EVALUATION OF AQUEOUS AND METHANOL ROOT EXTRACTS OF *CALOTROPIS PROCERA* AIT (ASCLEPIADACEAE) FOR ANTI-ASTHMATIC ACTIVITY ON WISTAR RATS AND GUINEA PIG ILEUM

BY

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DECLARATION

I declare that the work in this thesis entitled "**EVALUATION OF AQUEOUS AND METHANOLIC ROOT EXTRACTS OF *CALOTROPIS PROCERA (AIT) ASCLEPIADACEAE* FOR ANTIASTHMATIC ACTIVITY ON EXPERIMENTAL WISTAR RATS AND GUINEA PIG**" has been carried out by me in the Department of Pharmacology and Therapeutics. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

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CERTIFICATION

This thesis entitled "**EVALUATION OF AQUEOUS AND METHANOL ROOT EXTRACTS OF *CALOTROPIS PROCERA (AIT) ASCLEPIADACEAE* FOR ANTIASTHMATIC ACTIVITY ON EXPERIMENTAL WISTAR RATS AND GUINEA PIG ILEUM**" by **Ibrahim Mu'azzamu ALIYU** meets the regulation governing the award of the Degree of Masters of Science of the Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Asthma, which affects an estimated 300 million people worldwide, is an incurable health disorder of a major public health concern globally. The present orthodox therapy for asthma has several drawbacks including many undesirable side effects and high cost of management (especially challenging for low-income / developing countries). In a bid to develop a cheaper and better antiasthmatic agent with less side effects, this study was carried out to evaluate the effect of aqueous and methanol root extracts of *Calotropis procera* used in folkloric medicine as an antiasthmatic plant. The study involves *In-vitro* model on isolated guinea pig ileum preparation. *In-vivo* models like carageenan-induced leucocytosis in rats, passive paw anaphylaxis, carrageenan induced rat paw edema and haloperidol induced catalepsy. Median Lethal Dose (LD_{50}) determination was conducted using the method as described by Lorke's (1983). *In vitro* studies on isolated guinea pig ileum preparation was carried out to investigate for bronchospasmolytic activity of the extracts. Bioassay of histamine 10 µg/ml in the presence and absence of *Calotropis procera* extract 10 mg/ml was done. The normalization effects of the extracts were studied in carrageenan-induced total leucocyte count (TLC) after parenteral administration of carrageenan. Thirty five (35) Wistar rats were divided into 7 groups, five aminals per group. Group 1 was not given any treatment, but blood sample was used to establish the reference standard for TLC in rats. Groups 2 – 7, received respectively, distilled water (2 ml/kg), chlorpheniramine maleate (2 mg/kg), *Calotropis procera* (100 mg/kg), *Calotropis procera,* CP (200 mg/kg), *Calotropis procera* (100 mg/kg), and *Calotropis procera* (200 mg/kg). Immuno-modulatary / antiallergic antiasthmatic activity of the plant was studied using passive paw anaphylaxis model as described by Patil (2010). Wistar rats were sensitized subcutaneously with 100 mg fresh egg albumin for 10 days, after which serum was collected. A fresh set of thirty (30)

animals was divided into six (6) groups each containing five (5) rats. Groups 1 – 6 received distilled water 2 ml/kg, dexamethasone 0.27 mg/kg, CP 250 mg/kg aqueous extract, CP 350 mg/kg aqueous extract, CP 250 mg/kg methanol extract and CP 350 mg/kg methanol extract orally respectively. Anti-inflammatory antiasthmatic activity of CP was studied using carrageenan induced rat paw edema model as described by Anita and Babita (2008). Dopaminergic and/or adrenergic antiasthmatic study was carried out using haloperidol-induced catalepsy on wistar rats as described by Patil (2010). Preliminary phytochemical screening of the extracts was carried out as described by Trease and Evans. Extraction of powdered plant gave yields of 15.64% $^{w}/_{w}$ and 13.76%$^{w}/_{w}$ methanol and aqueous extracts respectively. Oral LD_{50} in Wistar rats for both aqueous and methanol CP extracts were found to be >5,000 mg/kg. Both aqueous and methanolic extracts inhibited histamine induced contraction of isolated guinea pig ileum ($p < 0.001$) carrageenan-induced leucocytosis ($p < 0.05$) egg albumin-induced passive paw anaphylaxis (0.05) and carrageenan induced rat paw edema ($p < 0.05$) However, only methanolic extract inhibited haloperidol-induced catalepsy in wistar rats ($p < 0.05$) Hence it is concluded that both aqueous and methanolic extracts possess antiasthmatic activity that may be responsible for the antiasthmatic activity and may have potential role in the treatment of asthma.

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ABBREVATIONS, DEFINITIONS, GLOSSARY AND SYMBOLS

AAAAI: American Academy of Allergy, Asthma and Immunology

AB: Antibody

AD: After Death

AG: Antigen

AIDS: Acquired Immunodeficiency Syndrome

ANOVA: Analysis of Variance

GINA: Global Initiative for Asthma

H_a: Alternate Hypothesis

H_0: Null Hypothesis

IgE: Immunoglobulin E

ISAAC: International Studies for Asthma and Allergies in Childhood

LD_{50}: Median Lethal Dose

NHLBI: National Heart Lungs and Blood Institute

SPSS: Statistical Package for the Social Science

Th2: T-Helper type 2

WHO: World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Statement of Research Problem

Asthma is currently a worldwide problem, with increasing prevalence in both children and adults; a prevalence rate of 5 – 10% has been reported for Nigeria (Ezike *et al*., 2008). Prevalence of asthma in Nigeria is 10.7% for children (Falade *et al*., 1998), 14.2% for adolescents (Ibe *et al*., 2002) and 5.1-7.5% for adults (Irusen, 2004). An estimated 300 million people worldwide suffer from asthma and 250,000 annual deaths are attributed to the disease. Almost all of these deaths are avoidable. The annual economic cost of asthma is $19.7 billion. Direct cost makes up $14.7 billion of that total, and indirect cost such as loss of productivity add another $5 billion. The Global Initiative for Asthma (GINA) management goals (Sapra *et al.,* 2009) have not been achieved as demonstrated in the report of the various surveys around the world that investigated the levels of asthma control (Masoli *et al.,* 2004; Neffen *et al*., 2005; Rabe *et al.,* 2004). Despite the availability of oral and inhaled medications, the prevalence of asthma is on the rise (NHLBI/WHO 1995). In a study in University of Benin Teaching Hospital (Oni *et al*., 2010) prevalence of asthma among respiratory unit patients was 6.6% and associated with a female preponderance of (F: M) 1.5:1. The majority (70%) of these patients used short acting β2-agonists and 38.2% were using combined inhaled long acting β2- agonists/steroid for the relief and control of asthma. There was an associated high asthma burden that could not be explained despite improved knowledge of the pathophysiology and management of asthma. With regard to morbidity, there is considerable evidence that a regular use of beta-agonist drugs as a class could potentially lead to worsening asthma control because of the effects on bronchial hyperresponsiveness, the development of tolerance and reduced protection against

provoking stimuli, an increased allergen load, and masking of the symptoms of deteriorating asthma (Beasley *et al*, 1999). Clinically significant complications of inhaled corticosteroids includes local effects such as dysphonia and oral candidiasis and potentially serious systemic effects associated with cortisol suppression including growth retardation, glucose intolerance and increased risk for osteoporosis and fracture (Chritie, 2004).

1.2 Justification

Plants have served as valuable starting materials for drug development even in advanced countries of the world (Sofowora, 1993). The drugs which are currently used for the treatment of this disease in modern medicine are far from satisfactory as they provide only symptomatic relief, produce several adverse effects and may lose effectiveness on continued use. Muscle tremor and hypokalemia are major adverse effects of β_2 agonists (Haalboom *et al*., 1985; Nelson, 1986). Theophyline has narrow therapeutic index and requires monitoring of drug levels (Nasser and Rees, 1993; Stoloff, 1994). Adverse effects of corticosteroids include fluid retention, increased cell mass, increased appetite, weight gain, osteoporosis, capillary fragility, hypertension, peptic ulceration, diabetes, cataract, and phychosis (Dajani *et al*., 1981). The challenge of developing new effective, safe and long lasting antiasthmatic drugs from natural products appears inevitable. Hence the search for an effective, relatively safe, cost-effective and broad spectrum antiasthmatic drug for which *Calotropis procera* has been selected due its reported antiasthmatic activity in Nigerian folkore medicine.

1.3 Theoretical Framework

The selections of inappropriate animal models have been identified as one of the common problems associated with ethnobotanical researches (Etuk, 2010). Several animal models have been developed for studying asthma or testing anti-asthmatic agents. Five animal models were adopted in this research.

Spasmolytic Activity on Isolated Guinea Pig Ileum Chain Preparation was used to determine whether the extracts possess H_1 receptor blocking property and to confirm its ileum spasmolytic (i.e. bronchospasmolytic) effect. This model is used to detect β-sympathomimetic, H_1-receptor blocking and leuko-triene receptor blocking properties of test drugs. Intracellular mechanism of smooth muscle relaxation regardless of which part of the body the muscle is found involves increase in intracellular cyclic adenosine monophophate (cAMP) and Adenylase cyclase resulting in release of nitric oxide a potent smooth muscle relaxant (Gerhard, 2002).

Formulations used in the treatment of asthma include some anti-stress herbs to enable adoption to stress since excessive stress or nervous debility may aggravate symptoms of asthma. Carrageenan-induced leucocytosis model was adopted to determine the Antistress (Adaptogenic) property of the extracts. The number of white blood cells (WBC's) (particularly eosinophil) is typically elevated when the body is fighting a severe infection or stressed by metabolic toxins. The normalization effect of an adaptogen can be observed in milk-induced leucocyte count (increase in leucocyte count) after parenteral administration of milk (Bhargava *et al.,* 1981; Pandit *et al*., 2008).

