Adverse Ocular Outcomes in the Management of Allergic Conjunctivitis and the Efficacy of a Herbal Intervention

by

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DECLARATION

I hereby declare that this thesis is an original work by me towards obtaining a Master of Philosophy Degree in Clinical Pharmacology, and that it contains no material previously published by another person or accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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To my parents; Mr Ebenezer Abokyi and Mrs Akosua Addae.

ABSTRACT

Allergic conjunctivitis (AC) is on the rise and pharmacological intervention is the mainstay of its treatment. However, reports on adverse ocular effects have been observed in the use of orthodox medicines in managing AC. This study, therefore, sought to investigate the outcomes of conventional anti-allergic medications use and the efficacy of a herbal intervention in management of AC.

A cross-sectional study was first conducted to determine the prevalence, prescription pattern and ocular complications in AC management, followed by a retrospective cohort study to determine the underlying cause of the commonest ocular complication observed in the management of AC by reviewing medical records of cases of AC diagnosed from January to December 2010.

The results indicated a high prevalence 9.1% of AC, with the acute forms of AC accounting for 82.5% of the total AC cases. The commonest prescribed medications were topical steroids 69.7% (1198) mainly prescribed for atopic keratoconjunctivitis ($P = 0.002$), and systemic antihistamines 48% (839) for the acute types of AC ($P \leq 0.001$). Dry eyes was the commonest disorder (5.2%) found accompanying AC, and the use of systemic antihistamines in managing AC was the most significant risk factor (OR: 2.79; $P \leq 0.001$) to this condition after adjusting for patient's age and occupation.

Anecdotal reports suggests *P. stratiotes,* a traditional medicinal plant, is potent in managing allergies. There is, therefore, the need to confirm or otherwise the efficacy of this herb in treating AC since conventional orthodox treatment is ineffective and/or associated with adverse ocular effects. Ovalbumin-sensitized ICR mice were pretreated with different doses (10, 50 and 100 mg/kg) of an aqueous leaf extract of *P. stratiotes* (ALPS) 1h before

multiple topical challenges in eyes by instillation of ovalbumin to induce AC. ALPS significantly and dose-dependently lowered ($P \leq 0.05$-0.001) ocular signs of AC (including conjunctival redness, lid edema, tearing and lid scratching), serum ovalbumin-specific IgE, and mast cell infiltrations and degranulations in ocular tissue of mice.

Managing AC with conventional orthodox drugs results in decreased tear secretion and stability (i.e. dry eyes). It was hence important to evaluate the effect of *P. stratiotes* on tear secretion and tear film stability. Phenol red thread test and fluorescein tear breakup time, for the assessment of tear secretion and tear film stability respectively, were performed before and after oral administration of ALPS for 7 consecutive days. No significant change ($P > 0.05$) in tear secretion and tear film stability was found following treatment with ALPS, while mice treated with prednisolone and cetirizine showed significant decline in tear secretion and/or stability ($P \leq 0.05$- 0.001).

AC is a prevalent eye disorder in Ghana and caution should be exercised in the use of systemic antihistamines as it could precipitate dry eyes. ALPS exhibited potent anti-allergic activity and did not affect tear secretion and tear film stability; hence may be useful in managing AC in humans.

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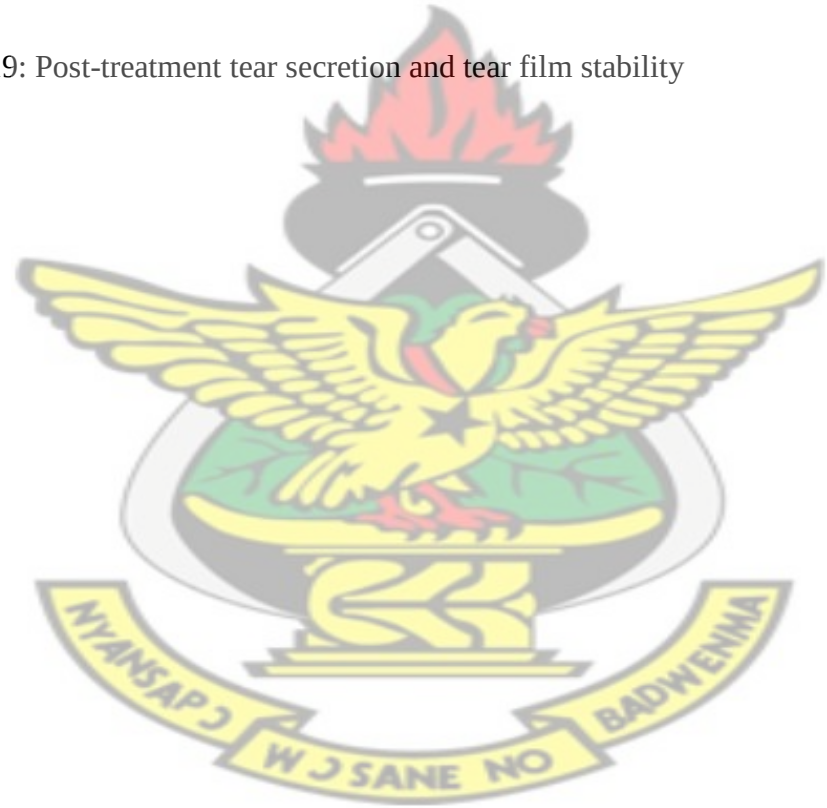

LIST OF ABBREVIATIONS

AC	Allergic conjunctivitis
AKC	Atopic keratoconjunctivitis
ALPS	Aqueous leaf extract of *Pistia stratiotes*
APCs	Antigen Presenting Cells
CD4	Cluster of differentiation 4
COX	Cyclooxygenase
CPT	Conjunctival provocation test
DCs	Dendritic cells
DE	Dry eyes
FcεRI	Fc epsilon RI/high-affinity IgE receptor
FTBUT	Fluoresceine tear break up time
GPC	Giant papillary conjunctivitis
HDC	Histidine Decarboxylase
ICR	Imprinting Control Region
IFN	Interferon
IgE	Immunoglobulin E
IL	Interleukin
LT	Leukotriene
MHC	Major histocompatibility complex
NSAID	Non-steroidal anti-inflammatory
OVA	Ovalbumin
OVA-s-IgE	Ovalbumin-specific Immunoglobulin E
PAC	Perennial allergic conjunctivitis
PGD2	Prostaglandin D2

PGE2	prostagladin E2
PGI_2	Prostacyclin
PRT	Phenol red thread
SAC	Seasonal allergic conjunctivitis
SANTH	Systemic Antihistamine
SCIT	Subcutaneous immunotherapy
SNAID	Systemic Non-Steroidal Anti-inflammatory Drug
SPT	Skin prick test
TANTH	Topical Antihistamine
TCR	T cell receptor
Th cells	T helper cells
TMCS	Topical M ast Cell Stabilizer
TNF	Tumor necrosis factor
TNSAID	Topical Non-Steroidal Anti-Inflammatory Drug
VKC	Vernal keratoconjunctivitis

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I am ultimately thankful to the Almighty God, the most gracious and most merciful, for the life and strength given me throughout the period of this study. It is true that for someone to make strides he will need the support of others. I therefore sincerely wish to express my gratitude to my supervisor for his immense contribution and guidance. In fact, his tutelage has given me the dexterity that I will utilize throughout my academic life and I will never forget him for this. To my elder brother, Godwin Abokyi, you have been instrumental in laying the educational foundation on which I thrive and I appreciate your effort.

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CHAPTER ONE

1.0 GENERAL INTRODUCTION

This chapter reviews relevant literature on allergic conjunctivitis and its management, as well as the anti-allergic potential of secondary plant metabolites, with emphasis laid on bioactive chemical composition of *Pistia stratiotes*. It also has the justification as well as the aim and objectives for the study.

1.1 INTRODUCTION

Allergic conjunctivitis is an undesirable inflammatory reaction of the conjunctiva resulting from acquired immune response against an allergen (a foreign antigen to which the individual has been previously sensitized) (Offiah and Calder, 2009). The conjunctiva is a thin transparent outermost membrane lining the eyeball surface. It is richly supplied by blood and easily gets inflamed when exposed to adverse environmental conditions.

Allergic conjunctivitis is estimated to affect about 15% of the world's population (Wood *et al.*, 1999) with variations observed among different geographical areas and age groups. Studies show that ocular symptoms of allergic conjunctivitis are presented in about 50% of individuals suffering from other allergic disorders (Williams *et al*., 2010).

A survey among adults in the United States revealed that about 40% of the respondents experienced recurrent symptoms of ocular itch and tearing which are characteristic of ocular allergy (Singh *et al.*, 2007).

A Tanzanian study was carried among 400 households in which was found that more than half of the households had one or more family members suffering from allergies and ocular allergy alone accounting for 10.8% of the total prevalence (Justin-Temu *et al.*, 2008). It has

been estimated that allergic conjunctivitis accounts for more than one-tenth of ophthalmic consultation (Katelaris, 2011). Sufferers experience disabling ocular symptoms which interfere with their quality of life and productivity (Palmares *et al*. 2010).

Due to the symptomatic nature of the disorder most patients present to eye care practitioners for remedy. Current management of this condition usually involves use of anti-allergic medications which are mainly targeted at alleviating the patient's symptoms. Most of these medications are used for a longer time due to the recurrent nature of symptoms. Patients are therefore burdened financially with the high economic cost of the medication (Pitt *et al.*, 2004). Unfortunately, some of these medications are ineffective (Rosa *et al.*, 2013) and may also be associated with more sight threatening complications including cataract, glaucoma and dry eyes (Abokyi *et al*., 2012a; Mohan and Muralidharan, 1989).

Since antiquity till now plants continue to be the richest resource of drugs for traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates as well as chemical entities for synthetic drugs (Fabricant and Farnsworth, 2001). Many of our present medicines are derived directly or indirectly from higher plants. While several classic plant drugs have lost much ground to synthetic competitors, others have gained a new investigational or therapeutical status in recent years (De Smet, 1997).

Medicinal plants are used as alternative medicines in management of numerous diseases (including allergic conjunctivitis). In developing countries, especially rural communities, where there is inadequacy of modern health care facilities or orthodox treatment is

unaffordable due to poverty, traditional medicinal plants usually remain the only available treatment.

The World Health Organization estimates that about 80% of the world's population relies on medicinal herbal products or their derivatives for their primary health needs (Doughari, 2012). In Ghana, it is reported that a majority (70%) of the population depend exclusively on traditional medicine for their health (WHO, 2001).

The continually increasing reliance on plants for cure has been partly because it is commonly available, less expensive, and considered safe and efficient with relatively fewer adverse effects (Yirga, 2010). In addition, secondary plant metabolites (phytochemicals) have demonstrated many prospects in management of several diseases including cardiovascular diseases, metabolic disorders, cancers and allergic disorders (Doughari, 2012).

Studies indicate that natural flavonoids usually occur as glycosides are commonly present in vegetables, fruits or teas (Hollman and Katan, 1999; Harborne and Williams, 2000) and exhibit anti-allergic activities by inhibiting histamine release, synthesis of IL-4 and IL-13 and CD40 ligand expression by basophils (Kawai *et al*., 2007). According to Hirano *et al*., (2004), of 45 flavones, flavonols and their related compounds that were studied, luteolin was one of the potent inhibitors of interleukin 4 (a cytokine involved in allergy), supporting an earlier finding which indicated that this phytochemical was the most potent inhibitor of histamine and cytokine release from human mast cells (Inoue *et al*., 2002). Luteolin is found in herbal preparations for asthma treatment in Japan (Alexander *et al.*, 2011).

Another plant flavonoid called rosmarinic acid found in perilla leaves has been effective in suppressing allergic reactions in mice (Makino *et al*., 2001) and, more recently, in human

(Takano *et al*., 2004). Rosmarinic acid relieves allergy symptoms by preventing the activation of immune responder cells and by inducing apoptosis, or cellular suicide, in already activated immune responder cells (Won *et al*., 2003). Thus, this phytochemical exerts a specific action only on allergy-activated T cells and neutrophils during allergic reactions without affecting the T cells or neutrophils in their resting state.

Pistia stratiotes, an aquatic macrophyte is also among the medicinal plants of huge unexploited potential. Some already reported medicinal attributes include antibacterial, antifungal, anti-helmenthic, antidiabetic, diuretic, antiprotease, antitubacular, emollient, antidysentry and antidermatophylitic (Tripathi *et al*., 2010).

Previous studies found that *Pistia stratiotes* L. contains large amount of two di-C-glycosylflavones of the vicenin and lucenin and lesser amounts of the anthocyanin cyaniding-3-glucoside and a luteolin-7-glycoside, and traces of the mono- C-glycosyl flavones, vitexin and orientin. Plant steroids including Stigmasta-4, 22-dien-3-one, stigmasterol, stigmasteryl stearate, and palmitic acids have also been extracted (Zennie and Mc Clure, 1977).

Luteolin-7-glycoside is a natural occurring glycoside of the flavonoid luteolin which is popular for its anti-allergic activity, indicating that this plant may be helpful in management of allergies. It is important to note that this plant is already included in herbal preparations used in the management of asthma (Alexander *et al*., 2011).

1.2 ALLERGIC CONJUNCTIVITIS

Allergic conjunctivitis (AC) is a collective term used to describe diverse ocular allergic disoders including seasonal allergic conjunctivitis (SAC), perennial allergic conjunctivitis (PAC), vernal kertoconjunctivitis (VKC), atopic keroconjunctivitis (AKC), gaint papillary conjunctivitis (GPC) and contact dermatoconjunctivitis (CDC). All these forms of allergic conjunctivitis can be broadly categorized into 2; namely acute allergic conjunctivitis and chronic allergic conjunctivitis depending on the underlying immunological mechanism (Leonardi *et al*., 2007).

1.2.1 Acute Allergic Conjunctivitis

SAC and PAC are acute forms of AC caused by immediate hypersensitive response in eyes of sensitized individuals after subsequent exposure to an already sensitized agent (allergen). (Offiah and Calder, 2009). Ocular itching is a charateristic symptom of AC and is often accompanied with tearing (du Toit, 2005). Other symptoms indicative of severe reactions may include ocular burning, foreign body sensation, photophobia and blurred vision although these are uncommon. Blurred vision and photophobia are reported by some patients due to alteration in composition of tear film (Suzuki *et al*., 2006). Common signs observed are conjunctival hyperemia and chemosis. Mucoid discharge, conjunctival papillae, red edematous eyelids and pseudoptosis are found in pronounced cases.

1.2.2 Chronic Allergic Conjunctivitis

VKC and AKC are chronic forms AC caused by both immediate and delayed hypersensitivity. Patients present with similar history of allergies (personal or familial) as

in the acute forms. The onset of VKC is in childhood and has a predilection for males (Neumann *et al*. 1959; Bonini *et al*. 2000) and the African race (Montan *et al*. 1999), and usually resolves spontaneously without complications in the late teens if well managed. Sufferers of VKC experience remission of ocular symptoms during cold seasons while AKC has no particular seasonal predilection (Majmudar, 2010). A significant sign aiding in the differential diagnosis of AKC from VKC is the component of periocular skin and lid changes which manifest as scaling and flaking of the lids (Ziskind, 2006).

GPC and CDC are also chronic and caused by persistent mechanical irritation and adverse reaction to topical medications or cosmetic products (Ziskind, 2006). The rationale in categorizing these conjunctival reactions under ocular allergic disease is due to evidence of an increased number of mast cells in the conjunctiva and increased levels of IgE in the tear film of patients (Donshik and Ballow, 1983).

1.2.3 Factors Associated to Allergic conjunctivitis

The increasing trend observed in the prevalence of allergic conjunctivitis is alarming and has led to investigations to provide insight and understanding into the development of this disorder. Researchers have identified several risk factors some genetic and others environmental inclined.

1.2.3.1. Family History

Several epidemiological studies indicate that a familial history of atopy is a significant risk factor for allergic disorders (Kaiser, 2004; Mingomataj *et al*., 2008) suggesting that there is an underlying hereditary factor.

1.2.3.2. Gender

Although controversial, studies have identified that gender (either male or female) is related to susceptibility to allergic disorders (Peroni *et al*., 2003; Alm *et al*., 2011). It is, however, important to note that population based studies on ocular allergies report that males are more susceptible to VKC than females (Neumann *et al*. 1959; Bonini *et al*. 2000). This also supports the role of genetic involvement in allergic diseases.

1.2.3.3 Socioeconomic status

Socioeconomic status includes aspects like education level, income and professional level. Socioeconomic status has been observed to be related to allergies. Almqvist *et al*. (2005) in their study on the relationship between socioeconomic status and allergies observed that asthma, rhinitis, and atopic sensitization were more common among persons with lower socioeconomic, which they attributed to differences in life style and environmental exposures between the groups.

1.2.3.4 Breastfeeding

Environmental exposures in the early months of life are critical for the development of the immune system. During breastfeeding immune cells from the mother are transmitted to an infant in breast milk, and that is clearly helpful to an infant as it serves as an irreplaceable passive and active immunity (Niers *et al*., 2007). Besides the effect of the active components in breast milk on the immune cells, also the intestinal microbiota is nourished by breast milk since it acts as a medium for the growth of bifidobacteria and lactobacilli

(Niers *et al*., 2007), thereby inducing Th1 responses serving as control against allergy-mediated Th2 immune response. Cross sectional studies in Estonia and Sweden observed that atopic infants had lower counts of lactobacilli, bifidobacteria as compared with non-atopic infants (Bjorksten *et al*., 1999; Bottcher *et al*., 2000).

1.2.3.5 Childhood use of antibiotics

The protective effect of early microbial exposure of the immune system cannot be overemphasized. Microbial infections in childhood stimulate the immune system towards a Th1 (non-allergic) response (Eggesbo *et al*., 2003). The use of antibiotics alters the intestinal microbiota resulting in a "cleaner" internal environment thereby depriving the development of Th1 immune response. A positive association has been observed between early childhood use of antibiotics and allergic disorders (Johnson *et al*., 2005).

1.2.3.6 Obesity

According to World Health Organization (2008) body mass index (relating the body weight of an individual to the square of body length) is a reliable indicator of overweight and obesity. A body mass index (BMI) above 25 indicates overweight, while a BMI between 18.5 and 24.9 is considered healthy. Research shows that a BMI above 30 (obesity) increases the risk of developing allergies (Huovinen *et al*., 2003). It has been observed that in obese people, not only hormonal levels (Guler *et al*., 2004) but also T cell functions (Mito *et al*., 2002) are impaired, which may explain for the associations between obesity and increased prevalence of allergic disorders.

1.2.3.7 Nutrition

Several dietary factors including higher consumption of processed foods and omega-6 fatty acids and a lower consumption of omega-3 fatty acids and fresh foods all have a potential impact on allergic reactions (Peat, 1996). Omega-3 fatty acids suppress inflammation, while omega-6 fatty acids aid in the development of inflammation (Peat, 1996). Westernized diets are relatively deficient in antioxidants which are essential in the removal of free radicals (causative agents of oxidative stress) and studies have found that oxidative stress is strongly associated with chronic inflammatory disorders (Lands, 2007).

1.2.3.8 Stimulant use

The use of stimulants like alcohol and drugs are investigated for their role in many diseases. Both chronic and acute intake of alcohol can cause powerful immune modulation (Cook, 1998; Nelson and Kolls, 2002; Szabo, 1999). It is shown that alcohol consumption leads to a shift in cytokine production by different T cell subsets in humans (Gonzalez-Quintela *et al*., 1999). Similar results are shown in mice that after alcohol intake results in skewing towards allergy promoting T cells or direct effects on B cells (Starkenburg *et al*., 2001; Waltenbaugh *et al*., 1998). Also the allergen-specific IgE levels in house dust mite-allergic individuals are found to be significantly higher in regular alcohol consumers compared to people that do not drink alcohol (Vidal *et al*. 2002).

1.2.3.9 Lack of microbial exposure (hygiene)

An inverse relationship has been found to exist between hygienic conditions and the magnitude of allergies. Reports indicate that prevalence of allergies in developing countries

with poorer hygienic conditions is much lower than in the developed countries (Penders *et al*., 2007). In addition, children that grew up on farms developed fewer allergies (Radon and Schulze, 2006). These studies support the hygiene hypothesis propounded by Strachan (1989) which states that a lack of early childhood exposure to microbes (e.g., gut flora or probiotics) and parasites due to the frequent number of vaccinations increases susceptibility to allergic diseases by suppressing natural development of the immune system (specifically the Th1 immune response). An imbalance between Th1 and Th2 immune responses is said to underlie several diseases including autoimmune disorders. Studies have found that well developed Th1 immune response keeps Th2 immune responses in check (Martinez, 2001).

1.2.3.10 Smoking

A direct link between smoking and allergies is controversial. While some researchers have shown that smoking is associated with increased total IgE levels (Jarvis *et al*., 1995; Oryszczyn, 1991; Warren *et al*., 1982), smoking has been found to be associated with reduced allergic sensitization to some allergens (Wuthrich *et al*., 1996).

1.3 THE IMMUNE SYSTEM

An allergic response is a consequential adverse inflammatory effect that accompanies the immune system's defense that is mounted to the eradicate substances which are considered foreign from the body. The immune system like all other systems of the body comprises of organs, tissues, specialized cells and chemical mediator molecules functioning together to rid the body from harmful materials in the external environment. The defensive systems of

the body is grouped into 2 namely; the innate immune system and the acquired immune system (Janeway *et al*., 2007).

1.3.1 Innate Immunity

The innate immune system is fully present and functional at birth and is the host's first line of defense against invading pathogens. Innate immunity is mounted by anatomical structures including the skin, nasal and conjunctival mucosal surfaces, cilia and other phagocytic cells (blood monocytes, neutrophils and tissue macrophages). Other physiological responses such as fever and low pH also play a key role in inhibition of microbial growth (Janeway *et al*., 2007).

1.3.2 Acquired Immunity

The acquired immune system comprises two main branches; the humoral immunity and the cell mediated immunity. The acquired immune system is however not fully active at birth and requires prior exposure to an antigen (foreign substance) either by infection or immunization. Emphasis is laid on the humoral immune system which is crucial in the development of the immediate hypersensitivity.

