ANTI-INFLAMMATORY AND HEPATOPROTECTIVE EFFECTS OF THE HOMOGENATE OF *CUCUMIS SATIVUS* (CUCUMBER) FRUITS

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TITLE PAGE

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE (M.Sc) IN PHARMACOLOGICAL BIOCHEMISTRY, UNIVERSITY OF NIGERIA NSUKKA.

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DECEMBER, 2014

CERTIFICATION

Agatemor, Mark-maria Uzuazokaro, a Postgraduate student with Registration Number PG/M.Sc/13/66023 in the Department of Biochemistry has satisfactorily completed the requirements and research for Masterôs degree in pharmacological Biochemistry (M.Sc). The work embodied in this report is original and has not been submitted in part or full for any other diploma or degree of this or any other University.

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DEDICATION

This research work is dedicated to Hon C.O. Agatemor. May Perpetual Light Continue to shine on You, My Friend, Father and Mentor.

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Ineffable Creator, from the treasures of your wisdom, you have established three hierarchies of angels, have arrayed them in marvellous order above the fiery heavens, and have marshalled the regions of the universe with such artful skill, you are proclaimed the true font of light and wisdom, and the true origin raised high beyond all things. Pour forth a ray of your brightness into the darkened places of my mind; disperse from my soul the twofold darkness into which I was born: sin and ignorance. You make eloquent the tongues of infants: refine my speech and pour forth upon my lips the goodness of your blessing. Grant to me keenness of mind, capacity to remembering, skill in learning, subtlety in interpreting, and eloquence in speaking. May you guide the beginning of my work, direct its progress, and bring it to completion. You are true God and true Man, and you live and reign, world without end. Amen. ST. THOMAS AQUINAS, Pray for us.

To God who always and constantly gives joy to my youth, the supreme intelligent, the maker of the universe, the director of the movement of the cosmos and the supreme director of this research work, *"Deo Omnis Gloria".*

It is said, *a tree cannot make a forest, and the strength of a broom is in its numbers.*It is a statement of fact that a herculean task of this nature could not have been successfully accomplished without the contributions and support from different people. Therefore, I wish to express my profound indebtedness to all who in one way or the other contributed to the successful completion of this work.

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ABSTRACT

Research on inflammation has become the focus of global scientific study because of its implication in virtually all human and animal diseases. Also, liver diseases have been on increase and of global concern.*Cucumis sativus* is believed to have anti-oxidant activity, high flavonoid content, anti-inflammatory and analgesic effect, which may be likely of use in the management of these diseases. The anti-inflammatory and hepatoprotective effects of the homogenate of *Cucumis sativus* fruit were therefore studied. The fresh fruit of *Cucumis sativus* was homogenized and used for all experimental analysis without further dilution. Acute toxicity tests of the homogenate of *Cucumis sativus*fruit were carried out. The phytochemical analyses and proximate compositions of the fruit homogenate were carried out. 1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH) radical scavenging activity of the fruit homogenate was determined. The effects of the fruit homogenate on agar-induced paw oedema in rats were investigated. The effects of the fruit homogenate on liver function enzyme (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) activities, total bilirubin concentration and lipid profile (total cholesterol, high density lipoprotein, triacylglycerol and low density lipoprotein concentrations) in rats intoxicated with carbon tetrachloride (CCl_4) were evaluated using standard biochemical methods.The effects of the fruit homogenate on hypotonicity-induced haemolysis of RBC, phospholipase $A_2$and prostaglandin synthase activitieswere also studied. Data were analysed using SPSS and two-way ANOVA; the acceptance level of significance was p’ 0.05.The qualitative phytochemical tests on the homogenate of *Cucumis sativus* fruitrevealed the presence of flavonoids, alkaloids, terpenoids, glycosides, resins, steroids, saponins and tannins. The quantitative phytochemical analysis of the homogenate of*Cucumis sativus* fruit showed that,reducing sugars (574.36 ± 3.88 mg/g) was highest amount when compared to other phytochemicals, alkaloids (2.22 ± 0.96 mg/g) and flavonoids (2.14 ± 0.56 mg/g) were moderately present while cyanogenic glycoside (0.21 ± 0.13 mg/g) was the lowest in quantity.Proximate analysis showed that*Cucumis sativus* fruit contained the following - fibre (1.30 ± 0.01%), moisture (94.6 ± 0.08%), protein (3.11 ± 0.07%) and ash (1.07 ± 0.24%)contents. The acute toxicity test showed no toxicity up to 5ml/kg (ſ 5000mg/kg) body weight which indicated the possible safety of the fruit to the users. There was relative increase in the percentage inhibition of DPPH radical scavenging activity with increased amount of the homogenate. At doses of 2ml and 4ml/kg b.w., the fruit homogenate significantly (p ’ 0.05) inhibitedagar-induced raw paw oedema relative to control. Studies on membrane stabilization using hypotonicity-induced red blood cell haemolysis revealed that the fruit homogenate significantly (p’ 0.05)inhibited haemolysis when compared to indomethacin (a known standard drug).The homogenate exhibited a significant (p’ 0.05) dose (0.5ml and 1.0ml) related inhibition of prostaglandin synthase activity (79.9% and 81.0% respectively), compared to 0.4mg/ml of indomethacin, standard drug (82.0%). The fruit homogenate like prednisolone significantly (p’ 0.05) inhibited phospholipase A_2 activity.Treatment of rats with the homogenate of *Cucumis sativus* fruits significantly (p’ 0.05) decreased CCl_4-inducedelevated levels of the liver enzymes ALT, AST and ALP and of total bilirubin in the serum when compared to positive control. The homogenate also attenuated the CCl_4-induced elevation of LDL, total cholesterol and triacylglycerol amounts and ameliorated the induced depletion of HDL. The results indicated that the homogenate of *Cucumis sativus* fruits possesses anti-inflammatory activities and hepatoprotective effects.

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LIST OF ABBREVIATIONS

µg/ml =Microgramme per mililitre

ALP = Alkaline phosphatase

ALT = Alanine aminotransferase

ANOVA = Analysis of variance

AST = Aspartate aminotransferase

CCl_4 = Carbon tetrachloride

CHOL = Cholesterol

COX= Cyclooxygenase

HDL = High density lipoprotein

IF =Interferon

IL =Interleukin

IU =International units

LD_{50}= Median lethal dose

LDL = Low density lipoprotein

MAC = Membrane attack complex

mg/kg = Miligramme per kilogramme weight

mg/ml = Miligramme per mililitre

mPGEs1 = Microsomal prostaglandin E synthase 1

NSAIDs = Non-steroidal anti-inflammatory drugs

PG = Prostaglandins

PLA = Phospholipase A_2

RBC = Red blood cell

ROS = Reactive oxygen species

SPSS = Statistical product and service solutions

SRS-A = Slow reacting substances of anaphylaxis

T.Bil = Total bilirubin

TNF-Ŭ=Tumur necrosis factor alpha

TRIG = Triacylglycerol

CHAPTER ONE

INTRODUCTION

In most rural communities of developing countries, plant materials are sources of shelter, food and medicinal compounds (Oduola*et al.,* 2005). Herbal medicine is fast emerging as an alternative treatment to available synthetic drugs for the treatment of disease possibly due to lower cost, availability, fewer adverse effect and perceived effectiveness (Ubaka*et al.,* 2010). The World Health Organization (WHO) has shown great interest in plant derived medicines which have been described in the folklore medicines of many countries (Mukherjee, 2002). However, the historic role of medicinal plants in the treatment and prevention of diseases and their role as catalyst in the development of pharmacology do not assure their safety for uncontrolled use by an uninformed public (Matthew *et al.,* 1999). It is thus, imperative that plant products, which have been used from ages, have scientific support for their efficacy. Medicinal plants with anti-inflammatory activity are considerably employed in the treatment of several inflammatory disorders (Iwueke*et al.,* 2006). Research on inflammation has become the focus of global scientific study because of its implication in virtually all human and animal diseases. Many anti-inflammatory plants and agents modify inflammatory responses by accelerating the destruction or antagonizing the action of the mediators of inflammatory reaction (Anosike *et al.,* 2009). The inflammatory responses involve a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair. These different reactions in the inflammatory response cascade are therapeutic targets which anti-inflammatory agents including medicinal plants interfere with to suppress inflammatory responses usually invoked in such disorders as rheumatoid arthritis, osteoarthritis, in infection or injury (Abebe, 2002).

1.1 Inflammation

The process of inflammation is one of the most essential reactions of cells and tissues to injury among the key homeostatic mechanisms of higher organisms. Sanderson (1971) defined inflammation as the succession of changes which occur in a living tissue when it is injured. On the other hand, inflammation can be defined as a response of the tissue to an infection, irritation or foreign substance (Guyton, 1981). It is a part of the host's defence, but if the response becomes great, it may be worse than the disease state that elicited the response. In extreme cases, the response becomes fatal. As a defensive reaction, inflammation is useful to the body but, often leads to tissue damage. Though inflammation is a defence mechanism, the complex events and mediators involved in the inflammation reactions can induce, maintain or aggravate many diseases (Sosa *et al.,* 2002). Inflammation is part of our innate immunity. Our innate immunity is what is naturally present in our bodies when we are born, and not the adaptive immunity we get after an infection or vaccination. Innate immunity is generally non-specific, while adaptive immunity is specific to one pathogen. Inflammation is a mechanism of innate immunity. The body immune system (defence system) triggers an inflammatory response in autoimmune diseases, when there are no foreign substances to fight off, causing damage to its own tissues (Coussens and Werb, 2002). Inflammation gets rid of any irritant either by flushing out or diluting the irritant with the fluid produced. Inflammation also takes care of irritants produced by antibody of some of the infiltrating cells and finally by phagocytosis. It rids the body of foreign matter, disposes damaged cells and initiates wound healing.

1.2 Classification of inflammation

Inflammatory reaction can be classified based on the type of exudates produced by the body. These are serous type, in which the serous fluid produced is used to flush out the invading irritant; mucus type, in which watery slimy fluid produced is to get rid of the invading irritant and fibrinous type, where the exudates contain fibrin that cover the area being irritated. Also, haemorrhagic inflammation exists, when there is production of bloody fluid to fight the irritant. Others are purulent and proliferative types that are characterized by formation of pus and connective tissues respectively.

On the other hand, it could be classified as either acute or chronic, depending on the type and duration of the antigen challenge and is mediated by some chemical substances such as histamine, serotonin, slow reacting substances of anaphylaxis (SRS-A), prostaglandins and some plasma enzyme systems such as the complement system, the clotting system, the fibronolytic system and kinin system.

1.2.1 Acute inflammation

Acute inflammation is usually of sudden onset, and characterized by the classical signs in which the vascular and exudative processes predominate (Dorland, 1982). It is the initial response of the body to harmful stimuli produced by the infiltration of plasma and leukocytes from the blood into an injured tissue. Histologically, acute inflammation is characterized by a complex series of events which include: vasodilation of the blood vessels leading to excess local blood flow, increased capillary permeability leading to leakage of fluids and blood into the interstitial space, clotting of the fluids in the interstitial space because of excess fibrinogen leaking from the capillaries, leukocyte migration (granulocytes and monocytes) into the injured area and swelling of the tissue cells (Ferrero-Miliani*et al.,* 2007). As long as the injurious stimulus is present, acute inflammation occurs and ceases once the stimulus has been removed, broken down, or walled off by scarring (fibrosis). Three main processes occur before and during acute inflammation; Dilation of arterioles,increased permeability of the capillariesand migration of neutrophils, and possibly some macrophages out of the capillaries and venules.When the skin is scratched (and is not broken), one may see a pale red line. Soon the scratched area goes red; this is because the arterioles have dilated and the capillaries have filled up with blood and become more permeable, allowing fluid and blood proteins to move into the space between tissues.This is further explained in figure 1 below.

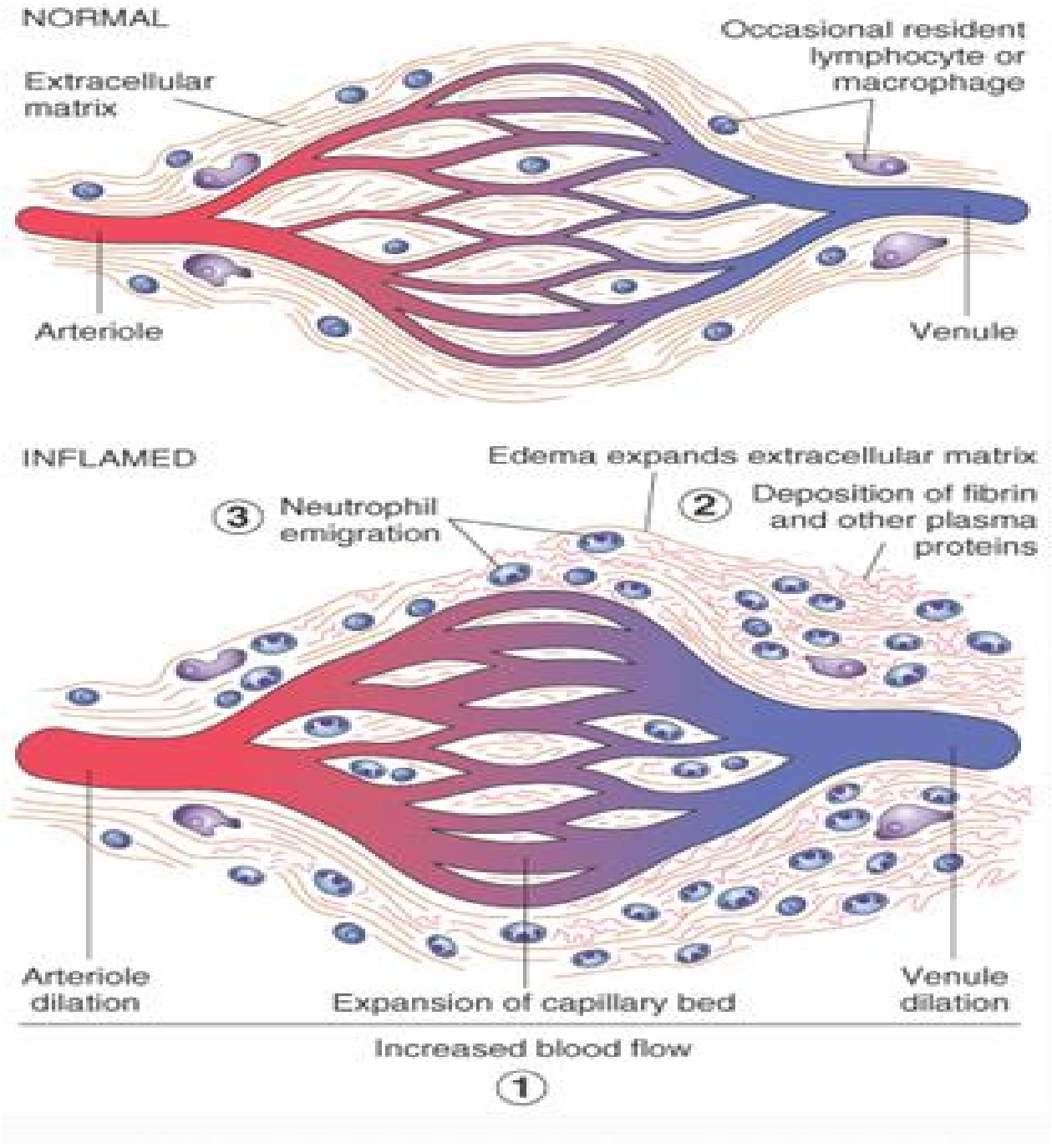

Figure 1: Overview of vascular changes in acute inflammation

Source:(Levison*et al*.,2008)

Acute inflammation is characterized by five cardinal signs ï ñPRISHò, that is;

Pain - The inflamed area is likely to be painful, especially when touched. Chemicals that stimulate nerve endings are released, making the area much more sensitive.

Redness - This is because the capillaries are filled up with more blood than usual.

Immobility - There may be some loss of function.

Swelling - Caused by an accumulation of fluid.

Heat - more blood in the affected area makes it feel hot to the touch.

These signs develop as an acute response to a local inflammatory insult by the action of inflammatory mediators (Garrison, 2000). The inflammatory insults may be caused mechanically, e.g. by the presence of foreign bodies, or chemically, e.g. by toxin, acidity and alkalinity, or physically, e.g. by temperature, or by internal processes, e.g. uraemia, or by microorganisms, e.g. bacteria, viruses and parasites. The pain is often attributed to increased pressure on the nerve endings, the irritating effects of toxic products and the action of certain mediators of the inflammatory process. The redness is caused by an increase of blood volume in the inflamed area; the swelling is due to increase of blood and additional presence of substances which exude from the blood vessels (exudates) into the surrounding tissues. The heat results from the increased flow of blood. These five acute inflammation signs are only relevant when the affected area is on or very close to the skin. When inflammation occurs deep inside the body, such as an internal organ, only some of the signs may be detectable. Some internal organs may not have sensory nerve endings nearby, so there may be no pain, as is the case with some types of pneumonia(acute inflammation of the lung). If the inflammation from pneumonia pushes against the parietal pleura (inner lining of the surface of the chest wall), then there is pain. The desirable outcome of acute inflammation process, which at least initially is protective and homeostatic, is the isolation and destruction of the injurious agent and resolution of the inflammatory lesion so that normal tissue conditions are fully restored. If however, the challenging stimulus persists, the inflammation may become chronic and the micro-circulating changes characteristic of acute inflammation is replaced by lesions typical of the chronic-disease (Serhan, 2008). This is further explained in figure 2 below.

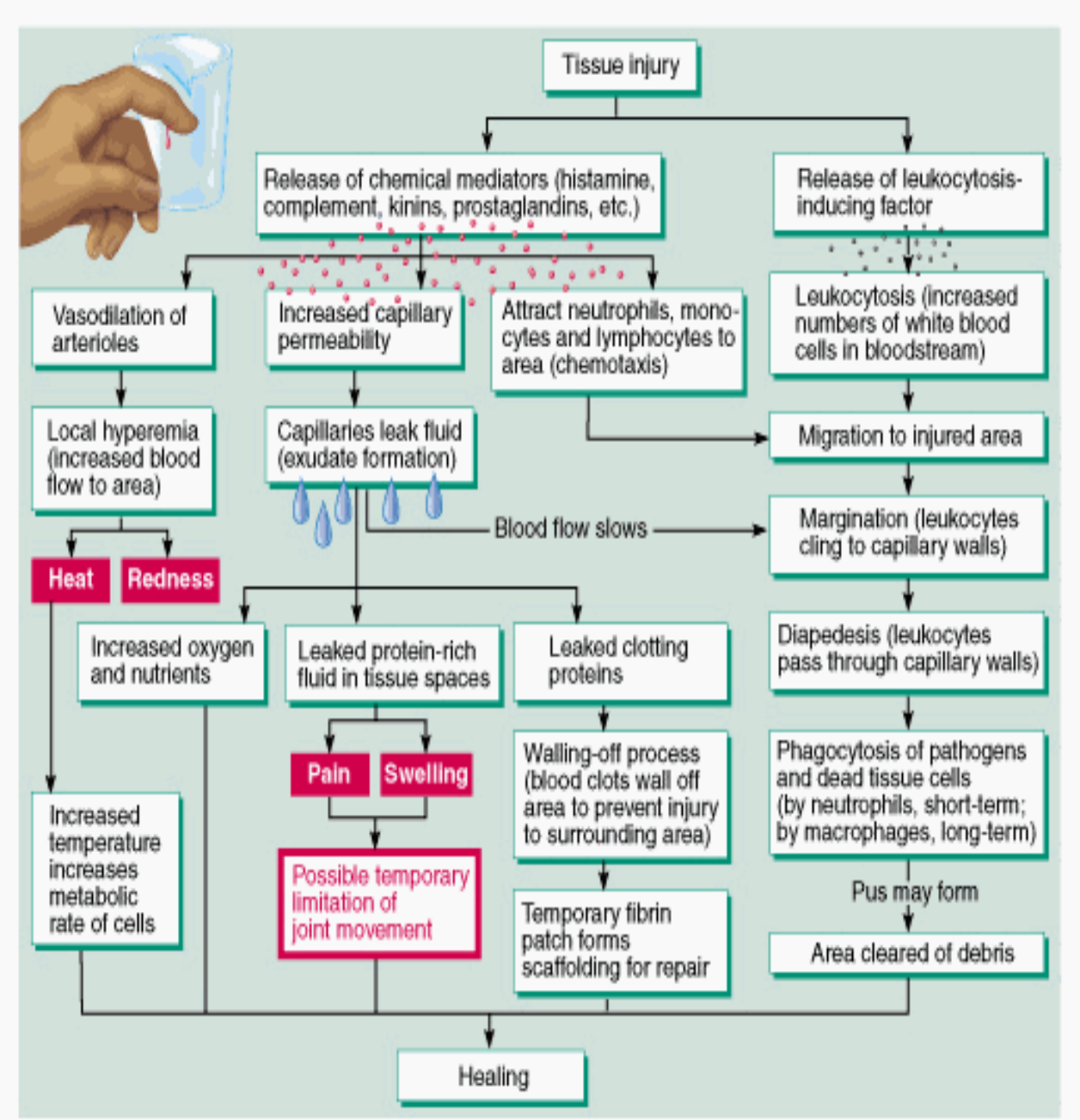

Figure 2: Schematic representation of inflammatory action

Source: (Marieb and Mitchell, 2007)

1.2.2 Chronic inflammation

Chronic inflammation is of slow progress and is marked chiefly by the formation of connective tissues. It may be a continuation of an acute form or a prolonged low grade form and usually causes permanent tissue damage (Dorland, 1982). It is characterized by simultaneous active inflammation, tissue damage and attempts at healing (repair) of the tissues from the inflammatory process (Eming*et al.,* 2007). Chronically inflamed tissue is characterized by infiltration of mononuclear immune cells (monocytes, macrophages, lymphocytes and plasma cells) tissue destruction and attempts at healing which include angiogenesis and fibrosis. These mononuclear immune cells are powerful defensive agents in the body, but the toxins they release (including reactive oxygen species) are injurious to the organismôs own tissues as well as to the invading agents. Consequently, chronic inflammation is almost always accompanied by tissue damage (Insel, 1996).

1.3 Inflammatory responses

1.3.1 Acute vascular response

Acute vascular response is the earliest gross event of response to injury by a transient arteriolar vasoconstriction, the narrowing of the blood vessels, which is caused by contraction of the smooth muscles of the blood vessel walls. It is seen on the skin as a transient blanching, the whitening of the skin. Acute vascular response which is a characteristic of acute inflammation, also includes vasodilation, increased permeability and increased blood flow, all of which are induced by the actions of various inflammatory mediators. Vasodilation occurs first at the arteriolelevel, progressing to the capillary level, and brings about a net increase in the amount of blood present, causing the redness and heat of inflammation.

Increased permeability of the vessels results in the movement of plasma into the tissues, with resultant stasisdue to the increase in the concentration of the cells within blood - a condition characterized by enlarged vessels packed with cells. Stasis allows leukocytesto marginate (move) along the endothelium, a process critical to their recruitment into the tissues. The movement of plasma fluid, containing important proteinssuch as fibrin and immunoglobulins (antibodies), into

inflamed tissue, is achieved via the chemically induced dilation and increased permeability of blood vessels, which result in a net loss of blood plasma. The increased collection of fluid into the tissue causes it to swell (oedema). This extravasated fluid is funnelled by lymphaticsto the regional lymph nodes, flushing bacteria along to start the recognition and attack phase of theadaptive immune system. Normal flowing blood prevents this, as the shearing forcealong the periphery of the vessels moves cells in the blood into the middle of the vessel. Acute vascular response follows within seconds of tissue injury and lasts for some minutes (Stvrtinova*et al.,* 1995).

1.3.2 Acute cellular response

Acute cellular response occurs in inflammation if there has been sufficient damage to the tissues or if infection has occurred. It takes place over the next few hours. During acute cellular response, inflammatory cells (mainly neutrophils) are normally contained in the central or axial part of the blood volume, thereby appearing in the tissues and attaching to the endothelial cells within the blood vessels, then crossing over into the surrounding tissue (diapedesis). The process is facilitated by the stasis of the blood. As a result of stasis, the red and white blood cells tend to come together. In this response, erythrocytes may leak into the tissues and haemorrhage can occur (e.g. a blood blister). If the blood vessel is damaged, fibrinogen and fibronectin are deposited at the site of injury, platelets aggregate and become activated, and the red cells stack together in what is called ñrouleauò to help stop bleeding and aid clot formation. The dead and dying cells contribute to pus formation (Stvrtinova*et al.,* 1995).

1.3.3 Chronic cellular response

When the tissue damage is severe and occurs over few days, chronic cellular response may follow. In it, mononuclear cell infiltrate, composed of macrophages and lymphocytes. The macrophages involved are activated by several cytokines, including IL-2 (interleukin-2), and their migration is stimulated by components of the extracellular matrix (i.e. collagen, elastin, fibronectin), TGF-ϐ, and complement cascade products. They are the most important cells present in the later stages of the inflammatory process (48-72 hours), acting as the key regulatory cells for healing and repair. Their subsequent production of inflammatory cytokines (IL-1 and

TNF [tumour necrosis factor]) and growth factors (mainly TGF-ɓ and PDGF) appears to be the most critical cell-driven event of the entire phase of inflammation. Releasing these products into the wound recruits fibroblasts, keratinocytes and endothelial cells to repair the damaged blood vessels. The absence of macrophages is associated with failure to progress to normal fibroblast recruitment and function.

Macrophages are also capable of releasing proteolytic enzymes (e.g. collagenase) that can debride tissue and extracellular matrix. Apart from wound debridement, other important functions of these cells include nitric oxide synthesis, phagocytosis of bacteria, and stimulation of angiogenesis (Doherty *et al.,* 2002). Additional growth factors such as TGF-Ů, HB-EGF (heparin-binding EGF), and bFGF (basic fibroblast growth factor), secreted by both PMNs and macrophages, may further stimulate the inflammatory response. On the other hand, the depletion of circulating monocytes and tissue macrophages can cause severe changes in wound healing, leading to poor wound debridement, delayed fibroblast proliferation, inadequate angiogenesis and poor fibrosis (Enoch and Price, 2004).The last cell type to enter the wound during the inflammatory phase (>72 hours after injury) is the lymphocyte. Lymphocytes may be attracted by IL-1, IgG and complement products. Since IL-1 is believed to play a key role in the regulation of collagenase, lymphocytes may be involved in collagen and extracellular matrix remodelling (Sedgwick *et al.,* 1981).