Haloperidol induces catalepsy by inhibiting dopamine D_2 receptors and dopamine secretion. Catalepsy is an extrapyramidal side effect of drugs that inhibits dopamine transmission or histamine release in the brain. Haloperidol is an antipsychotic drug that inhibits dopaminergic transmission in the brain. Dopamine is an agonist for adrenaline on beta adrenergic receptors, it releases adrenaline from storage vesicles and it is a biosynthetic precursor for adrenaline. Adrenaline is a physiological antagonist of histamine. Haloperidol induced catalepsy model was adopted to screen the extract for adrenergic and/or antihistamine activity by acting either as an antagonist on beta adrenergic receptor or inhibiting histamine release (Patil, 2010).

Egg albumin-induce passive paw anaphylaxis model is used to screen for immune-modulating properties of substances by demonstrating their ability to inhibit the Antigen-Antibody (AG-AB) reaction involved during an anaphylactic reaction as observed in asthmatics.

Carrageenan-induced rat paw edema model is used to screen agents for anti-inflammatory property due to their ability to inhibit synthesis and release of inflammatory mediators (i.e. prostaglandin, histamine and serotonin). The model is sensitive to cyclooxygenase inhibitors (Anita and Babita, 2008). Airway inflammation has been demonstrated in all forms of asthma. The degree of bronchial hyper responsiveness and airway obstruction is closely linked to the extent of inflammation (Bousquete *et al*., 2000).

1.4 Aim and Objectives of the Studies

1.4.1 Aim

The aim of this research is to scientifically evaluate the aqueous and methanol root extracts of *Calotropis procera* for antiasthmatic activity in guinea pig and rats.

1.4.2 Specific Objectives

1. To carry out phytochemical screening of the aqueous and methanolic root extracts of *Calotropis procera*.
2. To determine the median lethal dose (LD_{50}) of the aqueous and methanolic root extracts of *Calotropis procera*.
3. To establish the effect of aqueous and methanol extracts of *Calotropis procera* on histamine induced contraction of isolated guinea pig ileum preparation.
4. To screen the extracts for adaptogenic activity in carrageenan-induced leucocytosis in rats.
5. To screen the extracts effect on haloperidol-induced catalepsy in rats.
6. To test effect of the extracts on passive paw anaphylaxis in rats.
7. To determine effect of the extracts on carrageenan-induced paw edema in rats.

1.5 Research Hypothesis

The aqueous and methanol root extracts of *Calotropis procera* possess antiasthmatic activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Traditional Medicine

In spite of advances in modern medicine, there are various disorders including tropical diseases, herpes, acquired immunodeficiency syndrome (AIDS), cancer, bronchial asthma, which still remain a challenge to present day drug therapy(Kumar *et al.*, 2009). Since ancient times humanity has depended on the diversity of plant resources for food, clothing, shelter, and traditional medicine to cure myriads of ailments (WHO, 2000). Early humans recognized their dependence on nature in both health and illness. Traditional Medicine is defined as 'the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness' (WHO, 2000). Traditional medicine that has been adopted by other population (outside its indigenous culture) is often termed alternative or complementary medicine (WHO, 2000). Traditional medical practice utilizes plants, animals, minerals and other method (WHO, 2008). A medicinal plant is defined by the World Health Organization as "any plant in which one or more of its part contains substance that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. Physical evidence of the use of herbal remedies has been found some 60,000 years ago in a burial site of a Neanderthal man uncovered in 1960 in a cave in northern Iraq. Here, scientists found great quantities of plant pollen, some of which came from medicinal plants still used today. The first written records detailing the use of herbs in the treatment of illnesses are in the form of Mesopotamian clay tablet writings and Egyptian papyrus (Kumar *et al.*, 2011). Herbal medicines remain the major source of health care for the world's population. During the past decades, public interest

in natural therapies, namely herbal medicine, has increased dramatically not only in developing countries but also in industrialized countries (Calixto, 2000). WHO has recognized herbal medicine as an essential building block for primary health care of many countries. The World Bank estimated global trade in medicinal and aromatic plants and their products at US $ 5 trillion by 2050 AD. Global herbal market is around $ 70.5 billion with an average annual growth of 10-12% per annum (Handa, 2007*)*.

2.2 Overview of Asthma

According to the Global Strategy for Asthma Management and Prevention (GINA, 2012) asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role and is associated with airway hyperresponsiveness, widespread, variable, and often reversible airflow limitation that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing.

Asthma generally refers to the common chronic inflammatory disease of the airways characterized by variable and recurring symptoms, reversible airflow obstruction, and bronchospasm (Jaydeokar *et al.,* 2011). Asthma occurs when the body's natural system of defense against bacteria, viruses, and other harmful microbes becomes overprotective. It misidentifies relatively harmless pollens, dust, and dander, setting up a reaction that narrows and inflames small airways in the lungs. This results in breathlessness, wheezing, tissue damage, and in the worst cases, death (Jaydeokar *et al.,* 2011).

Bronchial asthma is characterized by hyper responsiveness of trachea-bronchial smooth muscle to a variety of stimuli resulting in narrowing of air tubes, often accompanied by increased secretion mucosal edema and mucus plugging.

2.3 Epidemiology

The prevalence of asthma is feared to be on the increase in developing countries with great economic and humanitarian effects. There are, however, no longitudinal studies from Nigeria to support this. Data from developed countries clearly demonstrate this time trend (Eder *et al*., 2006). Efforts have been made to find asthma prevalence among various populations in Nigeria. In a recent survey among university undergraduates aged 15–35 years in Ife, Western Nigeria, 10.4% of males and 17.9% of females had probable asthma (Erhabor *et al*., 2006). There is therefore an urgent need for a national survey on asthma prevalence and determinants in developing counties.

Data obtained from the International Study of Asthma and Allergies in Childhood (ISAAC) showed the prevalence of asthma as 13.1% in Nigerian children aged 6 to 7 years (Asher *et al*., 2006). Prevalence values from African countries were generally lower than those obtained from developed nations with only the South African prevalence being close to that obtained in the United Kingdom. A longitudinal study carried out in Ghana, showed an increasing prevalence of childhood asthma over a 10-year period (Addo-Yobo *et a*l., 2008).

2.4 Symptoms

Symptoms include breathlessness, wheezing, sputum production, difficulty in talking, dyspnoea, tightness of neck muscles, chest tightness, and shortness of breath, coughing

after physical activity, whistling sound while breathing, frequent coughing, feeling frightened, exhaustion, chest tightness, greyish or bluish coloring of lips (Kumar *et al.*, 2011). For managing asthma attack symptomatic relief is foremost requirement.

2.5 Factors Associated with Asthma

Asthma is caused by environmental and genetic factors. These factors influence how severe asthma is and how well it responds to medication. The interaction is complex and not fully understood.

2.5.1 Environmental factors

Many environmental risk factors have been associated with asthma development and morbidity in children. Environmental, especially maternal cigarette smoking is associated with high risk of asthma prevalence and asthma morbidity, wheeze, and respiratory infections. Viral respiratory infections are not only one of the leading triggers of an exacerbation but may increase the risk of developing asthma (Yawn, 2008).

Psychological stress has long been suspected of being an asthma trigger, but only in recent decades has convincing scientific evidence substantiated this hypothesis; it is thought that antibiotics make children who are predisposed to atopic immune responses susceptible to development of asthma because they modify gut flora, and thus the immune system (as described by the hygiene hypothesis) (Yawn, 2008).

2.5.2 Genetic

Through the end of the year 2005, twenty five (25) genes had been associated with asthma in six or more separate population. Many of these genes are related to immune system or to modulating inflammation. Asthma susceptible genes includes: GSTM1, IL10, CTLA-4, SPINK5, LTC4S, LTA, GRPA, NOD1, CC16, GSTP1, STAT6, NOS1, CCL5, TBXA2R, TGFB1, IL4, IL13, CD14, ADRB2, HLA-DRB1, HLA-DQB1, TNF, F_{CE}R1-β, IL4R, ADAM33 (Ober *et al*., 2006).

2.5.3 Socioeconomic factors

The incidence of asthma is highest among low-income population worldwide. Asthma deaths are most common among low and middle income countries, and in the western world, it is found in those low-income neighborhoods whose populations consist of large percentage of ethnic minorities. Additionally asthma has been strongly associated with the presence of cockroaches in living quarters; these insects are more likely to be found in those same neighborhoods (Jaydeokar *et al.,* 2011).

2.5.4 Occupation

Asthma as a result of workplace exposure is a commonly reported occupational respiratory disease. Estimates by the American Thoracic Society (2004) suggest that 15-23% of new-onset asthma cases in adults are work-related (Schroeder and Fahey, 2002).

2.5.5 Population disparities

Asthma prevalence in US is higher than in most other countries in the world, but varies drastically between diverse US populations (Jaydeokar *et al.,* 2011).

2.5.6 Athletics

Asthma appears to be more prevalent in athletes than in the general population. One survey of participants in the 1996 Summer Olympic Games, in Atlanta Georgia, US, showed that 15% had been diagnosed with asthma, and that 10% were on anti-asthma medication (Barar, 2007).

2.6 Types of asthma

2.6.1 Extrinsic asthma

Make up 90% of asthma cases and is allergic asthma. Many times this type of asthma has a childhood onset. Symptoms develop only on exposure to specific allergens, when there is a history of hay fever or eczema. They are less prone to status asthmaticus. Patients show positive skin test.

2.6.2 Intrinsic asthma

Make up 10% of asthma cases. It usually has an onset during adulthood, or after age 30. Allergies are not typically related to this type of asthma, which makes treatment for this type of asthma more difficult. Chronic year round symptoms marks this type of asthma. More prone to status asthmaticus.

2.7 Diagnosis of asthma

Airflow in the airways is measured with a peak flow meter or spirometer. The latest guidelines from the U.S. National Asthma Education and Prevention Program (NAEPP) recommend spirometry at the time of initial diagnosis, after treatment is initiated and symptoms are stabilized, whenever control of symptoms deteriorates, and every 1 or 2 years on a regular basis (Fauci *et al.*, 2008).

The Global Initiative for Asthma (GINA, 2011) recommended the following procedure diagnosis of asthma:

- History and patterns of symptoms
- Measurements of lung function

- Spirometry
- Peak expiratory flow

- Measurement of airway responsiveness
- Measurements of allergic status to identify risk factors
- Extra measures may be required to diagnose asthma in children 5 years and younger and the elderly

2.8 Asthma Inflammation: inflammatory cells, structural cells and mediators

2.8.1 Inflammatory cells involved in asthma include

Mast cells, eosinophils, Th2 cells, basophils, neutrophils and platlets (GINA, 2011).