Upon the first encounter of an individual to an antigen results in a relatively weak, short-lived response called the primary immune response which is characterized by the production of plasma cells (antibody-secreting cells) and memory B cells. It takes averagely about 1 week from the time of contact to the offending agent to the realization of antibodies circulation. This period from exposure till when antibodies are produced is referred to as the Latent phase (Janeway *et al*., 2007). During the primary humoral response, IgM predominates. During subsequent encounter with the same antigen a

secondary response ensues which is characterized by a more rapid response facilitated by the presence of memory B cells. There is immunoglobulin switch to the production of antigen specific isotypes (IgG, IgA, IgE) other than IgM (Janeway *et al*., 2007).

1.3.3 Cellular Components of the Immune System

The cells of the immune system are basically those cellular components of blood referred to as the white blood cells. These include the granulocytes (neutrophils, eosinophils, basophils), macrophages, dendritic cells and mast cells all differentiating from the myeloid progenitor cell (Guyton and Hall, 2006). The lymphoid progenitor cells also differentiate into the three lymphocytic cells; namely the T cells, B cells and NK cells (Guyton and Hall, 2006). These two forms of progenitor cells originate from the hematopoitic stem cells in the bone marrow. All these cells play a crucial role in the defense of the body against antigens. However, the main cells that participate in allergic reactions are the dendritic cells and macrophages (referred to as antigen presenting cells), lymphocytes (which include the T cells and B cells), mast cells, basophils and eosinophils (Janeway *et al*., 2007).

1.3.3.1. Antigen Presenting Cells

Antigen presenting cells (APCs) are those cells of the immune system that express MHC II molecules on their surface and are capable of recognizing antigens, and then process the antigens through phagocytosis after which they are presented for the activation of a naïve T cell (Germain and Stefanova, 1999). The phagocytozed antigens are presented as peptide fragments bound to a class II MHC molecule, on the membrane. This antigen-class II MHC complex binds to T cell receptor (TCR) and this together with other co-stimulatory molecules expressed by the APC activates the T cell (Riley, 2009).

Three specialized immune cells that act as APCs include the dendritic cells, macrophages and B cells. Dendritic cells (DCs) are of either a myeloid lineage or lymphoid lineage and are found in the interstitum of all tissues with exception of the brain (Janeway *et al*., 2007). Other tissue such as the skin also has Langerhans cells which are unique DCs. The macrophages originate from the myeloid progenitor while B cells arise from the lymphoid progenitor.

1.3.3.2 Lymphocytes

Lymphocytes are white blood cells which are very important components of the adaptive immune response. There are three major types of lymphocytes-T cells, B cells and NK cells, and all these cells are derived from the bone marrow but mature at different locations. The T cells mature in the thymus, the B cells in the bone marrow and the NK cells in the primary (bone marrow and thymus) and secondary lymphoid tissues (spleen, lymph nodes, tonsils, and mucosa-associated lymphoid tissue) (Huw, 2008).

During the maturation of the T-cell, it expresses a unique receptor on its membrane, called the T- cell receptor (TCR) which is specific to an antigen that is associated with cell membrane proteins known as major histocompatibility complex (MHC) molecules. T-cells are of two types: T-helper (Th) cells (possess CD4 membrane glycoproteins), and T-cytotoxic cells (display CD8 glycoproteins on the cell surface membrane) (Rhoades and Tanner, 2003).

1.3.4 Chemical Mediators of the Immune System

1.3.4.1 Histamine

Histamine is stored in the granules of mast cells and basophils and is the main mediator of ocular allergy and inflammation. Histamine is synthesized by decarboxylation of histidine by L-histidine decarboxylase (HDC), which is dependent on the cofactor pyridoxal-50-phosphate (Maintz and Novak, 2007). Histamine regulation is dependent on the gene for HDC enzyme, which is expressed in cells throughout the body. The release of this mediator results in contraction of smooth muscles around the airways (producing wheezing in asthma), increased mucus production, and abnormal vascular reactivity, with fluid leak into tissues. Three histamine receptor subtypes have been found on the conjunctiva, but only two (H1 and H2) have been found to contribute to allergic symptoms such as itching, conjunctival redness, and conjunctival swelling (Bielory and Ghafoor, 2005).

1.3.4.2 Prostaglandins

Prostaglandins are among the chemical mediators synthesized *de novo* after mast cells degranulation. They are synthesized from arachidonic acid (a long chain fatty acid), and consists of several types. Prostaglandin D_2 (PGD_2) however is the primary prostaglandin produced by mast cells and recruits Th2 cells, eosinophils, and basophils. Increased levels of PGD2 result in ocular redness, chemosis, mucus discharge and eosinophilic infiltrate in the conjunctiva. Other prostaglandins (PGE_2 and PGI_2) decrease the threshold of the conjunctiva to histamine invoked pruritus (Bielory, 2000).

1.3.4.3 Leukotrienes

Leukotrienes do not exist preformed in cells but are formed from the breakdown of arachidonic acid by the enzyme 5-lipoxygenase and are classified into two types: cysteinyl and non-cysteinyl. Leukotrienes exert their actions by binding to two receptors, cysteinyl-LT1 receptor and cysteinyl-LT2 receptor, which are found in smooth muscle cells and macrophages and on other inflammatory cells (including eosinophils). Cysteinyl-LTs play a role promoting and maintaining chronic inflammatory responses in allergic respiratory and skin disease through their effects on chemotaxis (attracting immune cells), vasodilatation (widening blood vessels) and oedema (swelling as fluid leaks through the blood vessels). Eosinophils, basophils and mast cells are important sources of cysteinyl-LTs (Brink *et al*., 2003)

Greater tear concentrations of various leukotrienes (mainly LTB4 and LTC4) have been found in sufferers of seasonal allergic conjunctivitis and other chronic forms of ocular allergy including vernal keratoconjunctivitis and atopic keratoconjunctivitis (Leonardi *et al*., 2008), suggesting of a possible role in the development of allergic eye diseases.

1.3.5 Role of the Immune Cells

T-helper cells are the major driving force and the main regulators of the immune system. Their tasks include activation of B cells and Tc cells, and secretion of proteins called cytokines (Alberts *et al*., 2002). However, the T-helper cells themselves must be activated. This happens when a macrophage or dendritic cell, which has eaten an invader, travels to the nearest lymph node to present information about the captured pathogen. The phagocyte displays an antigen fragment from the invader on its own surface, a process called antigen

presentation. The T-helper cell is activated when its receptor (TCR) binds to the antigen coupled with additional stimulatory molecules (Alberts *et al.,* 2002).

Once activated, T-helper cells differentiate into specialized effector cell types producing proteins that activate B and T cells including other immune cells. The effector cells are of two major types (Th1 and Th2 cells) and the cytokine milieu determines the subtype of T-helper cell that is favored during the differentiation process.

Evidence indicates that interleukin 12 (IL-12) and interferon γ (IFNγ) are the critical cytokines initiating the downstream signaling cascade to develop Th1 cells while Interleukin 4 (IL-4) and interleukin 2 (IL-2) favor Th2 cell differentiation. Th1 cells participate in the elimination of intracellular pathogens as well as organ transplant rejection (del Prete, 1992).

Th1 cells secrete IFNγ which mediate cell-mediated immunity through activation of mononuclear phagocytes and cytotoxic T cells resulting in enhanced phagocytic and cytolytic activities (Murray *et al*., 1985). On the contrary, Th2 cells mounts humoral immune response to extracellular parasites, including helminthes, and are crucial in the induction of asthma as well as other allergic diseases (del prete, 1992; Sokol *et al*., 2009).

The secreted cytokines by Th2 cells include IL4, IL5, IL9, IL10 and IL13. Interleukin 4 is critical in allergic inflammation through activation of B cell to differentiate into IgE-producing plasma cells and memory B cells. Interleukin 4 has also shown receptor-regulation activity on high-affinity IgE receptor (FcεRII) on mast cells and basophils which leads to degranulation and release of inflammatory mediators (Steinke and Borish, 2001).

Studies have shown that a balance between Th1 and Th2 responses is crucial in ensuring a harmonious immune system function, as reciprocal inhibitory signals are sent to each other to regulate the activities of one another, and an imbalance is observed in several disorders

including allergies (Lee, 2008). Other Th cells have been identified which include T-helper 17 (Th17), follicular helper T cell, induced T-regulatory cells, and the regulatory type 1 cells as well as the potentially distinct T-helper 9 (Th9) (Luckheeram *et al*., 2012).

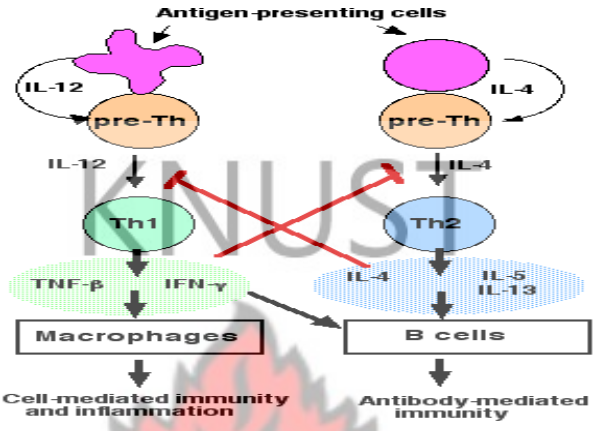

Figure 1: **Th1/Th2 balance. Depending on the cytokine milieu, pre-Th cells may develop to Th1 or Th2-cells that inhibit each other and drive cell-mediated immunity or humoral immunity responses (McGeady, 2004)**

1.4 PATHOGENESIS OF OCULAR ALLERGY

Allergic conjunctivitis is an immediate hypersensitive response of the conjunctiva and is Th2 cell driven culminating in the production of IgE by plasma cells which mediate the inflammatory process. The sequence of events leading to an allergic reaction is as described. When an allergen intrudes the body, it is bound by APC and presented as antigen-MHC-II complex molecule to a naïve T cells. The T cell is activated following three signals: 1) the association of the MHC-peptide complex with the T cell receptor (TCR), 2) the binding of CD28 to B7-1/2 (CD80/CD86) expressed on the APCs (also called co-stimulation), and 3) cytokine signaling (Alberts, 2002). Activated T cells differentiate into Th2 cells in a dominant IL-4 cytokine milieu (Romagnani, 2006).

An interaction is initiated between a protein called the CD40 Ligand found on the surface of the Th2 cell, and the CD40 protein on the B-cell surface resulting in activation of the B cell. In addition, Th2 cell also secretes cytokines (IL-4, IL-5 and IL-13) serving as co-stimulatory molecules in the B cell activation influencing the class-switching of B cells to IgE synthesis and the recruitment of mast cells (de Vries *et al*., 1993; Finkelman *et al*., 1990). Large amounts of IgE antibodies could be produced over several years and in the complete absence of allergen. Once formed and released into the circulation, IgE binds, through its Fc portion, to high affinity receptors (FCeRI) on mast cells, basophilic and eosinophilic granulocytes, leaving its allergen specific receptor site available for future interaction with an allergen. The process from the exposure to the allergen until the binding of IgE to the high affinity receptors is referred to as sensitization (Barnes and Marsh, 1998). Although, serum concentration of IgE is low (i.e. 0.05mg/mL), its binding to high affinity receptors prevents it from proteolytic cleavage and clearance which occurs in unbound IgE in circulation. Mast cell-bound IgE then remains in tissues for a long time and have the potential to react immediately to a specific allergen.

The challenge phase requires a subsequent exposure to the same allergen involved in the sensitization leading to the binding of the allergen to the allergen-specific receptor expressed on the IgE antibody molecule, already bound to a mast cell. The early phase of allergic reaction is initiated by cross linking of two or more neighboring IgE antibodies resulting in the activation of the mast cell. Activated mast cells undergo degranulation releasing preformed inflammatory mediators including histamine which are crucial in the observed clinical signs within 30 minutes of allergen contact. Some effects of histamine release include bronchial muscle contraction, vasodilation, increased vascular permeability, mucus secretion by goblet cells and stimulation of nerve endings (Lichtenstein, 1993).

Eosinophil chemotactic factor and neutrophil chemotactic factor are other preformed mediators in mast cells which upon secretion lead to infiltration of eosinophils and neutrophils to the inflammatory site. In addition, other secondary inflammatory lipid-mediators (leukotrienes, prostaglandins, bradykinin, platelet activating factor and cytokines) are synthesized *de novo* from arachidonic acid precursors within minutes of mast cell or basophil degranulation. Their effects are more pronounced and longer lasting than those of histamine and therefore are influential in modulation of the late phase reaction (Holgate, 2008). Late phase reactions are aftermaths of the immediate response that develop within 4-6 hours after exposure and may persist for 1-2 days. It is characterized by the infiltration of neutrophils, eosinophils, basophils, macrophages and Th2 cells, probably in response to cytokines released by activated mast cells (Holgate, 2008).

1.5 DIAGNOSTIC PROCEDURES

1.5.1 Clinical Diagnosis

A typical diagnosis of ocular allergy is usually made in the clinic during physical examination of patients. Patient history, forming part of the examination, is very important in arriving at this diagnosis since a subjective complaint of itching is considered essential, without which this diagnosis is erroneous (Moloney and McCluskey, 2007). In addition, other ocular symptoms including tearing, redness, ropy discharge, burning, pain and swollen lid could be reported. The clinician then performs assessment for signs such as conjunctival redness, chemosis, lid edema, papillae et cetera. However, occasionally it may be necessary to perform some additional laboratory testing either to support the clinical diagnosis or to identify the causative agent to ensure an effective management strategy.

1.5.2 Skin prick test (SPT)

Skin prick tests is an *in vivo* provocative test for identifying specific allergens to which an individual is sensitized and involves puncturing or intradermal injection of a standard extract of suspected allergens into the skin of the forearms or back. If the patient is allergic to the substance, then a visible inflammatory reaction (ranging from slight reddening of the skin to a wheal-and-flare response) will usually occur within 30 minutes. Testing is typically performed using the allergens relevant to the patient's environment (e.g., pollen, animal dander, moulds and house dust mites) (Small and Kim, 2011). Reports indicate that the use of SPTs alone in the clinical setting may lead to over-diagnosis due to the high sensitivity of the test (Boyce *et al*., 2010). The accuracy of SPTs vary depending on the type of ocular allergy in that with seasonal allergic conjunctivitis SPT are positive in 96% of subjects but decreases to 55% in VKC, 33% in AKC and 16% in GPC (Martin *et al*., 2003; Leonardi, 2005).

1.5.3 Serum IgE Tests

The test measures the concentration of IgE antibodies in the blood, which could be the total IgE tests or antigen-specific IgE (s-IgE). Although an elevated total IgE level in serum is frequently found in atopic individuals, it could also be found in non-atopic individuals (Klink *et al*., 1990). However, s-IgE tests can be especially useful when SPTs cannot be done (examples are presence of skin disorders and usage of antihistamine medications). Two common tests based on this principle are the radioallergosorbent test and, the much sensitive test, enzyme-labeled immunosorbent assays (Homburger, 2007).

1.5.4 Tear IgE measurements (Lacrytest)

Lacrytest is a qualitative and a rapid immunoassay procedure for determining whether or not the total tear IgE level is above the normal value (<2KU/L, 3ng/mL) observed in healthy subjects (Nomura and Takamura, 1998). This requires placing a strip in the lower conjunctival fornix to be wet with tears. Total IgE reacts with a gold-labelled antibody and is immobilised with the uptake of anti-IgE antibody. Signal intensity is dependent on the total IgE. For normal values, below 2.5 KU/L, no line is obtained (Monzón *et al*. 2009).

1.5.5 Atopy patch tests

The atopy patch test is of significant importance in the diagnosis of AKC and CDC (Leonardi, 2005). The procedure involves epicutaneous application of adhesive patches impregnated with suspected allergens. This is followed by evaluation for eczematous skin lesions at least twice, usually at 48 hours after application of the patch and again two or three days later (Boyce *et al*., 2010).

1.5.6 Conjunctival Provocation Test (CPT)

This test incorporates both diagnostic and therapeutic protocols and requires topical instillation of serial dilutions of suspected allergen determined by clinical examination and SPT into one eye and the other eye (control) instilled with balanced salt solution. Slit- lamp examination of eyes is done at different times. Criteria for a positive test are congestion of the conjunctival mucosa, itching, and eye watering. After the ocular allergy has been induced, topical antihistamine is applied to control allergic reaction. CPT therefore serves as a clinical protocol for inducing ocular allergy or determining ocular response to allergens, and evaluation of efficacy of anti-allergic medications. (Mortemousque, 2007)

1.6 CURRENT TREATMENT AND CLINICAL TRIALS

Management of allergic conditions can be categorized broadly into non-pharmacological and pharmacological treatment.

1.6.1 Non-pharmacological treatment

Evidence indicates that allergic conjunctivis could be effectively managed by relying on prophylactic measures (Rosa *et al*., 2013). Avoidance of the allergen is sometimes possible through lifestyle modification such as frequent washing of the hands, keeping the hands away from the eyes and isolation of pets from the home. Also, mechanical rubbing of the eyes lead to mast cell degranulation and further release of inflammatory mediators and individuals are normally discouraged from this act (Greiner *et al*., 1985). Another non-pharmacological means of managing ocular allergies is by performing cold compress to ameliorate the extent of allergic inflammation and soothe ocular discomfort through vasoconstriction.

1.6.2 Pharmacological treatment

For a sensitized individual, if avoidance of the allergen could not be achieved and the conjunctival mucosa (physical barrier) is traversed, then there is the need to prevent the acquired specific immune response which is antibody-mediated. Intervention at this stage usually requires the usage of pharmacological (anti-allergic) agents. However, there are instances where the provision of artificial tears as substitute for inadequate tear secretion (as is usually seen in ocular allergy sufferers) is able to dilute the allergens and stabilize the

tear film integrity on the ocular surface (Moloney and McCluskey, 2007). The mechanisms of action by which anti-allergic medications exert their effects are numerous and target specific sites of the immune system, mainly the humoral, to disrupt the sequential processes that are prerequisites for the development of an allergic response. The traditionally used pharmacological agents are mainly mast cell stabilizers, antihistamines, various classes of anti-inflammatory drugs and scarcely immunosuppressive agents (Duvall and Kershner, 2005).

The general mechanisms of action of anti-allergic medications can be broadly categorized as: 1) agents inhibiting synthesis or release of the chemical mediators (such as histamine, prostaglandins and leukotrienes), and 2) agents inhibiting chemical mediators from their target sites.

1.6.2.1 Mast Cell Stabilizers

The specific mechanism of action of mast cells stabilizers involve blocking a calcium channel, thereby inhibiting the influx of Ca^{2+}, a prerequisite for mast cell degranulation and release of chemical mediators (Cook *et al*., 2002). However, the main drawback for mast cell stabilizers is that after degranution has already occurred, as in an acute allergic conjunctivitis, mast cell stabilisers are ineffective. This class of anti-allergic agents are therefore only effective as a prophylactic measure.

1.6.2.2 Antihistamines

Topical antihistamines are the most preferred option for the treatment of ocular allergies. In allergic disease, it is the H1 antihistamines which are of primary benefit, although H2

antihistamines may also play a therapeutic role. Antihistamines act as antagonists to histamines by competitively binding to histamine receptors (mainly, H1 receptors) and by this mechanism are able to quickly decrease smooth muscle contraction, mucous secretion, itching, vasodilation and blood vessel permeability (Origlieri and Bielory, 2009). Drugs such as chlorpheniramine or cyclizine are classified as first generation antihistamines. The second generation H1 receptor antagonists are less sedating and commonly employed in the treatment of acute allergic conjunctivitis. The most common drugs in this class are levocetirizine, loratidine, cetirizine, azelastine and emedastine (Simons, 2004). The main ocular side effect of antihistamines is their antimuscarinic effect manifest in decreased tear secretion.

1.6.2.3 Steroids

Steroids refer to a class of anti-inflammatory drugs that obstructs the pathway leading to the synthesis of prostaglandins and leukotrienes by inhibiting phospholipidase A2 (Duvall and Kershner, 2005). Steroids have been found to also possess immunosuppressive and anti-proliferative properties since they can hinder the transcription factor that regulates the transcription of Th2-derived cytokine genes and differentiation of activated T-lymphocytes into Th2-lymphocytes making this class of pharmacological agents the most potent (Newton, 2000; Nandakumar *et al*., 2009). However, steroids are to be used with caution due to the numerous ocular side effect noted including melting of the cornea, cataract, increased intra-ocular pressure and risk of secondary infections.

1.6.2.4 Non-steroidal anti-inflammatory drugs (NSAID)

NSAID, another class of anti-inflammatory drugs, act as nonselective inhibitors of the enzyme cyclooxygenase (COX), inhibiting both the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes needed in synthesizing prostaglandins but not lipoxygenase, thus they have virtually no effect on the production of leukotrienes (Blaho, 1992). They are considered safer, though less effective, than corticosteroids. In addition, it has been established that NSAIDS are able to decrease histamine-induced itch by raising the threshold required for nerve end stimulation. Some possible ocular complications have been associated with NSAID including corneal deposits and melting (Flach, 2000; Lin *et al*., 2000).

1.6.2.5 Immunosuppressive agents

These are immunomodulatory agents with immmunosuppressing effects on the immune system. Though similar to corticosteroids in action, have few fewer side effects and are preferred over corticosteroids in the treatment of allergic conjunctivitis. Common examples include Cyclosporine A and tacrolimus. Cyclosporine A is an immunosuppressant that acts by inhibiting eosinophilic infiltration by interfering with the type IV allergic reactions in the conjunctiva (Fukushima *et al*., 2006). Findings in a study by Ebihara *et al*. (2009) have shown this agent to be effective in managing VKC and AKC. Also, tacrolimus acts by inhibiting the action of T-cells, and Ohashi *et al* (2010) has demonstrated the efficacy of this immunosuppressive in managing severe allergic conjunctivitis.

1.6.2.6 Subcutaneous Immunotherapy

Subcutaneous immunotherapy (SCIT) involves repeatedly administering subcutaneous injections of the specific allergen that the individual is sensitized to, starting with minute doses and increasing gradually to larger doses until desensitization is achieved. Such repeated introduction of allergen causes a shift toward IgG production or to induce T cell-mediated suppression that turns off the IgE response. Evidence supporting the efficacy of SCIT in managing allergies is found in the promotion of formation of T regulatory cells, which suppress Th2 responses through secretion of cytokines (IL-4, IL-10) regulating naïve T cell priming (Fujita *et al*., 2012; Boquete-París, 2013). The duration for this desensitization process takes about 3 to 5 years, but the improvement is usually noticed during the first 3 to 6 months of treatment.

