CHAPTER ONE

1.0 INTRODUCTION

The gastrointestinal tract is committed to the transformation and absorption of food nutrients and fluids required for optimal physiology functions of the body system (Martinez-Augustin *et al*, 2009). It carries out this vital role majorly through the maintenance of strict balance between the process of fluid transport and the motility of the intestine. Fluid transport is regulated through the control of fluid absorption via intestinal villous epithelial cells and secretion across the intestine via intestinal crypt cells (Martinez-Augustin e*t al*., 2009). The latter process is regulated by both the nervous and hormonal systems (Hansen, 2003). The ENS which mediates nervous regulation releases neurotransmitters that contract or relax gut muscles through their interactions with receptors located on them. Relaxing motor neurons of the ENS releases VIP and NO, while contracting motor neurons releases tachykinins, Ach and 5-HT (Furness, 2007). The autonomic nervous system do not fully regulate motor activity of the ENS, however, they do modulate its activity. The parasympathetic fibers of the ANS send their commands *via* acetylcholine release to accelerate motility. The sympathetic fiber slows down motility through somatostatin, neuropeptide Y (NPY) and norepinephrine (NE). Hormonal mechanism contributes a significant part in the compounded control of GI motility. Receptors involved in hormonal regulation of motility are found on most part of the smooth muscle cells and ENS where they are acted upon by gut hormones such as serotonin and histamine to modify gastrointestinal motility.

Upset of any of these mechanisms of motility and fluid transport processes results in intestinal disorders clinically marked as diarrhea (Vitalis *et al*, 2006).

Diarrhoea is defined as the change in usual bowel movement with features of increased frequency, volume, and water content of stool (Baldi *et al*, 2009). This often results in excessive fluid loss accompanied by electrolytes into the faeces (Chitme *et al*., 2004). Epidemiologists usually classify a frequency of GI transit of 3 stools per day or more as

diarrhea. (Chitme *et al*.,2004) The clinical features associated with diarrhea depend on pathophysiology (Guerrant *et al*., 2001). The pathophysiology of diarrhea could be contagious (Hodges and Gill, 2010), emotional stress (Soderholm and Perdue, 2001), immunity disorders (Schulzke *et al*., 2009), dysfunctional GIT and neurohumoral mechanisms (Vitali *et al*., 2006) Other diseases such irritable bowel syndrome, inflammation and colitis (Baldi *et al.,* 2009), malaria (Gale *et al*., 2007) and diabetes mellitus (Forgacs and Patel, 2011) also features diarrhea. The mechanism causing diarrhea can be imbalance between the absorptive and secretory processes, hypermotility or combination of these mechanisms (Vitalis *et al*, 2006). Diarrhoea resulting from hypermotility is usually characterized by increased movement that transports fluid to colon. This presents fluid loss in the stool (Hope *et al*., 2001). When diarrhea is caused by hypermotility they are not accompanied with loss of blood in the stool.

Diarrhoea is a health problem of large concern especially in developing countries contributing increased child death in Africa. Each year, there are approximately 4 billon cases of diarrhoea world wide leading to 4 million deaths especially among children in this age group (Azubuike and Nkagineme, 2007) In Nigeria, diarrhoea is probably rated first among diseases that kills children around the age of 5 years (Magaji *et al*., 2010).The most accessible modern method of managing diarrhoea is targeted at preventing dehydration and fluid loss through oral rehydration with salts and zinc tablets (Thapar and Sanderson, 2004). In the situation of severe fluid loss additional pharmacological agents such as anti-spasmodic or anti-motility agents are used to prevent further fluid loss (Wynn and Fougere, 2007). Traditionally, diarrhea is managed with the use of herbs. The use of herbs formulation in the diarrhoea management is a regular activity in most part of African nations. These nations still rely on herbal concoction from plants for their health management despite the brilliant progress and discovery in medical sciences (Agunu *et al*., 2005)

The issue of affordability, availability, efficacy and fewer side effects of herbs might justify the continual usage of herbs in the management of diarrhea. Today, rather than standing in the way of herbal medicine, the WHO now encourages researches on plants that prevents diarrhoea diseases (Alta and Mouneir, 2004) It is expected that through scientific research

usual questions that are always raised on the issues of the effective dosage and mechanism of actions of these herbs will be unraveled.

Anacardium occidentale is one of the most common herbs use in managing diarrhea in local community of Nigeria (Etuk, *et al, 2009*) and Africa. *Anacardium Occidentale* (A.O) is a member of the Anacadiacae popularly known as cashew. Scientific reports on the extract has demonstrated its protection against inflammation (Olajide *et al,* 2004), bacterial (Akinpelu, 2001) and diarrheal (Omoboyowa *et al*, 2013). There is an overwhelming evidence of the use of its various parts (leaves, stem, bark, kernel, flowers, fruits and roots) in disease management such as colic and diarrhoea (Leslie, 2005). The bark decoction is particularly used locally in management of severe diarrhoea (Ayepola and Ishola, 2009).

Scientific reports have validated the antidiarhoea effect of the leaves (Udedi *et al*, 2013), gum (Araujo, 2015) and kernel (Omoboyowa *et al*, 2013) of the plant.

However, there is no available information to explain the mechanisms of the antidiarheal effect. The work therefore will like to focus on the effect of the stem bark of *Anacardium occidentale* on the motility of gastrointestinal tract of laboratory rodents.

STATEMENT OF THE PROBLEM

Diarhoea is a leading cause of childhood mortality in Africa. Gut motility is crucial to pathogenesis of diarrhea. Anti-motility drugs are frequently limited by side effects (Henry *et al, 2001)* Going by the folkloric belief that the bark decoction of *Anacardium occidentale* is used locally in management of severe diarrhea (Ayepola and Ishola, 2009) there are no available information that explains this mechanism(s).

JUSTIFICATION

The possibility of inherent anti-motility or anti-spasmodic property in the stem bark of *Anacardium occidentale* and the mechanism(s) of action have been rarely researched and may provide evidence for its use in management of diarrhoea.

AIM

To study the effect of the stem bark extract of *Anacardium occidentale (*AoME*)* on smooth muscle preparations using rat stomach strip preparation (SSP), Guinea pig ileum strip preparation (GPI) and isolated rat uterus strip preparation (IRUS) and to also examine the extract effect on *in-vivo* activity in rats and mice.

SPECIFIC OBJECTIVES

1. To investigate the acute toxicity of AoME
2. To evaluate gastrointestinal activity of methanol extract of the stem bark of *Anacardium occidentale* (AoME)
3. To study the effect of AoME on guinea pig ileum strip preparation (GPI)
4. To investigate the effect of AoME on rats stomach strip preparation (SSP)
5. To study the effect of AoME on isolated rat uterus strip preparation (IRUS)
6. To produce the fractions of AoME
7. To investigate the gastrointestinal activity of fractions of AoMe using *in vitro* and *in vivo* procedure
8. To determine the probable mechanisms of action using functional *in vitro* and *in vivo* procedures.

CHAPTER TWO

2.0 LITERATURE REVIEW

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2.1 TAXONOMY OF *Anacardium occidentale*

Kingdom: Plantae

Division: Magnoliophyta (Flowering plant)

Class: Magnoliospsida

Order: Sapindales

Family: Anacardiaceae

Genus: *Anacardium*

Species: *occidentale*

Botanical name: *Anacadium occidentale* (Evans, 2001)

Local Names: *Anacardium occidentale* has different names in different language groups

English:Cashew

Igbo:Kashu

Hausa:Fisa

Yoruba Name: Kaju (Orwa *et al*., 2009)

2.2 BOTANY

Anacardium occidentale (Cashew) is a branched tree that can grow up to 120 centimeters. The root of a fully grown cashew tree has a huge root that is extended laterally and deep into the soil. Its leaves are simple and glabrous (Orwa *et al*., 2009) Flowers are small, yellow in colour and bisexual (Adeigbe *et al*, 2015). The cashew fruit has a thin skin and yellow flesh that is edible with an acidic and astringent taste. It has smooth grey bark when tender but develops cracks when they are fully grown. The fruit has a nut on top of it. The nut is ash in colour. Inside this nut is a seed that is kidney-shaped.

Fig 2.1: Cashew tree

2.3 HISTORY AND DISTRIBUTION OF CASHEW

Cashew emanated from the North eastern Brazil (Pimentel *et al*., 2009) Portuguese travelers brought it to Asia and Africa. It was from these continents that it found its way to other part of the world. Cashew is now grown in thirty two (32) nations that have adequate humid and warm climatic condition. At present in Africa, cashew is largely grown in Brazil, Ghana, Nigeria Benin republic, and Cote de'ivore. The first plantation of Cashew in Nigeria was in Agege, Lagos State, Nigeria in during the 16th century. Since then it has been distributed to other part of the country; the largest plantation is found in the South West. (Adeigbe *et al.*, 2015)

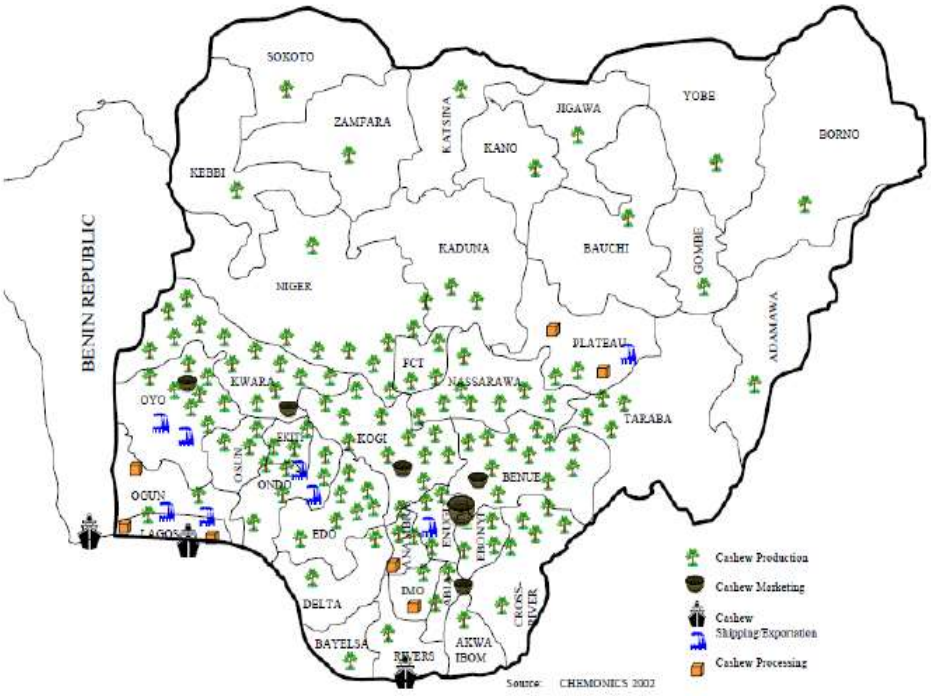

Fig 2.2: Distribution of Cashew In Nigeria (Chemonics, 2002)

2.4 FOLKLORIC USES OF *Anacadium occidentale*

Anacardium occidentale is generally regarded in folk lore system of medicine in tropical countries as a medicinal plant. Different parts of it are used either singly or collectively to manage various type of diseases (Evan, 2001)

2.4.1 The Leaf

The leaf extract are used orally to cure diabetes, skin diseases and diarrhea in Brazil and Thailand and West Indies. (Evan, 2001; Orwa *et al*., 2009)

2.4.2 Cashew seed

The seeds of *Anacardium occidentale* are used as aphrodisiac and to cure impotency. In Columbia, decoction of the seed is consumed orally whereas in Cuba, roasted seed powder is mixed with sugar before oral comsumption. Boiled extract of the seed is also employed in Peru to cure dysentery, inflammation, haemorrhagic conditions and warts (Evan, 2001).