2.8.2 Structural cells include

Epithelial cells, smooth muscle cells, endothelial cells, fibroblast and nerve cells (GINA, 2011).

2.8.3 *Mediators*

Histamine, leucotrienes, prostanoids, Platelet Activating Factor (PAF), adenosine, endothelins, kinins, cytokines, nitric oxide, chemokines and growth factors (GINA, 2011).

2.9 Pathophysiology of Asthma

Chronic asthma is an episodic narrowing of the bronchi thought to be caused by an underlying chronic inflammatory disorder (Weiler *et al*., 1998). In allergic asthma, inhaled allergen initiates the inflammatory response by interacting with IgE bound to mast cells and basophils. IgE binds with high affinity to receptors (Fcε- receptors) that are present on basophilic granulocytes and most prominently on the closely related mast cells. These cells thus acquire a "borrowed" (as it is, of course, synthesized by B-lymphocytes) allergen specific receptor, which can persist on these cells for long periods, at least several months, perhaps years. If that happens an individual is "allergic", i.e. sensitized to an allergen without exhibiting any clinical symptoms and without knowing it. Upon re-exposure to this specific allergen, the mast cells (and the other IgE-bearing cells) can immediately recognize the allergen with its IgE antibodies. This results in the cross linking of the Fcε receptors that in turn triggers activation of the mast cells (or basophils). The consequence is rapid degranulation of preformed vesicles and release of a plethora of mediators into their surroundings, the most prominent mediator is histamine which is preformed and stored in vesicles and acts immediately upon release. Within minutes, further mediators like leukotrienes are synthesized. These mediators act in concert on cells in their vicinity which bear the appropriate receptors and thereby cause the clinical symptoms of bronchial asthma (Weiler *et al*.,1998).

2.10 Pathogenesis

The fundamental problem in asthma appears to be immunological: young children in the early stages of asthma show signs of excessive inflammation in their airways. Epidemiological findings give clues as to the pathogenesis: the incidence of asthma

seems to be increasing worldwide, and asthma is now very much more common in affluent countries (Ranjeeta *et al., 2009*).

Although there are subtypes of asthma (allergic *versus* non allergic), there are features of airway inflammation common to all asthmatic airways. Airway inflammation is thought to be triggered by innate and/or adapted immune responses. Although there may be multiple "triggers" for an inflammatory response (such as mast cell secretion), there is general agreement that a lymphocyte-directed eosinophilic bronchitis is a hallmark of asthma. The lymphocytes that participate in asthma pathology are biased toward the T-helper type 2 (Th2) phenotype, leading to increases in production of interleukin 4 (IL-4), IL-5, and IL-13. The IL-4 from Th2 cells and basophils provides help for IgE synthesis in B cells. The IL-5 provides support for eosinophil survival. The innate or adapted immune response triggers the production of additional cytokines and chemokines, resulting in trafficking of blood-borne cells (*i.e.,* eosinophils, basophils, neutrophils, and lymphocytes) into airway tissue and these cells further generate a variety of autocoids and cytokines. The inflammatory cascade also leads to activation of resident cells within the airways that, in turn, can produce an array of cytokines, growth factors, chemokines, and autacoids. The chronic inflammatory response, over time, leads to epithelial shedding and reorganization, mucous hypersecretion, and airway wall remodeling most often exemplified by subepithelial fibrosis and smooth muscle hyperplasia. How these processes lead to attacks of asthma, which most often are induced or exacerbated by respiratory viral infections, remains unclear (Ranjeeta *et al.,* 2009).

In addition to airway inflammation, asthmatics commonly exhibit bronchial hyperreactivity. Thus the concentration of a bronchial spasmogen, such as methacholine or histamine, needed to produce a 20% increase in airway resistance in asthmatics is often only 1 to 2% of the equally effective concentration in healthy control subjects. This bronchial hyperreactivity most often is nonspecific such that the airways are also inordinately reactive to stimuli such as strong odors, cold air, and pollutants. Little is known about specific mechanisms underlying this ambiguous hyperreactivity. In 1968 Andor Szentivanyi first described *The Beta Adrenergic Theory of Asthma;* in which blockage of the Beta-2 receptors of pulmonary smooth muscle cells, causes asthma.

In 1995 Szentivanyi and colleagues demonstrated that IgE blocks beta-2 receptors. Since over production of IgE is central to all atopic diseases, this was a watershed moment in the world of allergy (Ranjeeta *et al., 2009*).

2.11 Goals of asthma therapy

The American Academy of Allergy, Asthma, and Immunology (AAAAI) and the American College of Allergy, Asthma, and Immunology (ACAAI) have developed guidelines for getting asthma under control. They list the goals of asthma treatment as:

- To achieve and maintain clinical control.
- Preventing long-term (chronic) symptoms that interfere with daily living, such as coughing or shortness of breath during the night or after exercise.
- Maintaining lung function near the personal best measurement.

- Allowing the person to participate in all activities of daily living, including work, school, and exercise. Treatment to decrease nighttime symptoms and achieve uninterrupted sleep also is important.
- Preventing repeated asthma attacks.
- Providing the best medicine treatment with the fewest possible side effects.
- Meeting your or your family's expectations for your or your teen's asthma care. Effective treatment for asthma involves a partnership between the person, his or her family, and the doctor.

2.12 Treatment of bronchial asthma

A specific, customized plan for proactively monitoring and managing symptoms should be created. Someone who has asthma should understand the importance of reducing exposure to allergens, testing to assess the severity of symptoms, and the usage of medications. The treatment plan should be written down and adjusted according to changes in symptoms.

2.12.1 Non pharmacological interventions to asthma include:

a. Developing Patient/Doctor partnership (GINA, 2011).
b. Lifestyle modification: cigarette smoke, indoor and outdoor pollution, and common allergens
c. Dietary changes: avoiding food additives and processed foods, nitrates/nitrites and sulfites (cheese, hotdogs, wine and beer).
d. Nutritional supplements: with omega-3s (fish oil and flaxseed oil are excellent source), antioxidants (Vit A, C, E, quercetin, bioflavonoids, N-acetyl cysteine and bromelain), and magnesium supplement.

e. Influenza vaccination should be provided to patients with asthma when vaccination of the general population is advised (GINA, 2011).

2.12.2 Pharmacotherapy of bronchial asthma

Stepwise approach to the pharmacological management of asthma is based on asthma severity (adapted from global strategy for asthma management and prevention: 2002 workshop report and confirmed in 2010 updated GINA report).

There are six (6) steps to treatment approach with one or more of oral or inhaled forms of the following classes of drugs:

I. *Bronchodilators:*

Sympathomimetics: salbutamol, terbutaline, bambuterol, salbuterol, formoterol, ephedrine.

Methylxanthines: Theophyline, aminophyline, choline theophylinate, hydroxyethyltheophyline, ethanolate of piperazine, Doxophyline.

Anticholinergics: Ipratropium bromide, Tiotropium bromide.

II. *Leukotriene antagonists:* Montelukast, zafirlukast.

III. *Mast cell stabilizers:* sodium chromoglycate, ketotifen.

IV. Corticosteroids:

Systemic corticosteroids: Hydrocortisone, Prednisolone & others.

Inhalational corticosteroids: Beclomethasone dipropionate, Budesonide, fluticasone propionate, flunisolide, ciclesonide.

V Anti – IgE antibody: omalizumab.

2.13 Important medicinal plants with anti-asthmatic potential:

The active principles of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents. Different species of medicinal plants are used in the treatment of asthma. There are many natural herbs and herbal supplements that can be used for asthma treatment. Natural Asthma treatment incorporates vitamins, minerals and herbs to relieve symptoms and prevent further attacks.

Calotropis procera (Family-Asclepiadaceae: Common name-Madaar/ Dead Sea Apple/Arka) in a traditional treatment of asthma patients arka flowers have been shown to have therapeutic effect. It is differentiated by other species by the color of its flower i.e. *Calotropis gigantean* (Williams and Evans, 2000).

Acorus calamus (Family-Araceae: Common name-Sweet flag). It is highly beneficial in the treatment of asthma; it removes catarrhal matter and phlegm from the bronchial tubes. About 65 centigrams of the herb is taken every 2 or 3 hours in this condition (Ranjeeta *et al*., 2009)

.***Asystasia*** **gangetica** (Family-Acanthaceae: Common name Foxglove). *Arystasia gangetica is* a traditional medicine which is used to treat a wide variety of diseases in Nigeria and other parts of world, commonly known as creeping foxglove. The leaf of *Asystasia gangetica T.* Adams is also used in many parts of Nigeria for the management of asthma. Therefore a study was performed to evaluate the antiasthmatic effect of the plant. Result indicated that hexane, ethylacetate, and methanol extracts of the leaves of *Arystasia gangetica,* obtained by successive sohxlet extraction inhibited the contraction evoked by spasmogens and the IC values were calculated (Szelenyl and Brune, 2002).

The extracts relaxed histamine-precontracted tracheal strips in the following degree of potency-ethylacetate extract>hexane extract=methanol extract. This study shows that the leaves of *Asystasia gangentica* have antiasthamatic potency (Akah *et al*., 2003).

Adhato davasica (Family-Acanthaceae: Common name - Adusa). The traditional healers are using this herb for the treatment of chronic asthma. Adusa is known as "Vasa" or "Vasak" in Sanskrit and is a reputed drug for asthma mentioned in Ayurveda (http://www.healasthma.com). *Adhato davasica is* considered in the East to be the best possible treatment for all chest diseases and used in India as an expectorant, antitussive and in other respiratory disease. It is also used widely to relieve asthma. *Adhato davasica* has been traditionally used in the management of allergic disorders and bronchial asthma. Research performed over the last three decades revealed that the alkaloids present in the leaves, vasicine and vasicinone, possess powerful respiratory stimulant activity (http://www.healasthma.com). Its essential oil exhibited antitussive on cats, expectorant on rats and rabbit, and antiasthmatic on guinea pig activity in *in-vivo* experiments (Szelenyl and Brune, 2002).