1.3.4 Resolution

Inflammation is often considered in terms of acute, inflammation involving all acute vascular and acute cellular response, and chronic inflammation, involving all the events of chronic cellular response and resolution or scarring. Resolution, that is restoration of normal tissue architecture may occur over the following few weeks after a tissue injury. It involves the removal of blood clots by fibrinolysis and if the tissue cannot be returned to its original form, it will result to scarring from in-filling with fibroblasts, collagen and new endothelial cells. Any infectious agent that was not completely destroyed and removed from the site of injury, would be walled off from the surrounding tissues in granulomatous tissue (Serhan and Savil, 2005). Resolution of inflammation occurs by different mechanisms in different tissues (Eming*et al.,* 2007). Mechanisms which serve to terminate inflammation include;short half-life of inflammatory

mediators *in-vivo*, production and release of transforming growth factor (TGF) beta from macrophages (Ashcroft, 1999) and production and release of interleukin 10 (IL-10) (Sato *et al.,* 1999). Serhan, 2008 reported production of anti-inflammatory lipoxins as a mechanism of termination of inflammationOther mechanisms include; down regulation of pro-inflammatory molecules, such as leukotrienes, up regulation of anti-inflammatory molecules such as the interleukin 1 receptor antagonist or the soluble tumour necrosis factor receptor (TNFR), apoptosis of pro-inflammatory cells, desensitization of receptors (Greenhalgh, 1998), production of resolvins, protectins or maresins, down regulation of receptor activity by high concentrations of ligands, increased survival of cells in regions of inflammation due to their interaction with the extracellular matrix (ECM) (Tender *et al*., 2002; Jiang *et al*., 2005). Cleavage of chemokines by matrix metalloproteinase (MMPs) might lead to production of anti-inflammatory factors (McQuibban*et al.,* 2000).

Acute inflammation normally resolves by mechanisms that have remained somewhat elusive. Emerging evidence now suggests that an active, coordinated programme of resolution initiates in the first few hours after an inflammatory response begins. After entering tissues, granulocytes promote the switch of arachidonic acidï derived prostaglandins and leukotrienes to lipoxins, which initiate the termination sequence. Neutrophil recruitment thus ceases and programmed cell death by apoptosis is engaged. These events coincide with the biosynthesis, from omega-3 polyunsaturated fatty acids, of resolvins and protectins, which critically shorten the period of neutrophil infiltration by initiating apoptosis. Consequently, apoptotic neutrophils undergo phagocytosis by macrophages, leading to neutrophil clearance and release of anti-inflammatory and reparative cytokines as transforming growth factor-ϐ1. The anti-inflammatory programme ends with the departure of macrophages through the lymphatics (Serhan and Savil, 2005).

1.4 Inflammatory cells

The different numbers of cells responsible for inactivation and removal of invading infectious agents and damaged tissues are recruited into area where there is a tissue damage, and they differ depending on the phase of the inflammation (which is mostly second and third phase), the type of inflamed tissue and factors triggering the inflammatory process (Anosike, 2010). In acute inflammation, neutrophils are the main cells involved. A pyrogenic bacterial infection occurs and

local depositions of immune complexes containing IgG are the cause of inflammation, neutrophils are the dominant cells (Wagner and Rothz, 2000). Fehervari*et al*.(2005) reported that the mononuclear phagocytes are the main infiltrating cells in sub-acute and chronic phase of inflammatory reactions and in cases of infection with intracellular parasitic microorganisms, and the eosinophils and basophils are predominant when inflammation is triggered by immediate allergic reactions or parasites. Lymphocytes are involved in specific immune responses and endothelial cells function in the regulation of leukocyte emigration from the blood into the inflamed tissue while platelets and mast cells are involved in the production of early phase mediators (Stvrtinova*et al.,* 1995).

1.5Oxidative damage in inflammation

During inflammation, free radicals are produced by neutrophils, phagocytes, macrophages, endothelial and other cells. Upon activation, neutrophils and mononuclear phagocytes have increased oxygen consumption, during which they release lysozyme and reactive oxygen species (ROS). These reactive oxygen species cause oxidative damage to biological tissues and are implicated in the development of inflammatory and other chronic disease conditions such as atherosclerosis, stroke and even ageing (Halliwell*et al.,* 1992). ROS include both oxygen free radical (superoxide radical (O_2), hydroxyl radical (OH), alkoxy radical (RO), peroxyl radical (ROO), hydroperoxyl radical (HOO) and oxygen non-radical that are reactive (hydrogen peroxide H_2O_2), hypochlorous acid (HOCl), ozone (O_3) and singlet oxygen (O_2) (Babu,*et al*., 2002). Upon activation of the respiratory burst, oxygen is univalently reduced by NADPH oxidase to superoxide anion, which is then catalytically converted by the action of superoxide dismutase to hydrogen peroxide. Hydrogen peroxide interacts with myeloperoxidase (MPO) contained in neutrophils azurophil granules to produce hypochlorous acid which is metabolised to hypochlorate and chlorine. Hydroxyl radical and hypochlorate are the most powerful substances involved in microbiocidal and cytotoxic reactions.

1.6 Antioxidants

Antioxidants are complex and diverse group of molecules that protect key biological sites from free-radical induced oxidative damage. They are molecules capable of showing or preventing the

oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radicals intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents. They can act by removing: oxygen or decreasing local oxygen concentration, catalytic metal ions, key ROS such as superoxide radical and $H_2O_2$and by breaking the chain of initiated free radical sequence (Sies, 1997). Antioxidants are classified into two broad divisions: Water soluble antioxidants which react with oxidants in the cell cytoplasm and lipid soluble antioxidants which protect cell membranes from lipid peroxidation (Sies, 1997). Antioxidants have anti-inflammatory properties. By scavenging radicals, they reduce inflammatory signals, thus reducing inflammation.

1.7 Inflammatory disorders

Inflammatory abnormalities are a large group of disorders which underlie a vast variety of human diseases. The immune system is often involved in inflammatory disorders, demonstrated in both allergic reactions and some myopathies, with many immune system disorders resulting in abnormal inflammation. Non-immune diseases with aetiological origins in inflammatory processes include cancer, atherosclerosis, and ischaemic heart disease (Contran*et al.,* 1999).A large variety of proteins are involved in inflammation, and any one of them is open to a genetic mutation which impairs the normal function and expression of that protein. Examples of disorders associated with inflammation include: acne vulgaris, asthma, autoimmune diseases, coeliac disease, chronic prostatitis, glomerulonephritis, hypersensitivities, inflammatory bowel diseases, pelvic inflammatory disease, reperfusion injury, rheumatoid arthritis, sarcoidosis, transplant rejection, vasculitis and interstitial cystitis.

1.8 Anti-inflammatory agents

Anti-inflammatory agents are compounds which act by several mechanisms to inhibit the various changes leading to inflammation. They modify inflammatory responses by accelerating the destruction or antagonizing the action of the mediators of the inflammatory reaction (Anosike *et al.,* 2009). They are divided into steroidal and non-steroidal anti-inflammatory agents. Steroidal

anti-inflammatory drugs are the glucocorticoids, they bind to cortisol receptors; they are called corticosteroids. Glucocorticoids suppress the expression of cyclooxygenase (COX-2), and thus prostaglandin production. This contributes to its anti-inflammatory effect. Examples of glucocorticoids includecortisone, cortisol, corticosterone, hydrocortisone, triamcinolone,betamethasone prednisone and prednisolone. Non-steroidal anti-inflammatory drugs which include aspirin, ibuprofen, diclofenac, and indomethacin are used primarily for the treatment of inflammatory diseases such as rheumatoid arthritis, pain and fever. They may act via single or combination of any of the mechanisms involving; inhibition of arachidonic acid metabolism, inhibition of cyclooxygenase (COX)/Inhibition of prostaglandin synthesis, inhibition of lipoxygenase (LOX), inhibition of cytokines (IL, TNF), inhibition of leukocyte migration/phagocytosis, uncoupling oxidative phosphorylation, release of steroidal hormone from the adrenals and stabilization of lysosomal membrane (Wallace, 2002)

1.8.1 Stabilization of lysosomal membrane

During inflammation, the lysosomes lyse to release their component enzymes which produce a variety of disorders. Since human red blood cell membranes are similar to lysosomal membranes (Goodman *et al*., 1982; Gandhisan*et al*., 1991), human red blood cell membrane stabilization has, therefore been used as a method to study the mechanism of action of anti-inflammatory drugs (Seeman, 1968; Murugesh*et al*., 1981). Stabilization of lysosomal membranes is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extracellular release (Chou, 1997). Some NSAIDs like indomethacin and acetylsalicylic acid are known to possess membrane stabilization properties (Murugesh*et al*., 1981; Furst and Munster, 2001) which may contribute to the potency of their anti-inflammatory effect.

Hypotonicity-induced haemolysis of red blood cells occurs due to osmotically coupled water uptake by the cells, and leads to swelling and lysis, resulting in the release of haemoglobin, hencehaemolysis. Haemolysis is a reflection of the stability of red blood cell membrane (Iwueke*et al*., 2006).The vitality of cells depends on the integrity of their membranes (Weissman, 1967).Feirrali*et al.* (1992)reported that the exposure of red blood cell to injurious

substances such as hypotonic medium, heat, methyl salicylate and phenyl hydrazine results in lysis of its membrane accompanied by haemolysis and oxidation of haemoglobin. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Such injury to RBC membrane will further render the cell more susceptible to secondary damage through free radical-induced lipid peroxidation. This notion is consistent with the observation that the breakdown of biomolecules lead to the formation of free radicals which in turn enhance cellular damage (Maxwell, 1995; Halliwell and Whiteman, 2004). The progression of bone destruction seen in rheumatoid patient for example, has been shown to be due to increased free radical activity (Pattison *et al*., 2004). It is therefore expected that compounds with membrane-stabilizing properties, should offer significant protection of cell membrane against injurious substances (Perenz*et al*., 1995; Shinde*et al*., 1999).

Compounds with membrane-stabilizing properties are well known for their interference with the early phase of inflammation reactions, namely the prevention of the release of Phospholipases that trigger the formation of inflammatory mediators (Aitadafoun*et al*., 1996). The stabilization of the red blood cell membrane prevents the release of lytic enzymes and active mediators of inflammation, such as 5-hydroxytrptamine, histamine and kinins (Phillips and Morrison, 1970).

1.8.2 Phospholipase A_2

The major structural feature of cell membrane is the lipid bilayer. The lysosomal enzyme, phospholipase A_2hydrolyses saturated or unsaturated lecithin dispersed as liposomes (Lewis *et al.,* 1979). Phospholipase A_2 is an enzyme that releases fatty acids from the second carbon group of glycerol. This particular phospholipase specifically recognizes the Sn-2 acyl bond of phospholipids and catalytically hydrolyses the bond releasing arachidonic acid and lysophospholipids. Lysophospholipids are powerful detergents that disrupt cell membranes, thereby lysing cells. Upon downstream modification by cyclooxygenases (Cox) ï (an enzyme that is responsible for the formation of prostanoids. The three main groups of prostanoids - prostaglandins,prostacyclins, and thromboxanes are involved in the inflammatory response). Arachidonic acid is modified into active compounds called eicosanoids. Eicosanoids include prostaglandins and leukotrines, which are categorized as inflammatory mediators (Dennis, 1994). In other words, the major action of the phospholipase A_2, an acyl hydrolase, during inflammation is to cleave from membrane phospholipids free fatty acids some of which are necessary precursors of prostaglandins. The activity renders the membrane leaky and the contents of the red blood cells flow out.

1.8.3 Prostaglandin synthase/Cyclooxygenase

Prostaglandins are important lipid mediators derived from arachidonic acid that control not only numerous physiological events such as blood pressure, blood clotting and sleep but also inflammation (Funk, 2001). Prostaglandin E_2 is a key player in pyresis, pain and inflammatory responses and the beneficial therapeutic effects of non-steroidal anti-inflammatory drugs (NSAIDs) are essentially attributed to the suppression of prostaglandins E_2 (Funk, 2001). The biosynthetic pathway to prostaglandin E_2 includes the release of arachidonic acid from membrane phospholipids by phospholipases A_2 followed by conversion via Cox-1 and -2 to prostaglandins H_2 and its subsequent isomerization by prostaglandins E_2 synthases (PGEs). $mPGEs_1$is induced by pro-inflammatory stimuli such as interleukin-1ɓ (IL-1ɓ) or lipopolysaccharide (LPS), and receives PGH_2 preferentially from Cox-2 (Murakami *et al.,* 2002). Thus inflammation, pain, fever and different types of cancer are closely linked to the increased prostaglandin E_2 formation originating from up-regulated $MPGEs_1$ (Samuelsson *et al*., 2007).

Prostaglandin E_2 is produced in small quantity under normal physiological conditions but in large quantity during inflammation (Girloy*et al*., 1999). The substantial increase in prostaglandins E_2 in inflammation is attributable to expression of Cox-2. Its (Prostaglandin E_2) implication in the majority of inflammatory reactions including pain, increased capillary permeability, vascular dilation and recruitment of inflammatory cells. Their ability to increase vascular permeability in man and animals and their ability to cause leucocyte emigration (Crook *et al*., 1976), suggest that they are important mediators of the acute phase of the inflammatory reaction. The reversal of the inflammatory reactions is caused by the inhibition of the biosynthesis by anti-inflammatory agents, the ability to inhibit the biosynthesis of prostaglandins E_2 underlie suppression of pain (analgesic), reversal of vasodilation, decreased capillary permeability and inhibition of migration of inflammatory cells. (Aba and Mensah-Attipoe, 2008). In addition to their involvement in the inflammatory response, prostaglandins sensitize the skin to painful stimuli probably because they sensitize pain receptors to mechanical and chemical stimulations (Roberts and Morrow, 2001) such as the pain-producing effect of mediators (e.g. histamine, kinins etc.) which are released in tissue injury and inflammation.

Prostaglandins manifested during strong physiological effects include regulating the contraction and relaxation of smooth muscle tissue (Nelson and Randy, 2005). Produced by almost all nucleated cells, prostaglandins are autocrine and paracrine lipid mediators that act upon platelets, endothelium, uterine and mast cells, synthesized in the cell from essential fatty acids.Prostaglandins level are increased by cox-2 in scenario of inflammation.

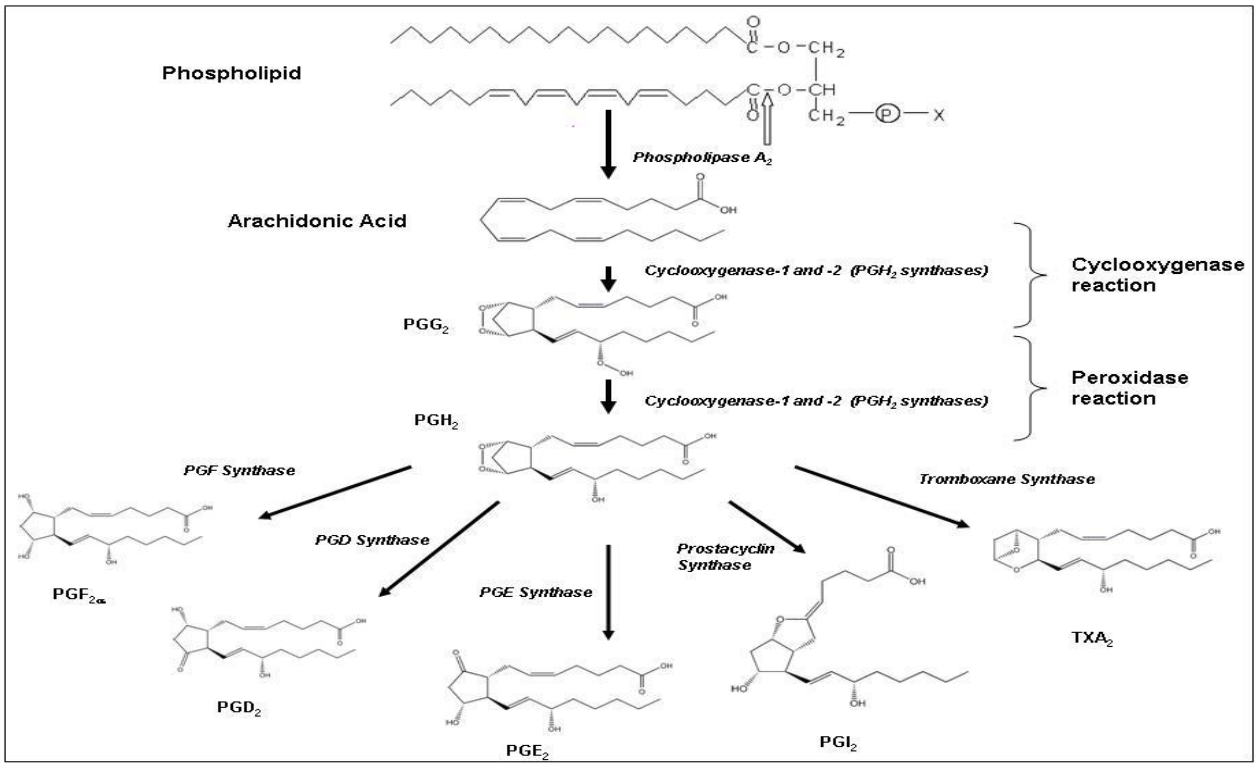

Figure 3: Synthesis of prostaglandins

Source: (Koch *et al*., 2002)

The production of prostaglandins begins with the formation of a cyclopentane ring in the linear fatty acid, as catalysed by prostaglandin H_2 synthase. The heme-containing enzyme (Commonly called COX, not to be confused with cytochrome c oxidase, which is also called COX) contains two catalytic activities: a cyclooxygenase that adds two molecules of O_2 to arachidonate, and a peroxidase that converts the resulting hydroperoxy group to an OH group.

Some of the major uses of prostaglandins include; induction of labour, regulation of calcium movement, control of hormone regulation, control of cell growth, decreasing intraocular pressure. Also, they act on thermoregularity centre of the hypothalamus to produce fever and on mesangial cells in the glomerulus of the kidney to increase glomerular filtration rate and on the pariental cells in the stomach wall to inhibit acid secretion, so as to maintain the integrity of the gastric lining of the stomach. They also sensitize spinal neurons to pains.

Inhibition of biosynthesis of prostaglandins and enzyme – cyclooxygenase

Cyclooxygenase (COX) is the pivotal enzyme in prostaglandin biosynthesis. It exists in two isoforms; constitutive COX-1 which is responsible for physiological functions and makes prostaglandins that protect the stomach and kidney from damage and inducible COX-2 which is involved in inflammation, inducing inflammatory stimuli such as cytokines and produces prostaglandins that contribute to the pain and swelling of inflammation. COX-2 is thought to be involved in ovulation and labour.

Inhibition of COX explains both the therapeutic effects (Inhibition of COX-2) and side effects (Inhibition of COX-1 of Non-Steroidal Anti-Inflammatory Drugs, NSAIDs) (Vane and Botting, 1996). It is important to note, that non-steroidal anti-inflammatory drugs which selectively inhibit COX-2 arelikely to retain maximal anti-inflammatory efficacy combined with less toxicity. The two isoforms (COX-1 and COX-2) share a high degree (60%) of sequence identity and structural homology (Voet *et al.,* 2013).

1.9Anti-inflammatory plants

The practice of traditional medicine is as old as the origin of man. The use of plants in traditional medicine referred to as herbalism or simply botanical medicine (Edeoga*et al*., 2005) falls outside the mainstream of the Western or orthodox medicine. In the field of ethnomedicinal plants or plants used as anti-inflammatory agents, a lot of information is available. Bagul*et al*.(2005) reported the anti-inflammatory activity of two Ayurvedic formulations. Bahattacharya*et al*.(2005) reported the anti-inflammatory potential of methanol extract of *Stepeniaglabra*of Menispermaceae family. Ammar*et al*.(1997) reported anti-inflammatory activity of bioactive fractions isolated from seeds of *Trigonellafoenumgracium*L., roots of *Glycyrhizaglabra*L. and fruits of *Coriandrumsativum*L. The anti-inflammatory and anti-ulcerogenic activity of ethanol extract of *Zingiberofficinale*was demonstrated by Anosike *et al*. (2009). Iwueke*et al.* (2006) demonstrated the anti-inflammatory activity of Vitexdoniana leaves, as well as their mechanism of action.The phytochemical analysis of the extract (Vitexdoniana) revealed the presence of flavonoids, glycosides, tannins and saponins. Some isolated flavonoids (quercetin, wogonin, nevadensin, and quercetinpentamethyl ether) possess strong anti-inflammatory activities (Reinhart, 1955). Biflavonoids in particular show advantages over certain classical non-steroidal anti-inflammatory drugs. Such advantages include high margin of safety and least ulcerogenicity (Rageeb and Barhate, 2011). This implies that flavonoids with non-

steroidal anti-inflammatory activity might decrease the risk of gastrointestinal damage (Palmer and Gosh, 1981).

The anti -inflammatory activity of the bioflavonoids of *Gareinia kola* have been demonstrated by Igboko (1987). Others are Azadirachtaindica (Winter *et al*., 1963; Okpanyi and Ezeukwu, 1981), Dashanasamskarachurna (Peiris*et al*., 2011) Cissusquadrangularis (Priyanka and Rekha, 2010). There is increased advocacy for the consumption of anti-inflammatory foods including fruits such as cucumber, vegetables, certain nuts for example coconut and some spices like onion (Hyman and Mark, 2006). Reports have shown that these plants have high chemical and nutrient profile such as vitamins, fats, oil, alkaloids, retinoids, bioflavonoids, tannins, saponins, and antioxidants some of which possess anti-inflammatory activity (Hyman and Mark, 2006).

There is evidence for both oxygen-centred free radical and products of complement activation acting as mediators of inflammation, and the generation and reaction of free radicals at sites of inflammation in several inflammatory conditions (Arora *et al.,* 2000). Antioxidants in these plants scavenge these free radicals and thus exert anti-inflammatory properties. The antioxidant chemicals found in many fruits and vegetables are the main benefits of high intake of these foods in the diet. These antioxidants scavenge excess free radicals produced during inflammation and also prevent the free radicals from oxidizing sensitive biological molecules and thus reduce the incidence of diseases (Cho *et al.,* 2005).

1.10Phytochemistry

Phytochemistry is the study of phytochemicals. Phytochemicals are secondary metabolites produced by plants.They occur in various parts of a plant. Their functions are diverse and include provision of strength to plants, attraction of insects for pollination and feeding, while some are simply waste products (Ibegbulem*et al.,* 2003).They give plants colour, flavour, smell and are part of a plantôs natural defence system (Agatemor *et al.,* 2009; Ejele and Akujobi, 2011). These compounds have been linked to human health by contributingto protection against degenerative diseases (Dandjesso*et al.*, 2012). Phytochemicals are present in varieties of plants utilized as important components of both human and animal diets. These include fruits, seeds, herbs and vegetables (Okwu, 2005). Different mechanisms have been suggested for the action of phytochemicals. They may act as antioxidants, or modulate gene expression and signal

transduction pathways (Dandjesso*et al.*, 2012). They may be used as chemotherapeutic or chemopreventive agents (Paolo *et al*., 1991).

Phytochemicals are formed during the plant normal metabolic processes. These chemicals are often referred to as ñsecondary metabolitiesò of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids (Harborne, 1973; Okwu, 2005). Phytochemicals are naturally occurring and are believed to be effective in combating or preventing disease due to their antioxidant properties (Ejele*et al*., 2012). The medicinal values of these plants lie in their constituent phytochemicals, which produce the definite physiological actions on human body. The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds (Iwu, 2000).Some of these naturally occurring phytochemicals are anti-carcinogenic and some others possess other beneficial properties, and are referred to as chemopreventers. Among the most investigated chemopreventers are some vitamins, plant polyphenols, and pigments such as carotenoids, chlorophylls, flavonoids, and betalains (Ejele*et al*., 2012).

1.10.1 Tannins

Tannins are an exceptional group of water soluble phenolic metabolites of relatively high molecular weight and having the ability to complex strongly with carbohydrates and proteins (Heldt and Heldt, 2005). Tannins are astringent, bitter plant polyphenols and the astringency from tannins is what causes the dry and pucker feeling in the mouth following the consumption of unripened fruit or red wine (Serafini*et al*., 1994). They are grouped into two forms, hydrolysable and condensed tannins (Nityanand, 1997). Hydrolysable tannins are potentially toxic and cause poisoning if large amounts of tannin-containing plant materials such as leaves of oak (Quercus*spp*.) and yellow wood (*Terminalia oblongata)* are consumed (Heldt and Heldt, 2005) and as such seen as one of the anti-nutrients of plant origin because of their capability to precipitate proteins, inhibit the digestive enzymes and decline the absorption of vitamins and minerals (Khattab*et al.,* 2010).

Several health benefits have been attributed to tannins and some epidemiological associations with decreased frequency of chronic diseases have been established (Serrano *et al*., 2009). Several studies have shown significant biological effects of tannins such as antioxidant or free

radical scavenging activity as well as inhibition of lipid peroxidation and lipoxygenases*in-vitro* (Amarowicz*et al*., 2000). They have also been shown to possess anti-microbial, anti-viral anti-mutagenic and anti-diabetic properties (Gafner*et al.,* 1997). The antioxidant activity of tannins results from their free radical and reactive oxygen species-scavenging properties, as well as the chelation of transition metal ions that modify the oxidation process (Serrano *et al*., 2009).

1.10.2 Phenols

Phytochemicals such as phenolics, which are present in foods have attracted a great of attention (Agatemor *et al.,* 2009). Phenols sometimes called phenolicsarea family of organic compounds characterized by a hydroxyl (-OH) group attached to a carbon atom that is part of an aromatic ring. Besides serving as the generic name for the entire family, the term *phenol* is also the specific name for its simplest member, monohydroxybenzene (C_6H_5OH), also known as benzenol or carbolic acid (Amorati and Valgimigli, 2012). They also are produced by plants and microorganisms, with variation between and within species. Organisms that synthesize phenolic compounds do so in response to ecological pressures such as pathogen and insect attack, UV radiation and wounding(Mishra and Tiwari, 2011). The largest and best studied natural phenols are the flavonoids, which include several thousand compounds, among them the flavonols, flavones, flavan-3ol, flavanones, anthocyanidins and isoflavonoids.Phenolics as secondary metabolites are present in plants and contribute to the development of colour, taste and palatability as well as the defence system of plants (Agatemor *et al.,* 2009).