1.6.2.7 Anti-IgE Treatment

A management modality for allergies currently undergoing scrutiny is anti-IgE treatment. This treatment procedure involves subcutaneous injection of Omalizumab (a humanized kappa IgG1 antibody) once every 2 or 4 weeks, and this inhibits the binding of IgE to the expressed FcεRI receptors on mast cells and basophils by binding to an antigenic epitope on the IgE (Incorvaia *et al*., 2008). The inhibited binding of IgE to FcεRI has profound effects on the attenuation of IgE-mediated allergic disorder. The antigenic epitope is the Cε3 domain of IgE, made up of six key amino acids that form a system of three loops; Cε3 is accessible only on free IgEs but not on the IgE bound to mast cells or basophils, where there is no access, as it is occupied by the FCεRI receptor (Schulman, 2001).

1.6.2.8 Leukotriene Antagonists

Leukotriene antagonists used in allergy management is of two kinds functionally; leukotriene receptor antagonist or inhibitors of 5-lipoxygenase (Berger, 1999). A study by El-Hossary *et al*. (2010) demonstrated that Montelukast (a specific leukotriene receptor antagonist) could be invaluable in managing allergic conjunctivitis. Zileuton, a leukotriene antagonist, is able to lessen allergic inflammatory responses not by acting on any leukotriene receptor but rather blocking the enzyme 5-lipoxygenase, to prevent the synthesis of leukotriene (Dubé *et al*. 1999). Intensive investigation is ongoing in discovering new ways of mitigating the effect of ocular allergies.

1.8 *PISTIA STRATIOTES* LINN.

1.8.1 Morphology and Distribution

Pistia stratiotes, popularly known as water lettuce or cabbage, is an aquatic macrophyte that is found submerging on polluted water surfaces. This plant belongs to the arum family, called Araceae. The genus name is derived from the Greek word "pistos", meaning "water", refers to the aquatic habitat of the plant. Pistia is the taxonomy of the genus and has a single species. The origin of *Pistia stratiotes* is speculated to be the Nile, near Lake Victoria in Africa, and is called by some as the Nile cabbage. However, it is now widely distributed in nearly all tropical and subtropical regions of Asia, Africa, and America (Tripathi *et al*., 2010).

Morphologically, it is seen as a uniquely floating, stoloniferous herb with a submerged mass of fibrous feathery-hairy roots. The leaves are approximately 13 cm long and 17 cm wide and of fan-shaped having parallel venation and together arranged to form a rosette (Holm *et al*., 1977). Leaves are green in color, odorless, and bitter in taste. It is propagated

by seeds or more rapidly by stolons and grows year round in warm climates. It forms a dense mat on the water surface and causes serious clogging on water ways. It is also responsible for harboring mosquito larvae, which carry the filarial parasites. It flowers in hot season and fruits appear after the rain.

1.8.2 Nutrients and Phytochemicals

The nutritional elements and phytochemicals found in *P. stratiotes* are diverse and several others continue to be discovered from this aquatic macrophyte. A biochemical studies carried out to determine the chemical composition of the leaves and stems revealed: moisture (92.9%), protein (1.4%), fat (0.3%), carbohydrate (2.6%), fibers (0.9%), ash (1.9%), calcium (0.2%) and phosphorus (0.06%). The leaves are rich source of vitamins (A, B and C) and ash is rich in potassium chloride and sulfate (Khare, 2005). It has been shown that *P. stratiotes* is rich source of cellulose and lignin (Chanda and Sardar, 1991).

Several phytochemicals have been extracted and identified. These include a novel stigmastane (11-hydroxy-24S-ethyl-5-cholest-22-en-3,6-dione) and three sterols (sitosterol-3-O-[2′,4′-O-diacetyl-6′-O-steraryl]-β-D-glucopyranoside, sitosterol-3-O-[2′-O-stearyl]-β-D-xylopyranoside and sitosterol-3-O-[4′-O-stearyl]-β-D-xylopyranoside) (Aliotta and Monaco, 1991; Monaco, 1991). Also, flavonoids stored in the form of glycosides such as two di-C-glycosylflavones of the vicenin and lucenin type, anthocyanin cyanidin-3-glucoside and a luteolin-7-glycoside, and traces of the mono-C-glycosylflavones vitexin and orientin (Zennie, 1997).

1.8.3 Therapeutic uses

Studies have shown that *P. stratiotes* posesses several therapeutic properties including antiseptic, antitubercular, and antidysentric activity. Analgesic effects have also been demonstrated in its use as an ocular anodyne for in the Gambia. Juice of plant is used by Mundas in ear complaints. The ash of plant is applied to the ringworm of the scalp. Leaves are used in eczema, leprosy, ulcers, piles, and syphilis. Juice of leaves boiled in coconut oil is applied externally in chronic skin diseases. The root is laxative, emollient, and diuretic. Leaves infusions have been mentioned in the folklore to be used for dropsy, bladder complaints, kidney afflictions, hematuria, dysentery, and anemia (Kirtikar and Basu, 2001).

1.8.4 Pharmacological Activites

Due to the numerous medicinal properties and wide usage of *Pistia stratiotes,* researchers have developed keen interest in this plant in order to unearth the mechanism by which this plant exerts its physiological effects on the body.

1.8.4.1 Antioxidant

The antioxidant property of Pistia stratiotes has been shown in a study by Megha *et al*., (2010). In their study they indicated that *P. stratiotes* leaf extract showed strong in vitro antioxidant activities in a dose dependent manner, by demonstrating its significant reducing power, superoxide anion scavenging and nitric oxide radical scavenging activities when compared with different standards such as ascorbic acid and butylated hydroxytolune.

1.8.4.2 Anti-inflammatory activity

Studies by Kyei *et al*. (2012a) investigated the efficacy of an extract of *P. stratiotes Linn* in a rat experimental model of arthritis and fever to ascertain its importance in the traditional management of these inflammatory disorders in Ghana. It was found that the extract significantly reduced paw thickness of formalin-induced arthritic animals and also lipopolysacharride-induced fever in rats treated with the effects comparable to standard drugs. Another study also indicated that both ethanolic and aqueous extracts of this plant was effective in treating uveitis, which is inflammation of the uveal tissue in the eye (Kyei *et al*., 2012b).

1.8.4.3 Calcium channel blocking activity

Achola and Indalo (1997) showed that methanolic extract of *P. stratiotes* has an *in vovo* calcium channel blocking activity by demonstrating that the extract was able to decrease both systolic and diastolic blood pressure in anesthetized rat by 18% and 10% respectively, and may be potentially capable to lower the level of thyroid hormones produced.

1.8.4.4 Antimicrobial activity

Also, the antimicrobial effect of *P. stratiotes* is reported by several investigators.

Ali *et al* (2011) indicated that ethanolic extract of this plant produced significant inhibition zones against gram positive and gram negative bacteria, and yeast compared to standard antimicrobial drugs such as tetracycline, vancomicin and nystatin.

1.8.4.5 Antimutational and antitumorigenic activity

Again, Megha *et al*. (2011) demonstrated the protective effect of pistia stratiotes against gamma radiation when extract was administered orally in mice. Treatment with *P. stratiotes* significantly reduced micronucleus formation and chromosomal aberration.

1.9 JUSTIFICATION OF THE STUDY

Allergic conjunctivitis has become very prevalent in developing countries, and huge proportion of patients present to health facilities with this ocular disorder. Sufferers of allergic conjunctivitis experience intense ocular discomfort and are often managed with conventional anti-allergic medications such as antihistamines, mast cell stabilizers and steroids. Due to the recurrent nature of symptoms, patients often are placed on these drugs which may be ineffective (Rosa *et al*., 2013), expensive (Pitt *et al*., 2004) and also associated with serious ocular adverse effects including cataract (Allen, 1989), glaucoma (Mohan and Muralidharan, 1989) and more often dry eyes (Ousler *et al*., 2004). This therefore necessitates investigations into the epidemiology of AC, prescription patterns for AC management, and to determine the adverse ocular effects resulting from management, as information regarding allergic conjunctivitis in Ghana is scanty.

Medicinal plants enjoy large usage in Ghana as a remedy for most diseases affecting all body parts including the eyes. *P stratiotes,* a folk medicinal plants, popularly known as ntranoa in the Akan language is purported to have several therapeutic potentials. It is already used as an ocular anodyne and some researchers have indicated that it possesses

anti-allergic properties and for that matter is incorporated in herbal preparations for the management of asthma. Zennie and McClure (1997) reported isolating luteolin-7-glycoside, a natural occurring form of luteolin (a phytochemical known for its potent inhibition of cytokines (Won *et al*., 2003; Hirano *et al*., 2004) and histamine (Inoue, 2002).This study will therefore validate the anti-allergic property and efficacy of *P stratiotes* in the management of allergic conjunctivitis.

1.10 AIMS AND OBJECTIVES OF THE STUDY

The aim of this study was to determine the prescription pattern and ocular adverse effects associated with the use of conventional anti-allergic medications. And subsequently, to indicate that *P stratiotes* could be useful in the management of allergic conjunctivitis.

1.9.1 Specific objectives:

The specific objectives of the study include:

- To determine the epidemiological profile of allergic conjunctivitis and the prescription patterns of conventional anti-allergic medications used in its management.
- To determine the common adverse ocular effects and ocular complications resulting from the use of conventional anti-allergic medications in the management of allergic conjunctivitis.
- To validate the anti-allergic potential of *P. stratiotes* in the management of a murine model of ovalbumin-induced allergic conjunctivitis.

- To establish that *P. stratiotes* is comparatively safer on the eye compared to the conventional anti-allergic medications.

CHAPTER TWO

2.0 EPIDEMIOLOGICAL PROFILE AND PHARMACOLOGICAL MANAGEMENT OF ALLERGIC CONJUNCTIVITIS: A STUDY IN GHANA

2.1 INTRODUCTION

Estimates on the prevalence of allergic conjunctivitis and the pattern of distribution this ocular disorder varies worldwide (Rosario and Bielory, 2011). The cause of the variations may be genetically (Bonini and Lambiase, 1999) and/or environmentally related (Bekibele and Olusanya, 2006). Allergic conjunctivitis, which is generally caused by pollen, animal dander and house dust mites, has been reported to be triggered by the consumption of local food substances such as ground nut (peanuts) and pineapple (Obeng *et al*., 2011).

While non-pharmacological management of AC is often used as a prophylactic measure, in situations of acute allergic conjunctivitis pharmacological agents are usually the intervention provided. However, improper use of the anti-allergic medications in the management of allergic conjunctivitis may results in complications including dry eyes, corneal ulceration and perforation, cataract and glaucoma (Garrity and Liesegang, 1984; Wakamatsu *et al*., 2011), which can significantly impair vision. Despite the greater burden of allergic conjunctivitis in the population, no information is available on the prescription pattern of anti-allergic medications in the treatment of allergic conjunctivitis in Ghana. The objective of this study was therefore to determine the epidemiological profile and pharmacological management of allergic conjunctivitis in Ghana.

2.2 MATERIALS AND METHODS

2.2.1 Study Area

A retrospective study was conducted in two eye clinics namely; St. Michael Hospital and Our Lady of Grace Hospital both run by the Catholic Mission in Ghana and supported by the Ghana Health Service. The St Michael Hospital is located at Pramso in the Bosomtwe District of the Ashanti Region of Ghana. The facility serves Kumasi the Capital City of the Ashanti Region and its environs. The eye clinic is the biggest in the District with one ophthalmologist, one optometrist, two ophthalmic nurses and an intern optometrist.

Our Lady of Grace Hospital is located at Asikuma-Odoben-Brakwa District in the Central Region of Ghana. This is the District Hospital designated to serving Asikuma, Odoben, Ahwhiam, Kuntanase, Jamra, Kokoso and Bedum and its environs. Over the years the great expertise of health care providers and Staff has made the Hospital a very important Centre for the people in the District. The eye care team comprises one ophthalmologist, two ophthalmic nurses, one enrolled nurse and two ward maids.

2.2.2 Ethical Considerations

The study was conducted in accordance to the recommendation of the Ethics committee of the Pharmacology departmentDepartmental Permission for this study was sought from the Hospital Directors of the health facilities and Kwame Nkrumah university of Science and Technology, Kumasi, Ghana. Confidentiality and anonymity was ensured with the patients' medical records and the information collected was used solely for the purpose of this study.

2.2.3 Study Conduct and Design

A cross sectional study design was employed and this involved purposive sampling of the medical records of 1718 patients diagnosed of allergic conjunctivitis out of the total 18,896 outpatient turnout from January to December 2010. Cases of allergic conjunctivitis sampled were based on diagnoses of the condition using clinical presentations which include ocular pruritus and discharge, chemosis, hyperemia, or papillae of the conjunctiva. Further classification into specific types of allergic conjunctivitis i.e. seasonal allergic conjunctivitis (SAC), perennial allergic conjunctivitis (PAC), vernal allergic conjunctivitis (VKC) and atopic keratoconjunctivitis (AKC) was based on patient's history of the allergic conjunctivitis and atopy and clinical signs observed. Information regarding allergic conjunctivitis such as: patient's symptoms, onset and duration of disease and associated topic diseases were also recorded. Patients' demographic data including gender, age, and occupation were taken.

2.2.4 Data Analysis

Data was compiled using the Statistical Packages for Social Sciences (SPSS) version 17 (SPSS Inc., Chicago, IL, 2008) and GraphPad Prism 5.0 for Windows (GraphPad Software, San Diego, CA, USA). Measures of central tendencies and dispersion, frequencies, and percentages were used in analysis of the patient's data. Fishers exact Chi Square (χ^2) test was used to determine significant associations in the categorical variables (type of allergic conjunctivitis, gender, occupation and age group). Binary logistic regression was used in analyzing the pattern of prescription of anti-allergic drugs within the various types of allergic conjunctivitis by computing Odds ratios (OR) to estimate the likelihood of

prescription of these drugs within the various forms of AC. An association was considered significant if $P \leq 0.05$.

2.3 RESULTS

2.3.1 Demographics of Patients

Of a total of 18,896 individuals who visited the two eye care facilities over the study period, 1,718 were diagnosed of allergic conjunctivitis; a prevalence of 9.1%. The population of patients with allergic conjunctivitis comprised 657 (38.2%) males and 1061 (61.8%) females. The mean age ± SD (years) of these patients was 21.92 ± 18.29 (Table 1). The patients diagnosed with VKC were the youngest 6.24 (95% CI: 5.59-6.90) of the four different forms of AC, while the AKC patients were the oldest 36.00 (95% CI: 27.13-44.87) (Figure 2). Occupations with higher prevalence of AC were students 782 (45.5%), traders [214 (12.4%)] and artisans [122 (7.1%)]

Distribution of types of Allergic Conjunctivitis

The acute forms of allergic conjunctivitis were the most prevalent [SAC: 993 (57.8%) and PAC: 425 (24.7%)]. The prevalence of chronic allergic conjunctivitis, comprising VKC and AKC, was 17.5% (300) (Table 1).

2.3.2 Patient Characteristics and Association with AC

Gender and Type of Allergic Conjunctivitis

Males were significantly susceptible to VKC ($P \leq 0.001$) but were less susceptible ($P = 0.003$) to PAC than females (Table 1).

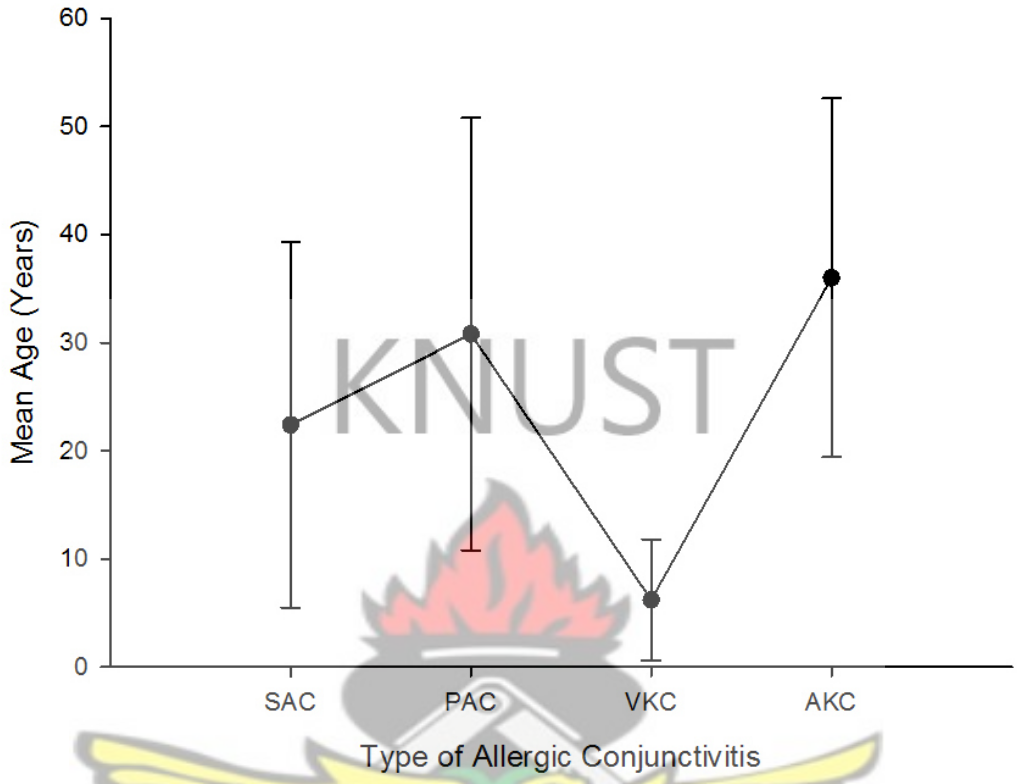

Figure 2: **The mean ages of patients with the various types of allergic conjunctivitis. Seasonal allergic conjunctivitis (SAC); perennial allergic conjunctivitis (PAC); vernal allergic conjunctivitis (VKC); atopic keratoconjunctivitis (AKC).**

Age and Allergic Conjunctivitis

Children (<15) constituted 88.7% of individuals with VKC indicating that they were the most susceptible ($P \leq 0.001$) being 8.11 times more susceptible of VKC compared to young adults (15-40). Most allergic conjunctivitis patients were below 40 years with VKC patients having the least mean age while patients with AKC had the highest mean age. Young ($P \leq 0.001$) and older adults ($P = 0.008$) were much more susceptible to SAC. Regarding PAC children and younger adults were the least susceptible ($P < 0.001$) (Table 1).

Occupation and Allergic Conjunctivitis

SAC was not significantly related to patients occupation (P >0.05), although artisans showed the highest susceptibility compared to all the others workers (RR: 1.15, 95%CI: 0.93-1.43) (Table 2). Student were the least susceptible to PAC (P = 0.009) but most susceptible to VKC ($P \leq 0.001$) (table 2).

2.3.3 Symptoms of AC reported by Patients

Of the total 1718 AC patients, itching was the commonest symptom and was presented by all the patients, followed by tearing 46.1% (627/1361), mild ocular pain 37.4% (480/1282) and ropy ocular discharge 37.2% (551/1480) in the hierarchy of importance (Table 3).

2.3.4 Coexisting Lid and Ocular Surface Disorders

Dry eyes was present in 89 (5.2%) and pterygium in 31 (1.8%) of the AC patients. The less prevalent conditions were blepharitis [10 (0.6%)] and stye [7 (0.4%)] (Table 3).

2.3.5 Ocular complications presented prior to treatment

Corneal abrasion [17 (1.0%)] was the most common ocular complication presented. Other sight threatening complications were corneal pannus [7 (0.4%)], keratoconus [1 (0.1%)] and steroid-induced glaucoma [1 (0.1%)] (Table 3).

Table 1: Patient demographic characteristics and the type of allergic conjunctivitis

	SAC (n = 993)			PAC (n = 425)			VKC (n = 284)			AKC (n = 16)		
Gender	Dist.	OR(95%CI)	p-value	Dist.	OR (95%CI)	p-value	Dist.	OR (95%CI)	p-value	Dist.	OR (95%CI)	p-value
Male	365 ns (36.8%)	0.94 (0.86-1.02)	0.145	137 ** (32.2%)	0.77 (0.64-0.92)*	0.003	150 *** (52.8%)	1.81 (1.46-2.23)	<0.001	5 ns (31.3%)	0.73 (0.25-2.10)	0.617
Female	628 (63.2%)	1.00	-	288 (67.8%)	1.00	-	134 (47.2%)	1.00	-	11 (68.8%)	1.00	-
Age												
<15.0	357 ns (36.0%)	1.23 (0.92-1.66)	0.154	86 *** (20.2%)	0.21 (0.16-0.28)	<0.001	252*** (88.7%)	8.11(5.70-11.54)	<0.001	2 ns (12.5%)	0.19 (0.02-2.03)	0.235
15-40	468 *** (47.1%)	1.57(1.17-2.10)	<0.001	209 *** (49.2%)	0.51(0.40-0.65)	<0.001	32 ns (11.3%)	1.00	-	9 (56.3%)	0.82 (0.11-6.33)	0.582
41-65	141 ** (14.2%)	1.43 (1.05-1.94)	0.008	93 * (21.9%)	0.69 (0.53-0.90)	0.011	0 ***	NA	<0.001	4 (25.0%)	1.09 (0.12-9.96)	1.000
>65	27 (2.7%)	1.00	-	37 (8.7%)	1.00	-	0	NA	0.101	1 (6.3%)	1.00	-

Data is presented as number of individuals with percentage distribution in parenthesis. Dist. = Distribution; CI = 95% Confidence interval; OR= Odds ratio; SAC=Seasonal allergic conjunctivitis; PAC=Perennial allergic conjunctivitis; VKC=vernal keratoconjunctivitis; AKC=Atopic keratoconjunctivitis. Significance in any relationship was established using Fishers Exact Chi-square test and the Exact p-value or Monte Carlo p-value was reported for all variables. *P ≤ 0.05* was considered statistically significant. ****P ≤ 0.001; **P ≤ 0.01; *P ≤ 0.05; ^{ns}P > 0.05;* NA= not applicable implies.