Aegle marmelos (Family-Rutaceae: Common name- Golden apple/ Bael fruit). Its leaf extract is being used in Indian system of medicine as an antidiabetic agent and traditional text of India prescribe it in the management of asthma. Therefore the effect of the alcoholic extract of the leaves of *Aegle marmelos* Corr. on guinea pig isolated ileum and tracheal chain was investigated using the isolated organ bath method. 1mg/ml and 2mg/ml doses of the alcoholic extract of this plant produced a positive relaxant effect in isolated guinea pig ileum and tracheal chain, respectively. In addition, they antagonized the contractions, which are produced by histamine. Because the alcoholic

extracts elicited the antagonistic effect against histamine and also relaxed the histamine-induced contractions, it can be concluded that relaxations induced by *A. marmelos* in both guinea pig ileum and tracheal chain were due to the depression of H_1-receptors. This study shows that *Aegle marmelos* can be effective in the treatment of asthmatic disorders (Ranjeeta *et al*., 2009)

Alstonia scholaris (Family-Apocynaceae: Common name-Sitwanchaal/ milky pine). The ethanol extract of *Alstonia scholaris* leaves, induced pronounced bronchodilator activity in anaesthetized rats with the probable involvement of prostaglandins. However, *in-vitro* preparations of guinea-pig trachea did not confirm this property, indicating that broncho-dilation is not due to the direct tracheal smooth muscle relaxation (Channa *et al*., 2005)

Andrographis paniculata(Family-Acanthaceae: Common name- Creat, Kalmegh, Indian Echinacea). Persistent activation of nuclear factor (NF) - kappa B has been associated with the development of asthma. Andrographolide, the principal active component of the medicinal plant *Andrograhphis paniculata* has been shown to inhibit NF-Kappa B activity. Findings implicate a potential therapeutic value of Andrographolide in the treatment of asthma and it may act by inhibiting the NF-kappa B pathway at the level of inhibitory kappa B kinase-beta activation (Bao *et al.*, 2009).

Astercantha longifolia (Family-Acanthaceae: Common name-Kulikhara, Kokilaksah). The methanolic extracts of *Astercantha longifolia* Inhibits the biosynthesis of leukotriene B_4 in bovine polymorphonuclear leukocytes and exhibited potent inhibitory

action with Half Maximal Inhibitory Concentration (IC_{50}) values of 20 (Sunil *et al*., 2000).

Allium cepa (Family-Lilliaceae: Common name- Pyaaz, Onion). Dorsch *et.al.,* in the year 2000, had studied the effect of onion oil on platelet-activating factor-induced bronchial obstruction by onion oils. In this study lyophilized onion extract and ether extracts of onions were separated by chromatographic methods into several subfractions and tested for their effects on asthmatic reactions of guinea pigs to allergen, histamine, acetylcholine and platelet-activating factor (PAF) inhalation as well as on thromboxane biosynthesis of human platelets and lung fibroblasts. Onion oils are counteracting the bronchial obstruction due to PAF inhalation. Thus onion oil can be effectively used in the treatment of asthma (Dorsch *et al*., 2000).

Acacia catechu (Family-Mimosaceae: Common name- Black catechu/Khair). The extracts of gum, flowering tops, leaves, young shoots, bark and fruits are used in asthma, cough, and bronchitis. (http://www.divineremedies.com/acacia_catechu.htm).

Albizia lebbeck (Family-Leguminosae: Common name-Pit Shirishshirisha). Albizia originates from India. The bark is used to treat asthma (http://botanical.com/botanical/mgmh/n/nighde05.html). Traditional Medicinal Use is in asthma, antihistamine, allergies, and bronchitis (Murugesa, 1998).

Atropa belladonna (Family-Solanaceae: Common name: Devils Cherries). Synonyms - Belladonna. It is a powerful antispasmodic in intestinal colic and spasmodic asthma. Occasionally the leaves are employed as an ingredient of cigarettes for relieving asthma.

Acalypha indica (Family-Euphorbiaceae: Common name: Kuppi). According to Siddha Materia Medica, the leaf powder when given in the dose of 950 mg to 1300 mg, cures respiratory diseases. Expressed juice of the leaves is useful in chronic bronchitis, asthma and consumption (Gupta and Gupta, 1998).

Boswellia serrata (Family-Bruseraceae: Common name: Indian olibanum tree). Boswellia is an Ayurvedic plant that contains antiinflammatory triterpenoids called boswellic acids. Boswellic acid and its derivatives have anti-carcinogenic, anti-tumor, and blood lipid lowering activities. Dried extracts of the resin of the *Boswellia serrata* tree have been used since antiquity in India to treat inflammatory conditions. It inhibits proinflammatory 5-lipoxygenase chemicals and blocks leukotriene synthesis and thus boswellia may helpful in medical conditions involved in inflammation including asthma (http://www.divineremedies.com/calamus.htm). From a clinical study involving 40 patients, it was concluded that gum resin of *Boswellia serrata was* quite beneficial in the management of asthma (Anil and Ramu, 2002).

Benincasa hispida (Family-Cucurbitaceae: Common name: Ash Gourd). Methanol extract of *Benincasa hispida* (MEBH) showed excellent protection in guinea pigs against the histamine induced bronchospasm even at a very low dose, 50mg/kg, p.o However at a higher dose of 400 mg/kg, MEBH did not offer any significant protection against acetylecholine challenge. Therefore it can be deduced that the MEBH is unlikely to have muscarinic action. Result suggested that the plant has a protective effect against bronchospasm induced by histamine. (http://www.sethayurvedics.com/ayurveda-herbs/index.html).

Blumea lacera (Family-Compositeae: Common name: Kukurmutta / kukronda). In case of acute asthmatic attack the patients are advised to inhale the fumes of dried Blumea leaves. For regular use, healers recommend to prepare herbal cigarette using this herb in combination with other herbs. In many parts of India, it is known as Janglimuli (http://www.botanical.com/site/column_poudhia/04_asthma.html).

Cuminum cyminum: (Family-Umbelliferae: Common name-Jeera). The relaxant effect of *Cuminum cyminum* is well established. It acts as a powerful bronchodilator. It makes breathing easy and free of blocks (http://www.healasthma.com).

Cinnamomum cassia: (Family-Laureaceae: Common name-.Chinese cinnamon / Dalchini). Enhance expectoration of fluids in lungs. It has powerful anti edemic properties - prevents stagnation of fluids (mucous) in lungs (http://www.healasthma.com).

Clerodendrum serratum: (Family-Verbenaceae: Common name- Bharnagi). *Clerodendrum serratum* is called as Bharnagi in Indian system of traditional medicine and the juice of bharangi root is given in cough and asthmatic conditions with some other drugs like ginger etc. It is given with ghee and honey in bronchial asthma and also gives with hot water when one suffers with high cough and asthma (http://www.divineremedies.com/cissus_quadrangularis.htm).

Cissusqu andrangularis: (Family-Vitaceae.: Common name-Hajora/ Asthisanhari). The leaves and stem are frequently eaten with curry in southern India The entire plant is considered to be an anthelmintic aphrodisiac, antiasthmatic Quadrangularins A, Band C,

resveratrol, piceatannol, pallidol and parthenocissin are present in the stem. Quadrangularins A, B and C, resveratrol, piceatannol, pallidol and parthenocissin are present in the stem (http://www.bhj.org/journal/2006_4804_oct/html/org_res_579_586.ht ml).

Tamarindus indica: (Family- Leguminosae: Common name- Tamarind, tamarind) The plant has powerful anti-inflammatory properties (Lantz *et al*., 2005). It instantly relieves inflammation in lungs and respiratory tract. It builds strong immunity to allergy. A decoction of bark of the tree is used in cases of gingivitis and asthma and eye inflammations (http://www.healasthma.com).

Zingiber officinale (Family- Zingiberaceae: Common name: Ginger) It is a powerful natural expectorant used widely in Chinese as well as Indian formulations for coughs, colds, and chronic bronchitis. The dried rhizome of ginger contains approximately 1-4% volatile oils. It is considered to be a powerful natural anti-allergy agent specially acting on respiratory system (http://www.healasthma.com).

Others include: ***Curcuma longa:*** (Family-Zingiberaceae : Common name- Turmeric/Haldi), ***Camellia sinensis :*** (Family-Theaceae : Common name- Tea), ***Cannabis sativa:*** (Family-Cannabaceae: Common name- Bhang), ***Ephedra sinica:*** (Family-Ephedraceae: Common name-Ma Huang), ***Glycyrrhiza glabra:*** (Family-Fabaceae: Common name- Mulethi, Liquorice), ***Moringa oleifera:*** (Family-Moringaceae: Common name- Sehjan, Drumstick Tree), ***Plantago major:*** (Family-Plantaginaceae: Common name- Plantain) (Ranjeeta *et al., 2009*).

2.14 The Family, *Asclepiadaceae*

Asclepiadaceae belong to the milkweed family of the flowering-plant order Gentianales. The name comes from the type genus *Asclepias* (milkweeds). They are mainly located in the tropics to subtropics, especially in Africa and South America. *Asclepiadaceae* are mostly herbs and shrubs with white sap comprising 250 genera and over 2000 species, many of which are lianous and some of which are cactus-like succulents with reduced leaves. The leaves are simple and nearly always opposite or whorled; minute stipules are present. The flowers are bisexual, nearly always actinomorphic, and usually include an elaborate crown or corona of nectariferous appendages between the corolla and sexual parts. The ovaries are distinct, nearly always superior, and each has a single locule with numerous marginal ovules. The fruit is a follicle. Seeds usually have a tuft of hairs at one end (Li *et al*., 1995).

2.15 *Calotropis procera*

Calotropis procera (Asclepiadaceae), called French cotton, Mudar plant, calotropis, rubber bush, apple of Sodom, mudar, madar, king's crown, and rooster tree in English; Tumpapiya in Hausa. *Calotropis procera* a spreading shrub or small tree (up to 4 m high), exuding copious milky sap when cut or broken; leaves opposite, grey-green, large up to 15 cm long and 10 cm broad, with a pointed tip, two rounded basal lobes and no leaf stalk; flowers waxy white, 5 petals, purple-tipped inside and with a central purplish crown, carried in stalked clusters at the ends of the branches; fruit grey-green, inflated, 8 to 12 cm long, containing numerous seeds with tufts of long silky hairs at one end (Kleinschmidt and Johnson, 1977).