1.10.3Flavonoids

Flavonoids area large family of polyphenolic compounds mainly of plant origin, ubiquitous in nature and are categorized according to their chemical structures into flavones, anthocyanidins, isoflavones, catechins, flavonols, chalcones and flavanones (Robak and Gryglewski, 1988). They occur mostly in vegetables, fruits and beverages like tea, coffee and fruit drinks. They accumulate in plants as phytoalexins defending them against microbial attack (Harborne, 1973); and fungal attack (Oloyede*et al*., 2010).

Flavonoids have been found to possess many useful effects on human health. They have been shown to have several biological properties including anti-inflammatory activity, enzyme inhibition, antimicrobial activity, oestrogenic activity (Malairajan*et al*., 2006; Atanassova*et al.,*

2011), antioxidant and free-radical-scavenging ability (Cook and Shamman, 1996). Flavonoids have also been shown to exhibit anti-leukemic properties and mild vasodilatory properties useful for the treatment of heart disease (Odugbemi*et al*., 2007).

1.10.4Anthocyanins

Anthocyanins are water-soluble vascular pigments that may appear red, purple, or blue depending on the pH.Anthocyanins occur in all tissues of higher plants, including leaves, stems, roots, flowers, and fruits (Andersen, 2001).In flowers, bright-reds and -purples of anthocyanins are adaptive for attracting pollinators. In fruits, the colourful skins also attract the attention of animals, which may eat the fruits and disperse the seeds. In photosynthetic tissues (such as leaves and sometimes stems), anthocyanins have been shown to act as a "sunscreen", protecting cells from high-light damage by absorbing blue-green and ultraviolet light, thereby protecting the tissues from photoinhibition, or high-light stress (Jack, 1998).In addition to their role as light-attenuators, anthocyanins also act as powerful antioxidants. However, it is not clear whether anthocyanins can significantly contribute to scavenging of free radicals produced through metabolic processes in leaves, since they are located in the vacuole and, thus spatially separated from metabolic reactive oxygen species. Some studies have shown hydrogen peroxide produced in other organelles can be neutralized by vascular anthocyanin.It may protect the leaves from attacks by plant eaters that may be attracted by green colour (Karageorgou and Manetas, 2006).

1.10.5 Alkaloids

Alkaloids play a very important role in organism metabolism and functional activity. They are metabolic products in plants, animals and micro-organisms. They occur in both vertebrates and invertebrates as endogenous and exogenous compounds. Many of them have a disturbing effect on the nervous systems of animals. Alkaloids are the oldest successfully used drugs throughout thehistorical treatment of many diseases (Aladesanmi*et al*., 1998) and are one of the most diverse groups of secondary metabolite found in living organism. They have an array of structural types, biosynthetic pathways, and pharmacological activities (Tanko*et al*., 2008). In plants and insects, toxic alkaloids are sequestered for use as a passive defence mechanism by acting as deterrents for predating insects (Eyong*et al*., 2006).However, they inhibit certain mammalian enzyme activities

such as those of phosphodiesterase, thus prolonging the action of cyclic AMP. At concentrations of these alkaloids in edible plants, they are usually non-toxic (Okaka*et al.,* 1992).

Alkaloids have been used throughout history in folk medicine in different regions of the world. They have been a constituent part of plants used in phytotherapy. Many of the plants that contain alkaloids are just medicinal plantsand have been used as herbs. Some alkaloids that have played an important role in this sense include aconitine, atropine, colchicine, coniine, ephedrine, ergotamine, mescaline, morphine,strychnine, psilocin and psilocybin (Aladesanmi*et al*., 1998).

Many alkaloids are known to have effect on the central nervous system. Some alkaloids act as antiparasitic (such as morphine, a pain killer). For example, quinine was widely used against *Plasmodium falciparum*. In this respect, it is found from the phytochemical screening that most plants traditionally used to treat malaria contain alkaloids among other things (Jeruto*et al*., 2011).

1.10.6 Glycosides

Glycosides play numerous important roles in living organisms. In plants, chemicals are stored in the form of inactive glycosides. These can be activated by enzyme hydrolysis (Brito-Arias, 2007), which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body.Glycosides can be classified by the glycone, by the type of glycosidic bond, and by the aglycone. By aglycone, glycosides are classified as anthraquinone glycosides, coumarin glycosides, cyanogenic glycosides, etc. Although glycosides form a natural group in that they contain a sugar unit, the aglycones are of such varied nature and complexity that glycosides vary very much in their physical and chemical properties and in their pharmacological action (Trease and Evans, 2002). From ancient times, humans have used cardiac-glycoside-containing plants and their crude extracts as arrow, ordeal, homicidal, suicidal and rat poisons, heart tonics, diuretics and emetics. In modern times, purified extracts or synthetic analogues of a few have been adapted for the treatment of congestive heart failure and cardiac arrhythmia.

1.10.7 Sterols

Sterols are triterpenes which are based on the cyclopentanehydrophenanthrene ring system (Harborne, 1973). Sterols in plants are generally described as phytosterols with three known types occurring in higher plants: sitosterol (formerly known as ɓ-sitosterol), stigmasterol and campsterol(Harborne, 1973). These common sterols occur both as free and as simple glucosides. Sterols have essential functions in all eukaryotes. Free sterols are integral components of the membrane lipid bilayer where they play important role in the regulation of membrane fluidity and permeability (Irvine, 1961). While cholesterol is the major sterol in animals, a mixture of various sterols is present in higher plants, with sitosterol usually predominating. However, certain sterols are confined to lower plants such as ergosterol found in yeast and many fungi while others like fucoterol, the main steroid of many brown algae is also detected in coconut (Harborne, 1973).

1.10.8 Resins

Chemically, resins are complex mixtures of resin acids, resins alcohols (resinols), resin phenols (resinotannols), esters and chemically inert compounds known as resenes. Resins are often associated with volatile oils (oleoresins), with gums (gum-resins) or with oil and gum (oleo-gum-resins).The resin produced by most plants is a viscous liquid, composed mainly of volatile fluid terpenes, with lesser components of dissolved non-volatile solids which make resin thick and sticky (Trease and Evans, 2002). The most common terpenes in resin are the bicyclic terpenes alpha-pinene, beta-pinene, delta-3 carene and sabinene, the monocyclic terpenes limonene and terpinolene, and smaller amounts of the tricyclic sesquiterpenes, longifolene, caryophyllene and delta-cadinene.

1.10.9 Terpenoids

Terpenoids, also known as isoprenoids are the major family of natural compounds, comprising more than 40,000 different molecules. The isoprenoid biosynthetic pathway produces both primary and secondary metabolites that are of great significance to plant growth and persistence (Trease and Evans, 2002). Terpenoids are secondary metabolites that have molecular structures comprising carbon backbones that are made up of isoprene (2-methylbuta- 1, 3-diene) units. The terpenoids are comprised of two isoprene units, containing ten carbon atoms. Among the primary metabolites produced by this pathway are: the phytohormones-abscisic acid (ABA); gibberellic acid (GAs) and cytokinins; the carotenoids; plastoquinones and chlorophylls involved in

photosynthesis; the ubiquinones required for respiration; and the sterols that impact membrane structure (Harborne, 1973). Many of the terpenoids are important for the quality of agricultural products such as the flavour of fruits and the fragrance of flowers like linalool (Singh, 2009). In addition, terpenoids can have medicinal properties such as anti-carcinogenic (e.g. taxol and perilla alcohol), antimalarial (e.g. artemisinin), anti-ulcer, antimicrobial or diuretic (e.g. glycyrrhizin) activity (Harrawijn*et al*., 2001). The steroids and sterols in animals are biologically produced from precursors of terpenoid and sometimes terpenoids are added to proteins to increase their attachment to the cell membrane, a process known as isoprenylation (Singh, 2009).

1.10.10 Saponins

Saponins are groups of secondary metabolites found widely distributed in the plant kingdom as plant glycosides, characterized by a skeleton of 30-carbon precursor oxidosqualene to which glycosyl residues are attached along with it, they have sturdy foaming property. (Harborne, 1973). They are subdivided into triterpenoids and steroid glycosides and are stored in plant cells as inactive precursors but are readily converted into biologically active antibiotics by plant enzymes in reply to pathogenic attack (Okwu, 2005).

Saponins protect plants against attack by pathogens and pets (Jeruto*et al*., 2011). These molecules also have substantial marketable value and are processed as drugs and medicines, foaming agents, sweeteners, taste converters and cosmetics (Kensil, 1996).They have the ability to haemolyse red blood cells and confer a bitter taste to fruits. Saponin containing plants are used as traditional medicines, especially in Asia, and are intensively used in food, veterinary and medical industries (Kensil,1996). The pesticidal activity of saponins has long been reported (Irvine, 1961). Saponin-glycosides are very lethal to cold-blooded organisms, but not to mammals (Kensil,1996). Plant extracts containing a high percentage of saponins are commonly used in Africa to treat water supplies and wells contaminated with disease vectors; after treatment, the water is safe for human drinking (Kensil,1996). Saponins induce a strong adjuvant effect to T-dependent as well as T-independent antigens and also induce strong cytotoxic $CD8^+$ lymphocyte responses and potentiate the response to mucosal antigens (Kensil, 1996). They have both stimulatory effects on the components of specific immunity and non-specific immune reactions such as inflammation (Chukwujeku*et al.,* 2005) and monocyte proliferation (Aggarwal and Shishodia, 2006).

Saponins have long been known to possess lytic action on erythrocyte cell membranes and this property has been used in their detection. The haemolytic actions of saponins are alleged to be due to their affinity for the aglycone moiety of membrane sterols, mainly cholesterol with which they form undissolvable complexes (Davies, 1995).

1.10.11 Reducing sugar

A sugar is classified as a reducing sugar only if it has an open-chain form with an aldehyde group or a free hemiketal group. A reducing sugar is one that reduces certain chemicals. Sugars with ketone groups in their open chain form are capable of isomerizing via a series of tautomeric shifts to produce an aldehyde group in solution (Campbell and Farrell, 2012). That is, saccharides bearing anomeric carbons that have not formed glycosides are termed reducing sugars, because the free aldehyde group that is in equilibrium with the cyclic form of the sugar reduces mild oxidizing agents. Identification of a sugar as non-reducing is an evidence that it is glycoside (Voet *et al.,* 2013).

1.11Hepatotoxicity

Hepatotoxicity implies chemical-driven liver damage. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Other chemical agents, such as those used in laboratories (e.g. CCl_4, paracetamol) and industries (e.g. lead, arsenic), natural chemicals (e.g. microcystins, aflatoxins) and herbal remedies (cascara, sagrada, ephedra) can also induce hepatotoxicity (Singh*et al*., 2012). Chemicals that cause liver injury are called hepatotoxins. These agents are converted into chemically reactive metabolites in liver, which have the ability to interconnect with cellular macromolecules such as protein, lipids and nucleic acids, leading to protein dysfunction, lipid peroxidation, DNA damage and oxidative stress. This damage of cellular function can dismiss in cell death and likely liver failure. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures. More than 75 percent of cases of idiosyncratic drug reactions result in liver transplantation or death (Ostapowicz*et al*.,2002).

1.11.1 The liver

The liver plays a pivotal role in regulating various physiological processes. It is also involved in several vital function, such as metabolism, secretion and storage. It has great capacity to detoxify toxic substances and synthesize useful principle (Domitrovic *et al*., 2013). It helps in the maintenance, performance and regulating homeostasis of the body. It is involved in almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. It aids metabolism of carbohydrate, protein and fat, detoxification, secretion of bile and storage of vitamins (Ahsan *et al*., 2009). The role played by the organ in the removal of substances from the portal circulation makes it susceptible to first and persistent attack by offending foreign compounds, culminating in liver dysfunction. These hepatotoxic agents activated some enzyme activities in the cytochrome P-450 system such as CYP2E1 leading to oxidative stress (Singh*et al*., 2012). Injury to hepatocyte and bile duct cells lead to accumulation of bile acid inside liver. This promotes further liver damage.

The liver is also the major reticula endothelial organ in the body as such has important immune function in maintaining body veracity. Damaging hepatocyte results in the activation of innate immune system like kupffer cells (kc), natural killer cells (NK) and natural killer T-cells (NKT)and result in producing pro inflammatory mediators such as tumour necrosis factor-Ŭ (TNF), interferon-ɔ (IFN), and interleukin-ɓ (IL) produced liver injury. Many agents which damage an intracellular organellemitochondria include drug accumulation, inhibition of electron transport and fatty acid oxidation or depletion of anti-oxidant defences. An indirect result ensuing from mitochondrial participation in programmes of cell death. These programmes lead to necrosis or apoptosis, they are mediated through signalling mechanisms arising at the cell membrane (e.g. death receptors) or in subcellular compartments (e.g. the endoplasmic reticulum or cell nucleus) (Sun *et al*., 2001; Friedman, 2000). Its dysfunction releases excessive amounts of oxidants which, in turn injure hepatic cells. Non-parenchymal cells such as kupffer cells, fat storing stellate cells, and leucocytes (i.e. neutrophils and monocytes) also and in the mechanism of hepatotoxicity (Patel *et al*., 1998). Hepatic injury leads to disturbances in transport function of

hepatocytes resulting in leakage of plasma membrane thereby causing an increased enzyme level in the serum (Ibid).

1.11.2 Assay associated with hepatotoxicity

When the integrity of the membrane of the hepatocytes is compromised, certain enzymes located in the cytosol are released into the blood. Their estimation in the serum is useful quantitative marker for the evaluation of liver damage (Pari and Kumar, 2002). Glutamate dehydrogenase activity is not found in normal serum but moderate elevation is found in most cases of acute hepatitis indicating cellular damage. Another demonstrable type of membrane damage involves injury to lysosomes which leads to the release of acid ribonuclease, acid phosphatases, and other liver enzymes such alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP), into the blood stream. These enzymes are elevated to distinguish and assess the extent and type of hepatocellular injury (Pari and Kumar, 2002). Other indicators used in hepatotoxicity studies are total bilirubin concentration, cholesterol concentration, low density lipoprotein (LDL) concentration, high density lipoprotein (HDL) and triacylglycerols concentrations.

1.12Carbon tetrachloride (CCl_4)

Carbon tetrachloride (CCl_4) was the formerly used for metal degreasing and as a dry-cleaning fluid, fabric-spotting fluid, fire extinguisher, grain fumigant and reaction medium. CCl_4 -induced liver damage has been lengthily used as an experimental model. It is used as a model drug for the study of hepatotoxicity in acute and chronic liver failure. (Weber *et al.,* 2003;Singh*et al.,* 2012). CCl_4 is metabolized by CYP2E1, CYP2ϐ and possibly CYP3A to form the tri-chloromethyl radical, CCl_3 (Poli, 1993). This CCl_3 radical can bind to cellular molecules damaging crucial cellular progression. This radical can also react with oxygen to form the tri-chloromethyl peroxy radical CCl_3OO, a highly reactive species. The metabolites of CCl_4 cause the hepatic injury in the CCl_4 liver injury model. Single dose of CCl_4 to a rat produces centrilobular necrosis and fatty changes. The poison reaches its maximum concentration in the liver in 3hrs of administration

(Dawkins, 1963). Non-lethal intoxication triggers liver tissue remodelling and healing through the activation of hepatic stellate cells (HSCs), leading to liver fibrosis (Friedman, 2000). Tri-chloromethyl free radical is believed also to initiate the biochemical processes leading to oxidative stress, a direct cause of many pathological conditions such as diabetes mellitus, cancer, hypertension, kidney damage, liver damage and death. Liver damage caused by acute exposure to CCl_4 shows clinical symptoms such as jaundice, swollen and tender liver and elevated levels of the liver enzymes -ALT, AST and ALP in the blood (Tirkey*et al.,* 2005).

1.13*Cucumis Sativus*(Cucumber)

1.13.1 Morphology

Cucumis sativus (Cucumber) is a widely cultivated plant in the gourd family of Cucurbitaceae, which also includes important crops such as melon, water melon, and squash. It is a creeping vine that roots in the ground and grows up trellises or other supporting frames, wrapping around supports with thin, spiralling tendrils. The plant has a large leaves that form a canopy over the fruit. The fruit of the cucumber is roughly cylindrical, elongated with tapered ends, and may be as large as 60 centimetres (24 inches) long and 10 centimetres (3.9 inches) in diameter. Having an enclosed seed and developing from flowers, botanically speaking, cucumber can be classified as an accessory fruits.

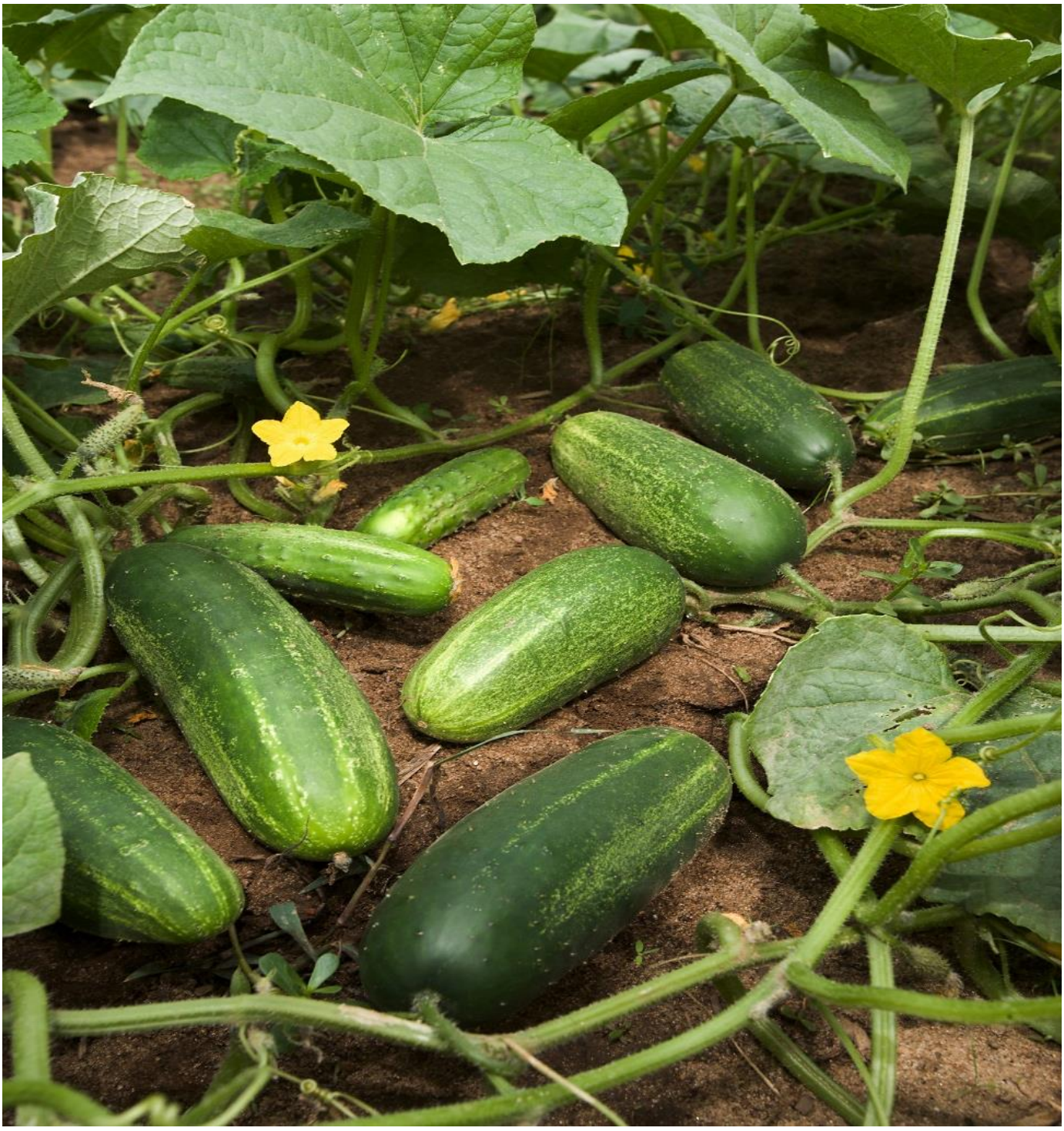

Figure 4: *Cucumis sativus* Fruits: Cucumber

Source: Prohens and Fernando, (2008).

1.13.2 Taxonomy and Nomenclature

Kingdom	-	*Plantae*
Unranked	-	*Angiosperms*
Unranked	-	*Eudicots*
Unranked	-	*Rosids*
Order	-	*Cucurbitales*
Family	-	*Cucurbitaceae*
Genus	-	*Cucumis*
Species	-	*Cucumissativus*

Source: William and Brigitta, (2010)

1.13.3Nutritional composition

There is increased consumption of *Cucumis sativus* fruits possibly because of their high nutritional value. The nutritional compositionof *Cucumis sativus* include protein, fat, and carbohydrate as primary metabolites; and dietary fibre which is important for the digestive system. *Cucumis sativus* contains some essential vitamins and anti-oxidants which are effective in human health (Grubben and Denton, 2004; Wang *et al*.,2007). Table 1 shows some basic nutritional composition of a cucumber fruit.

Table 1.Nutritional composition of 100g edible portion of cucumber fruit.

Compound	Amount
Water	95.23%
Energy	42kJ
Protein	0.65g
Fat	0.1g
Sugars	1.5g
Dietary Fibre	0.5g
Starch	0.83g
Ca	16mg
Mg	8mg
P	24mg
Fe	0.28mg
Zn	0.1mg
Mn	0.079mg
K	147mg
Na	2mg

F	1.3εg
Carotene	60mg
Thiamin	0.027mg
Folate	7εg
Ascorbic acid	2mg
Pantothenic acid	0.259mg
Vitamin B6	0.04mg
Vitamin K	16.4εg
Vitamin A	105 IU
Lutein + Zeaxanthin	23ug

Source: National Nutrient Database for Standard Reference, USDA

1.13.4.Uses of *Cucumis Sativus*

(a) In Medicine: Cucumber (*Cucumis sativus)* is used by native people to cure many illnesses in some countries. In Africa, ripe raw cucumber fruits are used as a cure for sprue, a disease that causes flattering of the villi and inflammation of the lining of the small intestine; and in Indo China, cooked immature fruits are used to treat dysentery in children (Grubben and Denton, 2004). It is also useful in fighting constipation, as the fibre content helps to overcome the hypotony which is the cause of constipation (Yohanna, 2013). Swapnil*et al*.(2012) reported the use of Cucumis Sativus in the treatment of patients with high blood pressure and with irritated skin as a result of sun burn.

(b) Food: As a fresh market vegetable in Europe, United States and many parts of the world, cucumber is mainly used in salads, but young and ripe fruits are used as cooked vegetables (Grubben and Dentons, 2004). It is a good health food for the diabetics (Sharmin*et al.,* 2013).

(c) In Cosmetics:*Cucumis sativus*-derived ingredients are reported to function in cosmetics as skin conditioning agents (Gottschalck and Breslawec, 2012). Products containing *Cucumis sativus* fruit extract are reported to be used on baby skin and may be applied to eye area or mucous membrane. Additionally, *Cucumis sativus* fruit extract is used in cosmetic spray products for face, neck, body and hand. (Rothe*et al.,* 2011).

1.14Rationale for study

It is believed that *Cucumis sativus*fruit has anti-oxidant activity, high flavonoid content, anti-inflammatory and analgesic effect (Kumar *et al*., 2010; Singh-Gill *et al*., 2010; Agarwal *et al*., 2012). It is therefore necessary to establish some of these properties and their application in management of inflammation and liver diseases.

1.15Aim of study

The aim of this research is to assess the effect of the homogenate of *Cucumis sativus* fruit on some inflammatory models and CCl_4-induced hepatotoxicity in rats so as to know the possibility of its implication in the management of diseases.

1.16Research objectives

This research work is set out to achieve the following specific objectives:

- To determine the phytochemical constituents of the homogenate of *Cucumis sativus* fruit.
- To determine the proximate composition of the homogenate of *Cucumis sativus* fruit.
- To determine the acute toxicityof thehomogenate of *Cucumis sativus* fruit.
- To determine effect of the homogenate of *Cucumis sativus* fruit on DPPH radical scavenging activity.
- To determine the anti-inflammatory effects of the homogenate of *Cucumis sativus*fruit on agar-induced paw oedema in rats.
- To determine the effect of the homogenate of *Cucumis sativus* fruit on hypotonicity-induced haemolysis of red blood cell.

- To determine the effect of the homogenate of *Cucumis sativus* fruit on phospholipase A_2 activity.
- To determine the effect of the homogenate of *Cucumis sativus* fruit on prostaglandin synthase activity.
- To determine the effects of the homogenate of *Cucumis sativus*fruit on some serum biochemical parameters such as liver marker enzymes and lipid profileof rats intoxicated with CCl_4.
- To carry out histopathological examination of the liver organ implicated in this study.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

2.1.1Plant Material

*Cucumis sativus*fruits were purchased from Nsukka main market, Nsukka, Enugu State, Nigeria, and was identified by Mr. Alfred Ozioko of Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu. The fruit of *Cucumis sativus*was homogenized (daily before administration) with Kenwood high speed blender and used without further dilution.

2.1.2 Animals

The animals used in this study weretwenty (20) albino mice of the Swiss strain (22 ï 28g) employed for acute toxicity study (LD_{50}) and fifty ï two (52) adult Wistar rats (120 ï 200g) purchased from the Animal House of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The animals were acclimatized to laboratory condition for seven days before experiments and maintained *ad libitum* on water and Growerôs mash rat pellets (Pfizer Feeds, Aba) bought from Nsukka market. The guide for the care and use of laboratory animals procedures were followed in this study.

2.1.3 Instruments

The instruments used for the study include; Blender (Kenwood, England), Oven (Gallenkamp, England), Centrifuge (Sigma-Aldrich, England),Weighingbalance (Vickas Ltd, England),Rotatory evaporator,Electrophoresis machine (Hilcon, England), Refrigerator (Newcastle, England), $_{P}$H meter, Sp 500 spectrophotometer,(PyeUnicam, England).