2.4.3 Bark

The bark is used either as hot or cold water extract to treat amenorrhoea, infertily and diabetes (Orwa *et al*., 2001). In Madagascar, the aqueous extract of the bark is taken via oral route to treat dysentery, hypotension and hypoglycemic conditions (Evan, 2001, Orwa *et al*., 2009).

2.4.4 Fruit

The ripe and unripe fruit are usually prepared in hot water to treat several diseases in several countries (Orwa *et al*., 2009). In Guinea, it serves as treatment for haemorrhage, diarrhoea, scurvy and hypotensive conditions (Evan, 2001).The decoction and hot water extract of the dried kernel are also use as aphrodisiac in India. (Orwa *et al*., 2009)

Table 2.1: Some scientific reports published on the stem bark of *Anacardium occidentale*

S/N		Part of the plant	References
1	Mutagenicity and antimutagenicity effect	**Stem bark**	Barcelos, 2007
2.	Anti-inflammatory actions	**the stem bark**	Olajide *et al*, 2004
3.	Sub-chronic Hepatotoxicity	**Inner stem bark**	Ana *et al*, 2003
4.	Hypoglycaemic effect	**Stem bark**	Alexander, 2004

2.5. DIARRHOEA

Diarrhoea is a change in normal GI movement with features of increased volume and frequency of loose stools. Factors causing diarrhea are impaired ions absorption and osmotic load within the intestine (Spiller and Garsed, 2009), increased intestinal movement and decreased motility (Vitali *et al.,* 2006).

Diarrhoeal disease can be classified as those resulting from pathogens and those that are non-infectious. Infectious diarrheoa are responsible for most occurrences nationwide. In infectious diarrhoea, the causative agents are bacterial fungi, virus and parasites (Mathabe *et al.,* 2006). Diarrhoea that is caused by drugs, allergy, toxins, inflammation and food poisons are regarded as non-infectious. These agents activate the release of neurohormonal substances from the enteric nervous system (ENS) such as secretagogues through which they activate mechanisms that manifest as diarrhoea (Wynn and Fougere, 2007).

2.5.1. Classification of Diarrheoa

1. Infectious Diarrheoa

Infectious diarrhea is responsible for the major total occurrence worldwide. The potential causative pathogens in infectious diarrhoea includes bacterial (Mathabe *et al.,* 2006), fungal (Robert *et al*., 2001), viral and parasite pathogens (Brijesh *et al*., 2006).

Bacteria are the most common cause of diarrhea worldwide, with up to 50 per cent of cases in the tropics. Common pathogens include *E. coli.* (Wheeler *et al*., 1999)

Viruses typically affect children, the most important being rotavirus, first discovered in 1973 and now known to be the single most important aetiologic agent of acute diarrhoeal illnesses of infants and young children in industrialised and resource-poor countries. It is estimated that one million cases of fatal diarrhoea worldwide are caused by rotavirus (Gopal *et al*., 2003)

Protozoa are a rarer cause of diarrhoea in the developed world, but are often imported by travellers. *Giardia lamblia, Cryptosporidium parvum* and *Cyclospora cayetanensis* all may cause an acute diarrhoeal episode, but are more commonly responsible for persistent diarrhea (Farthing *et al*., 1996)

2. Non-infectious diarrheoa

Non-infectious diarrhea is due to adverse reactions to drugs, toxins, allergy to food, poisons and acute inflammation which caused the release of secretagogues and some enteric nervous system (ENS) receptors (prostaglandin, serotonin, substance P, vasoactive intestinal peptides, and hormone) in the GIT (Wynn and Fougere, 2007).

3. Acute diarrheoa

Acute diarrhea is mostly caused by enteric pathogenic infections, intoxicants or food allergy. This type of diarrhoea is self-limiting without pharmacological intervention and usually resolves within two week from onset (Baldi *et al*., 2009)

4. Persistent diarrheoa

Persistent diarrhoea mostly result from a secondary cause such as enteric infections or malnutrition, and usually last for more than 14 days (Thapar and Sanderson, 2004).

5. Chronic diarrheoa

Chronic diarrhoea mostly results from congenital defects of digestion and absorption. This usually last for more than 30 days (Thapar and Sanderson, 2004).

6. Watery diarrhea

Watery diarrhoea typically referred to as secretory diarrhoea results from increased chlorine secretion decreased sodium absorption and increased mucosal permeability (Ravikumara, 2008).

7. Osmotic diarrheoa

Osmotic diarrhoea, also a watery form of diarrhoea, is caused by the ingestion of non-absorbable indigestible material or absence of brush border enzymes required for the digestion of dietary carbohydrates (Baldi *et al*., 2009)

8. Inflammatory diarrheoa

Inflammatory diarrhoea is characterized by the presence of mucus, blood, and leukocytes in the stool. It is usually induced by an infectious process, allergic colitis or inflammatory bowel disease (IBD) (Ravikumara, 2008).

2.5.2 Enteric nervous system in diarrhoea

The gut neural network is responsible for the control of propulsive transport and segmental peristalsis in the GIT, as well as secretion and absorption across the intestinal lumen (Bohn

and Raehal, 2006). As a unit, the ENS is a complicated physiological system with autoregulation being carried out by neurotransmitters such as acetylcholine, serotonin, substance P, histamine and endorphin (Farthing, 2003). Diarrhoea can result from the alteration of the smooth muscle contractility and intestinal motility.

2.5.2.1 Smooth muscle contractility

Many agonists and/or antagonists elicit contractility in GIT smooth muscle (longitudinal or circular) through activation of various receptors located within the muscle (Holzer, 2004). In some cases the activation of the smooth muscle receptors by neurotransmitters and inflammatory mediators include reactive oxygen species causes relaxation (spasmolytic) while in other cases, the process lead to increase in spontaneous or induced contraction (spasmogenic). Ionic channel (Ca^{2+} and Cl^{-}) are also known to play important roles in smooth muscle contraction (Giorgio *et al.,* 2007). The stimulation of the receptors on enteric secretomotor pathways caused secretion of anion and fluid secretion into the intestine.

2.5.2.2 Intestinal motility

Abnormal function of the motility of the intestine involves conditions in which the transit of material along the GIT is repetitive and rapid (diarrhoea). It could also be too slow as observes in pseudo- intestinal obstruction and constipation (Giorgio *et al*., 2007). Pathogenic bacteria overgrowth is common as a result of intestinal hypomotility or low transit time which may lead to mucosal inflammation, increased accumulation and absorption of toxins which are known pathophysiology of diarrhea as secretagogue**.** Activation of the macrophage of the immune system by the released cytokines and eicosanoids do occur. The activated macrophages release inflammatory mediators such as PGE2, LTE4, platelet activating factor (PAF), histamine, serotonin, adenosine resulting in cell damage mediated by T lymphocytes or proteases and oxidants generated (H_2O_2, $O2^{\cdot -}$, $OH^{\cdot}$, NO) by mast cells. Some of the inflammatory mediators (PGE2, LTB4, histamine) also serve as secretagogue causing secretory diarrhoea (Field, 2003).

2.6 THE SMOOTH MUSCLE OF THE GUT

2.6.1 Structure

The muscle of the gut is made up of longitudinal and circular layer. The inner circular layer is thicker compared to the outer longitudinal layer. The fibers of the two layers are divided by a laminar septae bundles usually about a millimeter long. They are fixed in a connective tissue matrix made up of collagen and elastic fibril (Olsson and Holmgren, 2001). The actin, thick myosin and desmin are the three myofilaments of the gut muscle. These filaments touch on each other. In the plasma membrane of the muscle fibre are two structures important to smooth muscle contraction. They are the caveolae and the dense bands. The caveolae which is the calcium store exist in groups which are detached from each other by dense bands. The dense bands serve as the attachment for the thin actin filaments. They are joined to the dense bodies by the desmin filaments. Through this connection, power generated by the contractile machinery is transmitted to the entire cell (Daniel *et al,* 2001)

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2.6.2 Electrical properties

When at rest, the electrical potential across the membrane of gut muscle cells oscillates between –40 and –80 mV. This is set up by Na^+-K^+ pump and K^+ channels activities. There are basically three classifications of ion channels situated on the plasma membrane of the smooth muscle of the gut: passive ion-selective, mixed selective ion and gated ion selective channels.

The mixed selective ion channel for both K^+ and Na^+ when activated depolarizes the muscle fibre. The gated ion-selective channels identified in the gut are the gated Ca^{2+} and K^+ channels. These channels generate action potential that maintains rhythmicity of the smooth muscle. The activities of ion-selective channels are controlled by certain neurohumoral agents and membrane potential.

2.6.3 Slow waves

The slow waves of the gut are referred to as basic electrical rhythms. These basic rhythms generate the rhythmicity in gut contractions particularly in the intestine. The frequency, duration and amplitude of slow waves vary in different segment of the gut.

The pacemaker tissues where the slow waves are generated are certain regions located in the circular muscles containing cells known as the interstitial cells of Cajal (ICC) (Huizinga *et al.* 2011*)* Although, the ICC cells are muscle-like, they are different functionally from the muscle fibre. They have large sized nuclei, many surface mitochondriae, caveolae, and endoplasmic reticulum. Each ICC cells are connected to one another and with nerve terminals and muscle cells. They act as pacemakers in GI muscles through initiation of the electrical activity that generate rhythmicity (Vanderwinden, 1999) Receptors for NO, tachykinin, acetylcholine and VIP are found on ICC cells (Camilleri, 2001)

The enteric nervous system inhibitory effect on intestinal motility is carried out by released NO and VIP (Keef *et al.* 2002). Consequently, the slow waves are kept below spike potential. This effect is called neural brake. When the neural brake is removed, the gut smooth muscle becomes excitable and is contracted. When there is loss or damage in ICC cells it usually manifest as abnormal gut motility such as stomach distension, abnormal motility, inflammatory bowel disease and anorectal malformations (Camilleri 2001).