Table 2: Bivariate analysis of patients' Occupation and the Type of Allergic Conjunctivitis

	SAC (n = 993)			PAC (n = 425)			VKC (n = 284)			AKC (n = 16)		
Occupation	Dist.	RR (CI)	p-value	Dist.	RR (CI)	p-value	Dist.	RR (CI)	p-value	Dist.	RR (CI)	p-value
Students	473 ns (47.6%)	1.01 (0.84-1.22)	0.906	172 ** (40.5%)	0.62 (0.45-0.86)*	0.009	135 *** (47.5%)	13.98 (1.98-98.67)	<0.001	2 ** (12.5%)	0.07 (0.01-0.41)	0.007
Artisans	84 ns (8.5%)	1.15 (0.93-1.43)	0.230	36 ns (8.5%)	0.83 (0.56-1.25)	0.444	1 ns (0.4%)	0.67 (0.04-10.60)	1.000	1 ns (6.3%)	0.22 (0.02-2.12)	0.305
Farmers	38 ns (3.8%)	0.86 (0.65-1.14)	0.334	33 ns (7.8%)	1.26 (0.86-1.86)	0.256	0 ns (0%)	NA	1.000	3 ns (18.8%)	1.11 (0.23-5.32)	1.000
Traders	127 ns (12.8%)	0.99 (0.81-1.22)	1.000	82 ns (19.3%)	1.08 (0.77-1.52)	0.688	3 ns (1.1%)	0.26 (0.04-1.50)	1.000	2 ns (12.5%)	0.26 (0.04-1.50)	0.132
Teachers	26 ns (2.6%)	0.95 (0.69-1.29)	0.852	16 ns (3.8%)	0.98 (0.60-1.61)	1.000	1 ns (0.4%)	1.78 (0.38-8.48)	1.000	3 ns (18.8%)	1.78 (0.38-8.48)	0.666
Others	74 ns (7.5%)	0.99 (0.79-1.25)	1.000	48 ns (11.3%)	1.09 (0.75-1.57)	0.769	1 ns (0.4%)	0.44 (0.08-2.56)	1.000	2 ns (12.5%)	0.44 (0.08-2.56)	0.387
None (Children)	122 * (12.3%)	0.75 (0.60-0.93)	0.023	9 *** (2.1%)	0.09 (0.46-0.19)	<0.001	142 *** (50%)	42.13 (6.0-296.4)	<0.001	0 * (0%)	NA	0.012
None (Adults)	49 (4.9%)	1.00	-	29 (6.8%)	1.00	-	1 (0.4%)	1.00	-	3 (18.8%)	1.00	-

Data is presented as number of individuals with percentage distribution in parenthesis. Dist. = Distribution; CI = 95% Confidence interval; RR= Relative risk; SAC=Seasonal allergic conjunctivitis; PAC=Perennial allergic conjunctivitis; VKC=vernal keratoconjunctivitis; AKC=Atopic keratoconjunctivitis. Significance in any relationship was established using Fishers Exact Chi-square test and the Exact p-value or Monte Carlo p-value was reported for all variables. p ≤ 0.05 was considered statistically significant. ****P ≤ 0.001; **P ≤ 0.01; *P ≤ 0.05*; ns implies *P > 0.05.* None (adults) are unemployed adults were used as there ference for occupation. NA=not applicable

2.3.6 Coexisting Atopic Diseases

Hay fever was the most prevalent atopic disease occurring in 342 (19.9%) patients across all types of allergic conjunctivitis. Asthma was reported by 57 (3.3%) patients with Atopic dermatitis found only 16 (0.9%) in patients with AKC (Table 3).

2.3.7 Anti-Allergic Medications Prescribed

Out of the total 1718 patients, only 23 were not placed on any anti-allergic agent (some were prescribed artificial tears). Thus 1695 prescriptions were made with a mean of 1.9 drugs per prescription. Polytherapy was routinely in the management of AC as 1171 (69.1%) were prescribed two or more anti-allergic medications. A total of 1198 (69.7%) were on topical steroids (dexamethasone 36.1%, prednisolone 29.3% and hydrocortisone 4.3%) and 789 (45.9%) patients on topical sodium cromoglicate; a mast cell stabilizers (Table 4). These two classes of drugs were the mostly used topical medications. Steroids and topical mast cell stabilizers were used by 277 (16.3%) and 168 (9.9%) respectively as monotherapy. For the systemic drugs, 839 (48.8%) patients were given oral antihistamines (comprising cetirizine 48.0%, chlorpheniramine 0.7% and promethazine 0.1%). This indicates an extensive use of oral antihistamines in the management of allergic conjunctivitis. NSAIDs were being used by 293 (17.1%) patients (Table 4).

2.3.8 Patterns of Prescriptions for AC

Topical Steroids

While sufferers of SAC constituted 59.9% of AC patients prescribed topical steroids, it was found that topical steroids were less prescribed (P =0.002) in all other forms of AC except AKC (Table 5).

Topical Mast cell stabilizers (TMCS)

A significant association ($P \leq 0.001$) existed between the type of AC and the pattern of prescription of TMCS. Patients with VKC (OR: 1.53, 95% CI: 0.56-4.19) were usually prescribed TMSC compared to all the other forms of AC (Table 5).

Topical non-steroidal anti-inflammatory drug (TNSAIDs)

Sufferers of SAC and PAC constituted 88.9% of AC patients prescribed TNSAIDs with PAC being often (OR: 1.40) prescribed TNSAID though no significant association was found (P =0.937) (Table 5).

Topical antihistamines (TANTHs)

Though most patients prescribed TANTHs belonged to SAC and PAC (94.5%), no significant association (P = 0.117) was found with the type of AC and the prescription of TANTHs (P =0.117). However, patients with VKC (OR: 0.29, 95% CI: 0.10-0.81) were less likely to be prescribed TANTHs compared to SAC (Table 6).

Table 3: Ocular symptoms and complications due to AC, other presenting ocular surface disorders and systemic atopic disorders

Ocular Symptoms presented	n (%)	Ocular complications *	n (%)	Ocular disorders *	n (%)	Associated Atopic disease	n (%)
Pain	480 (37.4%)	Corneal Abrasion	17 (1.0%)	Pingueculum	17 (1.0%)	Hay fever	342 (19.9%)
Redness	408 (29.4%)	Corneal pannus	7 (0.4%)	Pterygium	31 (1.80%)	Asthma	57 (3.3%)
Tearing	627 (46.1%)	Keratoconus	1 (0.1%)	Stye	7 (0.4%)	Atopic dermatitis	16 (0.9%)
Photophobia	205 (16.2%)	Steroid-induced glaucoma	1 (0.1%)	Chalazion	11 (0.6%)		
Foreign body sensation	277 (22.2%)			Blepharitis	10 (0.6%)		
Ropy Discharge	551 (37.2%)			Dry eyes	89 (5.2%)		
Burning Sensation	183 (14.5%)						
Swollen Eye Lids	126 (9.7%)						

Data is presented as number of individuals "n" with percentage distribution in parenthesis. AC=Allergic conjunctivitis,
*** Ocular complications and disorders recorded were those presented prior to treatment of allergic conjunctivitis**

Table 4: Anti-allergic medications prescribed for patients with allergic conjunctivitis

Systemic	n (%)	Topical	n (%)
Cetirizine	825 (48.0%)	Chlorpheniramine/naphazoline	72 (4.2%)
Chlorpheniramine maleate	12 (0.7%)	Diclofenac sodium	9 (0.5%)
Promethazine	2 (0.1%)	Sodium cromoglycate	789 (45.9%)
Diclofenac sodium	71 (4.1%)	Dexamethasone	621 (36.1%)
Ibuprofen	89 (5.2%)	Prednisolone	503 (29.3%)
Acetaminophen*	133 (7.8%)	Hydrocortisone	74 (4.3%)

Data is presented as number of individuals with percentage distribution in parenthesis. * Acetaminophen which is an analgesic for the purpose of this study was categorized under non-steroidal anti-inflammatory.

Table 5: Logistic regression analysis of pattern of prescribed anti-allergic medications within the various forms of AC

Type of AC	Steroid			TMCS			TNSAID		
	N (%)	*p*-value	OR (95% CI)	N (%)	*p*-value	OR (95% CI)	N (%)	*p*-value	OR (95% CI)
		0.002			<0.001			0.937	
SAC	718 (59.9)	0.432	0.60 (0.17-2.13)	390 (49.4)	0.387	0.65 (0.24-1.74)	5 (55.6)	-	1.00
PAC	266 (22.2)	0.139	0.38 (0.11-1.37)	220 (27.9)	0.897	1.07 (0.39-2.90)	3 (33.3)	0.645	1.40 (0.33-5.89)
VKC	201 (16.8)	0.383	0.57 (0.16-2.04)	171 (21.7%)	0.411	1.53 (0.56-4.19)	1 (11.1)	0.746	0.70 (0.08-6.02)
AKC	13 (1.1%)	-	1.00	8 (1.0)	-	1.00	0	1	NA

Legend: OR=Odds ratio. SAC=Seasonal allergic conjunctivitis; PAC=Perennial allergic conjunctivitis; VKC=vernal keratoconjunctivitis; AKC=Atopic keratoconjunctivitis TNSAID=Non-steroidal anti-inflammatory drug; TMCS=Topical mast cell stabilizer. AKC was used as reference for steroid and TMCS; SAC was used as reference for TNSAID; *P ≤ 0.05* was considered statistically significant.

Table 6: Logistic regression analysis of pattern of prescribed anti-allergic medications within the various forms of AC

Type of AC	TANTH			SANTH			SNSAID		
	N (%)	*p*-value	OR (95% CI)	N (%)	*p*-value	OR (95% CI)	N (%)	*p*-value	OR (95% CI)
		0.117			<0.001			<0.001	
SAC	47 (65.3)	-	1.00	504 (60.1)	<0.001	2.57 (1.93-3.42)	199 (67.9)	<0.001	4.18 (2.47-7.09)
PAC	21 (29.2)	0.874	1.04 (0.62-1.77)	247 (29.4)	<0.001	3.44 (2.50-4.75)	75 (25.6)	<0.001	3.57 (2.03-6.26)
VKC	4 (5.6)	0.018	0.29 (0.10-0.81)	81 (9.7)	-	1.00	16 (5.5)	-	1.00
AKC	0	1.00	NA	7 (0.8)	0.203	1.94 (0.70-5.38)	3 (1.0)	0.05	3.85 (1.0-14.90)

Legend: OR=Odds ratio. SAC=Seasonal allergic conjunctivitis; PAC=Perennial allergic conjunctivitis; VKC=vernal keratoconjunctivitis; AKC=Atopic keratoconjunctivitis. TANTH=Topical antihistamine; SANTH=Systemic antihistamine; SNAID= Systemic non-steroidal anti-inflammatory drug. SAC was used as reference for TANTH; VKC was used as reference for SANTH and SNSAID; $P \leq 0.05$ was considered statistically significant; NA=not applicable.

Systemic antihistamines (SANTHs)

SAC and PAC patients constituted 89.5% of AC patients that were prescribed systemic antihistamines. The pattern of prescription of systemic antihistamines was found to be very significantly associated to the form of allergic conjunctivitis ($P \leq 0.001$). SANTHs was usually prescribed for SAC (OR: 2.57) and PAC (OR: 3.44) (Table 6).

Systemic non-steroidal anti-inflammatory drugs (SNAIDs)

A very significant ($P \leq 0.001$) association was found between the pattern of prescription of SNAIDs and the type of AC. Persons with SAC and PAC alone contributed (93.5%; $P \leq 0.001$) of those that were prescribed SNAIDs (Table 6).

2.4 DISCUSSION

Allergic conjunctivitis is an ocular disorder that is influenced by both the genetic make-up of the individual and the environmental conditions such as pollution which comes along with urbanization (Bonini *et al*, 1999; Bekibele and Olusanya, 2006). These factors therefore are essential in the variations observed in the prevalence of AC worldwide. The prevalence of AC found in this study was 9.1%, with females constituting more than one-half of the total population of the AC patients. A hospital based study conducted in the Gambia by Wade *et al.* (2012) found the prevalence of AC to be 7.9%. Another clinical study conducted by Onakpoya and Adeoye (2009) in Nigeria reported a prevalence of AC to be 17.8%.Though Ghana and Nigeria have similar seasonal variations, the study in Nigeria included only children who were below 15 years old and allergies are known to be predominant among children. The high prevalence of this ocular disorder is an indication that AC is a public health problem.

Evidence from epidemiological studies regarding which sex (male or female) is more susceptible to AC has been controversial irrespective of the genetic factor that is agreed to be involved in this ocular allergic disorder. According to our study, it was observed that females had a higher burden of AC compared to their male counterparts. This finding is consistent with a study in Nigeria which found that of the total number of females, 59.3% had AC compared to 32% of the males (Ahuama and Emereole, 2005). There may be a genetic basis for the high subsceptibility of females to AC. However, it was observed during the study that females generally had a positive attitude to hospital attendance than males as they outnumbered the males reporting for eye care. This supports the findings by Hamilton *et al*., (2002) that males were worse culprits considering absenteeism to referrals. Perhaps it was also a reflection of the higher proportion of females in the Ghanaian population.

Patients' mean age for this study was 21.92 ± 18.29. This suggests that AC is essentially a disease of children and young adults. Our study indicated that the mean age for VKC patients was the least among all forms of AC and males had a higher burden of VKC. A study on VKC carried out in Uganda revealed that about 80% out of 420 children with VKC were below the age of 15 years (Kawuma, 2001).

Patients' occupation was related to the type of AC they suffered. While artisans more subseptible to SAC, traders were rather subsceptible to PAC. Traders are usually found in the market places, lorry stations, along the busy streets, and in areas where sporting and other social gatherings go on; here they are continuously exposed to dust from the environment and exhaust fumes from vehicles. Artisans are exposed to dust and fumes from materials and equipment they use to work or that generated from their working processes such as grinding, mixing, scraping, smoothening, gluing, polishing, and painting among

others. Considering AC holistically, farmers had a lower prevalence compared to traders and artisans. This finding was consistent with earlier studies which have found that farmers were less susceptible of asthma and other allergic disorders (Fuchs *et al*., 2012). Usually, farmers have past exposure during early-life to the farming environment, which exposes them to helminthic (Lampi *et al*., 2011), microbial infections (Van den Biggelaar *et al*. 2004), diet (such as unprocessed cow milk) which can be antigenic (Hagel *et al*.1993), and contacts with livestock and their fodders (Lima *et al*., 2002), which tend to activate and modulate innate and adaptive immune responses.

The association of AC with other allergic disorders has been widely reported in most studies with some observing higher prevalence of asthma in patients suffering from VKC (Ajaiyeoba, 2003; Bonini *et al*., 2000). According to this study, hay fever (allergic rhinitis) was the most prevalent with about one-fifth of the AC population suffering from this condition. According to a study conducted by Qiao *et al*. (2008), AC (especially SAC) mostly coexisted with rhinitis with over 90% of the patients presenting with either AC also having allergic rhinitis (AR) or vice versa.

This study found dry eyes and corneal abrasion to be the commonest ocular disorders presented by AC patients. This is consistent with the observation by Wakamatsu *et al*. (2011). Several factors have been associated with dry eyes and these include AC (Toda *et al*., 1995), systemic antihistamines usage (Ousler *et al*., 2007), meibomian gland dysfunction (Bron and Tiffany, 2004) and pterygium (Lee *et al*., 2002).

This study found that polytherapy was greatly used in the management of AC with topical steroids being the most commonly prescribed anti-allergic medication used as monotherapy. Steroids are the most potent anti-inflammatory drug and are therefore very effective in managing allergic reactions. The high likelihood of prescription of topical

steroids for patients with AKC and VKC found in this study may be due to the intense symptoms and inflammatory process experienced by patients with these forms of ocular allergy. These two chronic forms of ocular allergies are due to type I (attributed to the immunoglobulin E (IgE)) and type II hypersensitive reactions (cell-mediated reaction involving T-lymphocytes) which contribute to the inflammatory changes of the conjunctiva and the cornea. Steroids work rapidly to relieve the symptoms in allergic conjunctivitis. They affect the allergic response by inhibiting phospholipase A2, which is an essential enzyme in the synthesis of another group of chemicals which cause inflammation, such as the prostaglandins (Gherghel, 2002).

According to this study, there was monopoly in the choice of topical mast cell stabilizers (TMCS) in favor of sodium cromoglicate neglecting the newer and more effective TMCS such as lodoxamide, nedocromil sodium and olopatadine hydrochloride. This was because sodium cromoglicate was the only topical mast cell stabilizer that was covered under the NHIS (National Health Insurance Scheme). As a result, most pharmacies did not stock these other TMCS and patients have difficulty purchasing them when they were prescribed. Also, these other TMCS were usually inaccessible to patients due to the high cost which could be unbearable thereby making the newer TMCS infrequently prescribed by practitioners. TMCS were much prescribed for PAC than all other ocular allergies found in this study. This finding was expected looking at the mild and persistent nature of the symptoms experienced by patients with PAC. TMCS are the treatment of choice in the management of chronic AC as they act by preventing mast cell degranulation which culminates in the release of histamine and other mediators involved in the inflammatory process. TMCS therefore has a role to play in the management of VKC and AKC which are also chronic in nature. Topical non steroidal anti-inflammatory drugs (TNSAIDs) had a

limited use in the management of AC (0.5%) enjoying much use in treating PAC. This is consistent with literature as TNSAIDs only play an adjunct role to prevent the allergic response by inhibiting the enzyme cyclo-oxygenase, and can also decrease itching by raising the threshold of the conjunctival nerves (González-López, 2012; Moloney and McCluskey, 2007).

The first generation antihistamines were used topically in combination with the decongestants and these were prescribed mainly for the acute forms of AC and this may be due to the rapid onset of action and their efficacy in alleviating the symptoms. None of the second generation antihistamines which were known to be more effective and non-sedating were prescribed for topical use.

The increased use of systemic antihistamines in the management of AC found in this study was consistent with two studies, one in Spain by Del Cuvillo *et al*.(2007), and the other in France by Binder-Foucard *et al*.(2012), which have indicated that antihistamines were highly used in managing allergies (which is predominant in children). The frequent prescription of systemic antihistamines for the acute forms of AC may be due to the great advantage of treating concomitantly the symptoms in the eye, nose and throat in these patients. On the contrary to the sole prescription of first generation antihistamines that was found for local route of administration, prescriptions of systemic antihistamines were almost restricted to the second generation. The reason behind the prescription of second generation antihistamines for the systemic route may be due to their safety compared to the first generation antihistamines.

The frequent prescription of acetaminophen, an analgesic drug, for the acute forms of AC is an indication of the ocular discomfort (pain) that was presented by the patients.

2.5 CONCLUSION

Allergic conjunctivitis is the cause of the greater number of ophthalmic patient visits in Ghana, and steroids and antihistamines are the commonly used pharmacological agents in the management of this disorder.

2.6 RECOMMENDATION

There was the the need to conduct further investigaton to determine the cause of the high occurrence of dry eyes in the allergic conjunctivitis population.

CHAPTER THREE

3.0 DRY EYES: AN ADVERSE EFFECT OF SYSTEMIC ANTIHISTAMINE USE IN ALLERGIC CONJUNCTIVITIS MANAGEMENT

3.1 INTRODUCTION

Dry eyes (DE) is a symptomatic ocular surface disorder resulting from tear deficiency or loss of pre-ocular tear film (POTF) stability; causing ocular discomfort (International Dry Eye WorkShop [DEWS], 2007a). POTF overlies the conjunctiva and cornea and performs many functions including: moistening, protection, antibacterial, nutrition and optical (Lamberts, 1983; Pflugfelder *et al*., 1998; Norn, 1985). Therefore instability of the POTF could result in ocular surface disorders such as dry eyes, increased susceptibility to allergy and infection (Thoft, 1985; Suzuki *et al*., 2006).

The prevalence of DE reported by several studies worldwide has provided figures ranging from 5 to 30% (DEWS, 2007b). A clinical study by Hikichi *et al.* (1995) revealed that DEs was found in 15-30% of new patients reporting to eye centers in Japan. Very few studies have been conducted on dry eyes in Africa. However, a survey by Gillan (2009) which involved convenient sampling of 112 subjects and using questionnaire by the Ocular Surface Disease Index (OSDI) observed that about 64% experienced at least mild dry eye symptoms. Frequently reported complaints of patients suffering from DEs are burning, foreign body sensation, pain, tearing, ocular fatigue and itching (Perry and Donnenfeld, 2004; Schiffman *et al*., 2000). These symptoms affects the quality of life of patients by compromising their ability to read, drive, use the computer or watch the television resulting in loss of productivity. In addition to these, DE also imposes huge financial burden on sufferers (Clegg *et al*., 2006).

Allergic conjunctivitis (AC) is on the increase and accounts for a huge proportion of ocular consultations in Ghana (Abokyi *et al,* 2012b). Studies have shown that DE is often found in patients suffering from allergic conjunctivitis (Abokyi *et al*., 2012b; Toda *et al.* 1995; Hom *et al*., 2012). An increase in AC is therefore presumptive of a higher risk in the prevalence of DE. About one-half of cases of allergic conjunctivitis treated in Ghana involved the use of systemic antihistamines (Abokyi *et al*., 2012b). According to Al-Faris *et al.* (1999), systemic antihistamines were the most prescribed medications by practitioners and accounted for about one-fourth of all prescriptions. It is known however that systemic antihistamines decrease mucous and aqueous productions which are two components of the precorneal tear film and could therefore be implicated in DE (Ousler *et al*., 2007). Hence, despite the significant contribution of systemic antihistamines in managing AC, monitoring is required to prevent ocular complications arising from these drugs.

Although DE is a very common ocular surface disorder found in clinical practice, not much studies has been conducted in Ghana to estimate its incidence. This study therefore sought to determine the incidence of dry DE in patients with allergic conjunctivitis and the risk of DE associated with the management of allergic conjunctivitis using systemic antihistamines in Ghana.

3.2 MATERIALS AND METHODS

3.2.1 Study Area

This study was conducted in the eye clinics of St. Michael Hospital and Our Lady of Grace Hospital both run by the Catholic Health Secretariat, Ghana, and supported by the Ghana Health Service. The St. Michael Hospital, located at Pramso in the Bosomtwe District of the Ashanti Region of Ghana, serves Kumasi the Capital City of the Ashanti Region and its

environs. The eye clinic of the hospital, which has an Ophthalmologist, an Optometrist, two ophthalmic Nurses and one intern Optometrist, is the biggest in the District.

Our Lady of Grace Hospital, located at Asikuma-Odoben-Brakwa District in the Central Region of Ghana, is the District Hospital designated to serve Asikuma, Odoben, Ahwhiam, Kuntanase, Jamra, Kokoso and Bedum and its environs. Over the years the great expertise of health care providers and Staff has made the Hospital a very important Centre for the people in the District. The eye care team comprises an Ophthalmologist, two Ophthalmic nurses, an Enrolled nurse and two Ward Maids.

