin. Exp. Allergy.* **28**: (3): 57-63.

Moore, J.M., Earnest, M.A., DiSimone, A, G., Abumrad, N.A. and Fletcher, J. R. (1991). A PAF receptor antagonist,

BN52021, attenuates thromboxane release and improves survival in lethal canine endotoxemia. *Circulatory Shock* **35**: 53-59.

Nanji A.A. (1993). Role of eicosanoids in experimental alcoholic liver diseases. Review. *Alcohol* **10** (6): 443-6.

Nash, T. (1953). Colometric estimate of formaldehyde by means of the Hantzsch reaction. *Biochem. J.* **55**: 416-421.

Needleman, P., Moncada, S., Bunting, S., Vane, J.R., Samuelsson, B. (1976). Identification of an enzyme in platelet microsomes which generates thromboxane A₂ from PG endoperoxides. *Nature* **261**: 558.

Noamesi, B.K (1977). Power Tea (*Lippia multiflora*)- A potent hypertensive therapy. *W. Afr. J. Pharmac. Drug. Res.* **4**: 33-36.

Noamesi, B.K., Adebayo, G.I. and Bamgbose, S.O.A (1985). The vascular actions of the aextract of *Lippia multiflora*. *Planta Med.* 256-258.

Niewoehner, C.B. and Niewoehner, D.E. (1999). Steroid-induced osteoporosis: Are your asthmatic patients at risk? *Postgrad. Med.* **105**(3) 79-83, 87-8, 91.

Nyarko, A.K and Addy, M.E (1994). In vitro screening for the anti-anaphylactic agent in *Thonningia sanguinea*. *J. Ethnopharmacol.* **41**: 45-51.

Okpako, D.T. (1970). Antigen-dose-response relationship in guinea pig ileum anaphylaxis. *Int. Arch. Allerg. Appl. Immunology.* **38**: 474-481.

Payan, D.G, and Shearn, M.A. (1992). NSAID: Nonopoid analgesics; Drugs used in gout In 'Basic and Clinical Pharmacology. 5th ed. (katzung, BG ed). 491-496.

Piper, P.J. and Tippins, J.R. (1982). Advances in prostaglandin, thromboxane and leukotriene research. (Samuelsson, B and Paoletti, R. eds.) 183-185.

Roit, I., Brostoff, J. and Male, D. (1993) *Immunology*. 3rd Ed. Mosby-Year Book. Europe Ltd. 13.6-13.8.

Samuelsson, B. (1983) Leukotrienes: Mediators of immediate hypersensitivity reactions and inflammation. *Science* **220**. 568-575.

Samuelsson B. (1978). Prostaglandins and Thromboxanes. *Recent Prog. Horm. Res.* **34** 239-58.

Samuelsson, B., Dahlem, S.E., Lindgren, J.A. (1987). Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science.* **237**: 1171-1176.

Sirois, P., Harcey, M., Braguet, P., Borgeat, P., Maclouf, J and Pradelles, P. (1987). Advances in prostaglandin, thromboxane, and leukotriene research. **17**: 1033-1037.

Smith, W.L. (1992). Prostanoid biosynthesis and mechanism of action. *Am. J. Physiol.* **263**, F181-F191.

Sprangue, R.S., Stephenson, A.H. and Lonigro, A.H (1992). OKY-046 prevents increases in leukotriene B sub(4) and pulmonary edema in phorbol ester-induced lung injury in dogs. *J. Apply. Physiol.* **73**: 6:2493-2498.

Stadman, E.R. (1990). Metal ion-catalysed oxidation of proteins: biochemical mechanisms and biological consequences. *Free Rad. Biol. Med.* **9**: 315-325.

Stadman, E.R., Oliver, C.N. (1991). Metal-catalyzed oxidation of protein: physiological consequences *J. Biol. Chem.* **266**: 2005-2008.