2.1.4 Chemicals and Reagents

The chemicals and reagents used for this study were of analytical grade and they include:Methanol,Absolute Ethanol, N-hexane, Acetic acid glacial, Alkaline copper reagent, Nessler reagent , Acetone, Acetone nitrile, Hydroquinone, Ethylenediaminetetraacetate

(EDTA), Alkaline picrate reagent, FolinCiocalten reagent, Hydrochloric acid (HCL), Sulphuric acid, Ferric chloride, Phosphomolybdic acid, Sodium carbonate, Ammonia (BDH Chemicals Ltd., Pool England), Ethyl Acetate, Lead acetate, Formaldehyde, 1,1- Diphenyl -2- picryhydrazyl (DPPH), Citric acid, Potassium ferricyanide, Tris Buffer(Merck,Darmstadt, Germany), Sodium Chloride, Aluminum chloride, Sodium Nitrate, Chloroform, Tri-sodium citrate (May and Baker, Dagenham England), Potassium hydroxide, Calcium chloride, Gum acacia, Agar-agar (Riedel De HakingSeelze- Handover), Indomethacin (ElblePharmaVeranhandles, Germany),Phenylbutazone (Artesan, GMBH, Germany), Reduced Glutathione, Potassium hydroxide pellet (Qualikems Laboratory Reagent), Sucrose (Kermel, England), Total cholesterol Kit, Triacylglycerol kit, Aspartate aminotransferase kit, Total Bilirubin kit, Alanine aminotransferase kit, (RANDOX Lab. Ltd, U.K), Cholesterol LDL Precipitating Reagent (Biosystems S.A., Spain), Alkaline phosphatase Kit, (QuimicaClin. Aplicada., Spain).

2.2 Methods

2.2.1 Preparation of plant material

The fruits of *Cucumis sativus* were homogenized with Kenwood high speed blender, and the homogenates were used for biological and biochemical determination.

2.2.2 Qualitative phytochemical analysis of the homogenate of *Cucumis sativus*fruit

The qualitative phytochemical analyses of the fruits of *Cucumis sativus*were carried out according to the methods of Harborne (1998) and Trease and Evans (2002). The following test were done.

2.2.2.1 Test for alkaloids

A quantity, 2mlof the homogenate was boiled with 5ml of 2% HCl on steam bath. The mixture was filtered and 1ml aliquot of the filtrate was treated with two drops of the following reagents

(i) Dragendorffôs reagents: An orange precipitate indicated the presence of alkaloids.
(ii) Mayerôs reagents: A creamy-white precipitate indicated the presence of alkaloids.
(iii) Wagnerôs reagents: A reddish ï brown precipitate indicated the presence of alkaloids.
(iv) Picric acid (1%): A yellowish precipitate indicated the presence of alkaloids.

2.2.2.2 Test for flavonoids

A quantity, 2ml of the homogenate was heated with ethylacetate (10ml) on a water bath for 3 min. The mixture was filtered, and the filtrate was used for the following tests.

Ammonium test: About4ml of the filtrate was shaken with 1ml of dilute ammonium solution to obtain two layers. The layers were allowed to separate and a yellow colour observed in the ammonium layer indicated the presence of flavonoids.

Aluminium chloride test: Another4ml of the filtrate was shaken with 1ml of 1% aluminium chloride solution and observed for light yellow coloration that indicated the presence of flavonoids.

2.2.2.3 Test for glycosides

Dilute sulphuric acid (5ml) was added to 2ml of the homogenate, boiled for 15 min and then cooled and neutralized with 20% potassium hydroxide solution. A quantity (5ml) of the mixture of equal parts of Fehlingõs solutions I and II was added and then heated on a water bath for 5 min. A dense brick red precipitate indicated the presence of glycosides.

2.2.2.4 Test for steroids and terpenoids

Ethanol (9ml) was added to 2ml of the homogenate and refluxed for a few min and filtered. The filtrate was concentrated to 2.5ml on a boiling water bath, and 5ml of hot water was added. The mixture was allowed to stand for 1 hour, and the waxy matter filtered off. The filtrate was extracted with 2.5ml of chloroform using a separating funnel, these was divided into two equal parts, and the following tests carried out.

1) The first portion of the chloroform extract (0.5ml) in a test tube was mixed with 2ml of concentrated sulphuric acid carefully to form a lower layer. A reddish brown interface indicated the presence of steroids.

2) The second portion of the chloroform extract (0.5ml) was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10 min on a water bath. The formation of a grey colour indicated the presence of terpenoids.

2.2.2.5 Test for saponins

Distilled water (5ml) was added to2ml of the homogenate and boiled gently on a hot water bath for 5 min. The mixture was filtered while still hot. The filtrate was used for the following tests.

Emulsion test: To 1ml of the filtrate was added 2 drops of olive oil. The mixture was shaken vigorously and observed for the formation of emulsion. The formation of emulsion indicated the presence of saponins.

Frothing test: A quantity, 2ml of the filtrate was diluted with 4ml of distilled water. The mixture was shaken vigorously and then observed on standing for stable froth. A stable froth (foam) upon standing indicated the presence of saponins.

Fehling's test: To 5ml of the filtrate was added 5ml of Fehlingôs solution and the content heated on a water bath. A reddish precipitate which turned brick red on further heating with sulphuric acid indicated the presence of Saponins.

2.2.2.6 Test for tannins

A quantity, 2ml of the homogenate was boiled with 5ml of 45% ethanol for 5 min. The mixture was cooled and then filtrated. The filtrate was treated with the following solutions.

Lead acetate solution: To the filtrate (3ml), a few drops of lead acetate were added. A reddish colour indicated the presence of tannins.

Ferric chloride solution: A few drops of ferric chloride solution were added to 3ml of the filtrate; a greenish black precipitate indicated the presence of tannins.

2.2.2.7 Test for resins

(i) Precipitation test: The homogenate of *Cucumis sativus* fruit (4 ml) was extracted with 15ml of 95% ethanol. The alcoholic extract was then poured into 20ml of distilled water in a beaker. A precipitate occurring indicated the presence of resins.

(ii) Colour test: The homogenate of *Cucumis sativus* fruit (4 ml)was extracted with chloroform and the extract was concentrated to dryness. The residue was dissolved in 3ml of acetone and 3ml of concentrated hydrochloric acid was added to it. The mixture was heated in a water bath for 30 min. A pink colour which changed to red indicated the presence of resins.

2.2.3 Quantitative phytochemical analysis of the homogenate of *Cucumis sativus*fruit

2.2.3.1 Determination of tannin content

Quantitative determination of tannin was done using spectrophotometricdeterminationmethod described by Gupta and Verma, (2010).

Procedure

A quantity, 5g of homogenate was macerated in 20ml of methanol for 5 min then centrifuged for 10 min. The supernatant (5ml) was transferred into triplicate tubes and 0.3ml of 0.1M $FeCl_3$ in 0.1M HCl was added. To the mixture, 0.3ml of 0.0008M potassium ferricyanide was added. It was then mixed and the absorbance was taken at 720nm.

Calculation

$$\text{Tannin } \left(\frac{\text{mg}}{100\text{g}}\right) = \frac{\text{Conc.}\left(\frac{\text{mg}}{100\text{ml}}\right) \times 20\text{ ml}}{5\text{ g} \times 5\text{ml}}$$

$$\text{Conc.}\left(\frac{\text{mg}}{100\text{ml}}\right) = \frac{\text{Absorbance Reading}}{0.0328\text{ (Obtained from standard curve)}}$$

2.2.3.2 Determination of phenol content

The total phenol content of *Cucumis sativus* fruit was determined using a spectrophotometric method of Wolfe *et al.,* (2003).

Procedure

A quantity, 2g of the homogenate was macerated in 20ml of 80% ethanol for 5 min, and centrifuged for 10 min. The supernatant (1 ml) was transfer into triplicate tubes and 4ml of distilled water and 0.5ml of Folin-Ciocalteu reagent were added. To the mixture2ml of 20% $NaCO_3$ was added. It was allowed to stand for 30 min, and the absorbance taken at 650 nm for phenol content and at 760nm for polyphenol content.

Calculation:

$$\text{Conc.}\left(\frac{\text{mg}}{100\text{g}}\right)\text{of phenol or polyphenol} = \frac{\text{Conc.}\left(\frac{\text{mg}}{100\text{ml}}\right) \times 20\text{ml}}{2\text{g} \times 1\text{ ml}}$$

$$\text{Phenol}\left(\frac{\text{mg}}{100\text{ml}}\right) = \frac{\text{Absorbance} - 0.0634}{0.1759}\ \text{(From standard curve)}$$

$$\text{Polyphenol}\left(\frac{\text{mg}}{100\text{ml}}\right) = \frac{\text{Absorbance} - 0.0669}{0.1666}\ \text{(From standard curve)}$$

2.2.3.3 Determination of cyanogenic glycoside content

Cyanogenic glycoside was determined using alkaline picrate method of Onwuka and Olopade(2005).

Procedure

The homogenate of *Cucumis sativus* fruit (2 g) was macerated with 20ml of water for 10 min and allow to stand for 1hr with intermittent shaking every 10 min. It was then centrifuge for 10 min. A known volume, 1ml of the supernatant was transferred into triplicate tubes, and 4ml alkaline picrate reagent was added. The mixture was boiled for 5 min, and cooled to room temperature. Absorbance was taken at 490nm against blank containing 1ml distilled water and $4cm^3$ alkaline picrate solution.

Calculation

$$\text{Cyanogenic Glycoside } \left(\frac{\text{mg}}{100\text{g}}\right) = \frac{\text{Conc. } \left(\frac{\text{mg}}{100\text{ml}}\right) \times 20\text{ ml}}{2\text{ g} \times 1\text{ ml}}$$

$$\text{Conc. } \left(\frac{\text{mg}}{100\text{ml}}\right) = \frac{\text{Absorbance}}{0.1937\text{ (Obtained from standard curve)}}$$

2.2.3.4 Determination of glycoside content

Spectrophotometric determination of glycoside content was carry out with a method described by Qasheesh (1937).

Procedure

A mixture of 2g of the homogenate, 20ml of water and 2.5ml of lead acetate were macerated for 5 min, and 10ml of chloroform was added. After 5 min of centrifugation, 2.5ml of the lower layer was transferred into triplicate tubes, then evaporated to dryness. Acetic acid (3 ml) and 0.1 ml of ferric chloride in 0.2ml of sulphuric acid were added. It was then kept in the dark for 2 hr after shaking before absorbance reading was taken at 530nm.

Calculation

$$\text{Glycoside } \left(\frac{\text{mg}}{100\text{mg}}\right) = \frac{\text{Conc. } \left(\frac{\text{mg}}{100\text{ml}}\right) \times 20\text{ ml}}{2\text{ g} \times 2.5\text{ ml}}$$

$$\text{Conc. } \left(\frac{\text{mg}}{100\text{ml}}\right) = \frac{\text{Absorbance}}{0.3133\text{ (Standard curve)}}$$

2.2.3.5 Determination of flavonoid content

The flavonoid content was estimated using ferric chloride colorimetric method of Mattila and Kumpulairen(2002). The method is based on formation of flavonoid ï ferric complex.

Procedure

A quantity, 2 g of homogenate in 20 ml of ethyl acetate was macerated for 10 min, then centrifuged for 5 min. A known volume, 5ml of the supernatant was transfer into triplicate tubes and 0.3ml of 5% $NaNO_2$ was added, then 0.3ml of 10% ferric chloride. After 5 min, 4ml of 4% NaOH was added. Absorbance was taken at 510nm after 15 min of incubation at ambient temperature.

Calculation

$$\text{Flavonoid } \left(\frac{\text{mg}}{100\text{g}}\right) = \frac{\text{Conc. } \left(\frac{\text{mg}}{\text{ml}}\right) \times 20 \text{ ml}}{2 \text{ g} \times 5 \text{ ml}}$$

$$\text{Conc. } \left(\frac{\text{mg}}{100\text{ml}}\right) = \frac{\text{Absorbance}}{0.0162 \text{ (Standard curve)}}$$

2.2.3.6 Determination of saponin content

Saponin content was quantitatively estimated by spectrometric determination method of Uematsu*et al* (2000).

Procedure

The homogenate of *Cucumis sativus* fruit (2g) was macerated in 20ml of methanol and centrifuged for 5 min. A known volume, 2ml of the supernatant was then transferred into triplicate tubes. It was evaporated to dryness over a water bath. Ethyl acetate (2 ml) was added to the brownish residue, and allow to dissolve. Then, 1ml of 50% Sulphuric acid in ethyl acetate and 1ml of 0.5ml formaldehyde in ethyl acetate was added. It was incubated at 60^0cfor 20 min and the absorbance was taken at 430nm.

Calculation

$$\text{Saponin } \left(\frac{\text{mg}}{100\text{g}}\right) = \frac{\text{Conc.} \left(\frac{\text{mg}}{100\text{ml}}\right) \times 20\text{ ml}}{2\text{ g} \times 2\text{ ml}}$$

$$\text{Conc.} \left(\frac{\text{mg}}{100\text{ml}}\right) = \frac{\text{Absorbance}}{3.0225\text{ (From standard curve)}}$$

2.2.3.7 Determination of alkaloid content

Determination of alkaloid content was carried out by the method described by Harborne (1998).

Procedure

A known quantity, 2g of the homogenate with 10ml of 20% Sulphuric acid and 10ml of ethanol was macerated and allowed to stand for 1hr. It was centrifuged at 3000 rpm for 5 min. Thesupernatant (1 ml) was transferred into each of the triplicate tubes containing 5 ml of 60% sulphuric acid with 5ml of 0.5% formaldehyde. It was allow to stand for 3 hr and absorbance taken at 565nm.

Calculation

$$\text{Alkaloid } \left(\frac{\text{mg}}{100\text{g}}\right) = \frac{\text{Conc.} \left(\frac{\text{mg}}{100\text{ml}}\right) \times 20\text{ ml}}{2\text{ g} \times 10\text{ ml}}$$

$$\text{Conc.} \left(\frac{\text{mg}}{100\text{ml}}\right) = \frac{\text{Absorbance}}{0.012\text{ (From standard curve; Appendix VIII)}}$$

2.2.3.8 Determination of steroid content

The amount of steroid was determined by the method described by Edeoga*et al.* (2005).

Procedure

A known quantity (2g) of the homogenate was filtered and the filtrate was eluted with 0.1N NH_4OH (pH 9). The eluent (2ml) was put into a test tube and mixed with 2ml of chloroform. Ice-

cold acetic anhydride (3ml) was added to the mixture in the flask and 2 drops of conc. H_2SO_4 were cautiously added. A Standard sterol solution was prepared and treated similarly. Absorbance of standard and prepared sample were measuredat 420nm using a PyeUnicam spectrophotometer.

Calculation

$$\text{Steroid } \left(\frac{\text{mg}}{100\text{g}}\right) = \frac{\text{Conc.} \left(\frac{\text{mg}}{100\text{ml}}\right) \times 5\text{ ml}}{2\text{ g} \times 2\text{ ml}}$$

$$\text{Conc.} \left(\frac{\text{mg}}{100\text{ml}}\right) = \frac{\text{Absorbance}}{0.029\text{ (From standard curve)}}$$

2.2.3.9 Determination of reducing sugar content

Quantitative determination of reducing sugars was carried out using Folin and Wu method (1920).

Procedure

A known quantity, 2g of the homogenate with 20 ml of water was macerated for 5min, and centrifuged for 10min. The supernatant (1 ml) was transferred into triplicate tubes and 1ml of alkaline copper reagent was added. It was then boiled for 5 min. The hot mixture was cooledin cold water. Phosphomolybdic acid reagent (1 ml) was added and then diluted with 7ml of water and the absorbance was taken at 680nm.

Calculation

$$\text{Reducing sugar } \left(\frac{\text{mg}}{100\text{g}}\right) = \frac{\text{Conc.} \left(\frac{\text{mg}}{100\text{ml}}\right) \times 20\text{ ml}}{2\text{ g} \times 1\text{ ml}}$$

$$\text{Conc.} \left(\frac{\text{mg}}{100\text{ml}}\right) = \frac{\text{Absorbance}}{0.0195\text{ (From standard curve)}}$$

2.2.3.10 Determination of resin content

Resin content was determined quantitatively by the UV absorption method of Harborne (1998)

Procedure

A quantity, 2g of homogenate mixed with 10ml of acetone was macerated for 10 min, and allowed to stand for 1 hr before centrifugation at 3000 rpm for 5 min. The supernatant (1 ml) was transferred into triplicate tubes. After evaporation to near-dryness in a water bath, 2ml of aceton nitrite was added, and absorbance was taken at 272nm.

Calculation

$$\text{Resin } \left(\frac{\text{mg}}{100\text{g}}\right) = \frac{\text{Conc.} \left(\frac{\text{mg}}{\text{ml}}\right) \times 10\ \text{ml}}{2\ \text{g} \times 1\ \text{ml}}$$

$$\text{Conc.} \left(\frac{\text{mg}}{100\text{ml}}\right) = \frac{\text{Absorbance}}{0.050\ (\text{From standard curve})}$$

2.2.3.11 Determination of terpenoid content

Quantitative estimation of terpenoid content was carried out using oxidation method of Harborne (1998).

Procedure

The homogenate (2 g) in 20ml of methanol was macerated, and then centrifuged for 5 min. A known volume, 2.5ml of supernatant was transferred into triplicate tubes, and 2.5 ml of 5% phosphomolybdic acid was added with 2.5 ml of concentrated sulphuric acid, mixed and allowed to stand for 30 min. Thereafter 5ml of methanol was added to make up to 12.5ml. Theabsorbance was taken at 700nm against the blank.

Calculation

$$\text{Terpenoid (mg/100g)} = \frac{\text{Conc.} \left[\frac{\text{mg}}{\text{100ml}}\right] \times 20\ \text{ml}}{2\ \text{g} \times 2.5\ \text{ml}}$$

$$\text{Conc. (mg/100ml)} = \frac{\text{Absorbance}}{0.043\ \text{(From standard curve)}}$$

2.2.3.12 Determination of anthocyanin content

Anthocyanin content was estimated quantitatively with pH differentiation method of Harborne (1998).

Procedure

A quantity, 2 g of the homogenate was macerated in 20ml of citrate buffer (2g of citric acid dissolved in water, and 1M of sodium citrate to adjust pH 3.4, and then make up to 100ml) for 2 min. Centrifuged for 5 min, and the supernatant (2ml) was transferred into two sets of triplicate tubes. To each tube of the triplicate, was added 4ml of citrate buffer and allowed to stand for 1 hr. To the other set, 1:1 HCl ï H_2O was added. Absorbance was taken at 500nmafter 1hr against water as blank.

Calculation

$$\text{Anthocyanin} \left[\frac{\text{mg}}{\text{100g}}\right] = \frac{\text{Conc.} \left[\frac{\text{mg}}{\text{100ml}}\right] \times 20\ \text{ml}}{2\ \text{g} \times 2\ \text{ml}}$$

$$\text{Conc.} \left[\frac{\text{mg}}{\text{100ml}}\right] = \frac{\text{Absorbance}}{0.063\ \text{(From standard curve)}}$$

2.2.3.13Determination of chlorophyll content

Chlorophyll content was determined using Harborne (1998). A known quantity of homogenate (2 g) in 20ml of hexane was macerated for 5 min, and centrifuged at 3000 rpm for 10 min.

Absorbance of the supernatant was taken at 663nm and 645nm. The Chlorophyll content was calculated as follow.

$$\text{Chlorophyll a } \left(\frac{\text{mg}}{\text{g}}\right) = \frac{(12.3 \times \text{Absorbance @ } 663) - (0.86 \times (\text{Absorbance @ } 645)}{1000 \times \text{W}} \times \text{V}$$

$$\text{Chlorophyll b } \left(\frac{\text{mg}}{\text{g}}\right) = \frac{(19.3 \times \text{Absorbance @ } 645) - (3.6 \times (\text{Absorbance @ } 663)}{1000 \times \text{W}} \times \text{V}$$

Where

V = Volume of solvent

W = Weight of Homogenate

2.2.4 Proximate analysis

The proximate analysis of the homogenate of *Cucumis sativus*fruits for moisture, ash and carbohydrate were determined as described by AOAC (2000). The concentration of crude protein and fibre were determined using methods described by Pearson (1976). All determinations were done in triplicates and the results were expressed as means of percent values on dry weight basis.

2.2.4.1 Determination of crude protein content

The crude protein content was determined using Kjeldahl method. The method is generally used to determine nitrogen (N) in substances which contain N as ammonium salts, nitrates or organic N compounds. Since it measures the total amount of N in a compound only a rough indication of the total protein content (a measure of N quantity and not quality) can be obtained and termed crude protein. The quantity of N measured is then multipled by 6.25 to calculate the crude protein content of the substance. The multiplication factor can vary with some materials (AOAC).

A known quantity of the homogenate (4 g) was weighed into a Kjeldahl flask with 2ml of digestion mixture (sodium sulphate/copper sulphate) added. A volume of concentrated sulphuric acid (20ml) was added to the flask and the content was gently heated. The heating was increased until the contents of the flask were completely digested giving a clear solution. The content of the flask was washed with 20ml distilled into a distillation flask and cooled. An aliquots (5 ml)

of each were transferred into triplicate tubes. To each test tube, 0.5ml of Nesslerôs reagent was added, mixed and 1ml of 0.1% Gum acacia was added. The solution was made up to 10ml with water and the absorbance taken at 490nm against a blank (5ml of distilled water, 0.5ml of Nesslerôs reagent).

% Protein = % Nitrogen x 6.25.

NB: 6.25 = Conversion factor of nitrogen to protein.

2.2.4.2 Determination of moisturecontent

Moisture content was determined using the method of Harborne (1998). The*Cucumis sativus* fruit homogenate (10 g) was dried in the oven to a constant weight. The dish with the sample was cooled and reweighed and moisture content was calculated as W2 ï W3, and the percentage moisture content was calculated thus:

$$\% \text{ Moisture} = \frac{W2 - W3}{W1} \times 100$$

Where

W1 = Weight of sample

W2= Initial Weight of sample and dish

W3= Final Weight of dry sample and dish

2.2.4.3 Determination of ash content

Ash is defined as the mineral matter of a feed since it includes, for the most part, the inorganic or mineral components of the feed (Cullison, 1982). The sample was ashed at 600^0C to burn off all organic material. The inorganic material which does not volatilize at this temperature is called ash. A known quantity, 10g of the homogenate were weighed into a crucible and then placed in a muffle furnace at 600^0C for 3hr. The ash and the crucible were cooled in desiccators and reweighed. The percentage ash content was calculated.

$$\% \text{ Ash} = \frac{W3 - W1}{W2 - W1} \times 100$$

Where

W1 = Weight of empty crucible

W2 = Weight of crucible and sample before drying

W3 = Weight of crucible and ash after drying

2.2.4.4 Determination ofcrude fibre content

Fibre includes those materials in food which are of low digestibility namely cellulose, certain hemicelluloses and some of the lignin, if present. Some of the lignin, however, may be included in the nitrogen free extract. A moisture-free, ether extract is digested first with weak acid solution (1.25% H_2SO_4) and then with a weak base solution (1.25% NaOH). The organic residue left after digestion is collected. The loss of weight on ignition is called crude fibre (Anosike, 2010).

The fruit homogenate (10 g) was weighed into 500ml beakers containing pre ï heated dilute H_2SO_4 (40ml). The content was boiled for 30min and filtered. The residue was washed three times with hot water, then 150ml of pre ï heated KOH and drops of antifoam agent (loctanol) were added to the sample in the beaker. The mixture was boiled slowly for 30 min more, filtered and washed three times with hot water. Acetone was then used in washing it three times in a cold extraction unit and content dried at 130^0C for 1hr. The dry residue was weighed and then ashed at 600^0C in a muffle furnace. The ash was weighed and the percentage fibre calculated as follows.

$$\% \text{ Crude Fibre} = \frac{\text{Weight of Fibre}}{\text{Weight of Sample}} \times 100$$

2.2.5 Radical scavenging activity

The radical scavenging activity of *Cucumis sativus*fruit homogenate was measured using the stable radical scavenger, DPPH (2, 2 ï diphenyl ï 2 ï picrylhydrazyl hydrate) decoloration assay described by Choi (2002).The fruit homogenate (2g) was macerated in 20ml of ethanol. Different amountsof the mixture were transferred into test-tubes as shown in Table 2

Table 2: Radical scavenging activity reaction medium

Sample (ml)	Ethanol (ml)	0.3mM - DPPH (ml)
0.1	0.9	0.5
0.2	0.8	0.5
0.3	0.7	0.5
0.4	0.6	0.5
0.5	0.5	0.5

The absorbance was taken after 30 min of incubation at 518nm wavelength against a control (Blank) of Ethanol and DPPH. Inhibition of free radical by DPPH in percent % was calculated in the following way:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbaance of control}} \times 100$$

2.2.6 Acute toxicity studies

The acute toxicity studies of *Cucumis sativus*fruit homogenate was carried out by a modified method of Lorke (1983) to define the range of lethal dose and safe dose for the homogenate.Twenty (20) albino mice were utilized in the study. They were starved of food for 18 hr but allowed access to water prior to the study. The study involved two stages. In stage one, the animal were grouped into two (2) groups of fourmice each and were given 0.5 and 1 ml/kg body weight of the fruit homogenate respectively. In the second stage, three groups of four mice received 1.5, 3 and 5 ml/kg body weight of the homogenate respectively. The administration of the homogenate was done orally. The animals were observed for 24 hrfor nervousness, dullness, in-coordination and/or death.

2.2.7 Anti-inflammatory determination using agar-induced rat paw oedema formation

The rat paw oedama method of Winter *et al.* (1962) was used. Increase in the right hind paw volume induced by the sub-plantar injection of 2% agar-agar suspension (Ezekwesili and Nwodo, 2000) was used to assess oedema. Sixteen (16) adult Wistar rats of either sex (120-200 g) were divided into four groups of four animals each. They were fasted and deprived of water for 18hr before the experiment. Deprivation of water was to ensure uniform hydration and to minimize variability in oedematous response (Winter *et al.,* 1963).