PlateI: *Calotropis procera* in its natural habitat

2.16 Geographical Distribution of the Plant

It is widely distributed in tropical to dry parts of Africa, Arabia, Palestine, West Indies, Brazil, Columbia and Venezuela.

2.17 Ethnopharmacology

In India, in various traditional systems like Ayurveda, Unani and Siddha numerous herbs (including *calotropis procera*) were mentioned for therapeutic use in asthma. Important factors of the various parts of *Calotropis procera* have been widely reported. *Calotropis procera* latex has been used in leprosy, eczema, inflammation, cutaneous infections, syphilis, malarial and low hectic fevers, and as abortifacient (Kumar and Basu, 1994). Leaves are used in rheumatism, as an anti-inflammatory and antimicrobial and Roots are used as hepato protective agents, against colds and coughs, syphilis and elephantiasis, as an anti-inflammatory, analgesic, antimalarial and antimicrobial. Flowers are used as cytostatic, abortifacient, antimalarial, in asthma and piles and villagers in Bikaner district of Rajasthan state in west India ingest almost all plant parts in various dietary combinations for malarial fevers and pyrexias (Sharma and Sharma, 2000).

2.18 Some Pharmacological Properties of the Plant

The plant produces latex in the laticifers (elongated secretory cells). Dried latex and chloroform extract of roots has been reported to possess anti-inflammatory activity (Kumar and Basu, 1994). Aqueous extract of the flowers has been found to exhibit analgesic, antipyretic and anti-inflammatory activity. The alcoholic extract from different parts has been found to possess antimicrobial and spermicidal activity (Kamath and Rana, 2002).

The anti-inflammatory property of the latex of *Calotropis procera* was studied on carrageenin- and formalin-induced rat paw edema model. A single dose of the aqueous suspension of the dried latex was effective to a significant level against the acute inflammatory response (Basu and Chaudhuri, 1991; Kumar and Basu, 1994). A literature survey of *Calotropis procera* revealed that the plant contains mainly cardenolides besides steroids and triterpenes. From the hexane-insoluble fraction of this plant a new free cardenolide named proceragenin has been isolated. The medicinal importance of *Calotropis procera* prompted the studies on pharmacological screening of the antibacterial and anti-aggregating activities of proceragenin (Akhtar, 1992).

Procesterol, a new steroidal hydroxy ketone, has been isolated from the fresh and undried flowers of *Calotropis procera*. Mosquito control by Calotropis latex has been reported by Girdhar *et al.*, 1984. The effect of crude fractions of *C procera*, its flower, bud and root were tested against a chloroquine sensitive strain, MRC 20 and a chloroquine resistant strain, MRC 76 of *Plasmodium falciparum* (Sharma and Sharma, 1999).

The dry latex (DL) of *Calotropis procera* (Asclepiadaceae), a potent anti-inflammatory agent has been evaluated for anti-diarrhoeal activity. Anti-diarrhoeal effects of *C. gigantea* used traditionally in Indian system of medicine were recorded. The plant contains several useful enzymes. Pretreatment with an ethanolic latex extract of *Calotropis procera* at a dose of 300 mg/kg body wt., administered orally thrice a day for 30 days, reduced significantly the elevated marker enzyme levels in serum and heart homogenates in isoproterenol-induced myocardial infarction. Histopathological

observation revealed a marked protection by the extract in myocardial necrotic damage (Mueen *et al*., 2004).

2.19 Models for Antiasthamtic Study

The models available for the study of anti-asthmatic activity in experimental animals among others include:

2.19.1 Spasmolytic activity in isolated guinea pig Ileum preparation

Purpose and Rationale

The isolated ileum strip of guinea pigs can be used to test for β-blocking activity. In addition, this model can be used to test compounds which inhibit bronchospasms. It is used to detect β-sympathomimetic, H_1-receptor blocking and leuko-triene receptor blocking properties of test drugs. Carbachol is a cholinergic agonist that produces contraction of bronchial smooth muscle by muscarinic stimulation. Histamine is an important mediator of immediate allergic (type 1) and inflammatory reactions. It causes bronchoconstriction by activating H_1-receptors. Calcium ionophores induce the release of leukotrienes via the 5-lipoxygenase pathway. Leukotrienes are powerful bronchoconstrictors that appear to act on smooth muscle via specific receptors. To investigate a compound's ability to inhibit carbachol induced bronchospasm via β-receptor activation, a β-receptor blocking agent (for example propranolol) must be added. If relaxation of bronchial smooth muscle is brought about by β-receptor activation, the spasmolytic effect will decrease following propranolol administration. It is also known that all pharmacological agents that increase the intracellular level of CAMP relaxes airway smooth muscles and inhibits the release of autocoids from the tissues and basophils. The effect of bradykinin can be abolished by bradykinin

antagonists. The effects of potassium channel openers can also be studied in this test (Gerhard, 2002).

2.19.2 Carrageenan-induced leucocytosis

Formulations used in the treatment of asthma include some anti-stress herbs to enable adoption to stress since excessive stress or nervous debility may aggravate symptoms of asthma. The number of WBC's is typically elevated when the body is fighting a severe infection or stressed by metabolic toxins. The normalization effect of an adaptogen can be observed in carrageenan-induced leucocyte count (increase in leucocyte count) after parenteral administration of carrageenan (Bhargava and Singh, 1981; Horn and Robin, 1975).

2.19.3 Passive paw anaphylaxis in rats

Allergic asthma is a chronic inflammatory process occurring due to exposure of allergen resulting in the activation of T-lymphocytes with subsequent release of inflammatory mediators. Immune-modulating agents are useful in the treatment of asthma by inhibiting the antigen – antibody (AG – AB) reaction and thereby inhibiting the release of inflammatory mediators (Pandit, 2008; Patil, 2010).

2.19.4 Haloperidol-induced catalepsy

Haloperidol induces catalepsy by inhibiting dopamine D_2 receptors and dopamine secretion. Dopamine is biosynthetic precursor of adrenaline; stimulates adrenaline release; and an agonist for adrenaline on beta adrenoceptors (beta 1 and 2 receptors). Adrenaline is physiological antagonist of histamine. So as there decrease in dopamine there is imbalance in neurotransmitters means high level of histamine (Tripathi, 2003).

Catalepsy is a sign of extra pyramidal effects of drugs that inhibits dopaminergic transmission or increase/release histamine (inhibitory neurotransmitter) in brain. It is most commonly measured by the bar method and consist of placing an animal, after administration of a neuroleptic such as haloperidol, in a position with its front legs resting on a bar suspended above the floor of the test apparatus. Intensity of catalepsy is measured as the length of time the test subject maintain this abnormal posture (Patil, 2010).

2.19.5 Carrageenan induced rat paw edema

Airway inflammation has been demonstrated in all forms of asthma. Even in mild asthma, there is an inflammatory response involving infiltration, particularly with activated eosinophils and lymphocytes, with neutrophils and mast cells. The airways are infiltrated with inflammatory cells, which release various mediators that promotes the growth of smooth muscles cells, tissue damage and bronchial hyper reactivity. The airway constricts and becomes congested with necrotized epithelial tissue, dead immune cells, mucous and plasma. In rational targets in asthma treatment reducing the inflammation is paramount and the arachidonic acid derived prostaglandins and leucotrienes are rational targets. The degree of bronchial hyper responsiveness and airway obstruction is closely linked to the extent of inflammation (Bousquete *et al*., 2000). Anti-inflammatory drugs suppress the inflammatory response by inhibiting infiltration and activation of inflammatory cells as well as their synthesis, or release of mediators. Carrageenan induced rat paw edema model is known to be sensitive to cyclooxygenase inhibitors (Anita and Babita 2008). Since serotonin, histamine and prostaglandins are common mediators of both bronchial asthma and inflammation; this model could be of benefit in studying antiasthmatic drug.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Plant Material

The whole plant of *Calotropis procera* was collected from the roadside locations of Saye Town of Zaria local government. It was identified and authenticated by a botanist at the Department of Biological Sciences, Ahmadu Bello University, Zaria. Voucher specimen number (900219) was deposited in the herbarium section for future reference. Plant material was preserved in Department of Pharmacognosy and Drug development, Faculty of Pharmaceutical Science, ABU, Zaria. The root of the plant was separated, air dried to constant weight and coarsely powdered.

3.2 Preparation of Plant Extract

0.45 kg of the dried root powder was extracted by cold maceration with distilled water for 48 h. Another 0.45 kg of the powdered plant was extracted with 90% $^{v}/_{v}$ methanol for 72 h using Soxhlet extraction apparatus. The extract obtained was concentrated in a rotavapor under reduced pressure and completely dried over a regulated water bath maintained at 60□C.

7.3 Experimental Animals

Wistar rats weighing 150 -250g and guinea pig of either sex bred at Animal House, Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were maintained in a well-ventilated room, fed on standard rodent feed (Grand Cereals Ltd, Jos) and granted access to water *ad-libitum*. They were kept in clean cages under normal light / dark cycle. All experimental procedures were followed in accordance with the guideline prescribed by

the Committee for the Purpose of Control and Supervision on Experimental on Animals (198/99/CPCSEA).

3.4 Drugs and Chemicals

Distilled water (Department of Chemistry, ABU, Zaria, Nigeria), Histamine (Sigma Chemical Company, Germany), Normal saline 0.5% (Dana pharmaceuticals, Minna, Nigeria.), Freshly laid egg albumin (Al-Ihsan Poultry, Zaria), Dexamethasone, Kreb's solution (Department of Pharmacology ABU, Zaria, Nigeria), Tyrode's solution (Department of Pharmacology ABU, Zaria, Nigeria), 95% oxygen and 5% carbondioxide, Carrageenan (Sigma Chemical Company, Germany), Haloperidol, Chlorpheniramine 10mg/ml (Greenfield Pharmaceutical, China.), Diclofenac sodium 20mg (Hovid pharmaceuticals).