Steinberg, D., Parthasarathy, S., Careaw, T.E, Khoo, J.C. and Witztum J.L. (1989). Beyond cholesterol. Modifications of low density lipo-protein that increase its atherogenicity. *N. Eng. J. Med.* **320**: 915-924.

Suarez, J., Harrera, M.D., and Marhuenda, E. (1998). *In vitro* scavenger and anti-oxidant properties of hesperidine and neohesperidine dihydrochalcone. *Phytomed.* **5**(6): 469-473.

Szeffler, S.J. and Nelkon, H.S. (1998). Alternative agents for anti-inflammatory treatment of asthma. *J. Allergy Clin. Immunol.* **102**: 523-35.

Szucs, T.D., Anderhub, H. and Rutishauser, M (1999). The economic burden of asthma: Direct and indirect cost in Switzerland. *Eur. Respir. J.* **13** (2): 281-6.

Takami, M. and Tsukada, W. (1998). Correlative alteration of thromboxane A₂ with antigen-induced broncho-constriction and the role of platelets as a source of TXA₂ synthesis in guinea

pigs: effect of DP-1904, an inhibitor of thromboxane synthase. *Pharmacol. Res.* **38** (2): 133-139.

Taoubi, K., Fauvel, M.T., Gleye, J., Moulis, C. and Fouraste, I (1997). Phenylpropanoid glycosides from *Lantana camara* and *Lippia multiflora*. *Planta Med.* **63**: 192-193.

Their, S.O. and Smith L.H. (1981). Pathophysiology: The Biological principle of disease (vol. 1) W.B. Saunders Company, USA pp 1450-1452.

Valentin, A., Pelissier, Y., Benoit, F., Marison, C., Kone, D., Mallie, M., Bastide, J-M and Bassiere, J-M. (1995). Composition and anti-malarial activity *in vitro* of volatile components of *Lippia multiflora*. *Phytochem.* **40**: 1439-1442.

Westcott, J.Y, Johson, K., Batt, R.A. *et. al.* (1990). Measurement of Peptidoleukotrienes in biological fluids. *Am. J. Physiol.* **322**: 2640-2648.

Whaley, K. and MacSween, R.N.M. (1992). Muir's textbook of Pathology 13th Ed. Arnold Hodden headline Group, London. Pp15, 112-149.

APPENDIX**PREPARATION OF SOLUTIONS AND REAGENTS*****Potassium phosphate buffer, 100mM, pH 7.4***

11.0233g KH_2PO_4 and 3.3094g K_2HPO_4 were dissolved in 1 liter of distilled water and the pH adjusted with concentrated KOH.

Kreb Hanson Solution

The Kreb's solution was prepared by dissolving 27.68g NaCl (118.4mM), 1.40gKCl (4.69mM), 1.16g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1.18mM), 0.64g KH_2PO_4 (1.18mM), 1.28g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (2.2mM), 8.4g NaHCO_3 (25.6mM), 8.0g glucose (11.01mM), in 1liters of ultra-pure water. The solution was prepared four times concentrated to avoid the danger of microbial contamination and kept at 0°C. It was diluted to the appropriate concentration on the day of experiment. The glucose component was added on the day of use.

Th Biorad reagent.

It was made up of 100mg Coumasie Blue, 50ml methanol and 150ml of 85% phosphoric acid. It was diluted with four parts distilled de-ionized water and was filtered through Whatman number 1 filter to remove particulates. The diluted reagent was stored at room temperature and used within two weeks.

Fe³⁺ -EDTA mixture.

The Fe³⁺-EDTA mixture was made up of solutions A and B prepared as follows:

Solution A: 0.2031g FeCl₃.6H₂O was dissolved in 5ml of potassium phosphate buffer (PB). 1ml of the solution was diluted to 100ml with PB

Solution B: 0.1675g (EDTA) Na₂ was dissolved in 10ml PB and 1ml diluted to 100ml with PB.

Solutions A and B were mixed in the ration 1:2