The animals were acclimatized to laboratory condition for seven days and randomly divided into four groups of four animals each. The control group (I) received normal saline (5ml/kg b.w), the reference group (II) received Diclofenac (standard drug for anti-inflammation ï 150mg/kg b.w) and group III and IV received 2ml/kg b.w and 4ml/kg b.w of *Cucumis sativus*fruit homogenate respectively. One hour post administration, inflammation of the hind paw was induced by injecting 0.1ml of 2% agar-agar suspension into the sub-plantar surface of the right hind paw. The right hind paw volumes of the rats were taken using volume displacement method immediately before the experiment (zero time) and 1.5, 3 and 5.5 hours after induction. The average oedema at every interval was assessed in terms of differences in volume displacement after injecting the 2% agar suspension and zero time volume displacement of the injected paw (V_tï V_o). The percentage inhibition of oedema was also calculated for each dose using the relation (Perez, 1996);

$$\% \text{ Inhibition of oedema} = 1 - \left(\frac{a-x}{b-y}\right) \times 100$$

Where

a = mean paw volume of treated rats after agar injection

x = mean paw volume of treated rats before agar injection

b = mean paw volume of control after normal saline injection

y = mean paw volume of control before normal saline injection

2.2.8 Biochemical tests

Studies on the effect of the *Cucumis sativus*fruit homogenate on serum biochemical parameters of rats intoxicated with carbon tetrachloride (CCl_4) was carried out. The animals, Wistar albino rats were acclimatized to laboratory condition for seven days and randomly divided into six groups of six animals each. They were maintained under optimal atmospheric and hygienic conditions and allowed access to both feed and water *ad libitum***.** Route of administration was oral with the aid of an oral intubation tube. The groups and doses administered are summarised below.

Group 1: Normal control, received Olive oil (5ml/kg b.w)

Group 2: Received CCl_4 (1.5ml/kg b.w) in olive oil (1:1) every 72hr for 10 days.

Group 3: Received *Cucumis sativus*fruit homogenate (2ml/kg b.w) for 10 days and 1 hr after, was administered with 1.5ml/kg b.w CCl_4 in olive oil (1:1) every 72 hr for 10 days.

Group 4: Received *Cucumis sativus* (4ml/kg b.w) for 10 days and 1hr after, were administered with 1.5ml/kg b.w CCl_4 in olive oil (1:1) every 72 hr for 10 days.

Group 5: Received 100 mg/kg b.w of silymarin (standard drug) and 1hr after, were administered with 1.5ml/kg b.w CCl_4 in olive oil (1:1) every 72 hr for 10 days

Group 6: Received*Cucumis sativus* (4ml/kg b.w) only for 10 days.

At the end of the administration/treatment period, all animals were fasted for 18 hr, then blood samples were collected into centrifuge tubes (non ï heparinised sample bottles) through rectobulba plexus in the eye. Each blood sample was allowed to clot and the serum obtained by centrifugation at 3000 rpm for 10min, to enable a complete separation of the serum from the clotted blood. The clear serum obtained as the supernatant was then carefully aspirated with syringe and needle and used freshly for the assessment of some biochemical and liver function tests.

2.2.8.1 Liver function tests

2.2.8.1.1 Assay of serum alanine aminotransferase (ALT) activity

In-vitro assay of serum ALT activity by the Reitman and Frankel (1957) colorimetric method was carried out using RANDOX Laboratories (Crumlin, United Kingdom) test kit.

Principle: ALT activity is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine. The colour intensity is measured against the blank at 540nm.

Method: The blank and sample test tubes were set up in triplicates. Then, 0.1ml of serum was pipetted into the sample tubes. To each tube was added 0.5ml buffer solution containing phosphate buffer, L-alanine and Ŭ-oxoglutarate. The mixture was thoroughly mixed and incubated for exactly 30 min at 37 ^{0}C. A volume, 0.5ml of reagent containing 2, 4-dinitrophenylhydrazine was later added to each tube while 0.1ml of sample was added to sample blank tube. The tubes were mixed thoroughly and incubated for exactly 20 min at 25 ^{0} C. Five millilitres of sodium hydroxide solution was then added to each tube and mixed. The absorbance was read against the blank after 5 min at 540nm.

Calculation: The activity of ALT expressed in International Units per litre (IU/l) was read up from Table A in Appendix III.

2.2.8.1.2 Assay of serum aspartate aminotransferase (AST) activity

In-vitro assay of serum AST activity by the Reitman and Frankel (1957) colorimetric method was carried out using RANDOX Laboratories (Crumlin, United Kingdom) test kit.

Principle: AST activity is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine. The colour intensity is measured against the blank at 546nm.

Method: The blank and sample test tubes were set up in triplicates. A volume, 0.1ml of serum was pipetted into the sample tubes and 0.5ml of reagent 1 was pipette into both sample and blank tubes. The solutions were thoroughly mixed and incubated for exactly 30 min at 37 ^{0}C. After that, 0.5ml of Reagent 2 containing 2, 4-dinitrophenylhydrazine was added into all the test tubes followed by 0.1ml of the sample into the blank tubes. The tubes were mixed thoroughly and

incubated at 25 ^{0} C for 20 min and 5.0ml of sodium hydroxide solution was then added to each tube and mixed. The absorbance was read against the blank after 5 min at 546nm.

Calculation: The activity of AST was read up from Table B in Appendix III.

2.2.8.1.3 Assay of serum alkaline phosphatase (ALP) activity

Phenolphthalein monophosphate method for the *in-vitro* determination of alkaline phosphatase in serum by Klein *et al.* (1960) and Babson *et al.* (1966) was done using QuimicaClinicaApplicada (QCA, Spain) test kit.

Principle: The principle of this method is based on the reaction of alkaline phosphate and a colourless substrate of phenolphthalein monophosphate, giving rise to phosphoric acid and phenolphthalein which at alkaline pH values, turn pink that can be determined spectrophotometrically.

p-Nitrophenylphosphate + H_2O--------→ ALP ------→ PO_4^{2-} + p-Nitro phenol (pink at pH=9.8)

Method: The blank and sample test tubes were set up in triplicates and 0.05ml of sample was pipetted into the sample test tubes. 0.05ml of distilled water was pipetted into the blank tube. Three milliliters (3.0ml) of substrate was pipetted into each tube respectively, which was then mixed and the initial absorbance taken at 405nm. The stop watch was started and the absorbance of the sample and the blank read again three more times at one min intervals.

Calculation: Alkaline phosphatase activity was calculated as follows:

$$\text{Activity of ALP } (\text{in } \frac{\text{IU}}{\text{L}}) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 30$$

2.2.8.1.4 Determination of serum bilirubin concentration

The colorimetric method based on that described by Jendrassik and Grof (1938) was used for the *in-vitro* determination of total serum bilirubin concentration using RANDOX (Crumlin, United Kingdom) Laboratories kit.

Principle: Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin concentration is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

Method: To 0.2ml of sulphanilic acid solution in a test tube was added 1 drop of sodium nitrite. Then 1ml of caffeine solution and 0.05ml of serum sample were added and mixed thoroughly. The solution was allowed to stand at room temperature for 10 min. Thereafter, 1ml of tartarate solution was added and left to stand at room temperature for 5 min. The absorbance of the solution was read at 578nm against sample blank. The blank solution was made up of all contents subjected to the steps above without sodium nitrite.

Calculation:

$$\text{Total bilirubin Concentration } \left(\frac{\text{mg}}{\text{dl}}\right) = \text{Absorbance of sample} \times 10.8$$

2.2.8.2 Lipid profile tests

2.2.8.2.1 Determination of serum total cholesterol concentration

The*in-vitro* determination of serum cholesterol concentration was done by the method of Abell *et al.,* (1952) using RANDOX Laboratories (Crumlin, United Kingdom) test kit.

Principle: Cholesterol concentration was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase

Method: Three (3) test tubes were set up in a test tube rack and labeled blank, standard and sample respectively. To the blank, was added 10 εl of distilled H_2O, 10εl of standard specimen to the standard test tube and 10εl of sample (serum) to the sample test tube. To each of these test tubes was added 1000εl of the cholesterol reagent. It was thoroughly mixed and incubated for 10min at room temperature (20-25^oC). The absorbance of the sample (A_{sample}) against the blank was taken within 60 min at 500nm.

Calculation:

$$\text{Cholesterol concentration } \left(\frac{\text{mmol}}{\text{L}}\right) = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 5.2$$

2.2.8.2.2 Determination of serum high density lipoprotein (HDL) concentration

Determination of the concentration of the serum total HDL concentration was done, using the method described by Kameswara*et al.* (1999).

Principle: Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the high density lipoproteins (HDL) fraction, which remains in the supernatant is determined.

Method: The precipitant solution 0.1ml was added to 0.3ml of the serum sample and mixed thoroughly and allowed to stand for 15 min. This was centrifuged at 2,000 x g for 15 min. The cholesterol concentration in the supernatant was determined.

Calculation:

$$\text{HDL concentration } \left(\frac{\text{mmol}}{\text{L}}\right) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 5.2$$

2.2.8.2.3 Determination of serum triacylglycerol concentration

Triacylglycerol (TAG) concentration was determined using the method of Tietz (1990).s

Clinical significance: Triacylglycerol measurements are used in the diagnosis and treatment of diseases involving lipid metabolism and various endocrine disorders e.g. diabetes mellitus, nephrosis and liver obstruction.

Principle

The Triacylglycerolare determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

$$\text{Triacylglycerol} + H_2O \xrightarrow{\text{Lipases}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerolkinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPOD}} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminophenazone} + 4\ \text{Chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + HCl + 4H_2O$$

Method: A quantity of the sample (0.1 ml) was pipetted into a clean labeled tube and 1.0 ml of trichloroacetic acid (TCA) was added to it, mixed and then centrifuged at 250 rpm for 10 min. The supernatant was decanted and reserved for use. The assay procedure was carried out as shown in Table 3

Table 3: Serum triacylglycerol concentration reaction medium

S/N	Blank	Standard	Sample
1. Distilled water	0.5	-	-
2. Standard solution (ml)	-	0.5	-
3. TCA (ml)	0.5	0.5	-
4. Supernatant (ml)	-	-	1.0
5. Reagent mixture (ml)	1.0	1.0	1.0

The mixtures were allowed to stand for 20 min at 25 ^{0}C and the absorbance of the sample and standards read against the blank was taken at 540 nm.

Calculation: The concentration of triacylglycerol in serum was calculated as follows:

$$\frac{\text{Absorbanceofsample}}{\text{Absorbanceofstandard}} \times \text{Standardconcentration}(\text{mmol/l}) = \text{mmol/l}$$

2.2.8.2.4 Determination of serum low density lipoprotein (LDL) concentration

Determination of serum LDL concentration was done with the method of Assmann *et al*. (1984) using RANDOX Laboratories (Crumlin, United Kingdom) test kit.

Principle:LDL-C was determined using the following relationship

$$\text{LDL cholesterol } \left(\frac{\text{mmol}}{\text{L}}\right) = \left(\text{Total cholesterol} - \frac{\text{Triacylglycerol}}{2.2}\right) - \text{HDL}$$

2.2.9 Hypotonicity-induced haemolysis of red blood cell

The effect of *Cucumis sativus*fruit homogenate on hypotonicity ï induced haemolysis of red blood cell wasinvestigated using a modification (Ezekwesili and Nwodo, 2000) of the method of Murugesh*et al.*(1981).

Principle

Hypotonicity-induced haemolysis of red blood cells occurs due to osmotically coupled water uptake by the cells, and leads to swelling and subsequently lysis. This results in the release of haemoglobins which absorbs maximally at 418nm. Hence the optical density at 418nmis reflection of haemoglobin concentration. Reflection of the stability of red blood cell membrane is thus measured by changes in optical density, changes in haemoglobin concentration in the medium.

Preparation of erythrocyte suspension

Fresh whole blood (3ml) was collected from healthy volunteer into plastic tubes containing 0.1 volume of 3.8% trisodium citrate and used within 8 hr. The blood sample was centrifuged at 3000 x g for 10min and the supernatant (plasma) discarded. The pellet was washed twice by resuspending it in a volume of normal saline equal to the volume of the supernatant (plasma) and centrifuge at 3000 x gfor 10min.The pellet (0.1ml) was resuspended in 2.5ml of normal saline and used as the red blood cell (RBC).

Procedure

A set of twelve tubes were used for the analysis. The reaction medium is shown in Table 4.

Table 4: Hypotonicity-induced haemolysis of RBC reaction medium

Tube	RBC (ml)	Distilled Water (ml)	Normal Saline (ml)	Fruit Homogenate (ml)	Total Volume
1	0.1	-	1.9	-	2.0
2	0.1	1.0	0.9	-	2.0
3	-	1.0	0.5	0.1	1.6
4	0.1	1.0	0.5	0.1	1.7
5	0.1	1.0	0.5	0.2	1.8
6	-	1.0	0.5	0.2	1.8
7	0.1	1.0	0.5	0.4	2.0
8	-	1.0	0.5	0.4	1.9
9	0.1	1.0	0.5	0.6	1.2
10	-	1.0	0.5	0.6	1.1
11	0.1	1.0	-	-	1.1
12	0.1	1.0	0.5	Indomethacin (0.4mg/ml)	1.6

The reaction medium was incubated at 37^{o}C for 1 hr. After incubation, each of the incubates was centrifuged at 3000xg for 10min to terminate the reaction. The absorptions of the respective supernatants were measured at 418 nm as a measure of extent of haemolysis.The percentage inhibition of haemolysis or membrane stabilization was calculated according to modified method described by Shinde*et al*. (1999).

$$\% \text{ Inhibition of haemolysis} = 100 \times \frac{OD1 - OD2}{OD1}$$

Where:

OD1 = Optical density of hypotonic-buffered saline solution alone

OD2 = Optical density of test sample in hypotonic solution

Blank reaction medium contained 1.2ml normal saline and 0.8ml water.

Scanning at 418nm, 540nm, 570nm and 630nm were done to determine the level of haemoglobin ratio to each of oxy-haemoglobin, deoxy-haemoglobin and met-haemoglobin.

2.2.10Assay of phospholipase A_2 activity

The effect of the homogenate of *Cucumis sativus* fruit on phospholipase A_2 activity was assayed according to the methods of Vane (1971) and Morimoto *et al*.(1979).

Principle

The major action of the phospholipase A_2, an acyl hydrolase, during inflammation is to cleave, from membrane phospholipids, free fatty acids, some of which are precursors of prostaglandins (mediators of inflammation). This activity renders the membrane leaky and the contents of the red blood cells flow out. The absorption measured at 418nm represents total haemoglobin lost from the RBCs. Consequently, changes in OD_{418} reflects on the amount of haemoglobin in the medium and is used to determine leakiness, hence the activity of phospholipase A_2.

Enzyme preparation

The enzyme preparation was obtained from *Bacillus cereus* strain culture. The organism was cultured in nutrient broth for three days. The culture was transferred into a test tube containing normal saline and centrifuged at 3000 x g for 10 min. The cells settled at the bottom while the supernatant contained the exuded enzyme. The supernatant was decanted and used for enzyme assay.

Assay of enzyme activity

Aliquots (0.5ml) of re-suspended erythrocytes were mixed with normal saline containing 2mM calcium chloride and the enzyme preparation and incubated either in the absence or presence of the *Cucumis sativus*fruit homogenate, as shown in Table 5.

Table 5: Reaction medium for assay of phospholipase A_2 activity

HRBC (ml)	Homogenate (ml)	Normal saline (ml)	Enzyme preparation (ml)
0.5	-	2.0	0.2
0.5	0.4	1.5	0.2

0.5	0.6	1.5	0.2
0.5	0.8	1.5	0.2
0.5	1.0	1.5	0.2
0.5	Prednisolone (1ml/mg)	1.5	0.2

The reaction was initiated by the addition of 0.2 ml of the enzyme preparation. The reaction mixture was incubated at 37^0C for 1 hr., and the incubates centrifuged at 3000 x g for 10 min and the absorption of the supernatant was read against the blank (containing the enzyme and the homogenate, but without RBC) at 418nm. Prednisolone, a known inhibitor of the enzyme was used as standard. The percentage inhibition of the enzyme activity was calculated with the relationship:

$$\% \text{ Inhibition} = 1 - \frac{\text{Absorbance of test sample}}{\text{Absorbance of control (Tube without homogenate)}} \times 100$$

2.2.11Assay of prostaglandin synthase activity

Prostaglandin synthase activity was assayed by a modification (Nwodo, 1981) of the methods of Yoshimoto *et al.*(1970) and Flower *et al.*(1973).

Principle

The synthesis of prostaglandins synthase is catalysed by the enzyme prostaglandin synthase which is a microsomal enzyme. This enzyme is responsible for the oxidation of the free fatty acid precursor (arachidonic acid) forming predominantly prostaglandin E_2 (PGE_2) and to a lesser extent prostaglandin F (PGF_2) and prostaglandin D (PGD). Alkaline treatment of the synthesized PGE results in the formation of PGB which absorbs maximally at 278nm.

Isolation of the enzyme-containing fraction

The enzyme - prostaglandin synthase was isolated from beef seminal vesicle by the method of Nugteren*et al.* (1966). The frozen beef seminal vesicle obtained from the local slaughter house (Ikpa Commodity Market, Nsukka) was thawed and freed of fat and adhering connective tissues. A known quantity was weighed out, sliced and homogenized in 5 volumes of sucrose ï EDTA for 1 min at 0.4^0C. The homogenate was centrifuged at 6000 x g for 10 min, the supernatant

decanted. The pellet was centrifuged for 10 min at 15000 x g and the supernatant was again decanted, centrifuged at 18000 x g for 10 min, and the supernatant used as the crude enzyme preparation.

Procedure

The reaction mixture contained 1.5ml cofactor solution (33mM hydroquinone, 21mM glutathione and 40nM haemoglobin, 0.3 ml buffer), 8 mg of enzyme preparation and 0.5 ml arachidonic acid as substrate. After incubating at 37^0C for 2 min, the reaction was stopped by adding 1.5ml of 0.2 M citric acid. The incubates was extracted twice with 5ml ethylacetate and centrifuged at 2,500g for 10 min. Each time, a 4ml aliquots of the top organic layer was pipetted into a clean test tube. The combined ethylacetate extract was evaporated to dryness under a stream of nitrogen gas. The residue was dried overnight in vacuum and then dissolved in 2ml methanol and 0.5ml 3M KOH solution was added to the solution and allowed to stand for 15 min. The absorbance of tests against blank (the blank contained everything in the reaction mixture and a boiled (denatured) enzyme in place of the active enzyme sample at 37^0C) was read at 278 nm. Triplicate determinations were made with the assay mixture containing 0.1, 0.5, 1.0 ml of the fruit homogenate. Indomethacin (0.4mg/ml) was used as standard drug and control.

Enzyme activity

Enzyme activity was quantified using the relationship:

Unit g^{-1} enzyme preparation = $\{(Abs_{278}\, min^{-1} \times 10 \times 2.5 \times 1000) / (25.6 \times 9 \times mg_{\,enzyme}\, test^{-1})\}$

The percentage inhibition of the enzyme activity was calculated with the relationship:

$$\% \text{ Inhibition} = 1 - \frac{\text{Enzyme activity of test sample}}{\text{Enzyme activity of control}} \times 100$$

2.2.12 Histopathological examination

The histopathological examination of the liver was done according to the method of Culling (1975).

A. Slicing, fixation and washing

A thin section of the tissue (about 1 to 2 cm in diameter) was trimmed with a sharp razor blade. Formalin was used as the fixative agent and for the purpose of preservation. The small pieces of the tissues were placed in 10% formalin, the container was shaken gently several times to make

sure that the fluid has reached all surfaces and the pieces were not sticking to the bottom. This was incubated for 24 hours, to allow proper fixing. The fixed tissue pieces were washed with running water for 24 hours to free them from excess fixatives.

B. **Dehydration**

All water was removed from the tissue before embedding the tissue in paraffin. The dehydration was achieved by immersing the thin section of the section of the tissue in automatic tissue processor containing 12 jars.

The first three jars contained 70%, 90% and 99.9% alcohol respectively. This was done to remove the water content in the tissues. The absolute alcohol reduced the shrinking that occurred in the tissue. The time for each step was 30 min. A second change of absolute alcohol was included to ensure complete removal of water. This was achieved in the second three jar of the automatic tissue processor. This is called a well-refining step.

C. Clearing

Xylene solution was used for clearing the tissue sections. This step was achieved in the third three jars of the automatic tissue processor. This was because the alcohol used for dehydration would not dissolve or mix with molten paraffin, the tissue was immersed in xylene solution which was miscible with both alcohol and paraffin for infiltration to take place. Clearing removed opacity from dehydration tissue, making them transparent. A period of 15 min was allowed before the tissue was removed from the solution for infiltration with paraffin.

D. Infiltration with paraffin

The tissue was transferred directly from the clearing to a bath containing paraffin (50 ï 52 ^{0}C). After 30 min to 1 hour incubation in the first bath, the tissue was then removed to a fresh dish of paraffin contained in the fourth set of three jars of the automatic tissue processor for a similar length of time.

E. Embedding (blocking) with paraffin

After tissue infiltration with paraffin, it was allowed to solidify around and within the tissue. The tissue was then placed in a small container already filled with melted paraffin and cooled rapidly with water which embedded the tissue sections.

F. Paraffin sectioning

The embedded blocks were trimmed into squares and fixed in the microtome knives for sectioning after which the sections were floated on a water bath.

G. Mounting

Glass slides were thoroughly cleaned and a thin smear of albumen fixative was made on the slides. After collecting the required section from the rest of the ribbon in the water. The section on the glass slide was kept moist before staining.

H. Staining with haematoxylin

The slides were passed through a series of jars containing alcohol of decreasing strengths and various staining solutions in the following order as shown in Table 6.

Table 6: Histopathological staining with haematoxylin

Reagent	Duration
Xylene	3min
Absolute alcohol	3min
95% Alcohol	2 min
70% Alcohol	2 min
Lugolôs solution	3 min
Running water	3 min
50% Sodium thiosulphate	3 min
Running water	5 min
Delafield haematoxylin	5 min
Running water	3 min
Scott solution	9 min
Running water	3 min

The counterstaining of the tissue with eosin was followed in the order as stated in Table 7.

Table 7: Histopathological staining with eosin

Reagent	Duration
70% Alcohol	1 dip
95% Alcohol	2 dips

Absolute Alcohol	3 min
Absolute Alcohol-Xylene (1:1)	3 ,min
Xylene	3 min

Mounting medium: The section was kept with xylene while cover glass was added on the glass slide.

I. Microscopic observation of slide

The slides prepared were mounted on a photomicroscope, one after the other and were then viewed under different magnification powers of the microscope. Photographs of each of the slides were taken.

2.2.13 Statistical analysis

The results were expressed as means ± SEM and tests of statistical significance were carried out using two-way analysis of variance(ANOVA). The Statistical Product and Service Solutions (SPSS) IBM version 20 was used. $p<0.05$ was considered significant.

CHAPTER FOUR

4.1 DISCUSSION

Fruits are important as essential building blocks of any diet. Not only are they loaded with vitamins and minerals which are essential for healthy living, but they also help as part of a balanced diet. Increased intake of fruits will benefit healthy state of living and will boost immune system, as well as building resistance to common illnesses and infections. Fruits in the daily diet have been strongly associated withreduced risk for someforms of cancer, heart disease, stroke, and other chronic diseases (Hyson, 2002; Goldberg, 2003). In this study, the anti-inflammatory activity and biochemical effects of the homogenate of *Cucumis sativus* fruits were investigated. The study revealed some pharmacological potentials of *Cucumis sativus* fruits in inflammation and diet.

On preliminary phytochemical screening, *Cucumis sativus* fruit homogenate revealed the presence of phenolic compounds, tannins, alkaloids, steroids, saponins, flavonoids, terpenoids, resins and glycosides as major compounds that might have contributed to its antioxidant activity. The quantitative phytochemical analysis showing that the homogenate of *Cucumis sativus* fruits contains alkaloids (2.22 ± 0.96 mg/g), flavonoids (2.14 ± 0.56 mg/g), saponins (2.01 ± 0.08 mg/g), steroids (11.69 ± 1.80mg/g), terpenoids (26.27 ± 1.37 mg/g), resins (50.70 ± 8.82 mg/g), polyphenols (8.51 ± 0.50 mg/g), phenols (7.72 ± 0.50 mg/g), tannins (1.26 ± 0.07 mg/g) and glycosides (32.23 ± 0.41 mg/g) indicates that the homogenate possesses biologically active compounds. Flavonoids in *Cucumis sativus* fruits might be part of the active anti-inflammatory activities of the fruit homogenate.

Flavonoidsô anti-inflammatory activities are due to their inhibitory effects on enzymes involved in the production of the chemical mediators of inflammation and metabolism of arachidonic acid (Oweyele*et al.,* 2005; Metowogo*et al.,* 2008). The presence of flavonoids also suggests that the fruit homogenate has the ability to scavenge free radicals as they are the chief sources of antioxidant (Cook and Shamman, 1996) in plants which have been known to play some role in free radical scavenging.The antioxidant activity of the phenolics, tannins, flavonoid compounds are attributed to their redox properties which can act as reducing agents, hydrogen donors and singlet oxygen quenchers (Gulcin*et al*., 2007). Polyphenolics having hydroxyl groups are very important plant constituents which can protect thebody from different types of oxidative stress

(Jing *et al.,* 2010) such as CCl_4 induced hepatotoxicity.Saponins detected in the fruit, is a known anti-nutritional factor, which reduces the uptake of certain nutrients including glucose and cholesterol at the gut through intra-lumenal physiochemical interactions (Shi *et al.,* 2004). It has been reported to have hypocholesterolemic effects and is thus useful in human diet in controlling cholesterol levels (Ibid). Hence, they may aid in lessening the metabolic burden that would have been placed on the liver during metabolism.The homogenate of *Cucumis sativus* fruits show trace amount of tannins. Tannins have shown to possess some medicinal properties (Ekeanyanwu*et al.,* 2010) although they are anti-nutrients (Doss *et al.,* 2011). This result is in line with the findings of Liener (1994) who stated that lower concentrations of tannins in plants are found to be desirable for human and animal consumption. It could be the reduced amounts of tannins in the homogenate of the fruit that enhanced the protective property rather than the side effects. The phytochemical screening result of this study is contrary to the report of Jony and Roksana (2012) who reported the absence of flavonoids in the ethanol extract of *Cucumis sativus.* It could be that flavonoids were not detected as a result of the extraction method used, as Kumar *et al.,* (2010) reported the presence of flavonoids in the aqueous extract, thus correlating the findings of this investigation. The homogenate of *Cucumis sativus* fruit also revealed the presence of significant amount of chlorophylls a and b. Chlorophyll is important in many plant metabolic functions such as growth and respiration. It is used in medicinal preparation for treating anaemia and hypertension, as a healing agent and in oral hygiene (John, 1955), indicating that *Cucumis sativus* can be used to reduce bad breath and as healing agent.