2.6.4 Spike potential

Spike potentials should not be confused with slow waves. They are shorter membrane depolarization when compared to slow waves having duration of 10 to 100 and amplitude of up to 50 mV. When spike potential occurs, the threshold potential must have been reached and are superimpose plateau phase of the slow wave. Spike potential is determined by membrane Ca^{2+} flux (Murphy 1998). Spike potential activities are enhanced by acetylcholine released (Makhlouf, 1995)

2.7 SMOOTH MUSCLE CONTRACTION

Contraction of smooth muscle is usually accompanied by induction of cellular mediators and stimulation of sequestration of calcium through the binding of the agonist on targeted receptors. The transformation of receptor-agonist complex into an intrinsic signal is accompanied step-wise activation of membrane proteins such as cyclic adenosine monophosphate (cAMP), protein phospholipase C and guanosine triphosphate binding. Cyclic adenosine monophosphate activates protein kinase A which affects the cytosolic level of Calcium ion and the sensitivity of contractile machinery to it.

The mechanisms of contraction in the longitudinal muscle layer are not the same to those of the circular layer. IP3 participation is little in longitudinal muscle cell, while DG seems to be involved in circular muscle (Murphy, 1998).

Basically there are two classes of tone in the GI tract. They are the neurogenic and myogenic tones. The neurogenic tone is activated by consistent low stimulation arising from the excitatory innervations while muscle inherent property is responsible for the myogenic tone (Gregersen and Christensen, 2000).

Tonic and rhythmic phasic contractions are two different types of contraction that are manifested in the smooth muscle of the GIT. Both contractions combines together to cause mixing and propulsive movements. However, they seem to be different in terms of their motility function, neurohumoral, electric, and cellular processes that governs them. The mixing and the propulsive movement of the GI content that takes place during fasting and the postprandial states are caused by the action of the rhythmical and phasic contractions of the gut. The progression and the magnitude of these rhythmical and phasic contractions are controlled by the slow waves. On the contrary, occurrence of smooth muscle contraction or increase in tone is solely dependent on the release of neurotransmitters from the motorneurons. Excitatory junction potentials are produced when excitatory neurotransmitter binds on receptors on the muscle leading to increase in muscle contraction. When excitatory transmitters such as acetylcholine and bradykinin bind on their target receptors, they are accompanied by increase in receptor sensitivity to the specific agonist and greater contractions (Sanders *et al.,* 1999). Direction and distance propagated by each phasic contraction is determined by the GI segment over which there is concurrent release of

excitatory neurotransmitter such as acetylcholine and the distances covered by the slow waves (Hansen, 2002).

2.8 GASTROINTESTINAL MOTILITY

Motility is movement observed in the GI wall as a result of sequence of alternating contraction and relaxation of the muscles of the gastrointestinal tract. During the contraction of tract being a tube it closes off such that the inside opening becomes smaller and squeezes on the content. The contraction in this tract is synchronized in manner that foods inside it are transported downstream although occasionally it goes up for brief distances. This is often regarded as peristalsis. At some other times, the motility patterns are oriented in such a manner that the muscles in opposite parts of the tract are squeezed independently of each other. This serves to mix the contents adequately but not moving them (William, 2005).

2.8.1 Motility of the stomach

On functional basis, the stomach divided into the gastric reservoir and pump. The gastric reservoir comprises of stomach fundus and upper region of the stomach body (corpus) while the gastric pump consists of the antrum and the distal portion of the stomach body. In the stomach both peristaltic contraction and slow wave activity are exhibited. In the gastric reservoir only tonic contraction are observed while in the gastric pump both peristaltic contraction and slow wave activity are generated (Hansen, 2003). The motility function of the stomach assist the stomach to play is role of storing, mixing and evacuation of chyme. The two distinct motility patterns that have been observed in the stomach are the postprandial and the interdigestive patterns. In the latter, the tonic pressure of the upper stomach muscle becomes very high. This is not so in the distal part. In the distal stomach, a continuous contraction pattern termed as migrating motor complex (MMC) is observed. The purpose of this complex is to remove debris, secretions and microbes from the stomach during fasting period. After food ingestion the inter-digestive motility pattern is altered to postprandial

pattern. The upper stomach becomes relaxed so as to initially accommodate the food. This is known as receptive relaxation. Thereafter, a tonic contraction arising from this segment moves the food distally where it is mixes and grinded properly by strong and regular peristalsis (Janssen, *et al.,* 2011)

2.8.2 Gastric Emptying

Gastric emptying is initiated during the mixing and digestive period. In the mixing period, the movement of chyme into the intestine is adjusted to a rate that will not exceed digestion and absorption machinery of the gut can cope with. In normal physiological state, 200 Kcal of chyme is emptied from the stomach in an hour. Following the filling of the stomach that stretches its wall, weak peristalsis that originated from the upper part of the stomach corpus spreads distally with accelerated intensity (Kim *et al*, 2005). When this wave reaches the antrum, its intensity becomes maximal. At this stage the pylorus is almost fully contracted. This wave generates a backward movement of the chime until the consistency of chime is achieved. This movement is often regarded as retro-propulsion movement. It is critical for the process of mixing, breaking and homogenization of foods. While this postprandial contractile activity is ongoing, food particles that have fully homogenized are pass through the pylorus (Marti *et al,* 2007) The duration of this motor activity changes (approximately 120 minutes) depending on the volume, physical and chemical nature of the ingested food material. In all, during the period of digestion, the volume of chyme evacuated from the stomach is influenced by the following factors; the volume of ingested meal, gastric secretions, degree of mixing, solubility and the consistency reached. At the end of postprandial period, gastric motility occurs but is entirely regulated by migratory motor complex. The Motor component of the MMC is in three phases. The Phase I account for about sixty percent of the complex. In this phase, few waves are observed, almost no activity with occasional contractile waves that do not translate to propulsion. Phase II account for about 20-30%. During this phase, frequency of wave increases, but not regular and do not result in any propulsive activity. It is only in Phase III of this complex the waves becomes regular and translate to propulsive movement. This kind of contractile waves allows the emptying of indigestible solids remaining in the stomach (Best and Taylor, 2007)

2.8.2.1 Inhibition of gastric emptying

Gastric emptying is regulated in such a manner that intricate reflex (enterogastric reflex) are initiated once the volume of chime leaving the stomach into the first part of the small intestine overwhelms digestive and absorption machinery could handle to reduce it. This reflex is initiated by neural enteric network in the duodenal wall. In addition to inhibition by this nervous mechanism, gastric emptying is inhibited via negative feedback mechanism by certain hormones. The release of these hormones is activated by fat digestion products in the upper intestine. The principal hormones are secretin, the gastric peptide inhibitor and cholecystokinin (CCK) (Alberto and Julio, 2010).

2.8.3 Motility of the small intestine

The circular layer of the small intestine is responsible for the regular basic contractile cycles required for the mixing and segmentation movement of the intestinal content. On the other hand, the longitudinal muscle show less active propulsive abilities; however, it reduces the length of the small intestine promote its transit (Hasler, 1995).

Contractions in the small intestine are largely phasic and are regulated by spike potentials (Kutchai, 1998)

2.9 REGULATION OF SMOOTH MUSCLE ACTIVITY

The regulation of smooth muscle activity and motility patterns of the gut take place at four main levels. They are

1. Enteric nervous system,
2. Autonomic nervous system
3. central nervous system
4. Hormonal influences.

2.9.1 Regulation by enteric nervous system

The enteric nervous system (ENS) has the widest population of nerve cells outside the brain (Liao *et al*, 2008). The ENS regulates gut motility, secretory, motor function and local blood flow (James, 2009). The ENS has sensory neurons that are activated by both chemical and mechanical stimuli. These sensory neurons are connected through interneuron with motor neurons. The neurotransmitters released through this connection stimulate the contraction of gut muscle and blood vessels as well as water and electrolyte secretion by the enterocytes (Furness, 2006). The ENS is subdivided into the Auerbach's (myenteric) and Meissner's (submucosal) plexi. The duos show certain differences in respect to the gut activity they control. Motility is controlled mainly by the myenteric plexus. The latter regulate secretion and blood flow locally. Myenteric plexus connects to neurons of submucous plexus, paravertebral ganglia, muscle layers and their own plexus. Neuron's within the Auerbach's plexus are grouped into two. The first group is VIP and NO containing neurons while the other group contains SP (Hansen, 2003). Receptors upon which neurotransmitters (table 2) released by these neurons binds have been indentify in the gut (Kuiken *et al,* 2002).

Two class of motor neuron have been identified in the ENS. The inhibitory motorneuron inhibit smooth muscle of the gut via PACAP, VIP, and NO while excitatory motornerons activates gut functions through the release of tachykinins, Ach and 5-HT (Kunze and furness, 1999)

Table 2.2: Net effect of neurohormones of ENS origin on GIT contraction *in vivo*

INHIBITORY	STIMULATORY
Neuropeptide Y; (NPY)	Acetylcholine
Pituitary adenylatecyclase activating	Histamine
Polypeptide (PACAP)	Serotonin
Peptide histidine isoleucine (PHI)	Opiods
Peptide YY (PYY)	Bombesin
Nuerotensin	Cholestockinin
Secretin	Gastrin releasing polypeptide (GRP)
Glucagon	Motilin
Somatostatin	Neurokinin A
Galanin	Adenosine
	Thyrotropin-releasing
	hormone (TRH)
	Prostaglandin E2 (PG_2)

2.9.2 Regulation by autonomic nervous system

Extrinsic neurons of the autonomic nervous system influence gut motility indirectly via the connection with the neurons of the Auerbach's plexus.

The parasympathetic fibres increase motility via the release of Acetylcholine (Kunze and Furness, 1999) while sympathetic fibre release majorly norepinephrine and other transmitters such as the neuropeptide Y and somatostatin to slow down motility (Olsson and Holmgren, 2001).