3.2.2 Study conduct and design

A retrospective cohort study which involved reviewing of past medical records of newly diagnosed cases of allergic conjunctivitis (AC) among the patients aged 12 years and above, from January 2010 to December 2010. A total of 1147 cases of AC were seen and managed mainly with anti-allergic medications. All patients were scheduled for a follow-up (a subsequent examination of a patient for the purpose of monitoring earlier treatment) one month after the first visit. Out of these, 738 cases were re-examined one month after the second visit (409 patients were lost due to absenteeism).

Information regarding patients' demographics (including gender, age, and occupation), case history and diagnosis were recorded. All patients underwent a basic eye examination as recommended. All cases of AC were diagnosed based on patients' complaint of ocular itching in addition to clinical signs which includes discharge, chemosis, hyperemia, or papillae of the conjunctiva. Diagnosis of dry eyes syndrome was made on the basis of patients' symptoms such as of ocular irritation and the Tear Break-Up Time (TBUT) as recommended by Toda *et al.* (1995) for patients with AC. Patient's eyes were stained with

fluorescein and observed with the cobalt blue filter under the slit-lamp whilst they avoid blinking. The appearance of dark spots on the cornea before 10 seconds was diagnosed as dry eyes.

3.2.3 Exclusion Criteria

1. New cases of allergic conjunctivitis in children below 12 years.
2. Newly diagnosed cases of allergic conjunctivitis but which were lost during follow-up or not followed up.
3. New cases of allergic conjunctivitis with concomitant dry eye on the first encounter of examination.
4. Cases of allergic conjunctivitis that were also suffering from glaucoma, chronic systemic diseases (including hypertension and diabetes) or being managed of these conditions.

3.2.4 Ethical Considerations

The study was approved by the Ethics Committee of the Department of Pharmacology, KNUST, Kumasi, Ghana. Permission was also sought from the Hospital Directors of the various eye care facilities. Confidentiality and anonymity was ensured in the use of information retrieved from the patients' medical records and the information collected was used solely for the purpose of this study.

3.2.5 Data Analysis

Data was compiled using the Statistical Packages for Social Sciences (SPSS) version 17 (SPSS Inc., Chicago, IL, 2008). Descriptive statistics such as measures of central

tendencies and dispersion, frequencies and percentages were used in analyzing the patient's demographics and other variables. Both crude odds ratios (ORs) and adjusted odds ratios (aORs) were computed using logistic regression to determine associations between dry eyes and other variables (such as gender, age, occupation, type of ocular allergy, presence or absence of a pterygium and previous medical history of antihistamine exposure). Also, relative risks (RRs) and Fisher's Exact Chi- square (χ^2) were computed to determine statistically significant differences between ocular symptoms presented by ocular allergic patients also suffering from dry eyes compared to those without dry eyes . An association was considered to be statistically significant if the *p*-value was found to be ≤ 0.05.

3.3 RESULTS

Of the 1147 cases of allergic conjunctivitis (AC) found in patients aged 12 years and above, only 738 (64.3%) cases were eligible for the study since the others failed to return within the scheduled follow-up period. The incidence of dry eye recorded on the second visit was 17.5% (129/738).

Males constituted the minority [228 (30.9%)] of the AC study population but no significant difference in susceptibility (aOR: 0.87; $P = 0.558$) was found between gender and dry eye (Table 7). Five hundred and ninety one (80.1%) of the AC patients were below 45 years with 89 (69.0%) in this age group having dry eyes (in patients above 45 years, 40 (31.0%) had dry eyes) (Table 7). Patient's age was a very significant associated risk factor (aOR: 1.02, $P \leq 0.001$) of dry eyes. The mean age (years) of participants was 30.6 ±16.9.

Students [304 (41.2%; $P \leq 0.001$)] were the largest occupational group with allergic conjunctivitis, while farmers had the least prevalence [51 (6.9%)].

A very significant association ($P \leq 0.001$) existed between patients' occupation and dry eyes. Teachers had the highest risk of dry eyes (aOR: 1.42) while students were significantly less susceptibility ($P \leq 0.001$) to dry eyes (Table 8).

Table 7: Univariate and multivariate analysis of potential risk factors relating gender and age to dry eyes

Risk factors	N (%)	Dry eye present (n=129)	Dry eye Absent (n=609)	OR (95% CI)	*p- value*	aOR(95% CI)	*p-value*
Gender							
Male	228(30.9)	31(24.0%)	197(32.3%)	0.77(0.50-1.17)	0.220	0.87(0.53-1.40)	0.558
Female	510(69.1)	98(76.0%)	412(67.7%)	1.00		1.00	
Age							
<45	591(80.1)	89(69.0%)	502(84.9%)	1.00		1.00	
≥45	147(19.9)	40(31.0%)	107(72.8%)	1.03(1.02-1.04)	<0.001***	1.02(1.01-1.04)	0.007**

Data is presented as number of individuals with percentage distribution in parenthesis. Mean age ±SD (30.6 ±16.9); OR=odds ratio; aOR= adjusted odds ratio; CI = 95% Confidence interval; p ≤ 0.05 was considered statistically significant. ***p ≤ 0.001; **p ≤ 0.01; SD=standard deviation.

Table 8: Univariate and multivariate analysis of potential risk factors relating occupation to dry eyes

Occupation	N (%)	Dry eye present	Dry eye absent	OR (95% CI)	*p- value*	aOR (95% CI)	*p-value*
					<0.001***		<0.001***
Trader	139(18.8)	35(27.1%)	104(17.1%)	0.85(0.46-1.55)	0.587	0.82(0.43-1.57)	0.815
Student	304(41.2)	25(8.2%)	279(45.8%)	0.19(0.10-0.36)	<0.001***	0.24(0.12-0.49)	<0.001***
Farmer	51(6.9)	10(7.8%)	41(6.7%)	0.45(0.18-1.10)	0.079	0.52(0.21-1.25)	0.142
Teacher	29(3.9)	8(6.2 %)	21(3.4%)	1.09(0.43-2.73)	0.858	1.42(0.54-3.74)	0.483
Artisan	80(10.8)	15(11.6%)	65(10.7%)	0.65(0.32-1.34)	0.242	0.84(0.39-1.79)	0.649
Unemployed	53(7.2%)	13(10.1%)	40(6.6%)	0.79(0.36-1.72)	0.547	0.91(0.40-2.06)	0.820
Professionals	82(11.1)	23(17.8)	59(9.7)	1.00	-	1.00	-

Data is presented as number of individuals with percentage distribution in parenthesis. OR=odds ratio; aOR= adjusted odds ratio; CI = 95% Confidence interval; * implies $P \leq 0.001$;*$P \leq 0.05$ was considered statistically significant.**

Seasonal allergic conjunctivitis (62.2%) and perennial allergic conjunctivitis (32.1%) were the commonest forms of ocular allergy with no case of giant papillary conjunctivitis found (Table 9). Univariate analysis indicated that the type of ocular allergy was significantly associated (*P* for trend = 0.039) to dry eye with PAC showing a significantly higher susceptibility (*P* = 0.046) (Table 9).

Also, of the 60 (8.1%) patients with pterygium, 26.7% (16/60) were found to have dry eyes and a statistically significant (*P* = 0.050) association existed during univariate analysis (Table 10).

A total of 441 (59.8%) AC patients were managed with Cetirizine (a systemic antihistamine) on the first visit out of which [23.4% (103/441); $P \leq 0.001$] had dry eyes (Table 10).

In relation to the symptoms of ocular irritation reported by AC patients coexisting with dry eye, several symptoms except photophobia frequently occurred among AC patients suffering from dry eye than AC patients without dry eye. Although tearing and pain were the commonly presenting symptoms, there were no statistically significant associations to dry eye ($P > 0.50$). Burning sensation was, however, found to be significantly ($P \leq 0.001$) associated to dry eyes (Table 11).

Table 9: Univariate and multivariate analysis of potential risk factors relating the type of ocular allergy to dry eyes

Risk Factor	N (%)	Dry eye present (n=129)	Dry eye absent (n=609)	OR(95%CI)	p-value	aOR (95%CI)	p-value
Ocular Allergy					0.039*		0.298
SAC	459(62.2%)	71(55.0%)	388(63.7%)	2.79(0.65-11.94)	0.166	1.26(0.28-5.71)	0.765
PAC	237(32.1%)	55(42.6%)	182(29.9%)	4.43(1.03-19.12)	0.046*	1.79(0.39-8.24)	0.454
AKC	10(1.4%)	2(1.6%)	8(1.3%)	1.67(0.14-20.58)	0.690	0.57(0.04-7.51)	0.669
VKC	32(4.3%)	1(0.8%)	31(5.1%)	1.00	-	1.00	-

Data is presented as number of individuals with percentage distribution in parenthesis. OR=odds ratio; aOR= adjusted odds ratio; CI = 95% Confidence interval; SAC=Seasonal allergic conjunctivitis; PAC=Perennial allergic conjunctivitis; VKC=vernal keratoconjunctivitis; AKC=Atopic keratoconjunctivitis. $P \leq 0.05$ was considered statistically significant. * implies $P \leq 0.05$.

Table 10: Univariate and multivariate analysis of the ocular drying effect of pterygium and systemic antihistamine (cetirizine)

Risk Factors	n(%)	Dry eye present (n=129)	Dry eye absent (n=609)	(OR 95%CI)	*p*-value	(aOR 95%CI)	*p*-value
Pterygium							
Yes	60 (8.1%)	16 (12.4%)	44 (7.2%)	1.82(1.00-3.34)	0.050*	1.16(0.61-2.21)	0.659
No	678 (91.9%)	113 (87.6%)	565 (92.8%)	1.00		1.00	
Antihistamines							
Yes	441 (59.8%)	103 (79.8%)	338 (55.5%)	3.18(2.00-5.03)	<0.001***	2.79(1.76-4.42)	<0.001***
No	297 (40.2%)	26 (20.2%)	271 (44.5%)	1.00		1.00	

Data is presented as number of individuals with percentage distribution in parenthesis crude OR=odds ratio; aOR= adjusted odds ratio; CI = 95% Confidence interval; mean duration of treatment ± SD (10.4 ± 2.2 days); $P \leq 0.05$ was considered statistically significant. * implies $P \leq 0.001$; * implies $P \leq 0.05$.**

Table 11: Symptoms of ocular allergy presented by AC patients with concomitant dry eyes

Symptoms of Ocular Allergy	Dry eye present	Dry eye absent	RR (95%CI)	*p*- value
Burning sensation	33***	71	2.08	<0.001
	(31.7%)	(68.3)	(1.48-2.92)	
Foreign body sensation	32**ns**	133	1.11	0.641
	(19.4%)	(80.6)	(0.77-1.59)	
Tearing	60**ns**	230	1.33	0.085
	(20.7%)	(79.3)	(0.97-1.84)	
Photophobia	10*	92	0.52	0.024
	(9.8%)	(90.2)	(0.28-0.95)	
Pain	46**ns**	207	1.04	0.836
	(18.2%)	(81.8)	(0.75-1.45)	

RR=Relative risk; CI=Confidence Interval; Relative risk was computed by comparing AC patients with dry eye to their cohort without dry eye. Statistically Significant differences between the two groups were established using Fishers Exact Chi-square test and the Exact *p*-value or Monte Carlo *p*-value was reported for all variables. $P \leq 0.05$ was considered statistically significant. ***implies $P \leq 0.001$; * implies $P \leq 0.05$; ns implies $P > 0.05$.

3.4 DISCUSSION

The incidence of dry eyes (DE) recorded on the second visit in patients suffering from allergic conjunctivitis in this study was 17.5% (129/738). The coexistence of allergic conjunctivitis and dry eye usually present extreme discomfort to patients, since adequate tear film serves as a barrier to allergens and dilutes them, as well as washes away inflammatory mediators. According to Asbell *et al.* (2006), cross-sectional surveys have been frequently conducted to investigate the risk factors associated to dry eyes due to the challenges in carrying out longitudinal studies on a sufficiently large population. However, cohort studies are superior to cross-sectional studies in the identification of risk factors. One noticeable hospital-based cohort study by Moss *et al.* (2004) in an older population (age range 43-84 years) recorded an incidence of 13.3% over a five year study period. A study by Nita *et al.* (2009) also reported of a much higher incidence of 63% but the subjects of their study were among patients that had presented with some symptoms of ocular irritation.

In this study gender was not found to be a significant risk factor for dry eye. The role of gender in DE is slightly controversial, with most studies noting a significantly higher susceptibility among women while a few others have observed no significant differences between the two sexes. A study on prevalence of DE among diabetic patients by Manaviat *et al.* (2008) and the five- year incidence study by Moss *et al.* (2004) supported the assertion that gender was not a significant risk factor. However, Sendecka *et al.* (2004) found out in their study that females were significantly at risk of DE. The biological factor underlying this preferential susceptibility of females to DE has been attributed to differences in the level of sex hormones produced by males and females. A study by Davison *et al.* (2005) has shown that there is a very steep decline in the level of androgens

with aging in females. Androgens (sex hormones) are known to promote normal functioning of the lacrimal and meibomian glands responsible for tear production (Sullivan *et al.,* 1999). According to Kathleen *et al.* (2000), a deficiency in these hormones could cause dry eyes.

Findings of this study reaffirms that ageing is a significant risk factor of DE in the AC population as seen in other studies (Moss *et al*., 2000; Sendecka *et al.,* 2004). Mathers *et al.* (1996) indicated that ageing was significantly associated with reduction of tear production and increased tear evaporation in the normal eye. In addition, studies have shown that ageing results in lacrimal gland dysfunction due to the obstruction of the secretory ducts of this gland (Damato *et al*., 1984; Obata *et al*., 1995).

An individual's occupation may have some impact on the health status of the eye. From the study, occupation was found to be very significantly related to DE. Adequate blinking is important in the distribution of tears to lubricate and moisten the conjunctiva and cornea. It is found that any activity such as constant reading, writing, or working with a computer that tends to decrease the rate of blinking could predispose the individual to DE (Cole *et al.* 1996). The daily activities of a teacher include all of the above, which causes them to blink less frequently, increasing the risks of dry eye. Again, teachers are continuously exposed to a health hazard from the dust particles from blackboard chalk in the class room. Chalk is a product of calcium carbonate, which is alkaline in nature. Calcium carbonate ($CaCO_3$) is considered toxic to the human eyes causing both chemical and mechanical injury to the eye resulting in inflammation (NIOSH, 1991).

Studies have indicated that sufferers of ocular allergy are usually prone to dry eye (Todal *et al.*, 1995; Hom *et al*., 2012). Our study also, discovered that PAC was associated with the highest risk of DE. PAC is mainly caused by house dust mite (HDM), animal dander and

cockroaches which are indoor allergens. HDM exhibits proteolytic activity capable of causing damage to ocular epithelial cells (Chapman *et al*., 2007). The chronic exposure to these allergens in sensitized individuals results in persistent inflammation of the ocular surface (a prerequisite for DE) (Choi and Bielory, 2008; Stern *et al.,* 1998; Nelson *et al.* 2000).

The uniformity of the ocular surface is vital in allowing an even distribution of tears. The presence of pterygium on the eye compromises this uniform distribution of tears resulting in desiccation of areas of the ocular surface. Our study observed that the presence of pterygium was associated with a higher risk of DE. Lee *et al.* (2002) noted that pterygium remained a very significant risk factor of DE after adjusted for age and sex. A clinical case-control study by Rajiv *et al.* (1991) observed a decrease in both values of TBUT and Schirmer test in cases of pterygium compared to controls.

The high use of systemic antihistamines in allergic conjunctivitis management is an indication that these medications play a crucial role in the management of ocular allergies. The use of systemic antihistamines instead of topical antihistamines perhaps suggests of the coexistence of nasal symptoms in patients (Del Cuvillo *et al*., 2009). According to Qiao *et al.* (2008) about 90% cases of AC were concomitantly associated with allergic rhinitis. The second generation antihistamines are much safer and therefore preferred in managing AC. However, evidence suggests that these drugs induce some extent of an ocular drying effect (Ousler *et al.*, 2007, Del Cuvillo *et al*., 2009). In our study, cetirizine (a second generation antihistamine) was the only systemic antihistamine prescribed in managing AC and patients that had been managed with systemic antihistamines in this short term were almost 3 times much likely to experience symptoms of DE ($P \leq 0.001$) after controlling for confounders. A few studies have investigated the effect of systemic antihistamines on the ocular surface,

although using the cross-sectional design. Consistently, a positive correlation has been observed between systemic antihistamines use and DE. One very important cohort population based 10-year dry eye study by Moss *et al*. (2008) discovered that exposure to systemic antihistamine was significantly associated with 1.24 times risk of DE. Their study only adjusted for age and gender, neglecting other potential risk factors which could have masked the actual risk due to systemic antihistamines. Our study therefore found that after controlling for other potential risk factors of DE, systemic antihistamine exposure was associated with the most risk and may account for the high incidence of DE in allergic conjunctivitis patients.

To diagnose DE in patients already suffering from allergic conjunctivitis is very essential for appropriate management of their condition. According to the Subcommittee of International Dry Eye Workshop (2007a), the symptom of ocular dryness should serve as guide to the differential diagnosing of DE. However, it is usually challenging to diagnose DE in patients with AC because apart from itching, AC presents with ocular symptoms (including tearing, foreign body sensation, photophobia, burning sensation and dryness) which overlap with DE symptoms. According to this study burning sensation was found to be very significantly related DE ($P \leq 0.001$) and therefore could prompt the likelihood of concomitant DE among AC sufferers. This is consistent to some literature indicating that burning is a primary symptom of DE (Atiya *et al*., 2007).

3.5 CONCLUSION

The management of allergic conjunctivitis using systemic antihistamines was associated to dry eyes. Practitioners should monitor patients being managed with these anti-allergic medications as dry eyes further complicates allergic conjunctivitis.

3.6 RECOMMENDATION

Studies on alternative treatment modalities for allergic conjunctivitis needs to be intensified as the conventional pharmacological management has been associated with more sight-threatening complications.

CHAPTER FOUR

4.0 ANTI-ALLERGIC EFFECTS OF AN AQUEOUS EXTRACT OF *PISTIA STRATIOTES* IN MURINE MODEL OF OVALBUMIN-INDUCED ALLERGIC CONJUNCTIVITIS

4.1 INTRODUCTION

A preliminary survey conducted in two reputable eye clinics in Ghana indicated that the most frequently prescribed antiallergic drugs for managing allergic conjunctivitis were associated with ocular disorders including dry eyes, cataract, glaucoma and corneal ulcer (Abokyi *et al*., 2012). It is therefore crucial to conduct further research on alternative treatment modalities for allergic conjunctivitis.

Traditional medicinal plants play a crucial role in complementing the health care needs as about 80% of the world's population is estimated to have used herbal products for their primary health needs (Doughari, 2012). In Ghana, reports indicate that majority (70%) of the population use herbal medicine for treating health disorders (WHO, 2001).

An aquatic macrophyte plant called *Pistia Stratiotes (Linn.)* has been reported to possess several medicinal properties (Tripathi *et al*., 2010). The topical application of *P. stratiotes* on the eye is known in the Gambia, where it is used as an ocular anodyne. Also, anti-inflammatory properties have been shown as the extract was effective in the management of uveitis (Kyei *et al*., 2012b). It is also documented that *Pistia stratiotes* is used in herbal preparations for the management of asthma (Alexander *et al*., 2011). It is on these premises that this study sought to investigate the anti-allergic effect of an aqueous extract of *P. stratiotes* in a murine model of ovalbumin-induced allergic conjunctivitis.

4.2 MATERIALS AND METHODS

4.2.1 Collection and Authentication of Plant

Pistia stratiotes was collected from the Fosu lagoon, in the Central Region of Ghana, in December 2010 and identified by Dr. George Henry Sam, Department of Herbal Medicine, College of Health Sciences, KNUST, Kumasi, Ghana. A voucher specimen (KNUST/ HM1/11/W002) has been deposited at the Department's herbarium.

4.2.2 Preparation of Aqueous leaf extract of *P. stratiotes* (ALPS)

Fresh leaves of *Pistia stratiotes* were picked, washed and air dried. The dried leaves were powdered using a hammer mill (Schutte Buffalo, New York, USA). A 700 g quantity of the powder was soaked in a liter of water for 24 h. Reflux filtration was performed at 80^{o}C. The collected filtrate was lyophilized into powder with a Hull freeze dryer /lyophilizer 140 sq ft (model 140FS275C, USA) , which was labeled aqueous leaf extract of *Pistia stratiotes* (ALPS) and stored at 4^{o}C until during experimentation when powder was constituted into the required concentrations with normal saline.

4.2.3 Phytochemical Screening of ALPS

Aqueous leaf extract of *Pistia stratiotes* was screened following recommended protocols described for the presence of phytochemicals (Trease and Evans, 1996).

4.2.3.1 Tests for Alkaloids

To 0.5 g of extract 5 ml of 1 % aqueous hydrochloric acid was added and the solution warmed and filtered. A 1 ml each of the filtrate was transferred into two test tubes. To one of the test tubes was added a few drops of Mayer‘s reagent and to the other test tube few drops of Dragendorff‘s reagent and observation made. An orange precipitate indicates the presence of alkaloid.

4.2.3.2 Test for Glycosides

To 0.5 mg of the extract was added 5ml of diluted sulphuric acid. This is followed by the addition of 2 ml of 20% sodium hydroxide and few drops each of Fehling‘s solutions A and B. The mixture was heated on water bath for two minutes. Appearance of a brick-red precipitate is an indication of the presence of glycosides.

4.2.3.3 Test for Steroids

A one milliliter of concentrated sulphuric acid was added to 10 mg of extract dissolved in 1.0 ml of chloroform. Appearance of reddish blue colour exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of steroids.

4.2.3.4 Test for Terpenoids (Salkowski test)

To 0.5 mg extract dissolved in 2 ml of chloroform was 1 ml of concentrated sulphuric acid. Appearance of reddish violet ring at the junction of the two layers confirms the presence of triterpenoids.

4.2.3.5 Test for Flavonoids

Extract weighing 0.5 mg was added 5 ml of ethyl acetate in a test tube and warmed. Few drops of dilute ammonia solution were added to the mixture. Appearance of yellowish colour at the bottom of the test tube is indicative of flavonoids.

4.2.3.6 Test for saponins

Five grams of P. stratiotes extract was dissolved in boiled water in a test tube, allowed to cool shaken to mix thoroughly. Appearance of froth is an indication of saponins.