The proximate analyses show that the homogenate of *Cucumis sativus* fruits have high concentration of moisture and relative amount of fibre, crude protein and ash.Dietary fibre helps to reduce the chance of gastro intestinal problems such as constipation and diarrhoea by increasing the weight, size and wetness of stool (Weickert and Pfeiffer, 2008; Aina*et al.,* 2012). The result of this study is in line with the report that *Cucumis sativus* is useful in fighting constipation, as the fibre content helps to overcome the hypotony which aids constipation (Yohanna, 2013).The result on the high concentration of moisture agrees with the report of Aina*et al.,* (2012), that fleshy fruits have high percentage of moisture which aids in digestion and acts as a solvent in chemical reactions in the body system. The high moisture concentration was in accordance with the report of Egan *et al.,* (1981) and Okoye (2013) which showed the

moisture content of *Cucumis sativus* as 96.4% and 97.8% respectively. The appreciable amount of ash recorded from the study, shows that *Cucumis sativus* fruit homogenate could be recommended as effective sources of mineral nutrients.

The acute toxicity (LD_{50}) test of the homogenate of *Cucumis sativus* fruits did not show any toxic or deleterious effects by oral route up to 5ml/kg body weight indicating that the fruit homogenate is safe for use even at high dose. Volume for weight, 5 ml of the homogenate was equal to 5000 mg of solid contents.

The1, 1-diphenyl-2-picryl hydrazyl(DPPH) radical scavenging activity study shows that *Cucumis sativus* has good antioxidant properties, as there was decrease in the mean absorbance value and increase in the percentage inhibition of DPPH radical scavenging activity with increased amount (ml) of the homogenate. The essence of DPPH method is that the antioxidants react with DPPH (1, 1-diphenyl-2-picryl hydrazyl) and convert it to 1, 1-diphenyl-2-picryl hydrazine with discoloration. The degree of discoloration at 518nm indicates the scavenging potential of the antioxidant as has been used as a measure of the absorbance value (Gupta and Verma, 2010). The reducing properties are generally associated with the presence of reductones (Pin-Der, 1998) whose antioxidant action is based on breaking of the free radical chain by donating one hydrogen atom (Gardon, 1990). Reductones also react with certain precursors of peroxide, thus preventing peroxide formation. The data (Fig 16 and Appendix II) indicated that the absorbance value of the homogenate of *Cucumis sativus* fruits may be due to presence of polyphenols, which may act similar to reductones by donating the electrons and reacting with free radicals to convert them to more stable product and terminate free radical chain reaction; the presence of such compounds in the homogenate of *Cucumis sativus* fruit endows it with anti-oxidant activity, making *Cucumis sativus* a good antioxidant fruit. The antioxidant activity of phenolics (polyphenols) is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Akula and Odhav, 2008). This observation on the DPPH radical scavenging activity of the fruit of *Cucumis sativus* homogenate is in agreement with that Agarwal *et al.*(2012).

In the inflammation test, the homogenate of the fruit-*Cucumis sativus* showed good anti-inflammatory activity, suppressing the rat paw oedema both at the early and later phases of the oedema. The evidence showing significant progressive reduction in the rat paw volume of groups administered with the homogenate of *Cucumis sativus* fruits revealed anti-inflammatory activity. Oedema formation results from the synergistic action of inflammatory mediators such as histamine, serotonin and bradykinin at the site of a local inflammatory insult (Harriot*et al.,* 2004) leading to increased vascular permeability and blood flow. Oedema formation due to agar suspension is a biphasic event. Agar causes inflammation of the rat paw similar to carrageenan (Ezekwesili and Nwodo, 2000; Iwueke, *et al.,* 2006) and the homogenate inhibited the development of paw oedema in the treated animals at 1.5hr, 3hr and 5.5hr post injection of irritant corresponding to the two phases of the inflammatory response.

The early phase of oedema which begins immediately after the administration of the irritant and lasting up to 2hr is probably due to the release of histamine, 5-hydroxyl tryptamine, kinins and serotonin, while the later phase which is from 3hr to 5hr after administration of the irritant is induced by bradykinin, protease, prostaglandins and lysosome (Wallace, 2002; Harriot*et al.,* 2004). Sub-plantar injection of agar suspension into the paw produces oedema resulting from extravasation, increased tissue water and plasma protein exudation along with neutrophil extravasation (Chatpaliwar*et al.,* 2002). The reduction of oedemogenesis in the first phase evinced by the *Cucumis sativus* fruit homogenate in this study suggests that it contains active constituents which inhibit the release or action of the early phase mediators that arrive first at the site of injury thereby reducing vascular permeability, fluid exudation and thus, suppressing oedema. Suppression of oedema in the second phase of inflammation suggests that the anti-inflammatory activity of the fruit may also be due to the inhibition of phlogistic mediators such as prostaglandins, antagonizing their interaction with their respective receptors or it may be due to general mechanism like increasing the membrane stability of the cell or suppression of kinin formation induced by the agar within this period, thereby indicating that *Cucumis sativus* has good anti-inflammatory properties.

Anti-inflammatory activities are commonly possessed by the non-steroidal anti-inflammatory drugs (NSAIDs). These NSAIDs exert anti-inflammatory effect principally by inhibiting the synthesis of prostaglandin (Vane, 1971), an eicosanoid mediator of inflammatory response

(Foegh and Ramwell, 2001). This study correlates the earlier report by Singh-Gill *et al.* (2010) that *Cucumis sativus* exerts anti-inflammatory activity by inhibiting carrageenan induced-rat paw oedema.*Cucumis sativus* anti-inflammatory activity may also be attributed to its concentration of flavonoid, which has been reported to possess potent inhibitory effect on enzymes involved in the production of the chemical mediators of inflammation (Oweyele*et al.,* 2005; Metowogo*et al.,* 2008).

Carbon tetrachloride (CCl_4) challenge caused a marked rise in the serum levels of the liver enzymes alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP), of the rats used in this study demonstrating severe hepatic damage. It also caused elevated levels of total bilirubin, serum low density lipoprotein (LDL), Total cholesterol, Triacylglycerols (TAG), and decreased level of serum high density lipoprotein (HDL), demonstrating oxidative stress. The treatment of the animals with the homogenate of *Cucumis sativus* fruits decreased the CCl_4 induced elevated levels of the liver enzymes and total bilirubin in the serum suggesting that *Cucumis sativus* possesses anti-hepatotoxic and liver protective activities.

Bilirubin, a major breakdown product of heamoglobin rises when there is liver injury or damage leading to the discolouration of the skin known as jaundice. Elevation of total bilirubin which results from decreased uptake of and conjugation of bilirubin by the liver is caused by liver cell dysfunction which is as a result of decreased secretion from the liver (Sanjiv, 2002). Reduction of CCl_4 induced elevated total bilirubin by the homogenate of *Cucumis sativus* fruit showed a protective effect against CCl_4 induced liver toxicity. This fruit perhaps protect the liver by enhancing bilirubin uptake and conjugation by the liver and subsequent secretion into the bile ducts. The *Cucumis sativus* fruit homogenate also assuaged the CCl_4-induced elevated levels of low density lipoprotein, total cholesterol andtriacylglycerols and ameliorated the induced depletion of high density lipoprotein. CCl_4 is a well-established hepatotoxin; inducing liver injury by producing free radicals (Kuriakose and Kurup, 2008). CCl_4-induced liver inflammation and damage can result in locally increased production of free radicals by inflammatory enzymes, as well as the release of inflammatory mediators.

Studies have shown that certain plants with antioxidants activity protect against the CCl_4-induced inflammation and impairment in hepatic function (Fadhel and Amran, 2002; Anosike *et al.,*

2008). The efficacy of any hepatoprotective drug is essentially dependent on its capability of either reducing the harmful effects of a hepatotoxin or of maintaining the normal physiological mechanism that are unbalanced by a hepatotoxin (Hsiao *et al.,* 2003). The presence of flavonoids, tannins, saponins, and terpenoids in the fruit homogenate of *Cucumis sativus* explains its role in hepatoprotection by inhibiting the free radicals mediated damage (Banskata*et al.,* 2000).Takeota and Dao (2003) claimed that flavonoids, triterpens and tannins are antioxidant agents and they interfere with free radicals formation. The mechanism of action of flavonoids involves suppression of a wide range of reactive oxygen, nitrogen and chlorine species formation by inhibition of enzymes or by chelating trace elements involved in free radical production(Mira *et al*.,2002; Halliwell and Whiteman, 2004). Food and fruits rich in flavonoids and other phenolic compounds have been associated with decreased risk of developing inflammatory and other related diseases (Sadik*et al.,* 2003). Though, it is uncertain whether this protective effect is attributable to the phenols or to other agents in the diet (Halliwell*et al.,* 2005), considerable data indicate that increased oxidative damage is associated with and may contribute to the development of all major age-related diseases, and it has been logical to attribute the alleged protective effects of flavonoids to their antioxidant ability (Halliwell*et al*., 2000). By extension, these effects are attributable to *Cucumis sativus* homogenate.

The observations in this study correlates the earlier reports by Gopalakrishan and Kalairasi (2013) that *Cucumis sativus* has significant hepatoprotective effect on paracetamol-induced hepatotoxicity. Dhande*et al.* (2013) also reported the anti-hepatotoxic potential of *Cucumis sativus* on CCl_4-induced hepatotoxicity.

In this study, the homogenate of *Cucumis sativus* fruit attenuated the CCl_4-induced elevated levels of low density lipoprotein (LDL), total cholesterol, and triacylglycerol and increased the level of high density lipoprotein (HDL). The reduction in total serum cholesterol and LDL observed after the administration of the homogenate of *Cucumis sativus* fruit could be attributed to its antioxidant properties and fibre content. Soluble fibres from fruits and vegetables attenuate cholesterol formation. They have cholesterol lowering effect, they bind to bile acids in the small intestines and enhance cholesterol metabolism and excretion (Srinivasin and Sambaiah, 1991).Theuwissen and Mensink (2008) reported that bile acids enhance the re-absorption of cholesterol level into the blood stream, but plant fibres slow the absorption process and increase

the excretion of cholesterol, thereby lowering the body cholesterol level and thus reducing the incidence of cardiovascular diseases. The reduction in total cholesterol observed at the administration *Cucumis sativus* fruit homogenate may also be attributed to the fruit saponins content in the *Cucumis sativus* fruit homogenate. Saponins have been reported to also reduce the uptake of cholesterol in the body by their interaction and binding with bile acids, thereby lowering the bodyôs overall cholesterol levels (Shi *et al.,* 2004).

Thompson and Grundy (2005) reported that phytosterols have a significant hypocholesterolemic effect, cholesterol-lowering potentials and Han *et al.,* 2008 reported the presence of phytosterol in *Cucumis sativus* fruit. Therefore, the cholesterol-lowering effect could be attributed also the presence of phytosterols. Reduction of low density lipoprotein concentration may be due to the antioxidant property of *Cucumis sativus,* that could prevent LDL peroxidation and retard the accumulation (Daugherty *et al.,* 1991), thereby decreasing the risk of DNA oxidative damage through lipid peroxidation. LDL oxidation causes accumulation of fat within the artery walls, thereby clogging up the arteries and increasing the risk of atherosclerosis and cardiovascular diseases (Anthony*et al.,* 1998, Subash*et al.,* 2006). Balanced cholesterol level reduces the incidence of LDL oxidation and the associated risk of atherosclerosis and other related heart diseases. Liver injury causes the accumulation of abnormal amounts of fats, predominantly triacylglycerol in the parenchymal cells into the systemic circulation (Ray *et al.,* 1990). The elevated serum triacylglycerol levels observed might have been partially due lipoprotein lipase. Modest hypertriacylglycerolmia occurs in association with alcohol, virus and drug induced hepatitis (Glickman and Sebesin, 1982). The mechanism of this process may involve inhibition of lipolytic enzymes, hepatic triacylglycerol lipase and lipoprotein lipase (Ray *et al.,* 1990).

The reduction of these enzymes may lead to decreased removal of triacylglycerol from serum and the accumulation of triacylglycerol in the tissues.Significant reduction in the levels of triacylglycerol in serum of *Cucumis sativus* treated rats observed, shown beneficial effect of *Cucumis sativus* fruit homogenate against CCl_4-toxicity. This could be attributed to the action of pectin from the fruit (Sudheesh and Vijayalakshmi, 1999). Pectin, also known as pectic polysaccharide is a structural heteropolysaccharide, rich in galacturonic acid. Sudheesh and Vijayalakshmi, (1999) reported that the oral administration of the pectin extracted from the fruit of *Cucumis sativus*decreased the activities of glucose-6-phosphate dehydrogenase and malate

dehydrogenase while it increased the activities of lipoprotein lipase and plasma LCAT (Lecithin-cholesterol acyltransferase). The depleted levels of serum high density lipoprotein(HDL) in the CCl_4-induced hepatotoxicity rats may be duehypertriacylglycerolmia induced by reactive metabolite formed during metabolism by CYP2E1, CYP2ɓ and possibly CYP3A to form the tri chloromethyl radical, CCl_3 (Castro *et al.,* 1974; Poli, 1993).

The HDL is a free radical scavenger and prevents peroxidation of beta lipoproteins (Chander and Kapoor, 1990). Decreased HDL may be due to diminished lecithin cholesterol acyl transferase (LCAT) activity and may also contribute to increased cholesterol level. HDL transport cholesterol from and other lipids from the tissues back to the liver. A circulating HDL particle acquires its cholesterol by extracting it from cell-surface membrane (Voet *etal.,* 2013). In this study, oral administration of the homogenate of *Cucumis sativus* fruit increased the level of HDL in treated groups comparable to that of the standard drug-silymarin, thereby indicating the antihyperlipidaemic effect of *Cucumis sativus.*

The homogenate of *Cucumis sativus* fruit was found to exhibit high membrane stabilization effect against hypotonicity induced haemolysis of the red cells as is shown by the level of inhibition of haemolysis. Protection against hypotonicity-induced haemolysis is related to membrane stabilization which is an anti-inflammatory index (Ojoghane and Nwodo, 2010). This inhibition of haemolysis was found to be dose dependent, increasing with increased amount of the homogenate in the medium and was comparable with that of indomethacin, a standard anti-inflammatory drug. Hypotonicity-induced haemolysis of human red blood cells (HRBC) occurs due to water uptake by the cells and leads to the release of haemoglobin which absorbs maximally at 418nm.Hence, the reduced optical density at 418nm obtained for the various *Cucumis sativus* test samples was a reflection of the stabilization of the red cell membrane caused by the fruit homogenate. The fruit may also inhibit processes which stimulate or enhance the efflux of intracellular components. The erythrocyte membrane is analogous to the lysosomal membrane (Gandhisan*et al*., 1991; Mounnissamy*et al.,* 2008). Its stabilization implies that *Cucumis sativus* may as well stabilize lysosomal membranes against the release of lytic enzymes.

Lysosomal enzymes play an important role in the development of acute and chronic inflammation. Most of the anti-inflammatory drugs exert their beneficial effects by either

inhibiting the release of the enzymes or by stabilizing the lysosomal membranes (Mounnissamy*et al.,* 2008). Stabilization of lysosomal membranes is important in preventing the leakage of serum protein and fluids into the tissue during the period of increased permeability caused by inflammatory mediators. The anti-haemolytic properties of *Cucumis sativus* fruit homogenate may be due to the presence of some active constituents such as flavonoids, tannins and saponins. It has been reported that flavonoids exert profound stabilizing effects on lysosomes both *in-vitro* and *in-vivo* in experimental animals (Middleton, 1996; David, 2007) while tannins and saponins have the ability to bind cations and other biomolecules, and are able to stabilize the erythrocyte membrane (El-Shanbrany, 1997; Oyedapo, 2001). The high membrane stabilizing activity of the homogenate of *Cucumis sativus* fruitobserved in this study may be due to its flavonoids and tannins contents.

In the scanning at 570 nm (deoxy-haemoglobin) and at 630 nm (methaemoglobin), there was a decrease in the absorbance values. The decrease was more at 630nm (evident from the conversion ratio; Appendix IV:E) indicating that the homogenate was able to repress the conversion of haemoglobin to methaemoglobin, that is due to oxidation of $Fe^{2+}$of haemoglobin to Fe^{3+}. This could be due to the presence some anti-oxidation constituents of *Cucumis sativus.*When haemoglobin is exposed to oxygen, the Fe^{2+} atom of isolated heme is irreversibly oxidized to Fe^{3+}, a form that cannot bind O_2 (Voet *et al.,* 2013). The protein portion of haemoglobin which contains four heme groups in four globin chain prevents the oxidation and makes it possible for O_2 to bind reversibly to the heme group. A distortion in the structural configuration of the heme group (haemoglobin membrane) enhances oxygen irreversible binding. The result of the study indicate that oxygen binding was reversible, thereby confirming the stability of the membrane.

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In this study, the effect of the homogenate on phospholipase A_2 activity was considered. *Cucumis sativus* was highly effective in inhibiting phospholipase A_2 activity. The inhibition of phospholipase A_2 may be either directly or by an action of *Cucumis sativus* on the membrane (Lewis *et al.,* 1979). The latter is more plausible as stabilization has been demonstrated in this investigation. Direct enzyme inhibition is equally probable. The activity of the enzyme was enhanced by calcium ion availability in the medium. Enzyme inhibitory activity may be due to

interference with calcium utilization. Calcium ion is bound to the catalytic site of the enzyme and directs coordination of substrate carbonyl oxygen atom (Robert and Michael, 2009). Phospholipase A_2 cleaves free fatty acid from erythrocyte phospholipids. The enzyme activity assayed using its actionon erythrocyte membrane, creates leakage thus causing haemoglobin to flow out into the medium. Inhibition of phospholipase A_2 implies that the homogenate of *Cucumis sativus* fruit may suppress the mobilisation of free fatty acids from membrane phospholipids. It was reported that anti-inflammatory and immunosuppressive steroids inhibit arachidonic acid and its metabolites (prostaglandins) by induction which inhibits phospholipase A_2 (Schimmer and Parker, 2001; Iwueke*et al.,* 2006), thereby suggesting that *Cucumis sativus* may be an immunosuppresor.

Prostaglandins released from membrane phospholipids in response to hormones and other signals, functionally vary in a tissue-specific manner, but several of them trigger pain, fever or inflammation (Foegh and Ramwell, 2001; Robert and Morrow, 2001). They are synthesized *de novo* by the action of prostaglandin synthase from the free fatty acid precursor, arachidonic acid which is released from membrane phospholipids by the action of phospholipase A_2.In this study, the synthesis of prostaglandin was effectively inhibited by the homogenate of *Cucumis sativus* fruit. Vane (1971) considers inhibition of prostaglandin synthesis as the mechanism by which aspirin-like drugs produce anti-inflammatory effects. By implication, the mobilization of substrate was inhibited, therebyaccounting for decreased availability of the mediators-prostaglandins. The sequential inhibition of prostaglandin synthesis leads to potent suppression of prostaglandins synthesis and possible amplification of the anti-inflammatory activity of the homogenate of *Cucumis sativus* fruit. Thus, the homogenate may exert anti-inflammatory effect by sequential inhibition of phospholipase A_2 and prostaglandins synthase.

Liver histopathology studies indicate that under the present experimental conditions, the homogenate of *Cucumis sativus* fruit showed hepatoprotective effects against carbon tetrachloride induced liver damage in rats. The homogenate showed a sign of protection comparable to that of standard drug-silymarin, as it was evident from the absence of necrosis, space formation. Group I (control) animals treated with olive oil showed normal hepatic cells and normal architecture of the liver; while CCl_4 treated liver showed disarrangement of normal hepatic cells, centrilobular hepatic necrosis, cell infiltration and spotty pyknosis.Literature report

byFishwick*et al*. (1997) indicated that *Cucumis sativus* has a major fatty acid-palmitic acid (23.6 ï 27.5%), such as n-Hexadecanoic acid. It is a haemolytic 5-Ŭ reductase inhibitor (Gopalakrishnan and Kalaiarasi, 2013). It may be responsible for the liver disorder curing effect, thereby indicating that the homogenate of *Cucumis sativus* fruits show a high degree of protection against the hepatotoxic effect of carbon tetrachloride.

4.2 Conclusion

The *Cucumis sativus* (Cucumber) homogenate stabilized erythrocyte membrane, and inhibited inflammation, phospholipase A_2, and prostaglandin synthase activity and it is evident that it exhibited anti-inflammatory activity. Protection against hepatotoxicity by the homogenate was shown by decreased CCl_4-induced elevated levels of the liver enzymes ALT, AST and ALP and of total bilirubin. It was also associated with attenuation ï CCl_4-induced elevation of LDL, total cholesterol and triacylglycerol amounts and ameliorated the induced depletion of HDL. Both sets of observation (inhibition of inflammation and the protection of the liver revealed significant anti-inflammatory potency.

4.3 Suggestions for further studies

- Comparative study of the *Cucumis sativus* leaves and fruits should be carried out to ascertain the part with more effectiveness.
- An activity guided fractionation of the *Cucumis sativus* homogenate should also be investigated to isolate the active constituents of the plant.

REFERENCES

A.O.A.C (2000). Official Methods of Analysis of the Association of Analytical chemists. Washington D.C. 18th Edn. pp. 12-13.

Aba, P. and Mensah-Attipoe, J. (2008). *In-vivo* inhibition of prostaglandin E_2 production by crude aqueous extract of the root bark of *ZanthoxylumXanthoxyloides*. *Ghana Medical Journal,* **42** (2): 85-88.

Abebe, W. (2002). Herbal medication: Potential for adverse interactions with analgesic drugs. *Journal of Chemical Pharmacy Therapy,* **27** (6): 391-401.

Abell, L.L., Levey, B.B., Brodie, B.B. and Kendall, F.E. (1952). Extraction of cholesterol. *Journal of Biological Chemistry,* **195** (1): 357-366.

Agarwal, M., Kumar, A., Gupta, R. and Upadhyaya, S. (2012). Extraction of polyphenol, flavonoid from *Emblicaoffinalis, Citrus limon, Cucumis sativus* and evaluation of their anti-oxidant activity. *Oriental Journal of Chemistry,* **28** (2): 993-998.

Agatemor, M.U., Esekhiagbe, M. and Agatemor, C. (2009). Phenolic content and antimicrobial potentials of *Xylopiaaethiopica*and *Myristicaargentea. Macedonian Journal of Chemistry and Chemical Engineering,* **28** (2): 159-162.

Aggarwal, B.B. and Shishodia, S. (2006). Molecular targets of dietary agents for prevention and therapy of cancer. *Biochemical Pharmacology*, **71**: 1397-1421.

Ahsan, M.R., Islam, K.M., Bulbul, I.J., Musaddik, M.A. and Hague, E. (2009). Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. *European Journal of Scientific Research*, **37**: 302-310.

Aina, V.O., Sambo, B., Zakari, A., Hauwa-Haruna, M.S., Umar, H., Akinboboye, R.M. and Mohammed, A. (2012). Determination of nutritional and anti-nutrient content of *Vitisvinifera*(Grapes) grown in Bomo (Area C) Zaria, Nigeria. *Advanced Journal of Food Science and Technology,* **4**(6): 445-448.

Aitadafoun, M., Mounien, C., Heyman, S.P., Binistic, C., Bon, C. and God-hold, S. (1996). Alkoxybenzamides as new potent phospholipase A_2 inhibitors. *Biochemical Pharmacology,* **51:**737-742.

Akula, U.M. and Odhav, B. (2008). *In vitro* 5-lipoxygenase inhibition of polyphenolics anti-oxidants from undomesticated plants of South Africa. *Journal of Medicinal Plants Research,* **2** (9): 207-212.

Aladesanmi, A.J., Nia, R. and Nahrsteel, A. (1998). New pyrazole alkaloids from the root bark of *NewbouldiaLeavis*.*PlantaMedica,* **64:** 90-91.

Amarowicz, R., Naczk, M. and Shahidi, F. (2000). Antioxidant activity of crude tannins of canola and rapeseed hulls. *Journal of American Oil Chemists' Society*, **77:** 957-961.

Ammar, N.M., Alokbi, S.Y. and Mohammed, D.A. (1997). Study of the anti-inflammatory activity of some medicine edible plants growing in Egypt. *Journal of Islamic Academy of Sciences*,**10:** 1-9.

Amorati, R. and Valgimigli, L. (2012). Modulation of the antioxidant activity of phenolsbynon-covalent interactions. *Organic and Biomolecular Chemistry*,**10**(21): 4147-4158.

Andersen, O.M. (2001). Anthocyanins. *"Encyclopaedia of Life Sciences"*. John Wiley and Sons, Ltd, Singapore, p. 2.

Anosike, C.A. (2010). Comparative studies of the anti-inflammatory and biochemical effects of the methanol extracts of garden egg *(Solanumaethiopicum)* and ginger *(Zingiberofficinale)* in rats, Ph.D Thesis. University of Nigeria, Nsukka.

Anosike, C.A., Obidoa, O., Ezeanyika, L.U.S. and Nwuba, M.M. (2009). Anti-inflammatory and anti-ulcerogenic activity of ethanol extract of ginger *(Zingiberofficinale).African Journal of Biochemistry,***3**(12): 379-384.

Anosike, C.A., Ugwu, U.B., Nwakanma, O. (2008). Effect of ethanol extract of *pyrenacantha staudii* on carbon tetrachloride induced hepatotoxicity in rats. *Biokemistri,* **20** (1): 17-22.

Anthony, M.S., Clarkson, T.B. and Willians, J.K. (1998). Effects of soy isoflavones on atherosclerosis: Potential mechanisms. *American Journal of Clinical Nutrition*,**6**: 1390s -1393s.

Arora, B., Basu, N., Kapoor, V. and Jain, A. (2000). Anti-inflammatory studies on *Curcumialonga* (turmeric). *Indian Journal of Medical Research*, **5**: 249-257.

Ashcroft, G.S., Gillian, S., Yang, X., Glick, A.B., Weinstein, M., Letterio, J.J., Mizel, D.E. and Anzano, M. (1999). Mice lacking Smad3 show accelerated wound healing and impaired local inflammatory response. *Natural Cell Biology,***1**(5): 260-266.

Assmann, G., Jabs, H.U., Kohnert, U., Nolte, W. and Schriewer, H. (1984). Determination of low density lipoprotein (LDL) ï cholesterol. *ClinicaChimicaActa*, **140:** 77-83.

Atanassova, M., Georgieva, S. and Ivancheva, K. (2011). Total phenolic and total flavonoid contents, anti-oxidant capacity and biological contaminants in medicinal herbs. *Journal of the University of Chemical Technology and Metallurgy.***46**(1): 81-88.

Babson, A.L., Greeley, S.J., Coleman, C.M. and Philips, G.E. (1966). Phenolphthalein monophosphate as a substrate for serum alkaline phosphatase. *Clinical Chemistry*,**12**(8): 482-490.