2.9.3 Regulation by central nervous system

The central nervous system is connected to the enteric plexus via the autonomic neurons. Through this axis, information from the receptors are send through the autonomic fibres

(Gaman and Kuo, 2008). The role of the CNS is more of modulation but not fully control gut motility. It has also been observed that hormones such as opiods, bombesin, dopamine, CCK etc released after stimulation of the CNS influence gut motility (Rogers *et al.,* 1995)

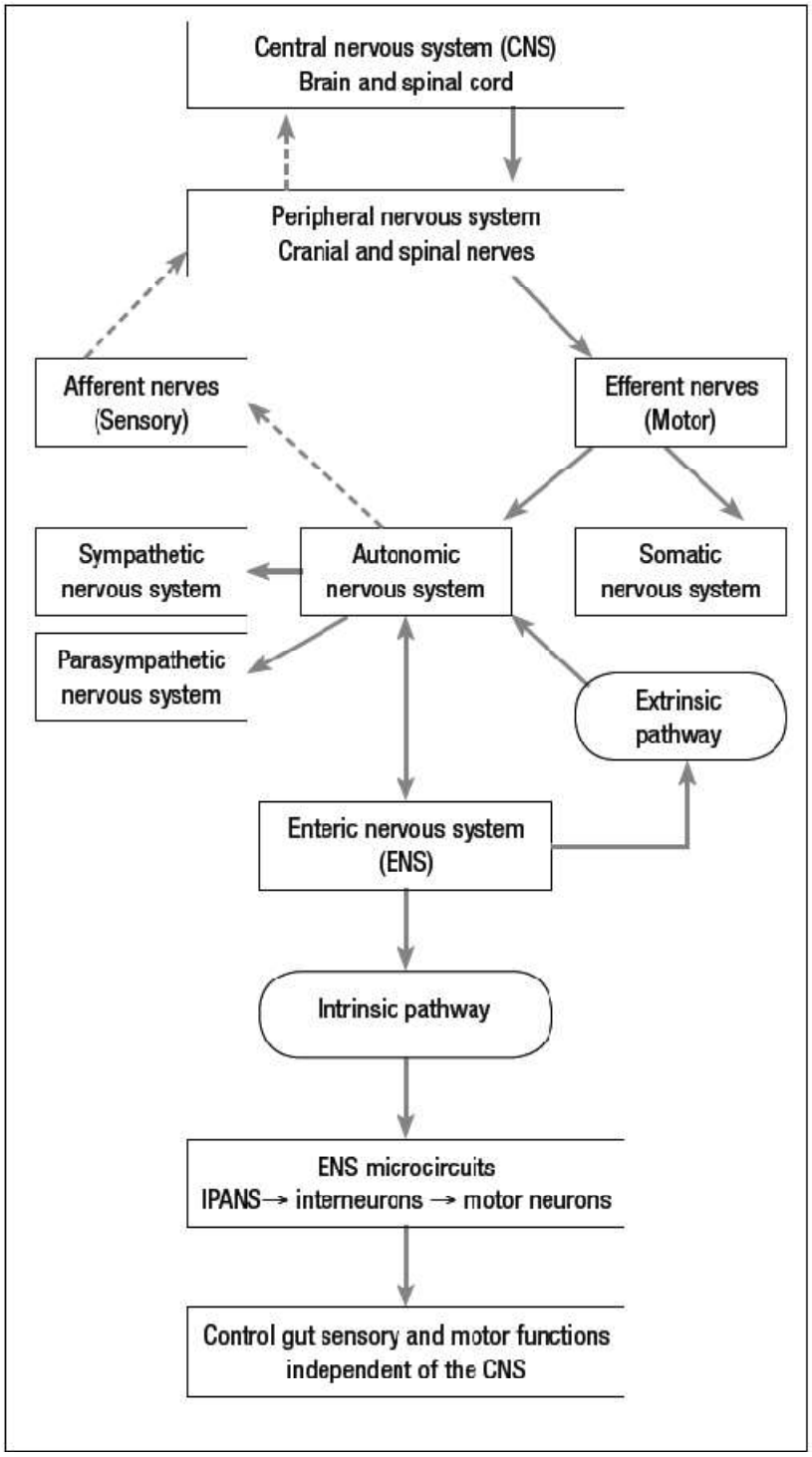


Figure 2.3: Integration of the control of gut motility

2.10 AGONISTS OF SMOOTH MUSCLE CONTRACTION

2.10.1 Acetylcholine

Acetylcholine is distributed widely and acts as a neurotransmitter in the CNS and peripheral nervous system. In the enteric nervous system, acetylcholine is the most common neurotransmitter that induce gastrointestinal tract contractions. In the gastrointestinal tract, acetylcholine is released from excitatory enteric motor neurons that have choline acetyltransferase and vesicular acetylcholine transporter, and mediates immediate smooth muscle contraction (Bornstein *et al*., 2004).

Acetylcholine signaling is mediated by nicotinic acetylcholine receptors and muscarinic acetylcholine receptors on the cell surface. Muscarinic acetylcholine receptors are known to control of many central and peripheral cholinergic responses (Wess, 2004). To date, five different muscarinic receptor subtypes (M1–M5) have been identified and characterized (Wess, 1996), all of which belong to the superfamily of the G-protein coupled receptor. M1, M3, and M5 receptors act preferentially to activate the phospholipase C pathway through selective coupling to G protein Gq/G11, whereas M2 and M4 receptors mainly mediate the inhibition of adenylate cyclase by coupling to G protein Gi/Go (Wess, 1996). Much research, including immunohistochemistry and *in situ* hybridization studies, has shown that muscarinic receptors are present in many organs and tissues (Dorje *et al*., 1991; Eglen *et al*., 1996; Ehlert *et al*., 1997; Levey, 1993; Wess, 2004). The M1 receptor is mainly distributed in the brain, M2 receptor in the brain, heart and smooth muscle organs, M3 receptor in the brain, smooth muscle organs, exocrine glands and eyes, M4 receptor in the brain and lung, and M5 receptor is expressed at rather low levels in both neuronal and non-neuronal cells.

In smooth muscle tissues such as the gastrointestinal tract, the M2 receptor is the major muscarinic receptor subtype, where it is coexpressed with a smaller population of M3 receptors (Eglen *et al*., 1996; Ehlert *et al*., 1997; Levey, 1993). In the gastrointestinal tract, more than 70% of muscarinic receptors are composed from the M2 receptor and about 20% of muscarinic receptors are from the M3 receptor. From a molecular study using the RT-PCR technique, both smooth muscle cells and interstitial cells of Cajal expressed both M2 and M3 receptors (Epperson *et al*., 2000). Interstitial cells of Cajal are the third cell types different

from smooth muscle cells and enteric neurons in the gastrointestinal musculature and are involved in muscle contraction and neuronal modulation of contraction.

2.10.2 Histamine

In the gut, histamine regulated and augments neuronal activities. Aside this role, it mediates inflammatory and allergic responses in the gastrointestinal tract (Dy and Schneider, 2004).

The major source of histamine in body is entero chromaffin-like cells. Other sources are gastric mucosa, parietal cells, basophils, mast and cells (Shahid *et al.*, 2009). In human GI tract, Four (4) histaminergic receptors (H1-H4) have been identified through which histamine carries out its actions (Byung *et al.*, 2013)

H1 receptors have been identified in several areas such as epithelial enterocytes, smooth muscle layer, immune and ganglion cells .The expression of H2 receptors are on parietal (within the mucosal of the fundus), epithelial and ganglionic cells (myenteric region) (Sander *et al*, 2006). The H3 receptors are assumed to be situated on the Meissner's neurons of human intestine (Breunig *et al.*, 2007)

Histamine effects on the motility of GI are intricate comprising of direct (neurally-mediated) and indirect effects. These biological effects are mediated by specific receptor subtype. The H1 receptor mediates smooth muscle contraction while H2 receptor is responsible for both relaxation and direct contraction of smooth muscle cells. The H3 receptor mediates both contractile and inhibitory effects. However, this inhibitory action is masked such that the contractile effect dominates. (Byung *et al*, 2013)

2.10.3 SEROTONIN

Serotonin is widely recognized for its actions in the GI tract as hormone, paracrine, autocrine, and endocrine agent. Aside this role, it promotes the growth central nervous system) and also mediate its activities (Gershon, 2013)

The biological effect of 5-HT are carried out by 15 distinct receptors differentiated into seven different subclasses. These receptors have been discovered in mammals. Six out of the seven subclasses are receptors that are linked to G-protein (Hannon and Hoyer 2008). Those

expressed and identified on the intestine are five classes of receptor. The 5-HT_3 and 5-HT_4 have been well-researched among these classes with respect to GI motility (Mawe and Hoffman 2013). The 5-HT_4 is found on presynaptic neurons while 5-HT_3 is situated on the myenteric and sensory neurons (Gershon 2004).

The binding of released 5-HT to its receptors initiate GI motility. Binding of 5-HT to 5-HT_3 results in the stimulation of postsynaptic potentials that initiate peristalsis in the gut (Hoffman *et al.* 2012). Activation of 5-HT_4 receptor augments an ongoing peristalsis and do not initiate new ones (Pan and Galligan *et al.,* 1994)

Serotonin (5-hydroxytryptamine; 5-HT) is best known as a neurotransmitter critical for central nervous system (CNS) development and function (Kepser and Homberg 2015; Brummelte *et al.* 2016). 95% of the body's serotonin, however, is produced in the intestine where it has been increasingly recognized for its hormonal, autocrine, paracrine, and endocrine actions (Gershon 2013).

Despite this historical presence in animals, 5-HT wasn't identified until the 1940s (Erspamer 1940) and its vast roles in gastrointestinal (GI) function have begun to be elucidated relatively recently. Intestinal 5-HT has been found to modulate enteric nervous system (ENS) development and neurogenesis, motility, secretion, inflammation, sensation, and epithelial development (Gershon 2013; Margolis *et al*., 2016; Hoffman *et al*., 2012)

5-HT synthesis begins with its precursor amino acid L-tryptophan that is converted by the rate-limiting enzyme tryptophan hydroxylase (TPH), to 5-hydroxytryptophan (5-HTP). 5-HTP is then converted by aromatic L-amino acid decarboxylase to 5-HT (Gershon 2013; Gershon and Tack 2007) There are two different isoforms of TPH that are separate gene products, tryptophan hydroxylase 1 (TPH1) and TPH2. In the intestine, TPH1-dependent 5-HT biosynthesis occurs in the enterochromaffin cells of the mucosal epithelium and in mast cells of mice and rats, and accounts for 90% of intestinal 5-HT production (Gershon and Tack 2007; Walther and Bader, 2003)

Once 5-HT is released, it then binds to specific receptors to initiate gut motility; the 5-HT_3 and 5-HT_4 receptors have been the most widely studied in regard to gut motility (Mawe and Hoffman 2013). 5-HT_3 antagonists and 5-HT_4 agonists both evoke peristaltic reflexes (Bertrand *et al.,* 2000). Both are present on neurons within the myenteric and submucosal plexuses of the ENS, intrinsic and extrinsic sensory neurons, and EC cells. The stimulation of 5-HT_3 receptors results in the activation of intrinsic and extrinsic afferent nerves and also the stimulation of a small number of excitatory postsynaptic potentials (EPSPs) (Paintal 1973; Hillsley and Grundy 1998; Hillsley *et al.*, 1998). 5-HT_4 receptor agonists augment the peristaltic reflex pathways by acting presynaptically on nerve terminals to enhance the release of acetylcholine (Fang *et al*.. 2008). By acting in this manner, 5-HT_4 receptor agonists are thought to enhance naturally occurring reflex activity rather than to generate neurotransmission (Hoffman *et al*., 2011). Further, colonic epithelial cells also express the 5-HT4 receptor where they have been demonstrated to activate 5-HT release, mucus discharge from goblet cells, and chloride secretion by enterocytes (Hoffman, *et al*.,2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. ANIMALS

Adult Wistar albino rats (160 -174 g) and Mice (15-21 g) used for this study were procured from the Animal House, University of Ibadan, Nigeria. While the Adult guinea pig (300-350 g) were purchased from the market. A good environment was provided for the animals and allowed to adjust to a new environmental condition for a period of ten (10) days. They were maintained under standard laboratory conditions (12 h light/dark cycle, temperature 22±2 ◦C). Standard pellet food and water were available *ad libitum*. All experimental protocols using animals were performed according to the "Principles of Laboratory Animal Care" (National Institute of Health publication No. 85-23) (1996).