3.5 Equipment and Other Materials

Macrodynanometer, small and medium rat cages, Top Loading and Champ II digital electronic weighing balances, thermo circulator, clock (timer), cotton wool, scissors, syringes (1 ml, 2 ml, 5 ml and 10 ml), spatulas, animal cages, pestle, mortar, and plastic containers.

3.6 Median Lethal Dose (LD_{50}) Determination in Rats

LD_{50} determination was conducted using the method as described by Lorke, 1983. This method is carried out in two phases. In the initial phase, three (3) groups of three rats each were treated with the extract of the plant at doses of 10, 100 and 1000 mg/kg body weight orally and observed for signs of toxicity and death for 24 hours. Based on the results obtained from phase one (1), in the second phase, three fresh groups of one rat

each were orally administered with plant extracts at doses of 1,600, 2,900 and 5,000 mg/kg.

The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1). The final LD_{50} was calculated as follows:

$LD_{50} = \sqrt{X \, x \, Y}$

Where X = Maximum dose that did not cause death

Y = Minimum dose that did cause death

3.7 Screening for Antiasthmatic Activity

3.7.1 *In vitro* studies on isolated guinea pig ileum preparation

Overnight fasted guinea pigs were sacrificed using cervical dislocation method. 3 cm long ileum was quickly dissected out and mounted in an organ bath maintained at 30 ± 0.5°C and containing 20 ml Tyrode's solution under basal tension of 500 mg. The solution was continuously aerated with air. The responses to drug were recorded on a macrodynanometer using isotonic transducer, which exerts a basal tension equivalent to 500 mg load on tissues. The tissue was allowed to equilibrate for 30 minutes, during which, the bathing solution was changed at every 10 minutes. The contractile response of ileum to histamine 10µg/ml was recorded in presence and absence of *Calotropis procera* extract 10 mg/ml. Percentage maximum contractile response was plotted to generate dose response curve of histamine, in the presence and absence of the extract (Pandit *et al.*, 2008).

3.7.2. Carrageenan-induced leucocytosis in rats

Forty two (42) albino rats were divided into seven (7) groups of six (6) rats each. Group 1 was the standard reference group. Group 2 (Control group) received distilled water (2 ml/kg), Chlorpheniramine (2mg/kg i.p.) was administered to group 3 (represents the standard drug). Groups 4 and 5 received single doses of aqueous extracts (100 and 200 mg/kg p.o.), while did group 6 and 7 (100 and 200 mg/kg p.o.) received methanolic extracts of the plant. Carrageenan-administered group served as a negative control. After 1 h of drug treatment, all groups received carrageenan injection at a dose of 1 mg/kg subcutaneously. Total leucocyte count for group 1 was determined initially so as to ascertain and compare the normal WBC count for rats; According to the Zoological Education Network normal WBC count for rats is 6.6-12.6 × 10^3/mm^3. Blood samples were collected from each rat 24 h after carrageenan injection and total leukocyte count were done using Sysmex Hematological Analyser in National Tuberculosis and leprosy Training Centre, Saye Zaria (Bhargava and Singh, 1981; Horn and Robin, 1975).

3.7.3 Haloperidol-induced catalepsy

Thirty six (36) adult wistar rats were grouped into six (6) groups of six (6) rats each. Group 1 received 2 ml/kg distilled water served as negative control. Group 2 received Chlorpheniramine maleate at a dose of 10 mg/kg served as positive control, groups 3 and 4 received single doses of aqueous extract, 200 and 300 mg/kg orally, respectively. Groups 5 and 6 received same doses of methanol root extracts 200 and 300 mg/kg orally, respectively. Groups 1 – 6 received haloperidol at a dose of 1 mg/kg, intraperitoneally 1 h after the drug administration. The severity of catalepsy was measured at 0, 30, 60, 90,120 and 150 min. Catalepsy of an individual rat was measured in a stepwise manner by a scoring method as follow:

Step I: Each rat was taken out of the cage and placed on a table. It was then pushed forward by a gentle touch on the back. If it failed to move when touched gently on the back or pushed, a score of 0.5 was assigned.

Step II: the front paws of the rat were placed alternately on a 3cm high block. If the rats failed to correct the posture within 15 seconds a score of 0.5 for each paw was added to the score of step I.

Step III: the front paws of the rats were placed alternately on a 9cm high block. If the rat failed to correct the posture within 15 seconds, a score of 1 for each paw was added to the scores in I and II Thus for an animal, the highest possible score was 3.5 (cut-off Score) and this reflects total catalepsy (Khisti *et al*., 1997; Sanberg, 1980).

3.7.4 Passive paw anaphylaxis in rats (antiallergic activity)

Wistar rats were sensitized by administering subcutaneously three doses of 100 mg of egg albumin on days 1, 3 and 5. On day 10 of sensitization, blood was collected and centrifuged to separate serum. A fresh set of thirty (30) rats were divided into six (6) groups each containing five (5) rats. Group 1 (Control) received distilled water at a dose of 2 ml/kg orally. Group 2 was administered with Dexamethasone at a dose of 0.27 mg/kg orally. Group 3 – 6 CP 250 mg/kg (low dose aqueous extract) orally, CP 350 mg/kg) high dose aqueous extract) orally, CP 250 mg/kg (low dose methanol extract) orally, and CP 350 mg/kg (high dose methanol extract) orally respectively. Prior to drug treatment animals were immunised with 0.5 mls of the previously collected serum subcutaneously. Next 24 h, after drug treatment animals again challenged with 10 mg egg albumin subcutaneously on the right hind paw and edema inhibition was calculated

at 0, 1, 2, 3 and 4 hours by measuring the paw diameter using a vernier calliper (Patil, 2010).

3.7.5 Carrageenan-induced rat paw edema (anti-inflammatory study)

Albino rats of either sex weighing 200 – 250 g were divided into six (6) groups of five (5) animals each. The following schedule of treatment was administered: Group 1received distilled water at a dose of 2 ml/kg. Group 2 and 3 received aqueous extracts of *C. procera* 200 mg/kg and 400 mg/kg orally respectively. Group 4 and 5 received methanol extracts of *C. procera* at a dose of 200 mg/kg and 400 mg/kg orally respectively. Group 6 were administered with diclofenac sodium at a dose of 20 mg/kg orally. Subsequently one hour after administering the extracts 0.1ml of 1% carrageenan was injected into the plantar region of the right hind paw to induce edema. The paw volume was measured initially and at 1, 2, 3, 4, and 5 hours after carrageenan injection using a vernier calliper. Percentage increase in paw volume from baseline was calculated and compared with control (Anita and Babita, 2008).

3.8 Preliminary Phytochemical Screening of *Calotropis procera*

The preliminary phytochemical screening of *C. procera* was carried out on the whole root of both aqueous and methanolic extracts. The tests were as follows:

3.8.1 Test for carbohydrates

Molisch test: 0.5 g of the extract was dissolved in water in a test tube. Three drops of 1%. - naphthol in 80% ethanol was added followed by 5 drops of concentrated sulphuric acid without mixing. Appearance of purple ring at the interface as a result of the reaction between - naphthol and furfural and 5- hydroxymethylfurfural aldehydes

produced by dehydration of saccharides would indicate the presence of carbohydrates (Silva *et al*., 1998).

3.8.2 Test for flavonoids

Shinoda reduction test (Cyanidin test): 3 ml of the alcoholic solution of the extract was evaporated to dryness. The residue was dissolved in 2 ml of 50% methanol with heat. Four drops of concentrated Hydrochloric acid was added followed by some chips of' magnesium metal. Immediate appearance of orange color denotes flavones, red - crimson color denotes flavonols and pink - magenta color denotes flavonones (Mahran *et al.,* 1980).

3.8.3 Test for cardiac glycosides

Keller-kiliani test: 1 ml of the extract was diluted with 20 ml of water and 1 ml of strong lead sub acetate solution was added to precipitate pigments, which were filtered off. The filtrate obtained was shaken with an equal volume of chloroform and allow separating into two layers in a small separating funnel. The chloroform layer was removed and evaporated to dryness on a water bath. The residue was dissolved in 3 ml of ferric chloride in glacial acetic acid, left for 1 minute and then transferred into a dry test tube. Six drops of concentrated sulphuric acid was added by the walls of the test tube. On standing, a brown colour at the interface (due to deoxy sugars) and a pale green colour at the upper layer (due to the steroidal nucleus) is a preliminary indication for cardenolides (Brain and Turner, 1975).

3.8.4 Test for cyanogenic glycosides

Guinard test: 0.5 g of the powdered drug was mixed with 0.1 ml of water in a test tube. A prepared damp sodium picrate paper was suspended at the mouth of the test-tube by means of a cork. The tube was placed in a water bath and allowed for an hour. A brick - red colour on the picrate paper indicated the presence of cyanogenic glycoside (Silva *et al*., 1998).

3.8.5 Test for saponins

Froth test: 2 ml of the aqueous methanol extract was diluted with twice its volume of water and shaken in a test tube for 15 minutes. The occurrence of foam column, of at least learn in height persisting for a minimum of 15 minutes would indicate the presence of saponins (Silva *et al*., 1998).

3.8.6 Test for tannins

Ferric Chloride test: 1 ml of the extract was diluted with 2 ml of water. Dilute solution of ferric chloride (3 drops) was then added. The occurrence of black-blue or black-green would show the presence of tannins (Evans, 1998).

Lead Sub acetate test: 1 ml of the aqueous methanol extract was diluted with 2 m18water. The dilute solution was mixed with lead sub acetate solution. Appearance of a light brown precipitate indicates the presence of tannins (Evans, 1998).

3.8.7 Test for steroids and terpenoids

Liebermann-Burchard's test: 5 mg of the powdered plant material was extracted with 5 ml of methanol and then filtered. The filtrate was evaporated to dryness on water bath. The residue was shaken with chloroform and filtered into a clean and dry test tube. 2 ml

of acetic acid anhydride was added to the filtrate and shaken. 1 ml of concentrated sulphuric acid was added carefully down the side of the tube to form a lower layer. A brownish-red or violet ring at the zone of contact of the two liquids and the upper layer turning green denote the presence of sterols and terpenes (Cuilei, 1990).