Babu, T.S., Huang, X. and Greenberg, B.M. (2002). Reactive oxygen species mediated toxicity of environmental contamination. *SETAC Globe Archive,***3:** 26-28.

Bagul, M.S., Shrinivas, H., Kanaki, M.S. and Rajani, M. (2005). Anti-inflammatory activity of two ayurvedic formulation containing guggul. *Indian Journal of Pharmacology,* **37:** 299-400.

Banskata, A.H., Tezuka, Y. and Adnyaa, I.K. (2000). Hepatoprotective effect of *Commbretum quadrang, ulare*and its constituents. *Biological Pharmaceutical Bulletin,* **23:** 456-460.

Bahattacharya, P., Lakshmi, S.M., Ashokumar, C.K. and Mandal, S.C. (2005). Conference Proceeding on Promotion and Development of Botanicals with International Coordination School of National Product. *JadavpurKolkota*,pp. 383-385.

Brito-Arias, M. (2007). Synthesis and Characterization of Glycosides. Springer Publishing, New York. p. 12.

Campbell, M. K. and Farrell, S. O. (2012). Biochemistry. USA: Mary Finch.pp. 183-185.

Castro, J.A., De-Ferrlyra, E.C., De-Catro, C.R., Fenoes, O.M., Sosame, H. and Gillette, J.R. (1974). Prevention of carbon tetrachloride-induced necrosis by inhibitor of drug metabolism-further studies on their mechanism of action. *Biochemical Pharmacology,***23:** 295-302.

Chander, R. and Kapoor, N.K. (1990). High density lipoprotein is a scavenger of superoxide anions. *Biochemical Pharmacology,***40:** 1663-1665.

Chatpaliwar, V.A., Johrapurkar, A.A., Wanjari, M.M., Chakraborty, R.R. and Kharkar, V.T. (2002). Anti-inflammatory activity of *Martyniadiandraglox*. *Indiana Drugs,***39:** 543-545.

Cho, E., Seddon, J.M., Rosner, B., Willet, W.C. and Hankinson, S.E. (2005). Prospective study of the intake of fruits, vegetables, vitamins and carotenoids and risk of age-related maculopathy. *Archives of Ophthalmology*,**122**(6): 883-892.

Choi, C.W. (2002). Anti-oxidant and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay guided comparison. *Plant Science,* **163**: 1161-1168.

Chou, C.T. (1997). The anti-inflammatory effect of *Tripterygiumwilfordii*Hook F on adjuvant-induced paw edema in rats and inflammatorymediatorôs release.*Phytotherapy Research*,**11**: 152-154.

Chukwujeku, J.C., Staden, J.V. and Smeth, P. (2005). Antibacterial, anti-inflammatory and antimalarial activities of some Nigerian medicinal plant. *South Africa Journal of Botany,* **71**(4): 316-325.

Contran, R.S., Kumar, V. and Collin, T. (1999). Acute and Chronic Inflammation. In: Robins Pathologic Basic of Diseases. 6th Edn Saunders Company, New York, pp. 50-88.

Cook, N.C. and Shamman, S. (1996). Flavonoids-chemistry, metabolism, cardio protective effects, and dietary sources. *Journal of Nutritional Biochemistry,* **7:** 66-76.

Coussens, L.M. and Werb, Z. (2002). Inflammation and cancer. *Nature*, **420**(6917): 860-867.

Crook, D., Collins, A.J., Bacon, P.A. and Chan, R. (1976). Prostaglandin synthetase activity from human rheumatoid synovial microsomes: Effect of ‘aspirin-like’ drug therapy. *Annals of the Rheumatic Diseases,* **35:** 327-332.

Culling, C.F.A. (1975). Handbook of Histopathological and Histochemical Techniques. 3rd Edn. Butterworths London, Boston Sydney Wallington, Durban Toronto. p. 26.

Cullison, A. E. (1982). Feeds and Feeding. 3rd Edn. Reston, Virginia: Prentice-Hall Company. pp. 54-57.

Dandjesso, C., Klotee, J.R., Dougnon, T.V., Segbo, J.M., Gbaguidi, F., Fah, L., Fanou, B., Loko, F. and Dramane, K. (2012). Phytochemistry and hemostatic properties of some medicinal plant sold as anti-hemorrhagic in Cotonou markets (Benin). *Indian Journal of Science and Technology,* **5**(8): 3105-3109.

Daugherty, A., Zweifel, B.S. and Schonfeld, G. (1991). The effects of probucol on the progression of atherosclerosis in mature watanabe heritable hyperlipidemic rabbits. *British Journal of Pharmacology,* **103:** 1013-1018.

David, S. (2007). Studies force new view on biology of flavonoids. *BioMedical,* **541:** 737-787.

Davies, K.J. (1995). Oxidative stress: The paradox of aerobic life. *Biochemical Society Symposia,* **61:** 1-31.

Dawkins, M.J. (1963). Carbon tetrachloride poisoning in the liver of the new born rat. *Journal of Pathology and Bacteriology,* **85:** 189-196.

Dennis, E.A. (1994). Diversity of group types regulation and function of phospholipase A_2. *Journal of Biological Chemistry,* **269**(18): 13057-13060.

Dhande, S.R., Dongare, P.P., Shah, P.R., Joshi, Y.M. and Kadam, V.J. (2013). Anti-hepatotoxic potential of *Cucumissativus*and *Pogostemon patchouli* against carbon tetrachloride induced hepatotoxicity. *Indo American Journal of Pharmaceutical Research,* **3**(11): 9213-9221.

Doherty, G.M., Lowney, J.K., Mason, J.E., Reznik, S.I. and Smith, M.A. (2002). *Washington Manual of Surgery.* Lippincott Williams and Wilkins, Philadephia, pp. 32-40.

Domitrovic, R., Skoda, M., Marchesi, V.K., Crijanovic, O., Pugel, E.P. and Stefan, M.B. (2013). Rosmarinic acid amehiorates acute liver damage and fibrogenesis in carbon tetrachloride intoxicated mice. *Food and Chemical Toxicology,* **51:** 370-378.

Dorland, D. (1982). Illustrated Medical Dictionary. 25th Edn.W.B Saunders, London,p. 5.

Doss, A., Pugalenthi, M., Valivel, V.G., Subhashini, G. and Anitha, S.R. (2011). Effects of processing technique on the nutritional composition and anti-nutrients content of under-utilized food legume *Canavaliaensiformis*L.D.C. *International Food Research Journal,* **18** (3): 965-970.

Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. (2005). Phytochemical constituents ofsome Nigerian medicinal plants. *African Journal of Biotechnology*, **4**(7): 685-688.

Egan, H., Kirk, R.S. and Swayer, R.R. (1981). Pearsonôs Chemical Analysis of Foods. 8th Edn. Churchill Livingstone, London, pp. 200-514.

Ejele, A.E. and Akujobi, C.O. (2011). Effects of secondary metabolites of *Garcinia kola* on the microbial spoilage of *Cajanuscajan*extract. *International Journal of Tropical Agricultural Food Systems,* **5**(1): 8-14.

Ejele, A.E., Duru, I.A., Ogukwe, C.E. and Iwu, I.C. (2012). Phytochemistry and antimicrobial potential of basic metabolities of *Piper umbellatum, Piper guineense, Ocimumgratissimium*and *Newbouldialeavis*extracts. *Journal of Emerging Trends in Engineering and Applied Sciences,* **3**(2): 309-314.

Ekeanyanwu, R.C., Njoku, O.U. and Ononogbu, I.C. (2010). The phytochemical composition and some biochemical effects of Nigerian Tigernut (*Cyperusesculentus L*) Tuber. *Pakistan Journal of Nutrition,***9** (7): 709-715.

El-Shannbrany, O.A., El-Gindi, O.D., Melek, F.R., Abdel-Khalk, S.M. and Haggig, M.Y. (1997). Biological properties of saponin mixtures of *Fagoniacretica*and *Fagonia mollis. Fitoterapia,***60** (8): 219-222.

Eming, S.A., Krieg, J. and Davidson, J.H. (2007). Inflammation in wound repair. Molecular and cellular mechanisms. *Journal of Investigative Dermatology*, **127**(3): 514-525.

Enoch, S. and Price, P. (2004). Current Concept in General Surgery. A Resident Review Georgetownn, Laidess, Dioserace, pp. 23 - 29.

Eyong, K.O., Folefoc, G.M., Kuete, V., Beng, V.P., Krohn, K., Hussain, H., Nkenyfack, A.E., Saeftel, M., Sarite, S.R. and Hoeranf, A. (2006). Newbouldiaquinone A: A napthoquinoneathraquinone ether coupled pigment, as a potential antimicrobial and antimalarial agent from *Newbouldialeavis. Phytochemistry,* **67**(6): 605-609.

Ezekwesili, C. and Nwodo, O.F.C. (2000). Anti-inflammatory activity of *VigniaUngiculata*seed extract. *Journal of Medical Laboratory Science,***9**: 141-145.

Fadhel, Z.A. and Amran, S. (2002). Effects of black tea extract on carbon tetrachloride induced lipid peroxidation in liver, kidney and testes of rats. *Phytotherapy Research,* **16**(1): 28-32.

Fehervari, Z., Philips, J.M., Dunne, D.W. and Cooke, A. (2005). Parasitic worms and inflammatory disease. *Parasite Immunology,***28**(10): 515-523.

Feirrali, M., Signorni, C.,Ciccolili, L. and Comporti, M. (1992). Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenylhydrazine, divicine and Isouramil. *Biochemical Journal,* **285:** 295-301.

Ferrero-Miliani, L., Nelson, O.H., Andersen, P.S. and Girardin, S.E. (2007). Chronic Inflammation: Importance of NOD2 and NALPZ in interleukin-1ϐ generation. *Clinical and Experimental Immunology*, **147**(2): 227-235.

Fishwick, M.J., Wright, A.J. and Galliard, T. (1997). Quantitative composition of the lipids of Cucumber fruit *(Cucumis sativus).Journal of the Science of Food and Agriculture,***28**(4): 294-398.

Flower, R.J., Cheung, H.S. and Cusham, D.W. (1973). Quantitative determination of prostaglandin and malondialdehyde formed by the arachidonate oxygenase (prostaglandin synthetase) system of bovine serum vesicle. *Prostaglandin*, **4:** 325-341.

Foegh, M.L. and Ramwell, P.W. (2001). The eicosanoids: Prostaglandins, thromboxanes, leukotrienes and related compounds. *In: (B.G. Kartzung Ed.). Basic and Clinical Pharmacology.* 8th edn. Lange Medical Books/McGraw-Hill, New York. pp. 311- 325.

Folin, O. and Wu, H. (1920). Blood Sugar Determination. *Journal of Biological Chemistry,* **41:** 367-374.

Friedman, S.L. (2000). Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *Journal of Biological Chemistry,* **275:** 2247-2250.

Funk, C.D. (2001). Prostaglandins and Leukotrienes: Advances in Eicosanoid biology. *Science*, **294:** 1871-1875.

Furst, D.E. and Munster, T. (2001). Non-steroidal anti-inflammatory drugs, disease-modifying anti-heumatic drugs, non-opoid analgesic and drugs used in gout. In: (B.G. Kartzunged). Basic and Clinical Pharmacology 8th edn. Lange medical Books/McGraw-Hill,New York pp. 596-623.

Gafner, S., Wolfender, J.L., Nianga, M. and Hostettmann, K. (1997). Phenylpropanoid glycosides from *Newbouldialeavis*roots. *Phytochemistry,* **44**(4): 687-690.

Gandhisan, R., Thamaraichelvan, A. and Baburaj, C. (1991). Anti-inflammatory action of *Lanneacoromandelica*HRBC membrane stabilization. *Fitoterapia*, **62**: 81-83.

Gardon, M.F. (1990). The mechanism of antioxidant action *in vitro*. *In: Food Antioxidant,* Hudson, BJF (ed). Elsevier, Applied Science, London, pp.1-78.

Garrison, J.C., (2000). In: The pharmacological basic of therapeutics. Goodman A., Gilman, A., Rall, T.H., Nies. A.S. and Taylor. P. (eds). Pengamon Press, New York, pp. 574-599.

Girloy, D.W., Coluille-Nash, P.R., Wilus, D., Chivers, J., Paul-Clarke, M.J. and Willoughby, D.A. (1999). Inducible cyclooxygenase may have anti-inflammatory properties. *Nature Medicine,***5**(6): 621-622.

Glickman, R.M. and Sebesin, S.M. (1982). Lipid metabolism. *In: I.M. Arias, D. Schachter, H. Popper, D.A. Shafritz (eds), The Liver Biology and Pathobiology.* Raven Press, New York, pp. 123-142.

Goldberg, G. (ed). (2003). Plants: Diet and health. *The Report of a British Nutrition Foundation. Task Force.* Blackwell Science, Oxford U.K., p. 347.

Goodman, A.S., Goodman, L.S. and Gihman, A. (1982). Analgesic, antipyretics and anti-inflammatory agents, Drugs Employed in the Treatment of Gout.6th edn. Macmillan Publishing Co., New York. pp. 682-728.

Gopalakrishan, S. and Kalaiarasi, T. (2013). Hepatoprotective activity of the fruits of *Cucumis sativus* (L.). *International Journal of Pharmaceutical Sciences Review and Research,***20** (2): 229-234.

Gottschalck, T.E. and Breslawec, H.P. (2012). International Cosmetic Ingredient Dictionary and Handbook. Personal Care Product Council. 14th Edn. Washington, D.C., p. 13.

Greenhalgh, D.G. (1998). The role of apoptosis in wound healing. *International Journal of Biochemistry and Cell Biology,* **30**(9): 1019-1030.

Grubben, G.J.H. and Denton, O.A. (2004). Plant Resources of Tropical Africa 2 Vegetables, Leiden, Wageningen, Backhuys Publishers, Netherlands. pp. 48-57.

Gulcin, I., Elmastaκ, M. and Aboul-Enein, H.Y. (2007). Determination of antioxidant and radical scavenging activity of basil (Ocimumbasilicum L. Family Lamiaceae) assayed by different methodologies. *Phytotherapy Research*, **21** (4): 354-361.

Gupta, C. and Verma, R. (2010). Visual estimation and spectrophotometric determination of tannin content and anti-oxidant activity of common vegetable. *International Journal of Pharmaceutical Science and Research*, **2**(1): 175-182.

Guyton, A.C. (1981). Textbook of Medical Physiology, W.B. Saunders Co., Philadelphia 6th Edn. pp. 65-73.

Halliwell, B. and Whiteman, M. (2004). Measuring reactive species and oxidative damage *in-vivo* and in cell culture: How should you do it and what do the results mean? *British Journal of Pharmacology,* **142**(2): 231-225.

Halliwell, B., Gutteridge, J.M.C. and Cross, E.C. (1992). Free radicals, antioxidants and human disease, where are we? *Journal of Laboratory Clinical Medicine*,**119:** 598-602.

Halliwell, B., Rafter, J. and Jenner, A. (2005). Health promotion by flavonoids, tocopherols, tocotrienols and other phenols: Direct or indirect effects? Antioxidants or not? *American Journal of Clinical Nutrition*,**81**: 268S-276S.

Halliwell, B., Zhao, K. and Whiteman, M.L. (2000). The gastrointestinal tract: A major site of antioxidant action? *Free Radical Research*,**33:** 819-830.

Han, J.H., Yang, Y.K. and Feng, M.Y. (2008). Contents of phytosterols in vegetables and fruits commonly consumed in China. *Biomedical and Environmental Sciences*, **21**(6): 449-453.

Harborne, J.B. (1973). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall Ltd, London. p. 279.

Harborne, J.B. (1998). Phytochemical Methods. A Guide to Modern Technology of Plant Analysis, 3rd Edn. Chapman and Hall, New York. pp. 88-185.

Harrawijn, P., Van-Oosten, A.M. and Piron, P.G.M. (2001). Natural Terpenoids as Messengers. A Multidisciplinary Study of Their Production, Biological Functions and Practical Applications. Kluwer Academic Publishers, Netherland. pp. 102-104.

Harriot, M., Marion, E., Martha, A., Wellford, S. and William, A. (2004). Inflammation induced by histamine, serotonine, bradykinin and compound 48/480 in the rat: Antagonists and mechanisms of action. *Journal of Pharmacology and Experimental Therapeutics,***191:** 300-305.

Heldt, H.W. and Heldt, F. (2005). Secondary Metabolites Fulfil Specific Ecological Functions in Plants. *In: Plant Biochemistry, 3rd edn.* Elsevier Academic Press, San Diego CA. pp. 402ï 412.

Hsiao, G., Shen, M.Y., Lin, K.H., Lan, M.H., Wu, L.Y., Chou, D.S., Lin, C.H., Su, C.H. and Sheu, J.R. (2003). Antioxidative and hepatoprotective effects of *Antrodia camphorate* extract. *Journal of Agriculture and Food Chemistry.* **51:** 3302-3308.

Hyman, T.M. and Mark, V. (2006). Ultra metabolism and inflammatory diseases. *Parasite Immunology.* **28**(10): 515-552.

Hyson, D. (2002). The health benefits of fruits and vegetables. *A Scientific Overview for Health Professionals.* Produce for Better Health Foundation, Wilmington DE, p. 20.

Ibegbulem, C.O., Ayalogu, E.O. and Uzohu, M.N. (2003). Phytochemical, anti-nutritional contents and hepatotoxicity of Zobo (*Hibiscus sabdariffa)* drink. *Journal of Agriculture and Food Science.***1**(1): 335-339.

Igboko, A.O. (1987). The anti-inflammatory biflavonoids and other constituents of Garcina kola-Heckel*(Guttiferae)*. Ph.D. Thesis, Pharmacology Department, University of Nigeria, Nsukka.

Insel, P.A. (1996). Analgesic, antipyretic and anti-inflammatory agents and drugs employed in the treatment of rheumatoid arthritis and gout. *In: The Pharmacological Basis of Therapeutics. Goodman A., Gilman, A., Rall, T.U., Nics, A.S. and Taylor, P. (Eds).* New York Pengamon Press; pp. 638-681.

Irvine, F.R. (1961). Woody Plants of Ghana with Special Reference to Their Uses. Oxford University Press, London. p. 81.

Iwu, M.M. (2000). Handbook of Africa Medicinal Plants, CRC Press, London, p.19.

Iwueke, A.V., Nwodo, O.F.C., and Okoli, G.O. (2006). Evaluation of the anti-inflammatory and analgesic activities of *Vitexdoniana* leaves. *African Journal of Biotechnology,* **5**(20): 1929-1935.

Jack, S. (1998). ñAnthocyaninò. *Carnivorous Plant Newsletter (CPN).* Retrieved 6 October 2009, **8:** 3-5.

Jendrassik, L. and Grof, P. (1938). Simplified Photometric methods for determination of bilirubin. *BiochemischeZeitschrift*,**277** (8): 1-9.

Jeruto, P., Mutai, C., Catherine, L. and Ouma, G. (2011). Phytochemical constituents of some medicinal plants used by the Nandis of South Nandi district, Kenya. *Journal of Animal and Plant Sciences*, **9**(3): 1201-1210.

Jiang, D., Fan, J., Yu, S., Chen, S., Luo, Y., Prestwich, G.D. and Mascarenhas, M.M. (2005). Regulation of lung injury and repair by toll-like receptors and hyaluronan. *Nature Medicine,* **11**(11): 1173-1179.

Jing, L.J., Mohamed, M., Rahmat, A. and Bakar, M.F.A. (2010). Phytochemicals, antioxidant properties and anticancer investigations of the different parts of several gingers species

(Besenbergia rotunda, Boesenbergiapulchellavarattenuata and Boesenbergiaarmeniaca). *Journal of Medicinal Plants Research*, **4** (1): 27-32.

John, C.K. (1955). Chlorophyll derivativesï Their chemistry? Commercial preparations and uses. *Economic Botany,***9** (1): 3-38.

Jony, M. and Roksana, A. (2012). Phytochemical screening and *in-vitro* evaluation of reducing power, cytotoxicity and anti-fungal activities of ethanol extract of *Cucumis sativus. International Journal of Pharmaceutical and Biological Archives,* **3**(3): 555-560.

Kameswara, R.B., Kesavulu, M.M. and Giri, C.H. (1999). Anti-diabetic and hypolipidemic effects of *Momordicacymbalania*hook fruit powder in alloxan-induced diabetic rats. *Journal of Ethnopharmacology,* **67:** 103-109.

Karageorgou, P. and Manetas, Y. (2006). The importance of being red when young: Anthocyanins and the protection of young leaves of *Quercuscoccifera*from insect herbivore and excess light. *Tree Physiology,* **26**(5): 613-621.

Kensil, C.R. (1996). Saponins as vaccine adjuvants. *Critical Reviews in Therapeutic Drug Carrier Systems,* **13**: 1-55.

Khattab, R., Goldberg, E., Lin, L. and Thiyam, U. (2010). Quantitative analysis and free- radical scavenging activity of chlorophyll, phytic acid and condensed tannins in canola. *FoodChemistry*, **122**: 1266-1272.

Klein, B., Read, P.A. and Babson, A.L. (1960). Rapid method for the quantitative determination of serum alkaline phosphatase. *Clinical Chemistry,* **6**: 269-275.

Koch, O. Kuiatkowski, D. P. and Udalova, I.A (2002). Genomics of inflammation. *Proceedings of Natural Academy of Science*. 99: 8167-8172.

Kumar, D., Kumar, S., Singh, J., Rashlimi, N., Vashistha, B.D. and Singh, N. (2010). Free radical scavenging and analgesic activities of *Cucumis sativus* L. Fruit extract. *Journal of Young Pharmacist,***2**(4): 365-368.

Kuriakose, G.C. and Kurup, G.M. (2008). Antioxidant activity of *Aulosirafertillisima*on CCl_4-induced hepatotoxicity in rats. *Indian Journal of Experimental Biology.* **46:** 52-59.

Levison, D.A., Reid, R., Bvit, A.D., Harrison, D.A. and Fleming, S. (2008). Muirôs Textbook of Pathology. Edward Arnold Publishers, New York. pp. 54-70.

Lewis, G.P., Piper, P.J. and Vigo, I. (1979). Mechanism of action of anti-inflammatory steroids on membrane fluidity and phospholipase activity. *British Journal of Pharmacology,* **66:** 453-436.

Liener, I.E. (1994). Implications of anti-nutritional components in soybean foods. CRC *Critical Reviews in Food Science and Nutrition,* **34:** 31-37.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, **55**: 275-287.

Malairajan, P., Grrtha, G., Jessi, K. U. and Nurasimban, S. (2006). Analgesic activities of some India medicinal plants. *Journal of Ethnopharmacology,* **119**: 425-428.

Marieb, E.N. and Mitchell, S. (2007). Human Anatomy and Physiology. 7th edn. Pearson Benjamin Cummings publishing company, San Francisco, p 7.

Matthew, H.B., Lucier, G.W. and Fisher, K.D. (1999). Medicinal herbs in the United States: Research needs. *Environmental Health Perspective,* **107:** 773-778.

Mattila, P. and Kumpulairen, J. (2002). Determination of free and total phenolic acids in plants-derived foods by HPLC with diode-array detection. *Journal of Agricultural and Food chemistry,* **50:** 3660-3668.

Maxwell, S.R.J. (1995). Prospects for the use of anti-oxidant therapics. *Drugs,* **49:** 345-361.

McQuibban, G.A., Tam, E.H., McCulloch, C.A., Clark-Lewis, I. and Overall, C.M. (2000). Inflammation dampened by gelatinase. A cleavage of monocyte chemo attractant protein-3. *Science,* **289**(5482): 1202-1206.

Metowogo, K., Agbonon, A., Eklu-Gadegbeku, K., Aklikokou, A.K. and Gbeassor, M. (2008). Anti-ulcer and anti-inflammatory effects of hydro-alcohol extract of *Aloe buettneriA. Berger* (Lilliaceae). *Tropical Journal of Pharmaceutical Research*, **7**(1): 907-912.

Middleton, J.E. (1996). Biological properties of plant flavonoids: An overview.*International Journal of Pharmacognosy,* **34**: 344-348.

Mira, L., Fernandez, M.T., Santos, M., Rocha, R., Florencio, M.H. and Jennings, K.R. (2002). Interactions of flavonoids with iron and copper ions: A mechanism for their antioxidant activity. *Free Radical Research,* **36:** 1199-1208.

Mishra, B.B. and Tiwari, V.K. (2011). Natural products: An evolving role in future drug discovery. *EuropeanJournal of Medicinal Chemistry,* **46**(10): 4769-4807.

Morimoto, S., Kazuna, S. and Kawai, K. (1979). Effects of some anti-rheumatic agents on copper-catalysed thermal aggregation of gamma albumin. *Agents Actions*,**9:** 375-380.

Mounnissamy, V.M., Kavimani, S., Balu, V., Drlin, I. and Quine, S. (2008). Evaluation of anti-inflammatory and membrane stabilizing properties of ethanol extract of *Canjerarehedi. Iranian Journal of Pharmacology and Therapeutics,* **6:** 235-237.

Mukherjee, P.K. (2002). Quality control of herbal drugs: An approach to evaluation of bacterial. *Indian Journal of Ethanopharmacology*, **58:** 207-213.

Murakami, M., Nakakani, Y., Tanioka, T. and Kudo, I. (2002). Prostaglandin E synthase. *Prostaglandins other Lipid Mediate,***68:** 383-399.

Murugesh, N., Vember, S. and Damondaran, C. (1981). Studies on erythrocyte membrane IV: *In-vitro*haemolytic activity of oleander extract. *Toxicology Letters,***8:** 33-38.

National Nutrient Database for Standard Reference, United States Department of Agriculture; Cucumber, 11205.

Nelson, H. and Randy, F. (2005). *An Introduction to Behavioural Endocrinology*. 3rd Edn Sunderland, Mass: Sinauer Associates. p. 100.

Nityanand, P. (1997). Textbook of Feed Processing Technology. Vikas Publishing House PVT Ltd., New Delhi, p. 281.

Nugteren, D.H., Beerthius, R.K. and Vandorp, D.A. (1966). Enzymic conversion of all ï cis ï 8, 11, 14ï eicosatrienoic acid into prostaglandin E. *Recueil Des TravauxChimiques Des Pays-Bas,***85:** 1757-1765.

Nwodo, O.F.C. (1981). Elucidation of the nature of pharmacologically active agents in *Abrus precatorius* seed. Ph.D Thesis, University of London.