3.2. CHEMICALS AND DRUGS

The drugs, chemicals and reagents of analytical grade employed in this work are the following:

Methanol, ethylacetate, hexane, potassium chloride; sodium bicarbonate, calcium chloride, sodium phosphate, sodium chloride, D-glucose, Magnessium chloride, distilled water, acetylcholine, histamine, serotonin, atropine, L-NAME, oxytocin, carbachol, loperamide, metoclopramide, ,activated charcoal, gum acacia, castor oil, phenol red, carboxy methylcellulose, hexamethonium, sodium hydroxide, tetraoxosulphate (vi) acid, potassium hydroxide, sodium hydroxide, activated charcoal , iodine, potassium iodide, ferric chloride, ammonium and nitric acid.

3.3. EQUIPMENT

Force transducer (model 7004; Ugo Basile varese, Italy) connected to Data capsule acquisition system model 17,400, Microdyanomometer (ugobasile Italy) tissue set up, chemical balance, oral cannulae, Observing cage, animal cages, dissecting kit, organ bath, homogenizer, cold centrifuge, syringes, ruler, Spectrophotometer, weighing balance rotatory evaporator, water bath.

3.4. COLLECTION OF PLANT MATERIAL

The stem bark of *Anacardium occidentale* was collected at Abeokuta, Ogun State. The plant was authenticated by Mr Esimelekuai D.P.O., a staff of the Department of Botany, University of Ibadan, Ibadan, Nigeria and deposited with herbarium no.: UIH-22599

3.5. METHODS

3.5.1. EXTRACT PREPARATION

The stem bark was dried under shade and pulverized. The obtained pulverised sample (500 g) was packed into a soxhlex extractor and extracted with methanol. Methanol was later removed from the resultant mixture with the aid of a rotatory evaporator under reduced pressure and temperature of 52^{o}C. The solid sample of the extract obtained was kept in the refrigerator. The crude methanol extract was designated as AoME

3.5.1.1 Fractionation of AoME

The protocol of Leila *et al*. (2007) was used in the fractionation of AoME. Briefly, 500g of the solid sample of the extract was grounded in a mortal into powdered form. Methanol was later added in control amount to the powdered form in the mortal until all the extract has exhaustively absorb the methanol to form a paste of the extract. Subsequently, the paste form of the extract was impregnated with dry sample of the stem bark of A.O. and silica gel. They were added to the paste form of the extract to remove the methanol and the resulting dry sample was also grounded into powdered form. At this stage the extract was ready for column packing/partitioning). The resultant mixture was packed into the Column and eluted by consecutive liquid/liquid partition with 500 ml of hexane, ethyl acetate and methanol. The hexane (AoHF), ethyl acetate (AoEF) and methanol fractions (AoMF) obtained were concentrated under reduced pressured evaporator.

3.5.2. PHYTOCHEMICAL SCREENING OF AOME AND ITS FRACTIONS

The protocols outlined by Trease and Evans (1989) were used for the screening of the AoME and AoEF. Saponins, tannins, flavonoids sterol, and alkaloids were assessed.

3.5.2.1 Test for Alkaloids

The extract (0.5g) was put in a clean test tube. This was followed by addition of Hydrochloric acid (5 mL) . The test tube was placed on a water bath such that the content boils for a period of 5 min. The test tube was later removed from the water bath. The boiled content was filtered. A small filtrate portion (about 1 mL) was introduced into two separate test tubes (A and B) and was used as follows:

a. Dragendroffs Reagent: An aliquot portion of this reagent dropped into test tube A. When alkaloids are present the filtrate in test tube "A" turns to an orange to a red precipitate.

b.Wagner's Reagent: An aliquot portion of this reagent dropped into test tube "B". When alkaloids are present the filtrate, a red to brown precipitate appears in the test tube.

3.5.2.2 Test for Saponins

The extract (0.25g) was put in a clean test tube. This was followed by addition of distilled water (20 mL). The test tube was placed on a water bath such that the content boils for two minutes. The test tube was later removed from the water bath. The boiled content was filtered. After cooling of the filtrate, 5 mL was removed and put into another test tube. Distilled water (15 mL) was added to the test tube and mixed vigorously. The test-tube was later allowed to stand. When stable foam appears in the test tube it is an indication of the presence of saponins.

3.5.2.3 Test for Tannins

The extract (1 g) was put in a clean test tube. This was followed by addition of distilled water (20 mL). The test tube was placed on a water bath such that the content boils for two minutes.

The test tube was later removed from the water bath. The boiled content was filtered and an aliquot portion of ferric acid was dropped into it. The presence of tannin is indicated by the appearance of a greenish black precipitate.

3.5.2.4 Test for flavonoids

The extract (0.5g) was put in a clean test tube. This was followed by addition of ethylacetate (10 mL). The test tube was placed on a water bath such that the content boils for a period of 3 min. The test tube was later removed from the water bath. The boiled content was filtered. After cooling of the filtrate, 4 mL of the filtrate was removed and put into another test tube. An aliquot portion of the dilute ammonia solution was later dropped into it and mixed with moderate force. The layers observed were allowed to separate adequately. When flavonoids are present, yellow colour in the ammonical layer appears.

3.5.2.5 Test for steroids

The extract (1 g) was put in a clean test tube. This was followed by addition of ethanol (9 mL). The test tube was placed on a water bath such that the content boils for a maximum period of 3 min until the content is reduced to 2.5 mL. The test tube was later removed from the water bath. Boiled water was later added to the test tube. The solution was vigorously mixed such that a waxy matter appears in the test tube. The waxy matter was removed using a separating funnel. An aliquot portion of chloroform (2.5 mL) was dropped into the filtrate. 0.5 mL of the mixture was later drawn by a syringe and introduced into a clean test tube. The test-tube was place on a water bath such that its content evaporated to dryness. The dry sample was boiled with concentrated sulphuric acid (3 mL) for 10 minutes on a water bath. The presence of steroid is indicated by the appearance of a reddish brown interface.

3.5.3. Preparation of Solutions

1. Charcoal meal

   Charcoal (10 g) was put into a clean beaker. Thereafter it was dissolved with distilled water (100 mL). The resultant solution was suspended in 10 % gum acacia.

2. Semisolid carboxymethylcellulose solution (CMC)

   0.0225 g of CMC solid was dissolved in 15 mL of phenol red.

3. Tyrode solution (5 litre)

   The composition of Tyrode solution used for the study is stated below:

   Nacl (40 g), KCl (1 g), NaH_2PO_4 (0.25 g), glucose (5 g), $NaHCO_3$ (5 g), $CaCl_2$ (1 g) $MgCl_2$ (0.05 g) were dissolved in distilled water (5 Liters)

4. De Jalon solution (10 liters)

   The composition of De Jalon solution used for the study is stated below:

   NaCl (90 g), D-Glucose (5 g), $CaCl_2$ Molar (2.7 mL), 10% KCl (42 mL) and $NaHCO_3$ (5 g) were dissolved in 10 liters of distilled water.

5. Acetylcholine (10^{-3} M): Acetylcholine (1.82 mg) was put inside a clean test tube. Distilled water (10 mL) was used to dissolve it.

6. Histamine (10^{-3} M): Histamine (1.11 mg) was put inside a clean test tube. Distilled water (10 mL) was used to dissolve it.

7. Acetylcholine (0.05 mg/mL): Acetycholine (10 mg) was added into distilled water (10 mL) in a test tube. The resultant solution (0.5 ml) was withdrawn with a syringe and added into another test-tube. The content of the test tube was made up to 10 mL with the addition of distilled water (9.5 mL)

8. Histamine (0.05 mg/mL): Histamine (10 mg) was put inside a clean test tube and dissolved with distilled water (10 mL). The solution (0.5 mL) was withdrawn with a syringe and added into another test-tube. The content of the test tube was made up to 10 mL with the addition of distilled water

9. Serotonin (0.05 mg/mL): Serotonin (10 mg) was put inside a clean test tube and dissolved with distilled water (10 mL). The solution (0.5 mL) was withdrawn with a syringe and added into another test-tube. The content of the test tube was made up to 10 mL with the addition of distilled water

10. Carbachol (1.0 mg/mL): Carbachol (5 mg) was put inside a clean test tube. Distilled water (5 mL) was added to dissolve it.

11. Serotonin (1.0 mg/mL): Serotonin (5 mg) was put inside a clean test tube. Distilled water (5 mL) was added to dissolve it.

12. Metochlopramide (1.0 mg/mL): Metochlopramide (5 mg) was put inside a clean test tube. Distilled water (5 mL) was introduced to dissolve it.

13. NaOH (0.025 M): NaOH (1g) was dissolved with distilled water (1 litre)

14. Hexamethonium (0.0051 g/mL): Hexamethonium (0.051 g) was put inside a clean test tube. Distilled water (10 mL) was introduced to dissolve it.

15. L-NAME (0.001 g/mL): L-NAME (0.01g) was put inside a clean test tube. Distilled water (10 mL) was added to dissolve it.

3.5.4. Acute Toxicity test

This test was carried on methanol extract of *Anacardium occidentale* (AoME) following Lorke's method (Lorke, 1983) with slight adjustment to give a resemblance of gradient doses method. A total number of eighteen mice were used for this study. They were distributed into six groups of three (3) mice each. Groups 1, 2 and 3 animals received oral treatment of the extract as shown in the table below. Thereafter, the animals were transferred into an observation cage where toxicity manifestations and death record were observed for a period of 24 hours. When there was no observed death of these animals, further specific doses of AoME were given orally to animals in group 4, 5 and 6 to establish the actual LD_{50} value.

Table 3.1: Animal grouping and treatment for acute toxicity test

GROUPS	DOSES (mg/kg)
1	**10**
2	**100**
3	**1000**
4	**1600**
5	**2900**
6	**5000**

3.5.5. Antidiarheal study on stem bark extract of *Anacardium occidentale* (AoME)

The antidiarheal study on AoME was done using the procedure explained by Musa *et al* (2015).