4.2.3.7 Tests for tannins

A 0.5 ml of extract was dissolved in 10 ml of boiled distilled water and hot-filtered. The filtrates were then allowed to react with 1.0 ml of 5% ferric chloride solution. Formation of greenish black coloration demonstrates the presence of tannins.

4.2.4 Ethical and Biosafety Considerations

Laboratory study was carried out in a level 2 biosafety laboratory. Protocols for the study were approved by the Departmental Ethics Committee. All activities during the studies conformed to accepted principles for laboratory animal use and care (EU directive of 1986: 86/609/EEC). Biosafety guidelines for protection of personnel in the laboratory were observed.

4.2.5 Experimental Animals

Eight-week old Imprinting Control Region (ICR) mice of either sex weighing 18-24g (mean: 21.08 ± 3.37g) were provided by the Animal House Unit of the Department of Pharmacology, KNUST, Kumasi, Ghana. These animals were kept in metallic cages under ambient conditions of temperature (25 ± 3°C), relative humidity (60–70%) and light/dark cycle throughout the study. Mice were given normal commercial mice chow pellet from AGRICARE, Kumasi, Ghana, and water *ad libitum* throughout the experimental period. Mice were made to acclimatize to the laboratory condition for at least one week before experimentations.

4.2.6 Induction of Allergic Conjunctivitis

Allergic conjunctivitis (AC) was induced using an active immunization protocol for the murine model as described below. During a sensitization phase, mice were injected intraperitoneally with 0.2 ml of emulsion solution containing 100 μg ovalbumin (Cayla-InvivoGen, Toulouse, France) and 0.01mg aluminium hydroxide (Hopkins and Williams Ltd., Chadwell Heath, Essex, UK) in phosphate buffer saline (PBS) of pH 7.4. Immunization was repeated after 7 days. In the challenge phase, each eye of the sensitized mice was topically instilled with 1.5 mg ovalbumin in 10μL PBS into the conjunctival sacs using a micropipette two times on day 15 and day 18 after first immunization. Conjunctival redness, lid edema, tearing and lid scratching in mice were indications of Ovalbumin-induced allergic conjunctivitis (OIAC).

4.2.7 Pretreatments of Ovalbumin-immunized mice

All mice were randomly assigned into eight groups (n=7) before immunization with ovalbumin. These were: the normal control group (mice not immunized and not treated); the untreated group (mice immunized but not treated); the normal saline group (mice immunized and pretreated with 2 ml/kg normal saline); the cetirizine group (mice immunized and pretreated with 5 mg/kg cetirizine); the prednisolone group (mice immunized and pretreated with 10 mg/kg prednisolone); and the other 3 ALPS groups (comprising of mice immunized and pretreated with ALPS at doses of 10, 50 and 100 mg/kg respectively). Treatments were provided orally 1 hour before topical challenges with ovalbumin on days 15 and 18. All animals were kept under the same experimental conditions.

4.2.8 Clinical Assessment of Ocular Allergy

Slit-lamp Examination

The extent of AC indicated by conjunctival redness, lid edema, tearing and lid scratching, in the eye of the mice was then scored 30 mins after the last topical challenge by observation under a SL500 Shin Nippon Slit Lamp (Ajinomoto Trading Inc., Tokyo, Japan).

Scoring of Allergic Conjunctivitis

Conjunctival redness, chemosis and tearing were assigned a score on the scale of 0 to 3 designating absent to severe allergic response as recommended in a previous study (Ozaki *et al*., 2005). Lid scratching was monitored for 30 s, and the frequency of scratching was

counted. Only one eye each was assessed for all mice and data for the clinical signs presented as the average per group.

4.2.9 Ovalbumin-specific Antibodies Assay

Protocol outlined by manufacturers of mouse ovalbumin-specific IgE ELISA kit (Biolegend, San Diego, CA) was strictly followed.

Obtaining the serum

Mice were anaesthetized with chloroform (VWR International Ltd, Leicester, UK) and blood collected by cardiac puncture into eppendorf tubes (Sigma-Aldrich, St. Louis, MO, USA) and allowed to clot. The clotted blood was centrifuged (temperature 25°C, speed 3000 g) for 5 minutes using a Mikro 220R machine (Hettich Zentrifuge, Tuttlingen, Germany). Serum obtained was stored at -70°C in eppendorf tubes for evaluation.

Serum Assay Procedure

A twofold diluted serum samples and dilutions of the standard stock solution provided in the ELISA kit were added onto antigen pre-coated plates and incubated at room temperature for 2 h while shaking. Plate content was discarded and washed with buffer after which was followed an addition of mouse IgE detection antibody (enzyme labeled second antibody) and incubation for 1 h at room temperature while shaking. Avidin-horseradish peroxidase (HRP) solution was pipetted into washed wells and incubated for 30 min to facilitate binding of enzyme labeled second antibody to Avidin conjugates. This was followed by washing of plate with buffer and pipetting of Substrate Solution F into wells and 15 min of incubation in the dark to develop coloration proportionate to IgE

concentration in sample and reaction stopped by adding Stop Solution to wells. Absorbance of each well was read at 450 nm by a plate reader (Thermo Scientific Multiskan EX, Thermo Fisher Scientific Oy, Vantaa, Finland) within 10 min.

4.2.10 Histopathological Assessment

Histopathological assessment of the conjunctival mucosal tissue was carried out at the Pathology Department of the Komfo Anokye Teaching Hospital, Kumasi, Ghana. The eyes including conjunctiva and lids were exenterated and fixed in 10% buffered formalin (Yash Chemicals, India). These were processed into formalin-fixed paraffin-embedded tissue blocks. Tissue blocks were sectioned at 3 micrometer and dewaxed in two changes of xylene for 5 minutes each, dehydrated industrial methylated spirit and washed in running tap water. Hydrated tissue sections were stained with Giemsa prepared from stock solution (at 1 in 10 dilutions) for 10 minutes. Stained slides were then rinsed in distilled water for 5 minutes and were differentiated briefly in acid alcohol. This was immediately followed by intense rinsing for 30 minutes. Sections were then dehydrated, cleared and mounted. Considering hot spots in each conjunctival tissue section, the degree of inflammation (i.e. the extent of mast cell infiltration and degranulation) was scored according to Table 12.

Table 12: Scoring of conjunctival inflammation in Ovalbumin-induced allergic conjunctivitis

Score	Degree of inflammation	Mast cell infiltrations per conjunctival section
0	Normal	< 30%
1	Mild	≥ 30% but < 50%
2	Moderate	≥ 50% but < 70%
3	Marked	≥ 70% but < 100%
4	Severe	100%

4.2.11 Data Analysis

Data obtained was presented as mean ± SEM. Statistical analysis was carried out using the unpaired t-test (two-tailed), and one-way analysis of variances (ANOVA) followed by Dunnetts' test *post hoc*, provided by GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA). Probability values found to be $P \leq 0.05$ were considered significant.

4.3 RESULTS

Phytochemical Screening

Preliminary screening of ALPS indicated the presence of alkaloids, glycosides, steroids, flavonoids and tannins (Table 13).

Table 13: Results for phytochemical screening in aqueous extract of *Pistia stratiotes*

Compound	Observation
Alkoloids	+
Glycosides	+
Steroids	+
Flavonoids	+
Saponins	-
Tannins	+
Triterpenoids	-

(-): Absent, (+): present

Clinically observed Anti-Allergic Effect

After sensitization and challenge with ovalbumin, there was about 7 fold increase in the mean clinical AC score for untreated group compared to normal control group (2.60 ± 0.07 vs. 0.40 ± 0.06, $P \leq 0.001$). Pretreatment with ALPS (10, 50 and 100 mg/kg) significantly and dose-dependently reduced ($P \leq 0.05$ - 0.01) conjunctival redness, lid edema, tearing and lid scratching i.e. the early allergic inflammatory response compared to normal saline treatment. Cetirizine and prednisolone-pretreatment groups showed significantly low mean clinical scores of OAIC ($P \leq 0.01$ - 0.001) compared to the other immunized groups of mice. No significant difference ($P > 0.05$) was observed comparing the untreated group to the normal saline group (Table 14).

Ovalbumin-specific Antibodies Assay

Serum concentration of ovalbumin-specific IgE was significantly high ($P \leq 0.001$) following induction of OIAC in the untreated group. ALPS (50, and 100 mg/kg) pretreatment before OIAC resulted in significantly low ($P \leq 0.01$ - 0.001) sera ovalbumin-specific IgE. The prednisolone-treated group showed the lowest significant serum ova-

specific IgE concentration ($P \leq 0.001$), while no significant difference ($P > 0.05$) was observed with cetirizine pretreatment (Figure 3).

Histopathology Assessment

The normal control group showed no sign of mast cell infiltration into the conjunctiva (Table 15; Plate 1). The untreated ovalbumin-sensitized mice and the normal saline pretreated mice showed significantly high ($P \leq 0.001$) mast cell infiltration and degranulation in the conjunctival epithelium and stroma after topical challenge with ovalbumin (Table 15; Plates 2 and 3). Mast cell infiltration and degranulation, however, was significantly low ($P \leq 0.05 – 0.01$) in the conjunctival stroma of ovalbumin-sensitized mice pretreated with cetirizine, prednisolone and ALPS (Table 15: Plates 4 – 8; Figure 4).

Table 14: Scores for clinical assessment of ocular inflammatory response due to OAIC in ICR mice following pretreatment.

Signs of AC	Normal	UT	2 ml/kg NS	5 mg/kg CET	10 mg/kg PRED	10 mg/kg ALPS	50 mg/kg ALPS	100 mg/kg ALPS
Conjunct Redness	0.29± 0.18	2.78±0.20 $^{\phi\phi\phi}$	2.93± 0.18 ^{ns,†††}	1.25± 0.25 **,††	1.02±0.45**,††	1.83± 0.20 *,††	1.59 ± 0.18 *,††	1.33 ± 0.18 **,††
Chemosis	0.43± 0.20	2.55± 0.18 $^{\phi\phi\phi}$	2.70± 0.20 ^{ns,†††}	1.14.± 0.14 **,†	0.74±0.25^{***,ns}	1.58± 0.18 *,†††	1.71 ± 0.18 *,†††	1.43 ± 0.20 **,††
Tearing	0.29± 0.18	2.85± 0.14 $^{\phi\phi\phi}$	2.54 ±0.25 ^{ns,†††}	0.57± 0.20 ^{***,ns}	0.94±0.74**,††	1.63± 0.20 *,†††	1.64 ± 0.14 *,†††	1.50 ± 0.00 *,†††
Lid scratch	0.57± 0.20	2.69±0.30 $^{\phi\phi\phi}$	2.81± 0.35 ^{ns,†††}	0.71± 0.18 ***,†††	1.45±0.60**,††	1.74± 0.25 *,†††	2.14 ± 0.20 ^{ns,†††}	1.43 ± 0.25 **,†††
Mean clinical AC Score	**0.40± 0.06**	**2.60±0.07** $^{\phi\phi\phi}$	**2.75±0.08** ^{ns,†††}	**0.92±0.16** ***,†	**1.04±0.75****,††	**1.69± 0.06** *,†††	**1.71 ± 0.20** *,†††	**1.42 ± 0.04** **,†††

Values are as mean ± SEM. Examination was carried out 30 min after induction of AC in ICR under a slit-lamp biomicroscope. Drug intervention was provided 1h before multiple topical challenges on days 15 and 18 after first immunization. Differences between Normal and Untreated: $^{\phi\phi\phi}$ $P \leq 0.0001$ (unpaired t-test, two-tailed). Differences between Untreated and Treatment groups: $^{ns}P > 0.05$,$^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$. Difference between Normal and Treatment groups: †† $P \leq 0.01$, ††† $P \leq 0.001$ (One-Way ANOVA followed by multiple Dunnett's post hoc test); untreated (UT), normal saline (NS), cetirizine (CET), prednisolone (PRED), aq. Leaf extract of *P. stratiotes* (ALPS), ovalbumin.

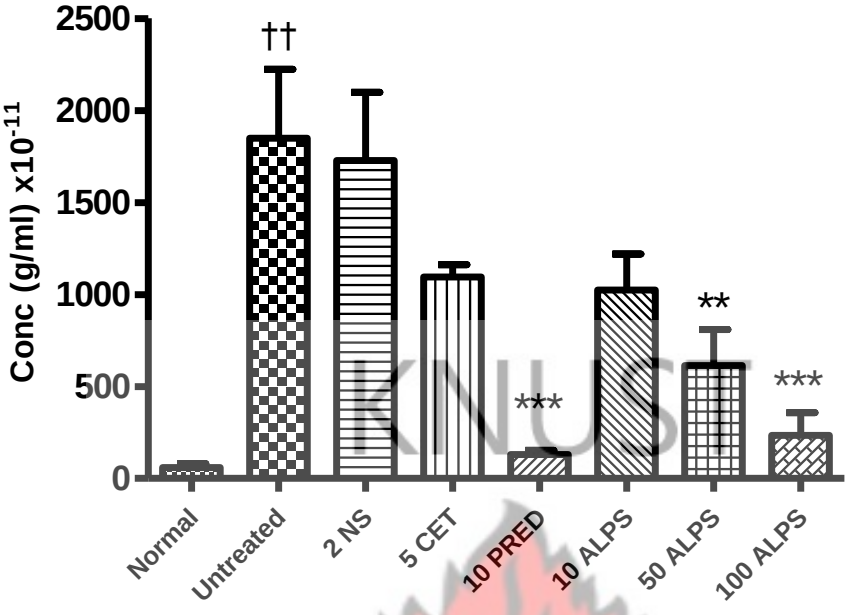

Figure 3: Sera concentration of IgE in ICR mice. Pretreatment with 2ml/kg normal saline (NS), 5mg/kg cetirizine (CET), 10 mg/kg prednisolone or 10, 50, and 100mg/kg of the aqueous leaf extract of *Pistia stratiotes* (ALPS) was given 1h in ova sensitized mice before topical challege. Normal vs. Untreated (unpaired t-test, two-tailed): †† $P \leq 0.01$. Untreated vs. Treatments (One-Way ANOVA followed by multiple Dunnett's *post hoc test*): **$P \leq 0.01$, ***$P \leq 0.001$.

Table 15: Histopathology of conjunctival tissue of normal and ovalbumin-induced allergic conjunctivitis (OIAC) in ICR mice

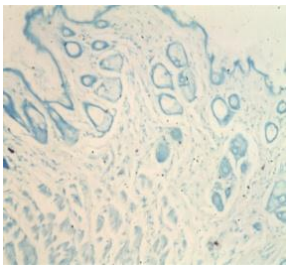

Plate 1: Normal conjunctival epithelium and stroma

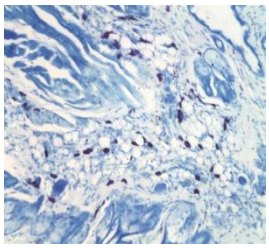

Plate 2: Untreated OIAC showing increased significant mast cell infiltration and degranulation in conjunctival stroma

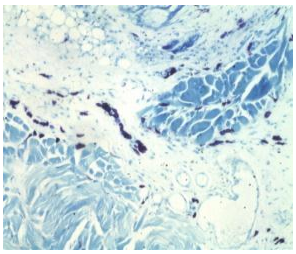

Plate 3: Normal saline-treated OIAC showed significant infiltration and degranulation of mast cells after treatment with normal saline

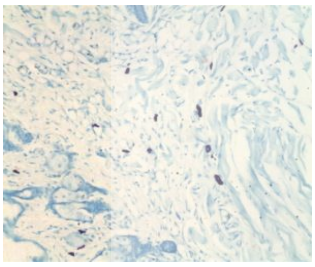

Plate 4: Cetirizine-treated OIAC showing minimal infiltration of intact mast cells in conjunctival stroma

Plate 5: prednisolone –treated OAIC showing significantly reduced mast cell population in conjunctival epithelium and stroma

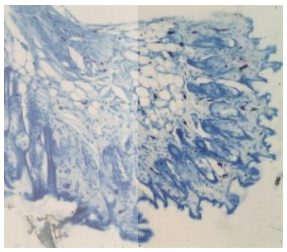

Plate 6: 10 mg/kg ALPS-treated OIAC showing minimal infiltration of intact mast cells in conjunctival epithelium and stroma

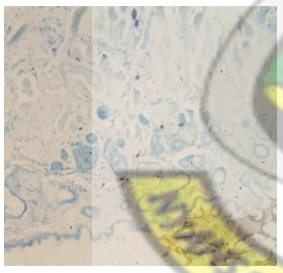

Plate 7: 50 mg/kg ALPS-treated OIAC showing significantly reduced mast cell population in conjunctival stroma

Plate 8: 100 mg/kg ALPS- treated OIAC showing significantly reduced mast cell population in conjunctival epithelium and stroma.

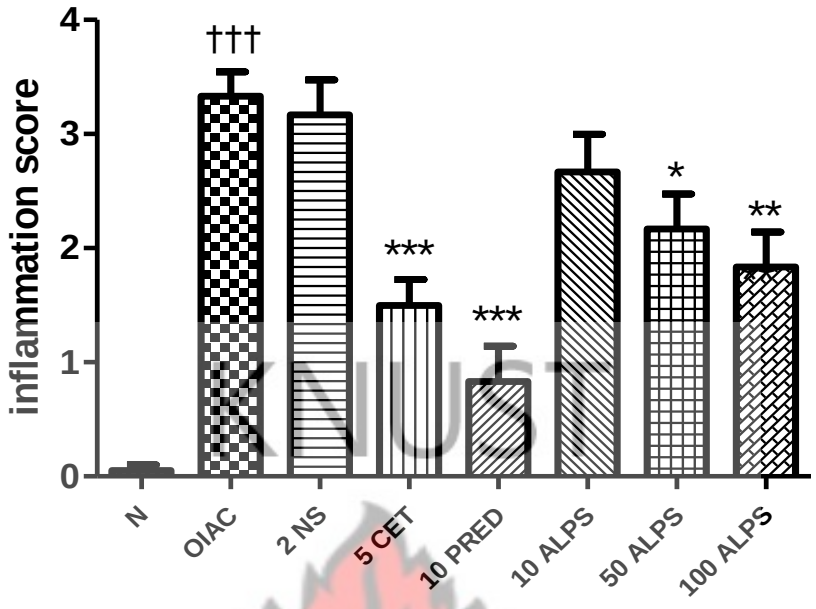

Figure 4: Ocular histological assessment of degree of mast cell infiltration and degranulation in ICR mice. Pretreatment with 2ml/kg normal saline (NS), 5mg/kg cetirizine (CET), prednisolone (PRED) or 10, 50, and 100mg/kg of the aqueous leaf extract of *Pistia stratiotes* (ALPS) was given 1h in ova sensitized mice before topical challenge. IOAC vs Normal (unpaired t-test, two-tailed): ††† $P \leq 0.001$. OIAC vs. Treatments (One-Way ANOVA followed by multiple Dunnett's *post hoc test*): $^{**}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

4.4 DISCUSSION

Allergic conjunctivitis (AC) was induced in ICR mice after sensitization and challenge with ovalbumin. Ovalbumin (OVA) is a phosporylated glycoptrotein with a molecular weight of approximately 45 kDa and 386 amino acid residues (Nisbet *et al*., 1981) and belongs to the serpin superfamily of proteins (Gettins, 2002; Stein *et al*., 1990). OVA has several mannose (carbohydrate) residues which binds to the mannose receptor (Huntington and Stein, 2001; Burgdorf *et al*., 2006; Autenrith *et al*., 2007) expressed by antigen presenting cells such as macrophages, dendritic cells (DCs), monocytes (Harris *et al*., 1992; Figdor *et al*.2002).

Studies have also identified some specific peptides of Ovalbumin containing both T and B cell epitopes (Janssen *et al*., 1999). Injection of ovalbumin in combination with Alum adjuvance enhances an immune response by induction of inflammation resulting in the release of uric acid from necrotic cells (HogenEsch, 2002) leading to recruitment and differentiation of monocytes to dendritic cells which phagocytose and presents it as linear peptides (having T and B cell epitopes) bound to class II MHC molecules. Naïve T helper (Th0) cells bind by their T cell receptors (TCR) to the MHC (II) complex resulting in the activation and differentiation into Th2 cells which are vital in allergic inflammatory process.

Th2 cells produce cytokines like IL-4, IL-5, IL-9 and IL-13 that are involved in the activation and differentiation of B cells to memory B cells and the class-switching of B cells to allergen-specific IgE synthesis as well as the recruitment of mast cells. These were evident from the increased sera IgE and massive mast cell infiltrations observed in the ocular tissues OIAC mice compared to the normal. Sensitization is completed after

IgE attaches to high-affinity IgE receptors (FCeRI) on mast cells. During the local ocular challenge, the allergen-specific IgE antibodies were cross-linked by IgE binding epitopes resulting in degranulation of the mast cells and the release of histamine. The consequences of histamine release from degranulated mast cell were characterized by manifestations of significantly increased lid scratching, conjunctival redness, chemosis and tearing observed in the OIAC mice.

While mast cells are considered to be principally effector cells in the pathogenesis of the early phase of allergic response, studies have found that mast cells also participate in the late phase of through the recruitment of inflammatory cells including Th2 lymphocytes and eosinophils through the action of prostaglandin D2 (Hirai *et al*., 2001). While eosiniphil infiltration usually characterizes the late phase of allergic induction, its absence has been noticed in some murine models indicating that it is not a prerequisite (Tournoy *et al*., 2000), but may rather exert regulatory functions instead of an effector or proinflammatory role in chronic allergies (MacKenzie *et al*., 2001; Mattes *et al*., 2002; Alam and Busse, 2004).

Cetirizine, very significantly reduced clinical signs of AC and mast cell infiltrations but not the sera IgE. This was because IgE synthesis and mast cell degranulation precede histamine release and stimulation of histamine receptors which is the target for antihistamines. Several studies have purported 3 subtypes (H1, H2 and H3) of histamine receptors on the conjunctiva (Bielory and Ghafoor, 2005) and a recent study has indicated H4 receptors may also be found on the conjunctiva (Hayashi *et al*., 2012). However, H1 receptor stimulation is chiefly responsible for nerve end stimulation and vasodilation (Bielory and Ghafoor, 2005) resulting in ocular symptoms such as itching, redness,

chemosis and tearing experienced by sufferers of ocular allergy. The preferential H1-selectivity, safety and rapid onset of action of cetirizine make it a favorable drug in the managing ocular allergy. Its efficacy in the management of allergic disorders including ocular allergy is widely documented (Curran *et al*., 2004; Masi *et al*., 1993). Apart from acting as histamine receptor antagonists, cetirizine and its derivatives have also been observed to exhibit anti-inflammatory activity (Walsh, 2000; 2009), which could explain for the decreased mast cell infiltration observed in the ocular tissue.