3.8.8 Test for alkaloids

1 ml of the extract of *C. procera* was treated with strong ammonia to make alkaline (pH 8) and then extracted twice 'with 10 ml portions of chloroform. The chloroform extract were combined and concentrated in vacuum to about 5 ml. The concentrate was then tested with the following reagents; Dragendorff's, Meyer, picric acid and Wagner. The presence of precipitate in most or all of the reagent tests indicates the presence of alkaloids (Evans, 1989), that is:

- Light brown to brownish precipitate - Wagner's reagent
- White or creamy white precipitate - Mayer's reagent
- Orange or orange-yellow precipitate -Dragendoff's reagent
- Yellowish precipitate - picric acid reagent.

3.8.9 Test for anthraquinones and their derivatives

2 g of the plant material was extracted with 10 ml of benzene and filtered. 5 g of 10% ammonia solution was added to the filtrate and shaken. The presence of a pink, red or violet colour in the ammoniacal (lower layer) phase indicates the presence of free hydroxyl anthraquinone. For the anthraquinone glycosides, 2 g of the powdered plant material was boiled with 10 ml of dilute sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml benzene. The benzene layer was then separated and to it, 3 ml of 10% ammonia solution was added and the mixture shaken. Appearance of a pink,

red or violet colouration in the lower layer indicates the presence of anthraquinones glycosides (Sofowora, 1993).

3.9 Statistical Analysis

All data were expressed as mean ± SEM and analysis was done using Statistical Package for the Social Sciences (SPSS) Version 19. One-way analysis of variance (ANOVA) or Friedman Test was used where appropriate. Dunnett post hoc test was used for experiments in which more than two (2) groups were compared. Differences in mean were considered to be significant when $p \leq 0.05$. Values were represented as tables and chart

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage Yield

900 g of the powdered plant (450 g each for aqueous and methanol extraction) gave a yield of 61.91 g aqueous and 70.37 g methanol extract. The percentage of methanol and aqueous soluble extractives were calculated with reference to air-dried plant material and the yield were determined to be 15.64% w/w and 13.76%w/w respectively (Table 4.1).

4.2 Chemical Constituents of Roots of *Calotropis procera*

Preliminary phytochemical screening of the methanolic fruit extract of *C.procera* revealed the presence of carbohydrate, cardiac glycosides, saponins, tannins, flavonoids, steroids, terpinoids and alkaloids. However, there were no free anthracene present (Table 4.2).

Table 4.1 Yield of Methanol and Aqueous Soluble Extractives of *Calotropis procera.*

Extracts	**Weight of Powdered plant (g)**	**Yield (in g)**	**Percentage Yield ($\%^{w}/_{w}$)**
Aqueous	450	61.91	13.76
Methanol	450	70.37	15.64

Table 4.2 Preliminary Phytochemical Screening of Aqueous and Methanolic Root Extracts of *Calotopis procera*

Chemical constituents	Methods	Remarks	
		aqueous	methanolic
Carbohydrate	Molish test	+	+
Steroids and terpenoids	Liebermann-Burchard's test	+	+
Anthracene (free)	Bontragers test	_	_
Cardiac glycoside	Keller killiani test	+	+
Saponins	Frothing test	+	+
	Haemolysis test		
Tannins	Ferric chloride test	+	+
	Lead sub acetate test	+	+
Flavonoids	Shinoda test	+	+
	Sodium hydroxide test	+	+
Alkaloids	Meyer's test	_	_
	Dragendoff test	+	+
	Wagner's test	_	_
	Picric test	+	+

+ = present, - = absent

4.3 Median Lethal Dose (LD_{50}) of Aqueous and Methanolic Root Extracts of *Calotropis procera* in Wistar Rats

Oral administration of both methanolic and aqueous root extract of *Calotropis procera* at doses of 10, 100, 1000, 1,600, 2,900, and 5,000 mg/kg did not produce any visible sign of toxicity or mortality in the animals over a period of 24 h for both the first and second phase of the Lorke's method. The oral LD_{50} was therefore found to be above 5,000 mg/kg body weight for both methanolic and aqueous extracts (Table 4.3).

4.4 Antiasthmatic Studies

4.4.1 Histamine-induced contractions of isolated guinea pig ileum chain preparation.

Both aqueous and methanolic *Calotropis procera* extracts (10 mg/ml) significantly ($p < 0.001$) inhibited histamine induced contraction of isolated guinea pig ileum chain preparation (Table 4.4).

4.3.2 Carrageenan induced leucocytosis in rats

The test standard reference obtained ($7.100 \pm 0.58 \times 10^3/mm^3$) falls within the range recommended by the zoological education network for rats ($6.6 - 12.6 \times 10^3/mm^3$). Control group gave a mean Total Leucocyte Count (TLC) above the mormal TLC for rats, meaning carrageenan has a propensity to result in an abnormal increase in total leucocyte count in rats. Both 100mg/kg doses of aqueous and methanolic extracts produced a significant ($p < 0.01$) inhibition of carrageenan induced TLC compared with standard Chlorpheniramine at a dose of 2 mg/kg. 200 mg/kg of aqueous extract gave the most significant ($p < 0.001$) difference in TLC from the control(Table 4.5).

Table 4.3: The Oral Median Lethal Dose (LD_{50}) of Crude Aqueous and Methanolic Extracts of *C. Procera* in Wistar Rats

Dose(mg/kg)	Route	No. of death/No. used	Phase	% Mortality
10	Oral	0/3	I	0
100	Oral	0/3	I	0
1,000	Oral	0/3	I	0
1,600	Oral	0/1	II	0
2,900	Oral	0/1	II	0
5000	Oral	0/1	II	0

LD_{50} was calculated to be > 5,000 mg/kg (oral route).

Table 4.4: The Effects of Aqueous and Methanolic Extracts of *Calotropis procera* on Histamine-Induced Contractions of Isolated Guinea Pig Ileum Chain Preparation.

Volume (ml)	**Maximum contraction (mm)**		
	Control (Histamine 10µg/ml)	Methanol Ext (10mg/ml)	Aqueous Ext (10mg/ml)
0.1	13.600 ± 0.87	2.600±0.40[a]	3.00 ± 0.32[a]
0.2	18.400 ± 0.51	4.800 ± 0.58[a]	6.400 ± 0.68[a]
0.4	22.000 ± 0.89	7.200 ± 0.37[a]	12.200 ± 1.02[a]
0.8	27.200 ± 0.58	8.800 ± 0.49[a]	15.60 ± 0.51[a]
1.0	26.600 ± 0.51	7.800 ± 0.58[a]	16.00 ± 0.51[a]

Data were analysed by one way ANOVA, "a" is significantly different at p□ 0.001; n=5. Ext = Extract, mm = millimeter, ml = milliliter

Table 4.5: Effects of Methanolic and Aqueous Extractsof *Calotropis procera*on Carrageen an Induced Leucocytosis in Rats

Treatment Groups	Doses (in mg per kg)	Total Leucocytes Count (TLC) (× 10^3/mm^3)
Standard Reference Group (for TLC in Rats)	__	7.100 ± 0.81
Normal saline	2ml/kg	12.920 ± 0.58
CPM	2mg/kg IP	7.840 ± 0.61[c]
Aqueous Extract	100mg/kg PO	5.580 ± 0.79[b]
Aqueous Extract	200mg/kg PO	5.300 ± 0.54[a]
Methanolic Extract	100mg/kg PO	7.600 ± 0.66[c]
Methanolic Extract	200mg/kg PO	12.840 ± 0.85

Data were analyzed by one way ANOVA. All treated groups were compared with control. a, b, and c are significantly different at p□ 0.001, p□ 0.005 and p□ 0.01 respectively; n=6. CPM = chlorpheniramine.

4.4.3 Haloperidol-Induced Catalepsy in Rats

Significant protection against haloperidol induced catalepsy was observed at 30 mins, 60 mins and 90 mins post haloperidol exposure in methanol extract group at doses 250mg/kg and 350mg/kg; and up to 150mins of protection for 350mg/kg dose of methanol extract. No significant protection was observed in the aqueous extract groups (Table 4.6).

4.4.4 Carrageenan induced paw edema in rats.

Results showed that all treatment groups except MCPE 400 mg/kg, inhibited significantly (p□ 0.05) inflammation of paw edema induced by carrageenan compared with standard Diclofenac sodium (20mg/kg), with the most significant anti-inflammatory activity across all the observed hours (1, 2, 3, 4, and 5 hours) observed in 200mg/kg MCPE group (Table 4.7).

Table 4.6 Effect of Methanolic and Aqueous Extracts of *Calotropis procera* on Haloperidol-Induced Catalepsy in Rats

Treatment	Dose (mg/kg)	Total Cataleptic Score				
		30 mins	60 mins	90 mins	120 mins	150mins
Distilled water	2ml/kg	3.200± 0.20	3.500 ± 0.00	3.500± 0.00	3.500± 0.00	3.500± 0.00
ACPE	250	2.500 ± 0.55	3.500± 0.00	3.100 ± 0.40	3.500± 0.00	3.500± 0.00
ACPE	350	2.000±0.55	3.100± 0.29	3.300± 0.20	2.300± 0.58	2.900± 0.40
MCPE	250	0.900 ± 0.40[a]	2.100± 0.51[c]	2.200± 0.25[b]	2.750± 0.75	2.600± 0.40
MCPE	350	1.200± 0.37[b]	1.500± 0.47[a]	2.300± 0.37[c]	3.300± 0.20	2.100± 0.37[b]
CPM	10	0.00	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.00

Data were analyzed using Friedman Test. a, b and b are significantly different at p□ 0.002, p□ 0.01 and p□ 0.02 respectively; n=5. CPM is Chlorpheniramine. ACPE is aqueous *Calotropis procera* extract, MCPE is methanolic *Calotropis procera* extract.

Table 4.7: Effects of Methanolic and Aqueous Root Extracts of *Calotropis procera* on Carrageenan Induced Rat Paw Edema.