On the commencement of this work, twenty five (25) healthy adult Swiss Mice (15-21 g) used for were deprived assess to food for a period 6 hours but allowed to drink water. They were later grouped and treated as shown in table 3.2. Thirty (30) minutes after drug and extract treatment, all animal were given 0.2ml of castor oil orally and thereafter placed in cages line with pre-weighted transparent paper and served as the first(1^{st}) weight of the paper (g). During

the 4-hour observation period the time of onset of diarrhea, total number of stool and the number of loose stool were recorded. After the experiment, the transparent paper containing the stool was weighed again served as the second (2^{nd}) weight of the paper (g). The transparent paper was later dried in an ovum for forty (45) minutes and weighted the third time. The Fresh weight of stool (g) and water content of stool were computed as follows:

Fresh weight of stool (g) = 2^{nd} weight of paper (g)-1^{st} weight of paper (g)

Water content of stool (g) = 2^{nd} weight of paper (g)- 3^{rd} weight of paper (g)

Table 3.2: Animal grouping and treatment for Antidiarrheal study

GROUP (N=5)	**TREATMENTS (p.o)**
1	Distilled water (0.1 mL/10 g)
2	Loperamide (2.5 mg/kg)
3	AoME 200 mg/kg (0.1 mL/10 g)
4	AoME 400 mg/kg (0.1 mL/10 g)
5	AoME 800 mg/kg (0.1 mL/10 g)

3.5.6. Gastrointestinal transit study on AoME

The procedure explained by Abdullahi *et al* (2001) was employed to study the effect of methanol extracts of A.O. on gastrointestinal transit (%). On the day of the work, thirty (30) Albino wistar rats were not allowed to eat for 18 h before the commencement of the experiment but were not denied drinking water. They were later distributed and treated as shown below.

GROUP (N=5)	**TREATMENTS (p.o)**
1	Normal saline (1.0 mL/100 g)
2	Carbachol (1 mg/kg)
3	Atropine (1 mg/kg)
4	AoME 200 mg/kg)
5	AoME 400 mg/kg
6	AoME 800 mg/kg

Thirty minutes after these administrations, 0.5 mL of charcoal meal was given to the all the animals and were later kept in their respective cages. After forty five (45) minutes of the administration of charcoal meal, the animals from the various groups were sacrificed by cervical dislocation and the small intestine from the level of the pylorus to caecum was carefully removed in each animal. The length of the small intestine, and the distance travelled by charcoal meal were measured.

For each animal, the percentage of the distance travelled by the charcoal meal against length of the small intestine is regarded as the gastrointestinal transit time (%).

3.5.7. Investigation of the effect of AoME on isolated tissue preparations

In this experiment, the effect of AoME was carried out on isolated rat stomach, guinea pig ileum and rat uterus strip preparations.

3.5.7.1. Preparation of Isolated rat stomach strip (SSP)

Adult wistar rats of both sexes used for this study were not allowed to eat for 18hrs before the onset of the experiment. They were sacrificed after cervical dislocation. Their abdomens were opened by a cut and their stomachs were removed. The pylorus was cut off from the fundus of the stomach. A strip of the stomach fundus was then cut. This was later mounted in a tissue bath filled with Tyrode solution up to the 15 mL mark. The solution was aerated and kept at 37°C. In all cases, the mounted tissues were suspended by a silk on a force transducer (model 7004; Ugo Basile, Varese, Italy) connected to a data capsule acquisition system model 17,400 captures that records tissue responses.

3.5.7.2. Preparation of Isolated guinea pig ileum (GPI)

Adult guinea pigs of both sexes used for this study were deprived of food for 18 h before the onset of the experiment but were not denied access to water. They were sacrificed via cervical dislocation and their abdomens were cut open. About 2 cm strip of the ileum was cut out and the attached mesentery dissected out. This was later mounted in a tissue bath filled with Tyrode solution up to the 15 mL mark. The solution was aerated and kept at 37°C. In all cases, the mounted tissues were suspended by a silk on a force transducer (model 7004; Ugo Basile, Varese, Italy) connected to a data capsule acquisition system model 17,400 which captures and records tissue responses.

3.5.7.3. Preparation of Isolated rat uterus strip (IRUS)

Adult Female Wistar rats used for this study were treated with Stilboestrol (1 mg/kg s.c.) forty eight hours before the commencement of the experiment.

Each rat was then sacrificed through cervical dislocation and the abdomen cut open to show the fallopian tubes (uterine horns). The horns were dissected in such a manner that adhering tissues were removed. About 2 cm strip of this horn was cut. This was later mounted in a tissue bath filled with De-Jalon's solution up to the 15 mL mark. The solution was aerated and kept at 37°C. In all cases, the mounted tissues were suspended by a silk on a force transducer (model 7004; Ugo Basile, Varese, Italy) connected to a data capsule acquisition system model 17,400 that captures and records tissue responses.

3.5.7.4. Protocol for testing the effect of AoME on isolated tissue preparations

In each of the tissue preparations, equilibration of 45-60 min was observed. During this time the physiological solution was replaced every 15 min and passive tension of 1.0 g readjusted. Thereafter, 0.025, 0.05, 0.1, 0.2 and 0.3 mg/ml (after final bath concentration calculation) of AoME were added cumulatively into the bath. The next addition were made when the peak response of the previous concentration are about waning. Tissues responses owing to each concentration of AoME were captured on the data capsule (Nwinyi and Kwanashie, 2009). Tissue responses were recorded on other freshly mounted strips from the same animal. This protocol was repeated on strips from two other animals. In all, tissues responses were measured in at least six independent experiments. Tissue responses of the activity of the extract were calculated as percentages of the maximum response. The percentage responses were plotted against log concentration of the extract to obtain a log concentration-response curve and the EC 50 was determined using Graph pad prism 5.01 software.

3.5.7.5. Protocol for interaction of AoME with standard agonists

The interaction of AoME with standard agonists was carried on a fresh strips from obtained from another set of animals (n=3). The following agonists were interacted with AoME :

Acetylcholine on SSP, GPI and IRUS

Histamine on SSP and GPI

Oxytocin on IRUS

After mounting the tissue following the procedure explained above. Tissue was exposed to graded concentration of agonists to determine a log concentration-response curve for the agonists. This was done by cumulative addition of graded concentration of the agonists to the bath (10^{-9}-10^{-5}M). Thereafter, the tissue were allowed to rest for thirty (30) minutes later, the tissue preparations were pre-incubated with 0.2 mg/mL of the extract. During this time no capturing of tissue response was made on the data capsule. After fifteen minutes, data capsule was put on for capturing while graded concentrations of the agonists were added to the bath cumulatively.

Tissue responses of the activity of the agonist only and agonist with AoME were calculated as percentage of the maximum response. The percentage responses obtained were plotted against log concentration to obtain a log-concentration-response curve. The EC_{50} values were determined using Graph pad prism 5.01 software. The EC_{50} values were compared and possible interactions were deduced.

3.5.8. Investigation of gastrointestinal activity of fractions of AoME (*in vitro* and *in vivo)*

Under this investigation, the following studies were carried

1. Effect of AoME fractions (AoEF, AoMF and AoHF) on Guinea pig ileum Strip (GPI)
2. Effects of AoME fractions (AoMF and AoEF) on gastrointestinal activity.

3.5.8.1 Preparation of Guinea pig ileum for the study of the effects of AoME fractions

Twelve (12) adult guinea pigs used for this study were fasted for 18 hrs prior to the experiment. They were distributed into four (4) groups (n=3). Group 1 was used for test the effect of AoEF, while group 3, 4 and 5 were used for non-cumulative study of the effect of AoHF, AoMF and AoME respectively. The same procedure of preparation of guinea pig ileum explained in 3.6.3.2 was adopted. However, the tissue set up was connected to microdynanomometer set at sensitivity of 2 mV and responses were recorded on the graph.

3.5.8.2. Procedure for testing the effects of the AoEF on Guinea pig ileum strip

0.1, 0.2, 0.4 and 0.8 mL of 1, 10, and 100 mg/kg of AoEF were added non- cumulatively to the ileum strip in the bath containing 15 mL of the Tyrode solution. After the tissue had

equilibrated, tissue responses were recorded covering 2 cm unit of the microdyanomoter graph. Once the tracing reached the 2 cm mark, specific volume of the AoEF was introduced into the bath and the tracing of the responses were allowed to cover 2 cm unit of the graph. After covering this point the pen was put off, the tissue was washed and allowed to rest for 30-45 min before testing the effect of the next volume of the AoEF. At each point of recording the tissue responses of a known volume of AoEF, the normal response of the ileum was always recorded. In all, six independent experiment (n=6) were carried out using animals in group 1.

The Final bath concentration of AoEF was calculated using the formular described below and was regarded as the concentration of AoEF

$$FBC = \frac{\text{Volume of AoEF added to the bath x concentration of AoE}}{\text{Volume of Bath}}$$

From the graph, the height of depression of the tracing from the baseline by each concentration of AoEF was measured in millimeters (mm) and regarded as the Height of relaxation (mm)

The height of relaxation (mm) was plotted against log concentration of AoEF to obtain a log-concentration-response curve and deduced the IC_{50} values using Graph pad prism 5.01 software.

3.5.8.3. Procedure for testing the effects of the AoMF on Guinea pig ileum

Same procedure as stated above for AoEF was adopted.

3.5.8.4 Procedure for testing the effects of the AoHF on Guinea pig ileum

Same procedure as stated above for AoEF was adopted.

3.6. Effects of AoEF and AoMF on Gastrointestinal transit

Ten (10) rats distributed into AoEF (400 mg/kg) and AoMF (400 mg/kg) groups containing five rats per group were used for this study following the procedure of Abdullahi *et al* (2010) described in **3.5.6.**

3.7. Investigation of the constituents of the crude and fractions and probable mechanisms of action (*in vitro* and *in vivo*)

3.7.1 Gas Chromatography Mass Spectrometer (GCMS) analysis of AoME and AoEF

The GC-MS analysis was carried out using Gas Chromatograph equipped and coupled to a mass spectrometer in National Research Institute of Chemical Technology, Zaria, Kaduna State.

3.7.2. Interaction of AoEF with standard agonist on Guinea pig ileum (In-vitro)

The AoEF (2.7 mg/mL) fraction was interacted with graded concentration (0.0033 µg/mL-0.267 µg/mL) of standard agonists (histamine, acetylcholine and serotonin) non-cumulatively. Interaction of AoEF with each agonist was done on the GPI preparation. After the preparation had equilibrated, dose response of the agonist was first carried out by adding 0.1, 0.2, 0.4 and 0.8 mL of 0.05 mg/mL concentration of AoEF non-cumulatively. The dose response of the agonist was repeated non-cumulatively with pre-incubation of 0.4 mL of 100 mg/mL of AoEF for 5 minutes before addition of each dose of the agonist. The dose responses of agonist only and agonist with AoEF on the graph were measured and regarded as plotted against log concentration to obtain a log-concentration-response curve. The EC_{50} values were determined using Graph pad prism 5.01 software. The EC_{50} values were compared and possible interactions were deduced.