Odugbemi, T.O., Akinsulire, O.R., Aibinu, I.E. and Fabeku, P.O. (2007). Medicinal plants used for malaria therapy in Ondo State, Southwest Nigeria. *African Journal Traditional, Complementary and Alternative Medicines,* **4** (2): 191-198.

Oduola, T., Avwioro, O.G. and Ayanniyi, T.B. (2005). Suitability of the lead extract of *Jatrophagossypifolia* as an anti-congulant for biochemical and haematological analyses. *Africa Journal of Biotechnology*, **4**(7): 679-681.

Ojoghane, E. and Nwodo, O.F.C. (2010). Comparison of extracts of *Cyphostemma glaucophilla*on total protein and membrane stabilization. *Journal of Chemical and Pharmaceutical Research,* **2**(4): 31-37.

Okaka, J.C., Enoch, N.C. and Okaka, N.C. (1992). Human Nutrition: An Integrated Approach.ESUT Publications, Enugu, pp. 57-58.

Okoye, N.R. (2013). Proximate analysis and protein solubility of four cucurbits found in Nigeria. *Pakistan Journal of Nutrition,***12** (1): 20-22.

Okpanyi, S.N. and Ezeukwu, G.C. (1981). Anti-inflammatory and anti-pyretic activities of *Azadiractaindica*. *PlantaMedica*, **44**: 34-39.

Okwu, D.E. (2005). ñPhytochemicals, vitamins and mineral contents of two Nigerian medicinal plants.ò *International Journal of Molecular Medicine and Advanced Science,* **1**(14): 372-381.

Oloyede, G.K., Onocha, P.A., Soyinka, J., Oguntokun, O.W. and Thonda, E. (2010). Phytochemical screening, anti-microbial and anti-oxidant activities of four Nigerian medicinal plants. *Annals of Biological Research*, **1**(2): 114-120.

Onwuka, S.K. and Olopade, J.O. (2005). Some aspects of the clinical anatomy of the mandibular and maxillofacial regions of the West African dwarf goat in Nigeria. *International Journal of Morphology,***23**(1): 33-36.

Ostapowicz, G., Fontana R.J., Schiodt, F.V., Larson, A., Davron J.T., Steven, H.B., Timothy, M. and Reish, J. (2002). Results of a prospective study of acute liver failure at 17 tertiary centres in the United States. *Annals of Internal Medicine,***137:** 947-954.

Oweyele, V.B., Oloriegbe, Y.Y., Balogun, E.A. and Soladoye, A.O. (2005). Analgesic and anti-inflammatory properties of *NelsoniaCanescens*leaf extract. *Journal of Ethnopharmacology,* **99:** 153-156.

Oyedapo, O.O. (2001). Biological activity of *Plyllanthusamarus* extracts on pragrow Dawley rats. *Nigerian Journal of Biochemistry and Molecular Biology*, **16**: 83-86.

Palmer, N.S. and Gosh, M.N. (1981). Gastric anti-ulcer activity of (+)-cyanidanol-(3), a histidine decarboxylase inhibitor. *EuropeanJournal of Pharmacology,***69:** 25-32.

Paolo, D.M., Michael, E.M. and Helmut, S. (1991). Anti-oxidation defence system: The role of carotenoids, tocopherol, and thiols. *American Journal of Clinical Nutrition,* **53:** 194-200.

Pari, L. and Kumar, N.A. (2002) Hepatoprotective activity of *Moringaoleifera*on antitubercular drug-induced liver damage in rats. *Journal of Medical Foods,* **5** (3): 171-177.

Patel, T., Robert, L.R., Jones, B.A. and Gores, G.J. (1998). Dysregulation of apoptosis as a mechanism of liver disease: An overview. *Seminars in LiverDisease,* **18:** 105-114.

Pattison, D.J., Silman A.J., Goodson N.J., Lunt, M., Bunn, D., Weich, A., Bingham, S., Khau, K.T., Day, N. and Symmons, D.P.M. (2004). Vitamin C and the risk of developing inflammatory polyarthritis: Prospective case-control study. *Annals of the Rheumatic Diseases,***63:** 843-847.

Pearson, D., (1976). The Chemical Analysis of Foods. 7th ed. London, Churchill Livingstone, pp 3-4.

Peiris, K.P.P., Ashok, B.K., Manjusha, R. and Ravishenkar, B. (2011). Anti-inflammatory and analysis activities of *Dashanasamskarachurna* and its paste form. *Indian Journal of Natural Products and Resources,* **2**(3): 363-368.

Perenz, R.M., Perenz, S., Zavala, M.A. and Salazer, M. (1995). Anti-inflammatory activity of the bark of *Hippocrateaexcelsa*. *Journal of Ethnopharmacology*, **47:** 85-90.

Phillips, D.R. and Morrison, M. (1970). The arrangement of proteins in the humanerythrocyte membrane. *Biochemical and Biophysical Research Communications*, **40:** 284-289.

Pin-Per, D. (1998). Antioxidant activity of budrock*(Arctiumlappa*Linn): Itôs scavenging effect on free radical and active oxygen. *Journal of the American Oil Chemists Society,***75:** 455-461.

Poli, G. (1993). Liver damage due to free radicals. *British medical bulletin,***49:** 604-620.

Priyanka, V. and Rekha, V. (2010). Analgesic, anti-inflammatory and anti-pyretic activity of *Cissusquadrangularis.Journal of Pharmaceutical Sciences and Technology,* **2**(1): 111-118.

Prohens, J. and Fernando, N. (2008). Handbook and plant breeding vegetables 1:*Asteraceae, Brassicaccae, Chenopodicaceac, and Curcumbitacene.* Valencia, Spain. Universidad Politeenica de Valencia. Http://two.springer.com (Accessed Decemer, 2013).

Quasheeh, M. (1937). Quantitative Analysis of Glycoside, Practical Guide. Department of Pharmacognsy, King Staub University, pp. 1-2.

Rageeb, M.U. and Barhate, S.D. (2011). Anti-inflammatory, analgesic and ulcerogenic activity of *VignaMungo* Linn-leaves. *International Journal of Phytopharmacy,***1**(2): 55-60.

Ray, S.D., Sorge, C.L., Raucy, J.L. and Corcoran, G.B. (1990). Early loss of large genomic DNA *in-vivo* with accumulation of Ca^{+} in the nucleus during acetaminophen induced liver injury. *Toxicology and Applied Pharmacology,* **106:** 346-351.

Reinhart, J.M. (1955). Anti-inflammatory effects of flavonoids. *Annals of the New York Academy of Sciences*,**61**: 684 -687.

Reitman, S. and Frankel, S. (1957). A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*. **28:** 56-62.

Robak, J. and Gryglewski, R.J. (1988). Flavonoids are scavengers of super oxide anions. *Biochemistry and Pharmacology*, **37**: 837-841.

Robert, S.R. and Micheal, H.R. (2009). Secretory Phospholipase A_2: A multifaceted family of proatherogenic enzymes. *Current Cardiology,* **11**: 445-451.

Roberts, J.L. and Morrow, J.D. (2001). Analgesic, antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout. I*n: (Eds; A.G. Gilman J.G, Hardman, L.E, Limbird Edition)* 10thEdn, Goodman and Gilmanôs: The Pharmacological Basis of Therapeutics. McGraw Hill Co., New York, pp. 687-731.

Rothe, H., Fautz, R., Gerber, E., Neumann, L., Rettinger, K., Schub, W. and Gronewold, C. (2011). Special aspects of cosmetic spray safety evaluations principles on inhalation risk assessment. *Toxicology Letter,* **205**(2): 97-104.

Sadik, C.D., Sies, H. and Schewe, T. (2003). Inhibition of 15-lipoxygenase by flavonoids: structure-activity relations and mode of action. *Biochemical Pharmacology.* **65:** 773-781.

Samuelsson, B., Morgenstern, R. and Jakobsson, P.J. (2007). Membrane prostaglandin E Synthase- 1: A novel therapeutic agent. *Pharmacological Reviews,* **59:** 207-224.

Sanderson, J.B. (1971). In A System of Surgery. 2nd Edn Edited by T. Holmes, Longmans, Green and Co. London Vol. 5, p. 728.

Sanjiv, C. (2002). The Liver Book: A Comprehensive Guide to Diagnosis, Treatment and Recovery. Atria Company, Ohio, p. 7.

Sato, Y., Ohshima, T. and Kondo, T. (1999). Regulator role of endogenous interleukin -10 in cutaneous inflammatory response of murine wound healing. *Biochemical and Biophysical Research Communications*, **26** (1): 194-199.

Schimmer, B.P. and Parker, K.L. (2001). Adrenocorticotropic hormone: Adrenocortical steroids and their synthetic analogues, inhibitors of the synthesis and actions of adrenocortical hormones. In: (A.G. Gilman J.G, Hardman, L.E, Limbird Edition) 10thEdn, Goodman and Gilmanôs: The Pharmacological Basis of Therapeutics. McGraw Hill Co., New York, pp. 1649-1677.

Sedgwick, A.D., Koh, M.S., Willoughby, D.A. and Peletier, M. (1981). Effects of sera and exudates on models of acute and chronic inflammation. *Agents Actions,* **11:** 477-481.

Seeman, P. (1968). *In:* Gandludasann, R., Thamasalchew, A. and Baburay, S. (1990). Anti-inflammatory actions of *Lanneacoromandelica* by HRBC membrane stabilization. *Fitoterapia,* **5:** 81-83.

Serafini, M., Ghiselli, A. and Ferro-Luzzi, A. (1994). Red wine, tea and antioxidants. *Lancet*, **344**: 626-631.

Serhan, C.N. (2008). Controlling the resolution of acute inflammation: A new genus of dual anti-inflammatory and pro resolving mediators. *Journal of periodontology,* **79**: 1520-1526.

Serhan, C.N. and Savil, B. (2005). Resolution of inflammation: The beginning programs the end. Nature Immunology, **6**(12): 1191-1197.

Serrano, J., Puupponen-Pimia, R., Dauer, A., Aura, A. and Saura-Calixto, F. (2009). Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. Molecular and Nutrition Food Research, 53: 310-329.

Sharmin, R., Khan, M.R.I., Akhter, M.A., Alim. A., Islam, M.A., Anisuzzaman, A.S.M. and Ahmed, M. (2013). Hypoglycemic and hypolipidermic effects of cucumber, white pumpkin and ridge gourd in alloxan-induced diabetic Rats. *Journal of Scientific Research,***5**(1): 161-170.

Shi, J., Arunasala, K., Yeung, D., Kakuda, Y., Mittal, G. and Jiang, Y. (2004). Saponin from edible legumes: Chemistry, processing and health benefits. *Journal of Medicinal Food.* **7** (1): 67-78.

Shinde, U.A., Phadke, A.S., Nair, A.M., Mungantiwan, A.A., Dikshirt, V.J. and Saref, V.O. (1999). Membrane stabilizing activity-a possible mechanism of action for the anti-inflammatory activity of *Cedrusdeodara* wood oil. *Fitoterapia,* **70:** 251-257.

Sies, H. (1997). Oxidative stress: oxidants and antioxidants. *Experimental Physiology*. **82**: 291-295.

Singh, G. (2009). Chemistry of Terpenoids and Carotenoids: Discovery Publishing House, New Delhi. pp. 1-2.

Singh, R., Kumar, S., Rana, A.C. and Sharma, N. (2012). Different models of hepatotoxicity and related liver diseases: A review. *International Research Journal of Pharmacy,***3**(7): 86-95.

Singh-Gill, N., Sood, S., Muthraman, A., Garg, M., Kumar, R., Bali, M. and Dev-Sharma, P. (2010). Antioxidant, anti-inflammatory and analgesic potential of *Cucumis sativus* seed extract. *Latin American Journal of Pharmacy,***29** (6): 927-932.

Sosa, S., Balick, M.J., Arvigo, R., Esposito, R.G., Pizza, C. and Altinier, G. (2002). A screening of the tropical anti-inflammatory activity of some central American plants. *Journal of Ethnopharmacology,* **8:** 211-215.

Srinivasin, K. and Sambaiah, K. (1991). The effect of spices on cholesterol 7-hydroxylase activity and on serum and hepatic cholesterol levels in the rats. *International Journal of Nutrition Research.* **61**: 364-369.

Stvrtinova, V. Jakubovsdy, J. and Hulin, I. (1995). Pathophysiology: Principles of Diseases. Academic Electronis Press, pp. 1-50.

Subash, S., Subramanian, P., Sivaperumal, R., Manivasagam, T. and Mohamed-Essa, M. (2006). Constant light influences the circadian oscillations of circulatory lipid peroxidation, antioxidants and some biochemical variables in rats. *Journal of Biological Rhythm Research,* **37:** 471-477.

Sudheesh, S. and Vijayalakshmi, N.R. (1999). Lipid-lowering action of pectin from *Cucumis sativus*. *Food Chemistry*, **67**(3): 281-286.

Sun, P., Hamagawa, E. and Tsutsui, C. (2001). Evaluation of oxidative stress during apoptosis and necrosis caused by carbon tetrachloride in rat liver. *BiochimicaetBiophysicaActa,* **1535:** 186-191.

Swapnil, S., Sachdev, Y., Gyanendra, S., Sarvesh, P. and Jaya, D. (2012). Laxative activity of *Cucumis Sativus.International Journal Pharmaceutical Sciences Review and Research,* **14**(2): 124-129.

Takeota, G.R. and Dao, L.T. (2003). Antioxidant constituent of almond *(Ptunusdulicis* (mill) D.A. Webb.) hulls. *Journal of Agricultural and FoodChemistry,* **51:** 496-501.

Tanko, V., Kamba, B., Saleh, M.I.A., Musa, K.Y. and Mohammed, A. (2008). Anti-nociceptive and anti-inflammatory activities of ethanolic flower extract of *Newbouldialaevis*in mice and rats. *International Journal of Applied Research in Natural Products,* **1**(13): 13-19.

Tender, P., Jiang, D., Liang, J., Cohn, L., Pure, E., Henson, P.M. and Noble, P.W. (2002). Resolution of lung inflammation by CD44. *Science,* **296**(5555): 155-158.

Theuwissen, E. and Mensink, R.P. (2008). Water-soluble dietary fibres and cardiovascular disease. *Physiological Behaviour.* **94** (2): 285-292.

Thompson, G.R. and Grundy, S.M. (2005). History and development of plant sterol estersfor cholesterol lowering purposes. *American Journal of Cardiology,* **96**: 3-9.

Tietz, N.W. (1990). Clinical Guide to laboratory tests. 2nd Edn. W.B. Saunders Company, Philadelphia, pp. 554-556.

Tirkey, N., Pilkhal, S., Kuhad, A. and Chopra, K. (2005). Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. *BMC Pharmacology*,**5**: 1471-2210.

Trease, G.E. and Evans, W.C. (2002). Textbook of Pharmacognosy. 15th Ed. Saunder Publishers, London. pp. 42-393.

Ubaka, M.C., Ukot, V.C., Okoye, C.T. and Adibe, O.O. (2010). Investigation into the anti-vice activity of aqueous leaf extract of *Aspiliaafricana.Asia Journal of Medicinal Science,* **2**(2): 40-43.

Uematsu, Y., Hirata, K. and Saito, K. (2000). Spectrophotometric determination of saponin in yucca extract used as food additive. *Journal of AOAC International,* **83** (6):1451-1454.

Vane, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *New Nature Biology,* **231**: 232-235.

Vane, J.R. and Botting, R.M. (1996). Mechanism of action of anti-inflammatory drugs. *Scandinavian Journal of Rheumatology*, **25**(102): 9-21.

Voet, D., Voet, J. and Pratt, C. (2013). Principles of Biochemistry, 4th Edn. John Wiley and Sons Inc., Singapore Pte Ltd. pp. 657-667.

Wagner, J.G. and Rothz, A.R. (2000). Neutrophils migration mechanisms with an emphasis on the pulmonary vasculature. *Pharmacology Review,* **52**(3): 349-374.

Wallace, J.M. (2002). Nutritional and botanical modulation of the inflammatory cascade: Eicosanoids, cyclooxygenase and lipoxygenase-as an adjunct in cancer therapy. *Integrated Cancer Therapy,* **1:** 7-37.

Wang, Y.H, Joobeur, T., Dean, R.A. and Staub, J.E. (2007) Cucurbits-Genome Mapping and Molecular Breeding in Plants 5. Vegetables, pp. 375.

Weber, L.W., Boil, M. and Stampfl, A. (2003). Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. *Critical Reviews in Toxicology,* **33:** 105-136.

Weickert, M.O. and Pfeiffer, A.F. (2008). Metabolic effects of dietary fibre consumption and prevention of diabetes. *Journal of Nutrition,* **138**(3): 439-442.

Weissman, G. (1967). The role of lysosomes in inflammation and disease. *Annual Review Medical,* **18:** 97-101.

William, J.J.O.D. and Brigitta, E.E.D. (2010). *Cucumis Sativus L.* Forma hardwickii and feral *Forma sativus*. *Thailand Forest Bulletin,* **38:** 98-107.

Winter, C.A., Risley, E.A. and Nuss, G.L., (1962). Carrageenan-induced oedema in hind limb paw of the rats as an assay for anti-inflammatory drugs. *Proceedings of the Society Experimental Biology and Medicine,* **111:** 544-557.

Winter, E.A., Risley, E.A. and Nuss, G.V. (1963). Anti-inflammatory and anti-pyretic activities of indomethacin. *Journal of Pharmacy and Experimental Therapeutics,* **141:** 369-376.

Wolfe, K., Wu, X. and Liu, R.H. (2003). Antioxidant property of apple peels. *Journal of Agricultural Food and Chemistry,* **51**(3): 609-614.

Yohanna, S. (2013). Healthy living for CEOs and VIPs. Cucumber, 2nd Edition. Snaap Press, Enugu. pp. 40-41.

Yoshimoto, A., Ito, H. and Tomita, K. (1970). Cofactor requirements of the enzyme synthesizing prostaglandin in bovine seminal vesicle. *Journal of Biochemistry,* **68:** 487-499.

Appendices

I. Preparation of reagents

5% (w/v) Ferric chloride solution

A quantity of ferric chloride (5.0g) was dissolved and made up to 100ml with distilled water.

Ammonium solution

A quantity, of the stock concentrated ammonium solution (187.5ml) was diluted in 31.25ml of distilled water and then made up to 500ml with distilled water.

Aluminium chloride solution

Aluminium chloride (0.5g) was dissolved made up to 100ml with distilled water.

Lead sub acetate solution

Fifteen percent lead acetate (i.e. 15.0g of lead acetate in 100ml of distilled water) was mixed with 20ml of absolute ethanol and made up to 100ml with distilled water.

Wagner's reagent

Known quantities, of iodine crystals (2.0g) and of potassium iodide (3.0g) were dissolved in minimum amount of water and then made up to 100ml with distilled water.

Mayer's reagent

A known quantity, of mercuric chloride (13.5g) was dissolved in 50ml of distilled water. Also, 5.0g of potassium iodide was dissolved in 20ml of distilled water. The two solutions were mixed and the volume made up to 100ml with distilled water.

Dragendorff's reagent

A known quantity, of bismuth carbonate (0.85g) was dissolved in 100ml of glacial acetic acid and 40ml of distilled water to give solution A. Another solution called solution B was prepared by dissolving 8.0g of potassium iodide in 20ml of distilled water. Both solutions were mixed to give a stock solution.

Molisch reagent

A quantity, (1.0g) of Ŭ-naphthol was dissolved in 100ml of absolute ethanol.

2% (v/v) Hydrochloric acid

A known quantity, of concentrated hydrochloric acid (2.0ml) was diluted with some distilled water and made up to 100ml.

1% (w/v) Picric acid

A quantity, of picric acid (1.0g) was dissolved in 100ml of distilled water.

Isotonic Sucrose(0.25M) solution, was prepared by dissolving 42.7g sucrose in 500ml of water.

Tris-HCL Buffer (0.02M) P^H 8.0, was prepared by dissolving 2.482g of tris-hydroxymethy amino methane in 1l of deionised water. The P^H was adjusted by the addition of 1M HCL.

Glutathione (2.1mM). This solution was prepared by dissolving 7.67mg of glutathione in 12 ml of phosphate buffer, P^H 7.4.

Hydroquinone (3.3mM). The solution was prepared by dissolving 5.5mg of hydroquinone in 15ml of buffer.

Haemoglobin (0.004mM) was prepared by dissolving 5.0mg of haemoglobin in 15ml of buffer.

Potassium Hydroxide (3M). This solution was prepared by dissolving 28.88g of potassium hydroxide in 250ml of 60% methanol.

Cofactor solution. It is was obtained by adding 1.5ml of hydroquinone solution and 1.5ml of haemoglobin solution to 12ml of glutathione.

II. Absorbance Values

(A): Absorbance and Activity of ALT in the Serum

Absorbance	U/L	Absorbance	U/L
0.03	4	0.28	48
0.05	8	0.30	52
0.08	12	0.33	57
0.10	17	0.35	62
0.13	21	0.38	67
0.15	25	0.40	72
0.18	29	0.43	77
0.20	34	0.45	83
0.23	39	0.48	88
0.25	43	0.50	94

(B): Absorbance and Activity of AST in the Serum

Absorbance	U/L	Absorbance	U/L
0.02	7	0.12	47
0.03	10	0.13	52
0.04	13	0.14	59
0.05	16	0.15	67

0.06	19	0.16	76
0.07	23	0.17	89
0.08	27		
0.09	31		
0.10	36		
0.11	51		

III. Effect of the homogenate of *Cucumis sativus* fruit on agar-induced rat paw oedema.

Groups	**Δ paw volume (oedema) ml and % Inhibition of oedema**		
	1.5 hr	**3.0hr**	**5.5hr**
Control	0.63 ± 0.03	0.63 ± 0.03	0.47 ± 0.12
Standard Drug (Diclofenac, 150mg/kg b.w)	0.13 ± 0.07*** (79.4)	0.03 ± 0.02*** (95.2)	0.03 ± 0.02* (93.6)
2ml/kg of *C. sativus*	0.30 ± 0.06* (52.4)	0.27 ± 0.03*** (57.1)	0.17 ± 0.12 (63.8)
4ml/kg of *C. sativus*	0.17 ± 0.09** (73.0)	0.10 ± 0.06*** (84.1)	0.03 ± 0.02* (93.6)

*Reduction in oedema is significant at p’ 0.05 compared to control, ** = p’ 0.01, *** = p’ 0.001.

Values of oedema shown are mean ± SEM (n = 3). Values in parenthesis ( ) are percentage inhibition of inflammation calculated relative to control

IV. Effect of the homogenate of *Cucumis sativus* fruit on hypotonicity-induced haemolysis of red blood cell.

(A): Descriptive

Tubes	**Sample**	**Volume (ml)**	**Mean Absorbance ± SD 418nm**	**Calculated Difference (^^ - ^)**
1 #	Control	-	0.280 ± 0.026	0.280
2 ##			1.367 ± 0.005	1.367
3 ^	Homogenate	0.1	0.074 ± 0.024	1.219
4 ^^			1.293 ± 0.022	
5 ^^		0.2	0.993 ± 0.020	0.856
6 ^			0.137 ± 0.015	
7 ^^		0.4	0.673 ± 0.025	0.479
8 ^			0.194 ± 0.021	
9 ^^		0.6	0.333 ± 0.015	0.076
10 ^			0.257 ± 0.021	
11 ##	Negative Control	-	1.533 ± 0.015	1.533
12	Indomethacin (0.4mg/ml)	1.0	0.254 ± 0.033	0.254

Description:
= Isotonic
= Hypotonic
^ = Without HRBC
^^ = With HRBC

(B): % Inhibition

Sample	**Volume (ml)**	**Mean OD**	**% Inhibition**
Control	-	1.367	-
Homogenate	0.1	1.219	10.8
	0.2	0.856	37.4
	0.4	0.479*	64.9
	0.6	0.076*	94.4

Indomethacin	1.0	0.254*	81.4

*Reduction in Optical density is significant at p’ 0.05 compared to control.

(C): Scanning at Different Wavelengths

(i) At 540nm (Oxy-heamoglobin)

Tubes	**Sample**	**Volume (ml)**	**Mean OD ± SD**	**Calculated Difference**
1	Control	-	0.16 ± 0.01	-
2		-	1.02 ± 0.00	-
3	Homogenate	0.1	0.04 ± 0.01	1.17
4			1.21 ± 0.03	
5		0.2	0.86 ± 0.03	0.76
6			0.10 ± 0.02	
7		0.4	0.56 ± 0.02	0.4
8			0.16 ± 0.01	
9		0.6	0.30 ± 0.01	0.11
10			0.19 ± 0.00	
11	Negative Control		1.27± 0.01	

(ii) At 570nm (Deoxy-heamoglobin)

Tubes	**Sample**	**Volume (ml)**	**Mean OD ± SD**	**Calculated Difference**
1	Control	-	0.17 ± 0.00	-
2		-	0.95 ± 0.03	-
3	Homogenate	0.1	0.04 ± 0.01	0.69
4			0.73 ± 0.01	
5		0.2	0.51 ± 0.01	0.44
6			0.07 ± 0.01	
7		0.4	0.52 ± 0.01	0.17
8			0.35 ± 0.01	
9		0.6	0.23 ± 0.00	0.03
10			0.20 ± 0.00	

11	Negative Control		1.12± 0.00	

(iii) At 630nm (methaemoglobin)

Tubes	**Sample**	**Volume (ml)**	**Mean OD ± SD**	**Calculated Difference**
1	Control	-	0.15 ± 0.02	-
2		-	0.80 ± 0.02	-
3	Homogenate	0.1	0.02 ± 0.00	0.37
4			0.39 ± 0.02	
5		0.2	0.20 ± 0.01	0.17
6			0.03 ± 0.00	
7		0.4	0.15 ± 0.01	0.08
8			0.07 ± 0.01	
9		0.6	0.09 ± 0.00	0.02
10			0.07 ± 0.01	
11	Negative Control		0.96 ± 0.04	

(D): Summary of scanning

Homogenate (ml)	@ 540 nm	@ 570 nm	@ 630nm
0.1	1.17	0.69	0.37
0.2	0.76	0.44	0.17
0.4	0.40	0.17	0.08
0.6	0.11	0.03	0.02

(E):Ratio

Sample	**SCANNING WAVELENGHT (nm)**	
Homogenate (ml)	**540:570**	**540:630**
0.1	1.69	3.16
0.2	1.72	4.47
0.4	2.35	5.00
0.6	3.66	5.50