Treatment	Dose (mg/kg)	% Change in Volume of Paw (mm)				
		1 h	2 h	3 h	4 h	5 h
Distilled water	2ml/kg	64.478 ± 2.95	104.333 ± 2.77	126.264 ± 3.02	99.156 ± 1.67	71.899 ± 4.38
ACPE	200	54.098 ± 7.30	78.779 ± 3.40[c]	114.627 ± 5.13[a]	76.701 ± 2.12[a]	57.056 ± 4.47
ACPE	400	34.498 ± 2.23[b]	39.246 ± 1.24	51.082 ± 2.71	39.114 ± 0.64	33.427 ± 1.37
MCPE	200	39.437 ± 1.26[c]	52.383 ± 1.94[b]	76.166 ± 0.53 [a]	63.975 ± 1.33[b]	52.915 ± 1.27[b]
MCPE	400	43.349 ± 0.83	48.540 ± 2.37	50.324 ± 2.90	44.620 ± 1.36	34.248 ± 1.41
Diclofenac sodium	20	33.861 ± 1.34	40.866 ± 0.69	60.089 ± 1.90[b]	45.592 ± 2.34	36.712 ± 0.80

Data were analysed by Repeated Measure ANOVA, a and b are significantly different at p□ 0.001and p□ 0.01respectively; n=5. ACPE = aqueous *Calotropis procera* extract, MCPE = methanolic *Calotropis procera* extract, mm = Millimeter.

4.4.5 Passive Paw Anaphylaxis in Rats

All treatment groups except ACPE 350 mg/kg resulted in a significant ($p < 0.05$) inhibition of anaphylactic inflammation compared with Dexamethasone (0.27 mg/kg) after five hours of drug administration (i. e. four hours after treatment with 10 mg egg albumin). Most significant ($p < 0.01$) inhibition of inflammation was observed with 250 mg/kg aqueous extract at four hours post albumin exposure (Table 4.8).

Table 4.8: Effects of Methanolic and Aqueous Root Extracts of *Calotropis procera*on Passive Paw Anaphylaxis in Rats

Treatment Dose (mg/kg)		Change in Paw Volume (mm)			
		1 h	2 h	3 h	4 h
D / Water	2 ml/kg	1.932 ± 0.29	1.974 ± 0.64[b]	2.068 ± 0.77	2.466 ± 0.21
Dexamethasone	0.27	1.840 ± 0.21	1.192 ± 0.26	1.056 ± 0.24[b]	0.764 ± 0.29[b]
ACPE	250	3.016 ± 0.09	1.848 ± 0.17[a]	1.630 ± 0.20[a]	1.262 ± 0.20[a]
ACPE	350	2.490 ± 0.30	2.040 ± 0.27	1.730 ± 0.12	1.310 ± 0.11
MCPE	250	1.800 ± 2.17	1.650 ± 0.26	1.448 ± 0.23	1.160 ± 0.15[b]
MCPE	350	1.800 ± 0.26	1.560 ± 0.20	1.280 ± 0.23	1.122 ± 0.23[b]

Data were analysed by Repeated Measure ANOVA. a and b are significantly different at p□ 0.01 and p□ 0.05 respectively; n=5.

CHAPTER FIVE

5.1 DISCUSSION

The oral LD_{50} was found to be above 5,000 mg/kg showing the possible safety of aqueous and methanol *Calotropis procera* extracts if used orally.

Syndrome of bronchial asthma is characterized by widespread narrowing of the bronchial tree due to contraction of the smooth muscles in response to multiple stimuli resulting in the release of chemical mediators such as histamine (Cabrera *et al*, 2008). Guinea pig ileum is used for screening antiasthmatic activity. The stimulation of H_1 receptors produces graded dose related contractions of isolated guinea pig ileum preparation (Pandit *et al*., 2008; Saraf and Patwardhan, 1998).

In the present study, significant (p 0.001) inhibition by aqueous and methanolic *Calotropis procera* extract (10 mg/ml) of contractions of the isolated guinea pig ileum induced by histamine depicts its H_1 receptor antagonistic activity. This confirms its bronchospasmolytic potential and therefore supports the anti-asthmatic property of the plant. Maximum protection was observed at 10 mg/ml for bronchi-relaxant study.

Most allergic and non-allergic asthmatics, including those with mild asthma, have bronchial eosinophilia and there is a significant association between eosinophil activation and asthma severity as well as bronchial hyper responsiveness (Horn and Robin, 1975). Therefore total leucocyte count was carried out for doses 100 and 200 mg/kg p.o. body weight (each for aqueous and methanolic axtracts).

Indeed, reviewing the forty four (44) new adaptogens from a phytochemical perspective was revealing. Isreal Brekhman, known as 'the father of adaptogens' had demonstrated that the adaptogens he identified contained saponins. Many of the Eclectic tonics also contained saponins (Panossian and Wikman, 2009). However, there were several other recurring compounds amongst the eclectic tonics including alkaloids, phenols, isoflavones, and tannins that are adaptogenic (Panossian and Wikman, 2009).

Significant ($p < 0.05$) protection against haloperidol induced catalepsy observed in methanolic extract group at doses 250 mg/kg and 350 mg/kg demonstrates its catecholamine antagonistic property and confirms its ability to protect against asthma.

Haloperidol induced catalepsy model of asthma is typical of a situation where one drug (e.g. adrenaline) antagonizes another (histamine) by producing the opposite physiological response.

Both type of drugs are agonists but acts at different receptors with different effector mechanisms, thus this seems likely eventually to converge at the final step involving effects on the intracellular concentration of calcium ions (Goodall *et al*., 1986; Lew 1995; Lew and Flanders 1999). Proof that *C. procera* extract protects against haloperidol induced catalepsy is evident by the propensity of only methanolic (and not the aqueous extract) to protect against the catalepsy, meaning methanolic extract (which is believed to contain lipid soluble phytoconstituents) is capable of penetrating the blood brain barrier to elicit the effect in question.

The same mechanism is believed to apply in the use of adrenaline as a drug of choice in anaphylactic shock. In this situation it acts as a physiological antagonist to the released

histamine i.e. it reverses all the main effects of histamine (vasodilation, bronchoconstriction) (Goodall *et al*., 1986; Lew 1995; Lew and Flanders, 1999).

Airway inflammation has been demonstrated in all forms of asthma. Even in mild asthma, there is an inflammatory response involving infiltration, particularly with activated eosinophils and lymphocytes, with neutrophils and mast cells. The degree of bronchial hyper responsiveness and airway obstruction is closely linked to the extent of inflammation (Bousquete *et al*., 2000).

Significant (p□ 0.05) inhibition of paw edema by all treatment groupsexcept MCPE 400 mg/kg, induced by carrageenan in anti-inflammatory studies which are comparable to standard diclofenac sodium (20 mg/kg P.O.) depicts the sayings of Anita and Babita, 2008 that anti- inflammatory drugs suppress the inflammatory response by inhibiting infiltration and activation of inflammatory cells as well as their synthesis, or release of mediators. Carrageenan induced rat paw edema model is known to be sensitive to cyclooxygenase inhibitors (Anita and Babita, 2008).

Since serotonin, histamine and prostaglandins are common mediators of both bronchial asthma and inflammation; the beneficial effects of both aqueous and methanolic extracts could be due to the inhibition of the release of these mediators, possibly due to the inhibition of the enzyme cyclooxygenase leading to prostaglandin inhibition.

In passive paw anaphylaxis (antiallergic model), subcutaneous administration of egg albumin to rats raises the anti-serum to egg albumin in the plasma and sub planter. Injection of plasma containing these antibodies then challenged with egg albumin leads

to passive anaphylaxis in rats (Pungle *et al*., 2003). Significant (p□ 0.05) inhibition of anaphylactic inflammation by both aqueous and methanolic extracts compared with the control group after five (5) hours of drug treatment (i.e. four hours after albumin administration) implies that the extract has the ability to significantly (p□ 0.05) reduce paw edema in animals pretreated with aqueous and methanolic *Calotropis procera* root extracts.

The protective effects of the extracts in reducing paw edema in passive paw anaphylaxis may be due to inhibition of antigen antibody reaction. The anti-anaphylactic and anti-allergic activity shown by *Calotropis procera* is probably due to the flavonoids present. The flavonoids have long been recognized to poses antiallergic, anti-inflammatory activities (Middleton and Kandaswani, 1993). One of the target pathways for flavonoids is effect on IgE mediated Hypersensitivity (Type I) as a result of inhibition of chemical mediator release and cytokine production by mast cells, basophils or T cells or due to Inhibition of signal transduction and gene expression in mast, basophils or T cells (Yuva *et al*., 2013).

Recently, the biphasic allergic reaction has become of interest because of the similarity to the clinical manifestation of chronic asthma. After challenge with relevant antigen, sensitized animals exhibit immediate responses, such as broncho-constriction of the airways, and late phase response, such as edema and mucous over production which usually persist over a six to twenty four (6-24) hour period (Charlesworth, 1994). Flavonoids are found to be active at both the phases of allergic response (Nagore *et al*., 2009).

The major limitations of the studies are the absence of highly sensitive and mechanized devices to undertake models like histamine induced bronchoconstriction guinea pigs and studies on guinea pig trachea.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The aqueous and methanolic root extracts of *Calotropis procera* was found to poses anti-asthmatic activities in *in vivo* and *in vitro* on animal models, thus support the folkloric use of the plant in inflammatory and allergic conditions including asthma. The high oral median lethal dose (greater than 5,000 mg/kg) results obtained for both aqueous and methanolic extracts of *C. procera* in wistar rats makes the plant roots relatively safe for use. Anti-asthmatic activity showed by *Calotopis procera* might be because of the chemical moieties it contains.

6.2 Recommendations

It is recommended that further research be carried out to determine:

1. The possibly pharmacologically active compound(s) responsible for the observed antiasthmatic activity
2. Sub-acute, sub-chronic and chronic toxicity studies should be carried out on the crude extract to determine the long term toxicity of the extract.
3. A more sensitive preliminary phytochemical screening method (e.g. Tin Layer Chromatography, Gas Liquid Chromatography, High Performance Liquid Chromatography e.tc.) should be used.

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