3.7.3. Interaction of AoEF with inhibitors of smooth muscle contraction on Guinea pig ileum (In-vitro)

The AoEF (2.7 mg/mL) of was interacted with standard antagonist (hexamethonium ($5x10^{-3}$ and 1 $x10^{-3}$ M), L-NAME (100 $x10^{-6}$ M x 200 $x10^{-6}$ M) and atropine (10^{-5} M and 10^{-4} M))

Interaction of each antagonist with AoEF was done on the GPI. After, the preparation had equilibrated; response of 0.4 mL of 100 mg/mL of AoEF was recorded. Thereafter, the tissue was pre-incubated with the specific concentration of the antagonist before the strip was exposed to AoEF (0.4 mL of 100 mg/mL of AoEF) The time of pre-incubation for hexamethonium, L-NAME, and atropine are ten (10), thirty (30) and five (5) minutes respectively.

In each case, the heights of relaxation (mm) of the normal tonus of the guinea pig ileum by AoEF alone and in the presence of the Antagonists were compared and possible interaction was deduced.

3.7.4. Interaction of AoEF with standard agonist in Mice

The Gastric emptying (GE) and Intestinal transit (IT) times are index of the gastrointestinal tract that can be measured and aids in the study of the mechanism by which substances affect motility.

The method described by Suchitra *et al*., (2003) that allows the assessment of GE and IT in the same animals was used in this study. In this method, pathways leading to increase in smooth muscle activity were selectively stimulated by certain drugs in the presence of the extract. Cholinergic pathway activation was by carbachol while serotonergic and dopaminergic pathways were achieved by serotonin and metoclopramide respectively.

Thirty five (35) male Swiss mice used for this study were distributed into seven groups (n=5). They were treated as follows:

Group 1, 2, 3 and 4 received AoEF (400 mg/kg p.o), Carbachol(10 mg/kg p.o) serotonin (10 mg/kg p.o), metoclopramide (30 mg/kg) (0.1 mL/10 g of mice) respectively.

In another groups of animals, where interaction were carried out,

Group 5, 6, 7 received AoEF (400 mg/kg p.o) thirty (30) minutes before they were treated with carbachol (10 mg/kg p.o), serotonin (10 mg/kg p.o), metoclopramide (30 mg/kg p.o) (0.1 mL/10 g of mice) respectively.

Thereafter, all groups received carboxyl methylcellulose semisolid solution (CMS) (0.5 mL/animal, p.o.). The animals were transferred into their cages. Fifiteen (15) minutes thereafter, each animal was sacrificed and the abdomen opened. The stomach along with the intestines was carefully removed immediately. The Stomach cut off from the intestine at the pyloric junction. The stomach was used for the assessment of gastric emptying (GE) while the intestine was used for the intestinal transit (IT).

Assessment of Gastric Emptying

The stomach was homogenized with 7 mL of distilled water in a sample bottle and later centrifuged at 3000rpm for duration of 15 mins. NaOH (1 mL,0.025 M) was added to 1 mL of the supernatant and shaken briefly. The absorbance of this mixture was measured using a spectrophotometer set at 560 nm.

Gastric emptying (GE) was deduced using this formula: **GE (%) =100 − (E×100/C)**

E = absorbance of supernatant of the homogenized stomach of experimental animals

C = the mean (n=5) absorbance of supernatant of the homogenized stomach of control animals (control animals were sacrificed at 0 min following administration CMS).

Assessment of Gastrointestinal Transit (GT)

For the assessment of Gastrointestinal Transit, the total length of the small intestine and the distance travelled by the marker were measured.

GT was later calculated using this formula:

$GT=P/L \times 100$

where P =distance traveled by phenol red and

L=total length of the small intestine

CHAPTER FOUR

RESULTS

4.1 Percentage yield of Methanolic extract of the stem bark of *Anacardium occidentale* and its fractions

The percentage yield of the extract (AoME) was 23.73% (Table 4.1) while the fractions yields are 0.7% (AoHF), 2.68% (AoEF) and 20.06% (AoMF) (Table 4.2).

Table 4.1. Crude extraction of the stem bark extract of *Anacardium occidentale*

Weight of dried stem bark	3 kg
Weight of dried Methanol extract	712 g
Yield (%)	23.73

Table 4.2. Percentage yields of the different fractions of AoME

Weight of dried AoME	500 g	-
Weight of Hexane fraction (AoHF)	3.5 g	0.7 %
Weight of Ethylacetate fraction (AoEF)	13.4 g	2.68 %
Weight of Methanolic fraction (AoMF)	100.3 g	20.06 %

4.2. Phytochemical composition of methanol extract of the stem bark of *Anacardium occidentale* and its fractions.

The qualitative phytochemical compositions revealed the presence of high amount of steroids, flavonoids and phenols in the methanolic extract of the stem bark of *Anacardium occidentale* (AoME). The level of alkaloids, saponins and tannins in AoME were as shown in Table 4.3. The Hexane fraction showed the presence of high level of flavonoids and steroids while phenol and tannins were absent. All the bioactive substances tested were present in the ethylacetate fraction with exception of saponin. The level of flavonoids, phenol and steroid were high while tannins and alkaloids were in moderate concentration. In the methanol fraction, the presence of steroids and phenol were exceptionally high when compared with the levels of saponin and flavonoids which are present in low concentration. Alkaloids and tannins are absent. (Table 4.3)

Table 4.3. Phytochemical analysis of AoME and its fractions

	ALKALOIDS	SAPONIN	STEROIDS	FLAVONOIDS	PHENOL	TANINS
AoME	++	+	++++	+++	++++	++
HEXANE FRACTION (AoHF)	++	+	++++	+++	-	-
ETHYLACETATE FRACTION (AoEF)	++	-	++++	+++	++++	++
METHANOL FRACTION (AoMF)	-	+	++++	+	++++	-

+ = Low; ++ = Moderate; +++ = High; ++++ = Extremely High

4.3. Acute toxicity study in mice

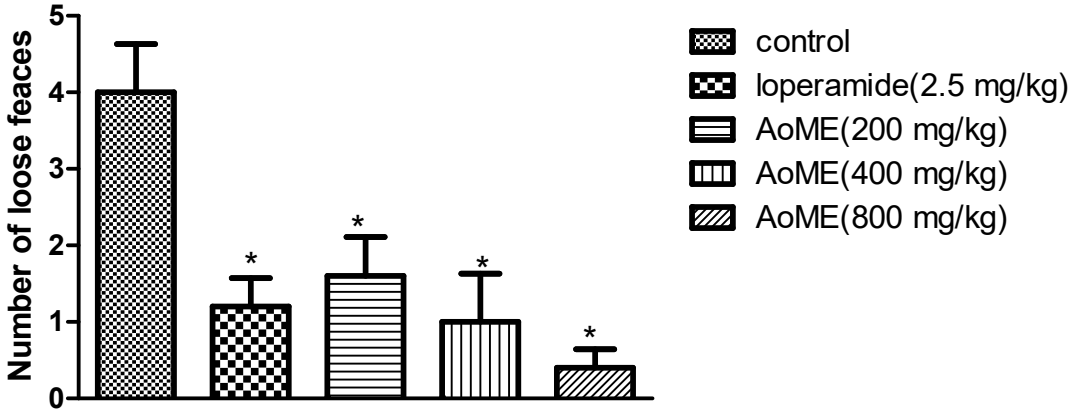
The toxicity test of the extract (AoME) on mice is represented on Table 4.4. There were no incidences of mortality recorded with all doses of the extract used. The $LD_{50} > 5$ g/kg body weight orally of the extract.

Table 4.4. Acute toxicity profile of the methanol extract of the stem bark of *Anacardium occidentale* (AoME) in mice

Phase I	
Doses (mg/kg p.o)	**Number of death**
10.0	0 (3)
100.0	0 (3)
1000.0	0 (3)
Phase II	
Doses (mg/kg p.o)	**Number of death**
1600.0	0 (3)
2900.0	0 (3)
5000.0	0 (3)

4.4. Effect of the methanol stem bark of *Anacardium occidentale* on number of loose faeces

The results obtained in this study (Fig 4.1) show that the administration of castor oil significantly induced diarrhea, and pretreatment with AoME at all tested doses (100, 200, and 400 mg/kg), significantly ($P<0.05$) reduced number of loose faeces (1.6±0.5 g; 1.0±0.6 g; 0.4±0.24, respectively) compared to the control group (4.0±0.6 g) after 3-hour administration of castor oil. The reduction in number of loose faeces is dose dependent. The standard antidiarrheal drug, loperamide (2.5 mg/kg, p.o.), also inhibited the diarrheal parameter.

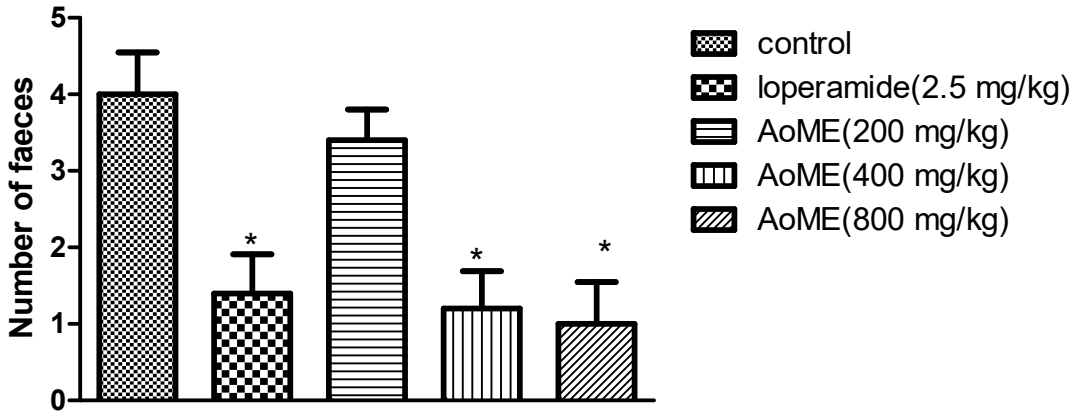


significantly different from control p<0.05

Fig 4.1: Effect of AoME on Loose feaces

4.5. Effect of the methanol stem bark of *Anacardium occidentale* on total number of faeces

The results obtained in this study (Fig 4.2) show that pretreatment with AoME at all tested doses (100, 200, and 400 mg/kg) reduced total number of faeces however, these reductions were significant ($P<0.05$) only in animals that received 400 and 800 mg/kg (3.4±0.4g; 1.2±0.5g, respectively) of AoME when compared to the control group (4.0±0.5g) after 3-hour administration of castor oil. The standard antidiarrheal drug, loperamide (2.5 mg/kg, p.o.), also inhibited this diarrheal parameter.