ALPS significantly reduced clinical symptoms, sera OVA s-IgE concentration and mast infiltration and degranulation, indicating anti-allergic and/or anti-inflammatory effect. A study that has already explored the anti-inflammatory activity of *P. stratiotes* observed that this extract was effective in the management of inflammatory disorders such as fever and arthritis with activity comparable to standard anti-inflammatory drugs such as methotrexate, diclofenac, and dexamethasone (Kyei *et al*., 2012a). A recent study involving the use of the extract in the successful management of a sight threatening intra-ocular inflammatory disease is also documented (Kyei *et al*., 2012b). While its anti-allergic potential may not be well known, *P. stratiotes* is already purported to be among some herbal preparations in the management of asthma (Alexander *et al*., 2011).

The activity of *P. stratiotes* could be due to the collective effects of alkaloids, glycosides, steroids, flavonoids and tannins; the secondary plant metabolites present. Studies have isolated and identified specific bioactive compounds from this plant including Steroids (such as Stigmasta-4,22-dien-3-one, stigmasterol, stigmasteryl stearate, and palmitic acids) and other flavonoid glycosides (such as two di-C-glcosylflavones of vicenin and

lucenin type, anthocyanin-cynidin-3-glucoside, luteolin-7-glycoside and mono-C-glcosyl flavones – vitexin and orientin) (Zennie, 1997).

While most classes of these phytochemicals have shown immunomodulatory activities the flavonoids have received a great deal of attention for their anti-inflammatory and anti-allergic properties. These effects are achieved through diverse mechanisms of actions proposed by these metabolites. For instance, quercetin, a flavonoid inhibits both the cyclooxygenase and 5-lipoxygenase pathways (Ferrandiz *et al.*, 1991, Laughton *et al.*, 1991) thereby regulating the release of arachidonic acid (Yoshimoto *et al.*, 1983). Cyclooxygenase and lipoxygenase are required in the synthesis of arachidonic acid, which is a starting point for a general inflammatory response. Quercetin has also been reported to inhibit histamine release by allergen-stimulated human basophils (Middleton and Kandaswami, 1992). Another unique flavonoid, rosmarinic acid, has been shown to induce apoptosis, or cellular suicide in allergy-activated T cells and neutrophils during allergic reactions without affecting the inactivated T cells or neutrophils (Hur, 2004).

Also, luteolin glycosides, which has already been isolated from this plant has been purported to be the most potent inhibitor of histamine release from mast cells (Inoue *et al.*, 2002). In addition luteolin has been reported to inhibit the CD40 ligand expression by basophils and mast cells (Hirano *et al.*, 2006). B cells express CD40 and activation and differentiation of B cells into IgE producing plasma cells requires costimulation by a cell expressing CD40 ligand and cytokines (including IL-4 or IL-13) (Yanagihara, 2003) . Therefore, inhibition of CD40 ligand expression and cytokine synthesis make flavonoids potential natural IgE inhibitors.

4.5 CONCLUSION

Oral administration of aqueous leaf extract of *P. Stratiotes* exhibited potent antihistamine, anti-IgE and anti-inflammatory activity in ICR mice sensitized and challenged by topical instillation of ovalbumin. *Pistia stratiotes* has a rich source of flavonoids stored in the form flavonoid glycosides and these polyphenolic plant metabolites may be the remedy to allergies.

4.6 RECOMMENDATION

Investigation of the effect of *P. stratiotes* on the ocular surface is warranted to determine whether or not this plant exhibits any adverse effect on tear secretion and/or tear film stability.

CHAPTER FIVE

5.0 EVALUATION OF THE EFFECT OF *PISTIA STRATIOTES* LINN (ARACEAE) ON TEAR SECRETION AND TEAR FILM STABILITY IN ICR MICE

5.1 INTRODUCTION

Drugs prescribed are of invaluable therapeutic benefit when associated adverse effects are minimal. Conventionally, management of allergic conjunctivitis (AC) involves the use of antihistamines, mast cell stabilizers, steroids and decongestants (Duvall and Kershner 1998; Rosa *et al*., 2013). Treatment with these drugs usually is targeted at managing the symptoms (aftermaths of the allergic response). AC is therefore recurrent, demanding sufferers to continuously rely on these medications. AC management with these drugs, however, may present with the challenge of adverse effects including cataract, corneal ulcers, glaucoma, and dry eyes (Ousler *et al*., 2004; Allen *et al*., 1989; Mohan and Muralidharan, 1989). Abokyi *et al.* (2012) in earlier studies on epidemiology of AC, drug-prescription patterns and adverse effects, established that dry eyes was the most common ocular disorder associated with systemic antihistamine use.

Currently, the recommend therapy for AC involves the use of anti-allergic medications; that have mast cell stabilization and antihistaminic effects, and have longer duration of action (Abelson *et al*. 2007; Beauregard *et al*., 2007; Torkildsen and Shedden 2011). It has, however, been reported by some researchers that the newer orthodox anti-allergic drugs that meet the above criteria also adversely impair tear secretion or decrease tear film stability, resulting in dry eyes (Villareal *et al*., 2006).

Studies have indicated that some herbs possess most of these qualities (Makino *et al*., 2001; Takano *et al*., 2004; Tachibana *et al*., 2001). Recent studies have revealed *Pistia stratiotes*, a traditional medicinal plant, to possess potent anti-inflammatory and anti-allergic properties, and has been used in the management of uveitis and arthritis (Kyei *et al*., 2012; Koffuor *et al*., 2012). A preliminary investigation conducted on the anti-allergic properties of *P. stratiotes* has shown that the plant is effective in the management of a murine model of ovalbumin-induced AC. It was observed that the herb effectively lowered the serum IgE concentration and also significantly reduced signs of acute allergic inflammation, suggesting its useful in the therapeutic management of AC in humans. Other studies have also indicated the beneficial role of this *P. stratiotes* in the management of asthma (Alexander *et al*., 2011).

While efforts are being channeled into exploring the potentials of this herb in the management of ophthalmic disorders, it is important to study the effect on tear secretion and tear film stability as a way of evaluating its tendency to causing dry eyes (as seen with conventional antihistamines) when used in the management of AC.

5.2 MATERIALS AND METHODS

5.2.1 Animals

Twenty eight ICR mice of either sex (10–12 weeks old) weighing 26–30 g obtained from the Department of Pharmacology, KNUST, Animal House were used in this study. All mice were housed in a constant room temperature 27 °C ± 5 °C and relative humidity 60% ± 10% with a ambient light and dark cycle. Mice were fed with normal commercial mice chow pellet from Ghana Agro Food Company Limited (GHAFCO), Ghana, and

water *ad libitum*. All procedures performed were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

5.2.2 Preparation of ALPS

Fresh leaves of *Pistia stratiotes* were picked, washed and air dried. The dried leaves were powdered using a hammer mill (Schutte Buffalo, New York, USA). A 700 g quantity of the powder was soaked in a liter of water for 24 h. Reflux filtration was performed at 80^{o}C. The collected filtrate was lyophilized into powder with a Hull freeze dryer /lyophilizer 140 sq ft (model 140FS275C, USA) , which was labeled aqueous leaf extract of *Pistia stratiotes* (ALPS) and stored at 4^{o}C until during experimentation when powder was constituted into the required concentrations with normal saline.

5.2.3 Experimental Drugs and Consumables

Cetirizine Hydrochloride (10 mg; Kinapharma Limited, Accra, Ghana), Prednisolone (5 mg; Ernest Chemists Limited, Accra, Ghana), ketamine (500 mg/10 ml; Laboratorio Sanderson SA, Santiago, Chile), Fluorescein solution (Sigma-Aldrich, St. Louis, MO), phenol red thread (Tianjin Jingming New Technological Development Co., Ltd., China).

5.2.4 Experimental Procedure

5.2.4.1 Randomization and Treatment

ICR mice were randomly assigned into 4 experimental groups (n=7) and treated as follows: Group1 (normal saline: 2 ml/kg), Group 2 (cetirizine: 5 mg/kg); Group 3 (prednisolone: 10 mg/kg), Group 4 (ALPS: 100 mg/ kg). Treatments were given consecutively for 7 days. Tear film secretion and tear film stability test were carried out before and after treatments using the phenol red threat test and the fluorescein tear breakup time. Animals were given intramuscular injection of 80 mg/kg ketamine to immobilize them for the assessment of tear film parameters. Tests were carried out at same time (12 pm) of the day in an air-controlled environment.

5.2.4.2 Phenol red thread (PRT) Test

The amount of aqueous tear produced was measured with a phenol red thread. For each mouse in a group, one eye was randomly selected for the test. The lower eyelid was pulled down slightly, and a 1 mm portion of the thread was inserted at the conjunctival cul-de-sac at a point approximately one-third of the distance from the lateral canthus of the lower eyelid. A time interval of 15 seconds was allowed after insertion of the thread for the wetting and color change to red. The wetting length was measured under a microscope, using a micron-scale digital ruler. This procedure was repeated three times for that eye and the average was considered as the final score. After the test, eyes were turned close to avoid excessive exposure and irritation of ocular surface.

5.2.4.3 Flourescein Tear breakup time (FTBUT)

The stability of the tear film on the ocular surface was assessed by the FTBUT. This started with the instillation of 1 microliter of 1% sodium fluorescein solution into the conjunctival cul-de-sac. Mice were allowed to blink three times following instillation of the solution, after which the lids were held open under a slit lamp (Ajinomoto Trading Inc., Tokyo, Japan) for the measurement of the FTBUT using the cobalt blue filter. This procedure was repeated three times for each eye and the average of these measurements was recorded as the final score.

5.2.5 Data Analysis

The variables measured were tear secretion by the PRT test and tear film stability by the FTBUT. Data was presented as mean ± SD. Baseline values for tear secretion and tear film stability were compared between groups using one-way ANOVA. Paired-t test was used to determine changes between the baseline measurements and post-treatment measurements of the two tear function tests for each group. $P \leq 0.05$ was considered statistically significant.

5.3 RESULTS

5.3.1 Baseline

No statistically significant differences were found in the tear secretion ($P = 0.721$) and tear film stability ($P = 0.876$), comparing all the experimental groups at the baseline (Figure 5A and 5B).

5.3.2 Post-treatment

Phenol red thread (PRT) test

Comparison of the PRT scores at baseline and post-treatment showed that mice treated with cetirizine and prednisolone had a significant decline in tear secretion (1.188 ± 0.4369 mm; $P \leq 0.05$, and 0.9380 ± 0.4422 mm; $P \leq 0.05$ respectively) (Figure 1B and 1C), while no significant changes ($P > 0.05$) were observed following treatment with normal saline (0.4800 ± 0.4211 mm) and LEPS (0.7050 ± 0.4236 mm) (Figure 6A and 6D).

Fluorescein Tear Film Break-Up Time (FTBUT)

No significant changes in tear film stability ($P > 0.05$) were observed with normal saline (0.1880 ± 0.5909 s), prednisolone (-0.1450 ± 0.6182 s), and LEPS (-0.9790 ± 0.6145 s) treatment in mice comparing FTBUT values at the baseline to post-treatment values (Figures 7A, 7C, and 7D). Cetirizine-treated mice however showed a significant decline (2.688 ± 0.6185 s; $P < 0.001$) in the stability of tear film (Figure 7B).

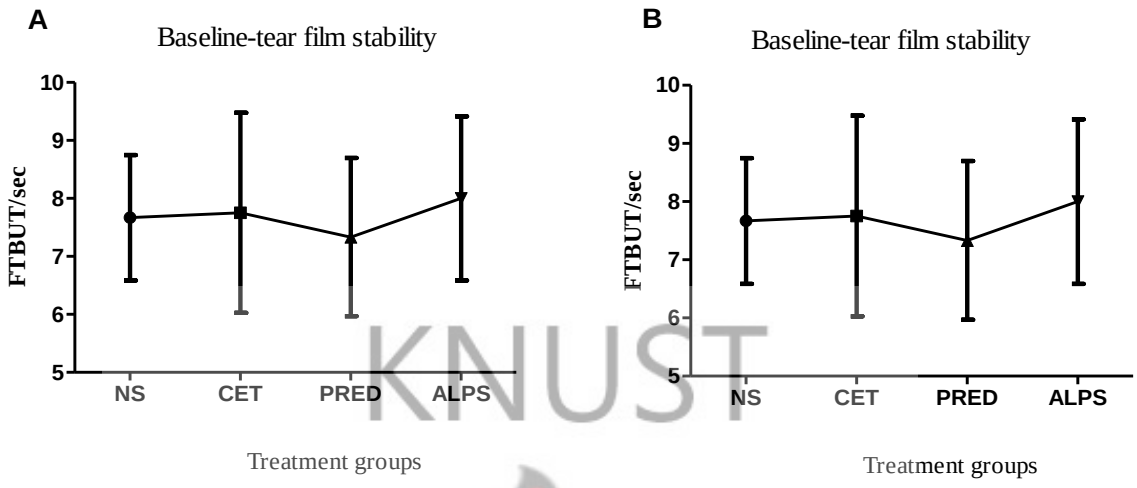

Figure 5: Comparison of (A) tear secretion and (B) tear film stability at the baseline between the experimental groups, using One-way ANOVA (*P* > 0.05). NS=normal saline, cetirizine (CET), prednisolone (PRED), aq. Leaf extract of *P stratiotes* (ALPS).

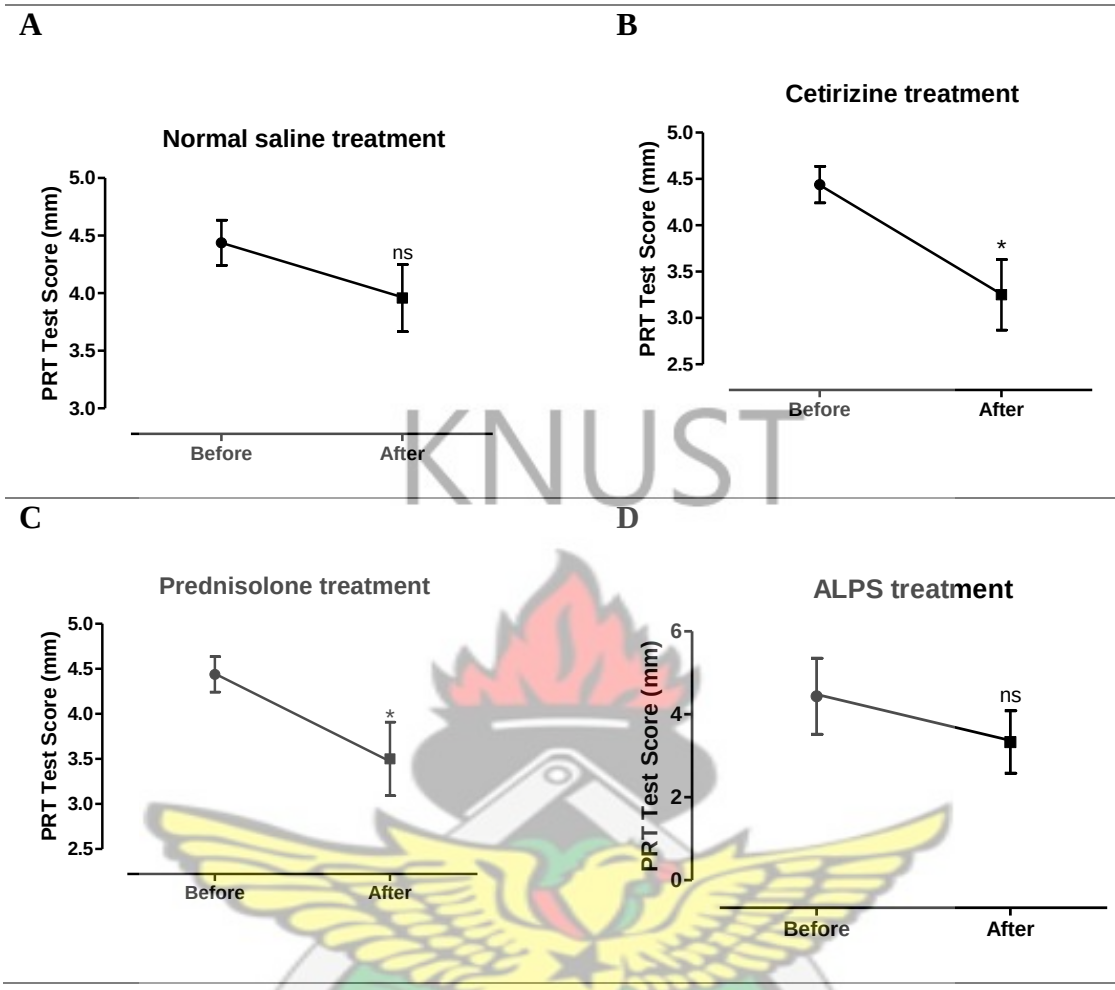

Figure 6: Tear secretion before, and after one week of treatment with (A) 2 ml/kg normal saline, (B) 5 mg/kg cetirizine, (C) 10 mg/kg prednisolone, (D) 100 mg/kg ALPS in a phenol red thread test. ^{ns} P > 0.05, * P ≤ 0.05; paired t-test (two-tailed). PRT=Phenol red thread.

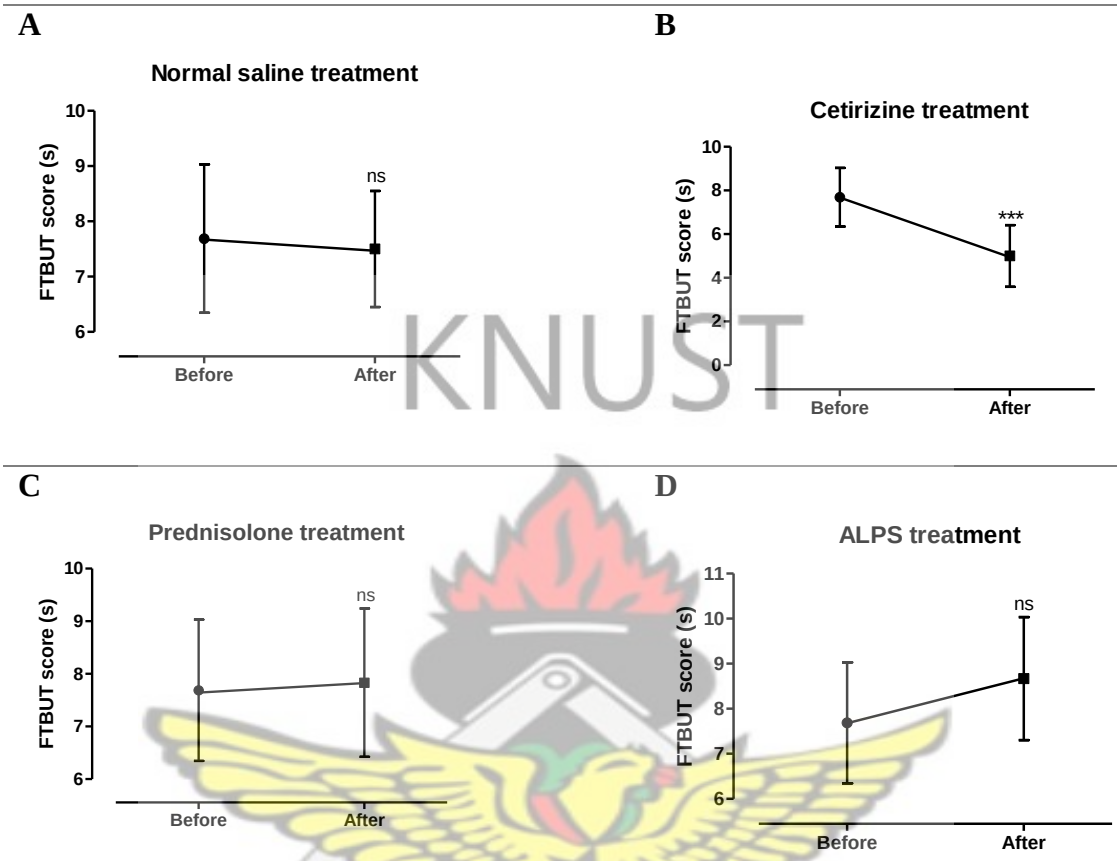

Figure 7: Tear film break-up time before, and after one week of treatment with (A) 2 ml/kg normal saline, (B) 5 mg/kg cetirizine, (C) 10 mg/kg prednisolone, (D) 100 mg/kg ALPS in a phenol red thread test. ns P > 0.05, *** P ≤ 0.001; paired t-test (two-tailed). FTBUT=fluorescein tear break-up time.

5.4 DISCUSSION

The preocular tear film is a three-layered physiological secretion by lacrimal glands in the eyelids and conjunctiva, and is found overlying the corneal surface. Each layer plays a peculiar role. For instance, the mucin layer which is closest to the cornea is involved in the wetting of the corneal surface; the middle aqueous layers is involved in the supply of oxygen to the cornea epithelium, washing away of debris and also has bactericidal activity; and lastly, the lipid layer involved in retarding evaporation of the tear film to about ten percent (Mishima and Maurice, 1961; Kanski, 2003).

Scientists have shown that a deficiency in one or more layers of this three-layered film underlies cases of dry eyes, but in most individuals (80% of all cases) it is attributable to a lipid layer deficiency (Dausch *et al.*, 2006). A deficient lipid layer would result in an evaporative dry eyes (EDE), in which case the volume of tear produced is enough but there is a significant decline in the stability of the tear film, while a deficient aqueous layer results in a tear deficient dry eye (TDDE) (DEWS, 2007). Phenol red thread test is used in the assessment of the quantity of tear secretion and provides information on the aqueous layer as this layer makes up over 90% of tear volume. Fluorescein tear film break-up time (FTBUT) on the other hand indicates the stability of tear film which is largely dependent on the integrity of the lipid layer.

Mice treated with normal saline did not show any significant decline in tear secretion and tear film. This is because normal saline treatment has no pharmacological activity on tissues, and is useful as placebo for comparisons purposes in most experimental studies.

Cetirizine treatment resulted in significant decline on both tear secretion and stability of tear film while LEPS had no significant influence on tear secretion and decrease tear film. This study, therefore, further corroborates reports of earlier studies indicating that

histamine antagonists (such as cetirizine) inhibited the secretion of tears (Abokyi *et al*., 2012; Ousler *et al*., 2004). Histamine, an autacoid synthesized from the amino acid histidine, exerts its effects on the body through the activation of four histamine receptor subtypes labelled H1, H2, H3 and H4. The existence of histamine receptors on the human conjunctiva has been confirmed by several investigators (Bielory and Ghafoor, 2005; Li *et al*., 2012). Histamine possesses stimulant effect on exocrine glands, which include the lacrimal gland and accessory lacrimal glands responsible for the secretion of aqueous tears (Danowski and Kmieć, 2002). Hence, histamine antagonists by competitively binding to histamine receptors, inhibit histamine from binding to these receptor molecules (Van Dyke and Woodfork, 2004) on the conjunctiva decreasing the tear secretion and consequently a decline in tear film break up time. LEPS did not adversely impair tear secretion, an indication that its anti-allergic properties may differ from the mechanism exhibited by ordinary histamine antagonists.

Prednisolone in this study also, showed a significant decline in aqueous tear secretion, but did not affect tear film stability. This could have been due to the compensatory action of an increased lipid-tear layer secretion by the meibomian glands. Generally, steroids (such as prednisolone) are known for their anti-inflammatory effect on the meibomian glands and are therefore crucial in managing meibomian gland dysfunctions (Ehler and Shah, 2008). Apart from the anti-inflammatory effect of steroids on the meibomian glands, some steroids (sex steroids) influence lipid secretion by meibomian glands through suppression of genes associated with keratinization and stimulation of those genes involved in lipogenesis (Sullivan *et al*., 2009; Schirra *et al*., 2006).

Compared to treatment with prednisolone, LEPS showed a better improvement on the tear film stability despite an insignificant decline in tear secretion. Since the tear-lipid layer is

largely responsible in retarding tear evaporation (i.e. increasing tear film stability), an increase of this layer will further stabilize tear evaporation. Various anti-inflammatory agents have been shown to be effective in the management of dry eyes (i.e. increasing tear film stability) (Avni *et al*., 2010; Nagelhout, 2005). It has also been revealed that specific anti-bacteria agents have the tendency to decrease bacterial lipolytic enzymes involved in the breakdown of normal meibum lipids into potentially inflammatory free fatty acid fragments, thereby improving tear film stability (Dougherty *et al*., 1991). Hence, this increase in the tear-lipid layer by the LEPS-treated mice could be attributed to the anti-inflammatory and anti-bacterial properties of *P. stratiotes*. It is noteworthy to indicate that preliminary phytochemical investigations conducted on LEPS indicated that this plant was enriched with several secondary plant metabolites including steroids, flavonoids, glycosides, tannins etc, as reported by other researchers (Aliotta *et al*., 1991). It is documented that these numerous phytochemicals confer the anti-inflammatory and antibacterial activities among other medicinal properties of *P. stratiotes* (Conti *et al*., 2013; Rathee *et al*., 2009).

Overall, *P. stratiotes* was safer on the precorneal tear film, manifesting in the maintenance of adequate tear secretion and tear film stability.

5.5 CONCLUSION

An aqueous extract of *Pistia stratiotes* does not reduce tear secretion, and does not affect tear film stability hence its use in the management of AC is not likely to be accompanied with the adverse effect of dry eyes as conventional antihistamines do.

CHAPTER SIX

6.0 GENERAL DISCUSSION

Allergic conjunctivitis is a very common ocular disorder and presents with symptoms such as itching, redness, tearing and burning sensation among others. It is the single cause of the increased turnout of patients seeking ophthalmic consultation. The allergic conjunctivitis population observed in eye care practice accounts for just a small fraction of the entire sufferers of this eye disease. It is estimated that about 20% of the total population in developed countries suffers from allergic conjunctivitis (Butrus and Portela, 2005). Although, allergic conjunctivitis also affects a substantial proportion of the developing countries, very few studies have been conducted into this disorder at this part of the world. Adegbehingbe *et al.* (2005) found in a population based study conducted in Nigeria that about half of the sample population suffered from allergic conjunctivitis. According to Ilechie (2008), ocular itch (a characteristic symptom of AC) was the most reported chief complaint by the pediatric population in Ghana.

The repeated episodes of ocular irritation experienced by allergic conjunctivitis patients makes it frustrating and unbearable. Allergic conjunctivitis affects the productivity of individuals since the ocular discomfort hinders comfortable vision required to execute daily tasks such as reading and computer usage (Pitt *et al.*, 2004). The academic performance of children and adolescents are mostly affected as they have the highest burden of allergic conjunctivitis (Mir *et al*., 2012; Gupta *et al*., 2004).

This study therefore holistically considered the various dimensions of allergic conjunctivitis including its epidemiological profile, management, pattern of prescription of anti-allergic

medications and adverse ocular effects likely to arise from improper use of those medications in management of allergic conjunctivitis.

Firstly, a hospital-based cross sectional was conducted to evaluate the prevalence of allergic conjunctivitis. In order to accurately determine the prevalence of allergic conjunctivitis in the Ghanaian population may require a population-based cross sectional survey, which will take a longer time to execute and also demand higher financial resource. Another approach is to conduct a hospital-based cross sectional survey that will provide some insight into the magnitude of this ocular disorder at a shorter time with less expenses. Also, in a hospital-based study, the health status of participants could be monitored over time, making it a preferable study setting to measure the treatment outcomes (adverse effects) of pharmacological management of allergic conjunctivitis. Hospital-based surveys have been used in estimating the prevalence of several disease conditions in Ghana including hypertension, diabetes and glaucoma, and continue to be widely used due to its convenience and robustness.

The findings of this cross sectional study were striking. The prevalence of AC was high and accounted for almost one-tenth of the patient turnout to those eye care facilities. Pharmacotherapy was the preferred treatment for allergic conjunctivitis in Ghana, and systemic antihistamines and corticosteroids were significantly used in allergic conjunctivitis management. Some adverse ocular outcomes arising from treatment included dry eye, corneal ulcer and steroid-induced glaucoma.

It is suggested that avoidance of contact to the allergen (foreign substance) helps prevent the allergic reaction and is the most effective way in managing allergic conjunctivitis. But for this to be achievable it will require investigation to determine the particular allergen to which the individual is sensitized. This procedure is recommended as the first line

treatment for AC (Prescribers' Journal, 2000) and in the advanced countries it is incorporated in their management routine. The use of pharmacological agents in allergic conjunctivitis management is sorted after only when the prophylactic measure of allergen elimination is unsuccessful and there is an allergic reaction.

However, treatment of allergic conjunctivitis in Ghana usually involve the use of topical mast cell stabilizers, systemic and topical antihistamines, topical steroids and topical decongestants. Often patients' symptoms are alleviated for a short while, if at all, necessitating continual use of the medications. However, prolong usage of these drugs may result in some adverse ocular effects further complicating the already disabling symptoms, and sometimes result in the loss of sight. Some common ocular complications arising from poor pharmacological management of allergic conjunctivitis include dry eyes, glaucoma and corneal ulcers. Due to the associated adverse effects of some of the anti-allergic medications, it is recommended that patients using them are monitored through follow-ups.

Several studies have cited systemic antihistamines to be one of the most used anti-allergic medications, and are usually prefered in the management of most allergic disorders (Angier *et al*, 2010). The frequent prescription of systemic antihistamines (mainly of the second generation) could be partly due to its rapid onset of action and efficacy in managing the discomforting ocular symptoms presented by patients. Also, second generation antihistamines are generally known to be safe (Philpot, 2000; Potter, 2004), and this may have have increased its usage. In addition, the easy accessibility as well as comparatively less cost of these drugs compared to the topical medications. In Ghana, the use of systemic antihistamines is further compounded by self-medication since these drugs are sold to patients without prescription. However, it has to be emphasized that these drugs have to be used with caution. Evidence based practice indicates that the duration of use of

anthistamines be for a short term (5-7 days) in allergic conjunctivitis management (Ciprandi *et al*, 1992). However, in this study it was found that the duration of treatment is much longer (ranging between 10-12 days) and may explain for the high prevalence of dry eyes observed in the study population. This frequent occurrence of dry eye in the AC population has been similarly observed by other researchers (Berdy and Hedqvist, 2000; Hom *et al*., 2012).

Corticosteroids are the most effective and potent pharmacological agents utilized in the allergic conjunctivitis management. Its use is, however, limited to managing acute symptoms of allergic conjunctivitis. Nonetheless, corticosteroids cause several adverse systemic effects that are well documented in studies. This is because they are analogous to essential chemical messengers in the human body called hormones. Misuse or prolong usage of corticosteroids has been also associated with severe ocular disorders that could cause blindness. Studies indicate that these drugs are implicated in some cases of glaucoma (Allen *et al.,* 1989) as was observed in our study. Other adverse ocular effects include cataract (Mohan and Muralidharan, 1989), ocular infections (Renfro and Snow, 1992) and, rarely, retinal and choroidal emboli (Carnahan and Goldstein, 2000).

Current investigations reveal that drugs such as olopatadine hydrochloride, azelastine, and ketotifen fumarate are quite effective and safe, hence mostly used in the advance countries as the first line pharmacotherapy for allergic conjunctivitis (Potter and Barney, 2004). Studies have indicated that these novel anti-allergic medications have dual mechanisms of action (Williams *et al.,* 2010; Lambiase *et al*., 2009; Rosenwasser *et al*., 2005). They act as mast cell stabilizers, thereby preventing further degranulation of mast cells, as well as possessing antihistamine properties which make them useful during acute symptoms of

allergic conjunctivitis. But in Ghana these medications are not accessible, influencing eye care practioners to abstain from prescribing such drugs.

Based on the findings of this earlier study, we therefore hypothesized that the high use of the systemic antihistamines in the management of allergic conjunctivitis may be the underlying risk factor to dry eyes which is common in the allergic conjunctivitis population. Investigations into the risk factors associated to dry eyes in the allergic conjunctivitis population was carried out by using a cohort study. Cohort studies have the ability to predict the risk associated to the exposure of disease-causing substances. This is because it starts with healthy subjects without any characteristic of the disease under study. Subjects once recruited are followed up until the (study outcome) disease state is attained and the reasearcher measures the extent of exposure to the likely causative agent.

Only the medical records of patients that were previously diagnosed of AC and reported again for follow-up within one month after treatment were reviewed (a total of 738 cases were found). Dry eyes was later diagnosed during subsequent visits in 17.5% of the AC population. While patient's age and occupation were among the risk factors found, systemic antihistamines was the most implicated as sufferers of allergic conjunctivitis who were treated with these medications were almost 3 times vulnerable to develop dry eyes after adjustement for confounders had been made. Systemic antihistamines have been found to possess anti-muscarinic property and can therefore cause drying of secretions (Liu and Farley, 2005), as is found in the dry eyes condition where there is inadequate tear. Many population based studies have also observed an association between systemic antihistamines and dry eyes (Moss *et al*., 2008; Welch *et al*., 2002), but could not establish a "cause and effect" relationship since they were mainly cross-sectional in design. Also, for the few cohort studies that have investigated this ocular effect of systemic antihistamines,

their subjects have usually been the general population instead of the targeted AC-population. Sufferers of ocular allergy are more susceptible to dry eyes compared to the normal population and therefore the true risk could only be assessed by a study conducted in this target group.

Since conventional pharmacotherapy for allergic conjunctivitis is ineffective and accompanied by adverse ocular effects, the interest of researchers have been geared towards looking for alternate treatment modalities for this ocular disorder. This growing investigation into anti-allergic therapy has been partly due to the inefficacy and adverse effects of the conventionally accepted medications such as antihistamines, mast cell stabilizers, decongestants and steroids. Attention of researchers has been drawn to medicinal plants which have been with man since antiquity. In fact, this recent ascendancy in the prevalence of allergies has occurred concurrently with the present age where there is a deliberate effort to discourage the use of herbs in their crude state. Studies have shown the importance of inclusion of some plant products, especially dietary polyphenols, in the prevention and treatment of allergic diseases (Han *et al*., 2007; Singh *et al*., 2011).

Evidence, however, suggests that plants may be suitable and effective in the treatment of allergic conjunctivitis (Inoue *et al*., 2002; Kawai *et al*., 2007). According Bellik *et al*. (2013), phytochemicals, which are secondary plant metabolites, exert anti-inflammatory and anti-allergic activities by engaging in radical scavenging activities; modulation of cellular activities of inflammation-related cells (mast cells, macrophages, lymphocytes, and neutrophils); modulation of pro-inflammatory enzymes (including phospholipase A2, cyclooxygenase, and lipoxygenase and nitric oxide synthase); molecules and gene expression.

A traditional medicinal plant popularly known as 'ntanowa' in Akan (Burkill, 1985) was selected after extensive review of literature on the several medicinal uses, as well as other well documented pharmacological activities of *P. stratiotes*. Alexander *et al*., (2011) has reported the inclusion of the plant in herbal preparations for managing asthma, coupled with other studies that have reported the anti-inflammatory activities of this plant (Koffuor *et al*., 2012; Kyei *et al*., 2012a; 2012b).

Despite the several medicinal properties of *P. stratiotes*, very few studies have so far been done on the toxicity profile of this plant. While all the toxicity studies so far agree that this plant is safe (Koffuor *et al.*, 2012; Alexander *et al*., 2011), it was ethically not advisable to evaluate the efficacy of this remedy on humans since those studies were mainly conducted in experimental animals. The available option left that was utilized, involved mimicking allergic conjunctivitis in an experimental animal. Several recommended protocols are available to induce allergic conjunctivitis in animals (Groneberg *et al.*, 2003). The use of ovalbumin to induce allergic conjunctivitis is widely used protocol due to the easy accessibility and safety of this product to humans. It was therefore chosen to investigate the efficacy of *P. stratiotes* in managing allergic conjunctivitis.

The anti-allergic property of *P.stratiotes* was investigated *in vivo* in ovalbumin-sensitized ICR mice by oral pretreatment with the aqueous extract of the plant 1hr before topical challenges with ovalbumin. The results of this study showed that mice pretreated with ALPS showed significantly less signs of induced allergic conjunctivitis and also had lower serum ovalbumin-specific IgE, as well as lower mast cell infiltration and degranulation.

Among the anti-allergic properties of *P stratiotes* found, of greater interest is its inhibition of mast cell infiltration and degranulation in ocular conjuctival tissue. This may be indicative of a potential deactivation of immune cells or interference with the cytokine

mileu favoring Th2 immune response. This property in addition to its antihistaminic activity, demonstrated by the lowering of acute signs of allergic conjunctivitis such as redness, chemosis, tearing and lid-scratch behavior makes it a potentially effective anti-allergic remedy. In this study, *P stratiotes* exhibited properties that were found in the novel anti-allergic drugs such as olopatadine and azelastine that have been recommend to replace the currently used medications.

These findings support the essential role that this plant could play in the management of allergic conjunctivitis. However, it was important to evaluate the effect of *P. stratiotes* on the ocular surface because ocular dryness was the commonest adverse ocular effects found in conventional management of AC. Only after it is found that *P stratiotes* does not significantly decrease tear secretion and tear film stability will it be considered as a better treatment modality compared to the conventional orthodox treatment of AC.

In order, therefore, to ascertain whether or not *P. stratiotes* had any adverse effect on the ocular surface, two tear function test were selected – namely, the phenol red thread test and the flouresceine tear break up time (FTBUT). These are standardized tests used in the assessment of tear secretion and tear film stability respectively (DEWS, 2007a). Since volume of tears secreted is largely determined by the aqueous-tear layer (Walsh and Hoyt, 2008), the PRT test measuring tear secretion directly provides important information on the adequacy of the aqueous-tear layer. Although, the Schirmer test is mostly used in assessing tear secretion (DEW, 2007a, 2007b), this study used PRT test to evaluate the effect of tear secretions due to the following reasons: PRT test offers some advantages over the schirmer test in that, there is little or no sensation upon insertion of the tiny cotton thread into the conjuctival cul-de-sac. Also, the test time is for PRT is much shorter (only 15 seconds compared to 5 minutes in Schirmer test), both of which result in providing an accurate

measure of tear secretion. Asbell *et al*. (1987) reported in their study that the tear secretion measurements by PRT tear test was more repeatable than the Schirmer test corroborating the superiority of PRT test over Schirmer. Lastly, the PRT test was preferred choice for measuring tear secretion because of the experimental animals used (mice) and is an acceptable protocol for measurement of tear secretion in such animals with small eyeball size (Barabino *et al*., 2004).

The stability of tears depends on the rate of evaporation of the tear film, measured by the flouresceine tear break up time (DEWS, 2007), hence the longer the time it takes for the tear film to evaporate, the more stable the tear and vice-versa. Because the outer lipid-layer is responsible for retarding tear evaporation (Mishima and Maurice, 1961; Walsh and Hoyt, 2008) a longer FTBUT points to the intergrity of this layer.

The experimental animals used for the entire study were mice. AC and other ocular disorders have been extensively studied in mice due to their easy accessibility and resemblance to humans. The murine model of AC and dry eyes are acceptable protocols used by researchers for the purpose of investigation into those disease entities (Groneberg *et al*., 2003). A randomized, single-blinded experiment was conducted using normal healthy mice to investigate the ocular drying effect of *P. stratiotes.* Laboratory experimental studies are useful in the establishment of "cause and effect" relationship because the investigator is able to manipulate the variables (intervention) to observe the effect of his actions. Biases in experimental studies are avoidable due to the possibility of "blinding" either the examiner or the subjects or both. This study used the single-blinded option whereby the experimenter was blinded to the specific intervention given to each group of mice until the end of the study period. Hence, researcher bias was eliminated and

any relationship observed between the two variables measured (i.e. treatment and tear functions) was real.

Each group of mice were given a specific interventions one of which was the leaf extract of *P. stratiotes* for 7 days. This treatment duration was adopted based on evidence-based management of AC with conventional orthodox medications (Owen *et al.,* 2004; Swamy *et al.,* 2007). At the end of this study, ALPS showed a comparatively better effect on tear secretion and stability than standard ant-allergic-medication such as cetirizine and prednisolone.

6.1 CONCLUSION

This study indicated that AC is a common eye disorder among Ghanaians, and caution should be taken in the use of systemic antihistamines in treating AC due to the risk of dry eyes. The traditional plant, *P stratiotes,* demonstrated potent anti-allergic activity in a murine model of ovalbumin induced allergic conjunctivitis and could be useful in treating allergic conjunctivitis. In addition to the efficacy of *P stratiotes* in treating AC, it offers an advantage over conventional therapy as it does not significantly affect the tear secretion and stability.

6.2 RECOMMENDATIONS

- Clinical trials are required to determine whether this herb will prove effective in managing allergic conjunctivitis in human.
- Although second generation systemic antihistamines are presumably safe, caution should be exercised in prescribing this medication in the management of allergic conjunctivitis.

- The National Health Insurance Scheme Drug List should be expanded to cover anti-allergic medications such as olopatadine hydrochloride and azelastine hydrochloride which have comparatively less ocular drying effect.
- Eye care practitioners should always investigate for the presence of dry eyes in their patients being managed with allergic conjunctivitis.

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APPENDIX

Table 16: Mean ages of sufferers of the various forms of allergic conjunctivitis

Type of AC	SAC	PAC	VKC	AKC
Mean age	22.38 ± 16.94†††	30.76 ± 20.01†††	6.24 ± 5.60	36.00 ± 16.64†††

Data presented as mean ± SD. ANOVA (Dunnet's Multiple Comparism test) was used to compare the mean ages of other ocular allergies to VKC ;†††P ≤ 0.001. AC= Allergic Conjunctivitis, SAC = Seasonal Allergic Conjunctivitis; PAC = Perennial Allergic Conjunctivitis; VKC = Vernal Allergic Conjunctivitis; AKC = Atopic KeratoConjunctivitis.

Table 17: Mice serum IgE concentration and ocular histological assessment of inflammation based on mast cell infiltrations and degranulations

Group	Normal	Untreated	2 NS	5 CET	10 PRED	10 ALPS	50 ALPS	100 ALPS
Serum IgE Conc (g/ml) x10^{-11}	59.6 ± 20.59	1848 ± 376.5†††	1728 ± 371.2	1095 ± 65.89	130.27 ± 43.42	1024 ± 195.0	612.8 ± 196.10**	234.6 ± 123.10***
Ocular histology (inflammation)	0.05 ± 0.10	3.33 ± 0.21†††	3.17 ± 0.31	1.50 ± 0.22***	0.83 ± 0.34	2.67 ± 0.33	2.17 ± 0.31*	1.83 ± 0.31**

Data presented as mean ± SEM. Pretreatment with 2ml/kg normal saline (NS), 5 mg/kg cetirizine (CET), 10 mg/kg prednisolone (PRED) or 10, 50, and 100 mg/kg of the aqueous leaf extract of *Pistia stratiotes* (ALPS) was given 1h in ova sensitized mice before topical challege. Normal vs. Untreated (unpaired t-test, two-tailed): †† $P \leq 0.01$. Untreated vs. Treatments (One-Way ANOVA followed by multiple Dunnett's *post hoc test*): $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

Table 18: Baseline tear secretion and tear film stability

TEAR MEASURE	NS	ALPS	CET	PRED
Mean Tear Secretion $\pm$ *SD* (mm.)	4.042 ± 0.7486	4.500 ± 1.0950	4.667 ± 0.9309	4.542 ± 1.1660
Mean Tear Film Stability $\pm$ *SD* (sec.)	7.667 ±1.080	8.000± 1.414	7.7501±.725	7.333±1.366

Comparison of the baseline tear secretion and tear film stability between different experimental groups using one-way ANOVA shows no significant differences (*P > 0.05*); NS=normal saline, CET=cetirizine, PRED=prednisolone, ALPS=aq. Leaf extract of *P stratiotes*.

Table 19: Post-treatment tear secretion and tear film stability

TEAR MEASURE	NS	ALPS	CET	PRED
Mean Tear Secretion ± SD (mm.)	3.958 ± 0.7144	3.500 ± 1.000	3.250 ± 0.9354*	3.333 ± 0.7528*
Mean Tear Film Stability ± SD (sec.)	7.500 ± 1.049	9.000 ± 3.464	5.000 ± 1.414***	7.833 ± 3.710

Paired-t test was used in comparing post-treatment values for tear secretion and tear film stability to their respective baseline values for each experimental group to determine changes in tear secretion and tear film stability after intervention. Normal saline (NS), cetirizine (CET), prednisolone (PRED), aq. leaf extract of *P. stratiotes* (ALPS); *$P \leq 0.05$, ***$P \leq 0.001$.