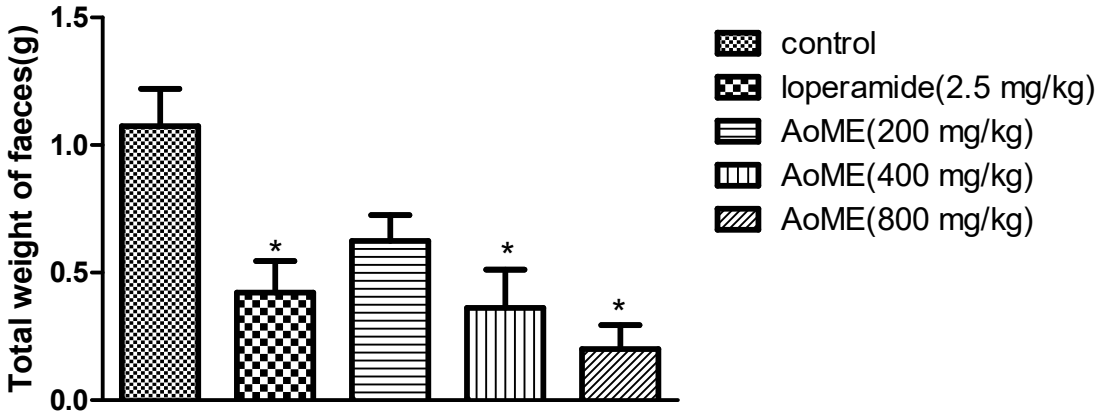


significantly different from control p<0.05

Fig. 4.2. Effect of AoME on total number of faeces

4.6. Effect of the methanol stem bark of *Anacardium occidentale* on total weight of faeces

The extract (AoME) in all the doses administered decreased the total weight of feaces after a four hour observation period when compared to the values obtained in the control animals (1.1±0.1g) as shown in Fig 4.3. However, this reduction was significant ($P<0.05$) only in animals that received 400 and 800 mg/kg of AoME (0.4±0.1g; 0.2±0.09g respectively). The reduction in animals that received high dose of the extract was more pronounced than the result obtained in animals treated with loperamide, an antidiarrheal drug (0.4±0.1g)

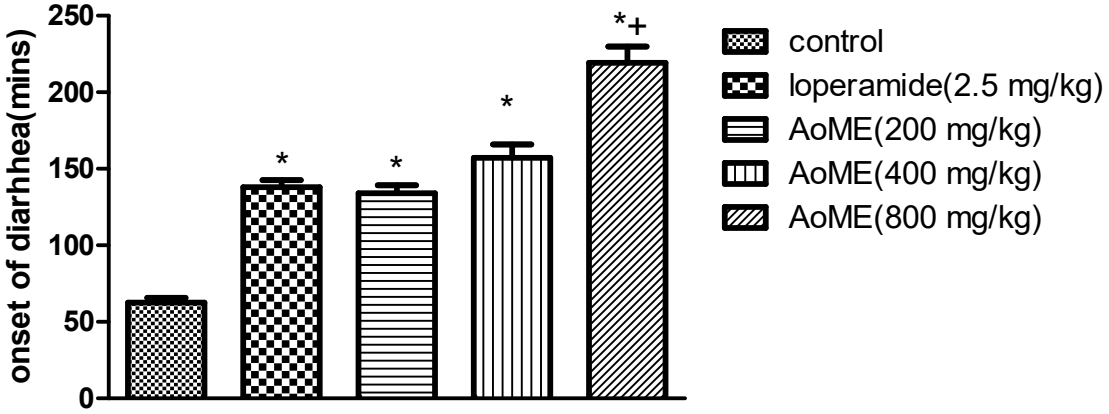


significantly different from control p<0.05

Fig 4.3. Effect of AoME on total weight of faeces

4.7. Effect of the methanol stem bark of *Anacardium occidentale* on onset of diarrhoea in castor oil induced diarrhea

The results obtained in this study (Fig 4.4) show that pretreatment with AoME at all tested doses (100, 200, and 400 mg/kg) significantly ($P<0.05$) delayed the onset of diarrhea after four hour administration (134.0±5.2; 157.0±8.8; 219±10.6 mins, respectively) when compared to the values obtained in the control animals (62.60±3.0 mins) The delay in the onset of diarrhea in animals who received 800 mg/kg of the AoME was significantly ($P<0.05$) higher than the result observed in animals that received loperamide (2.5 mg/kg) (138.0±4.6 mins)

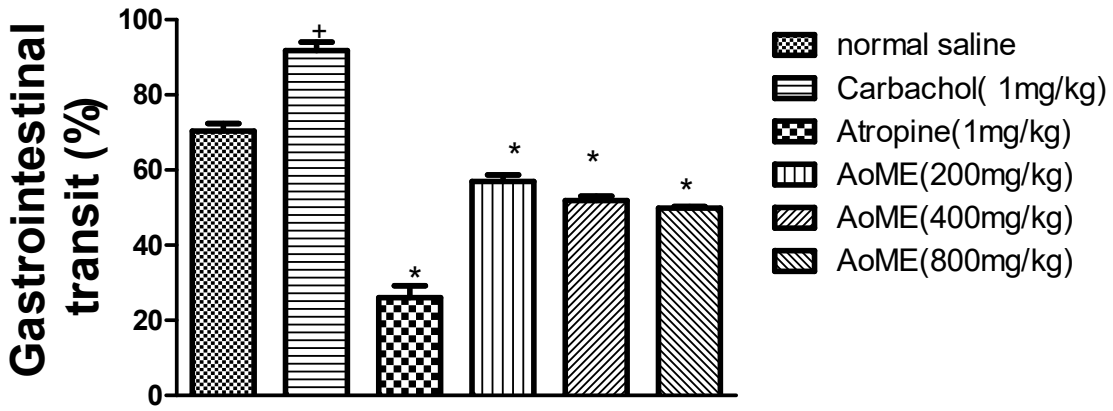


significantly different from control p<0.05, + significantly different from loperamide

Fig. 4.4. Effect of AoME on onset of diarrhoea

4.8. Effect of the methanol stem bark of *Anacardium occidentale* on Gastrointestinal Transit (%) in rats

The extract (AoME) at doses of 200, 400 and 800 mg/kg produced a significant decreased in the intestinal transit of the intestine when compared to the control animals which received 1 mg/100 g normal saline ($P<0.05$).This effect is presented in Fig 4.5. The reduction produced by the extract was not higher than that of atropine (an antagonist of cholinergic receptors). carbachol at 1 mg/kg significantly increased charcoal meal transit compared with normal saline (1 mg/100 g p.o) ($p<0.05$)



significantly different from control p<0.05

Fig 4.5. Effects of AoME on Gastrointestinal transit of charcoal meal in mice

4.9. Effect of the methanol stem bark extract of *Anacardium occidentale* (AoME) on stomach strip preparations (SSP)

Figure 4.6(a) shows a representative portion of the various recordings obtained for the effect of AoME on tonus activity of rat stomach strip preparation when added cumulatively. The AoME added cumulatively contracted the stomach strip. This is shown by the change in the height of the waves from the baseline with increasing doses of the extract. The Log concentration- response curve for the contractile effect of AoME on the stomach strip is shown in figure 4.6(b). The EC_{50} value of AoME determined from the curve is 0.54 mg/ml.

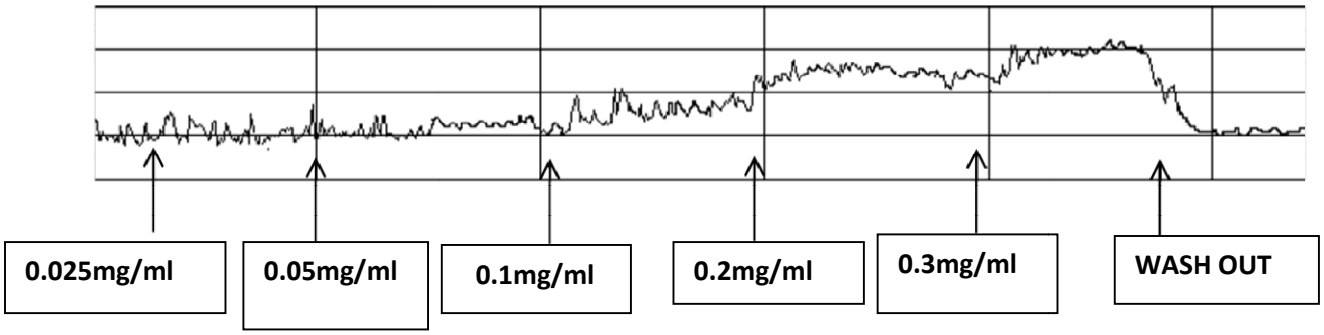


Fig. 4.6 (a). Typical tracing showing effects of graded concentrations of the methanol extract of AoME on rat stomach strip preparations. The AoME raised the tonic waves of the stomach strip from the baseline with increasing concentration of the extract added cumulatively. This is an indication of the contractile effect of the extract on the stomach strip.

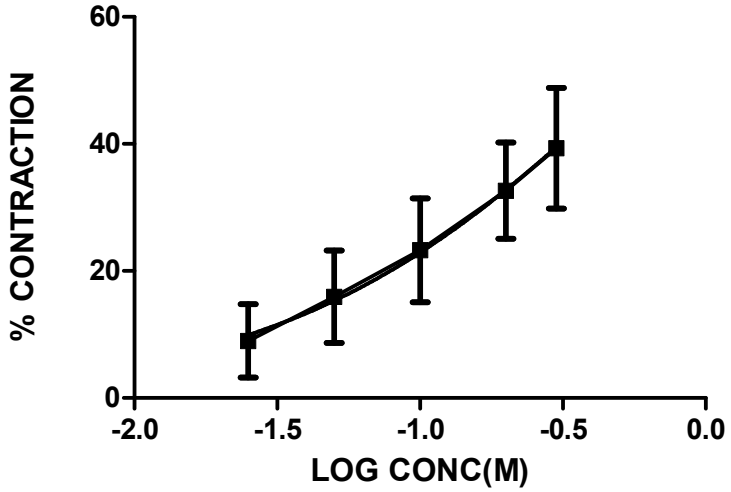


Fig.4.6 (b). Log concentration response curve of isolated rat stomach strips to cumulative addition of AoME. Each point represents Mean±SEM of six independent experiments.

4.10. Effect of Acetylcholine alone and in the presence of the methanol stem bark extract of *Anacardium occidentale* (AoME) on stomach strip preparations (SSP)

The findings are represented as shown in figures 4.7(a-c) Contraction of isolated stomach strip preparation by graded concentration of acetylcholine (cholinergic agonist) (Fig 4.7a) added cumulatively was inhibited by AoME pre-incubated at a concentration of 0.2mg/ml. This observation is demonstrated on the log concentration – response curves (Figures 4.7c). AoME shifted the log concentration of both acetylcholine and histamine to the right. The EC_{50} value of acetylcholine (6.76x10-9 M) was increased to 9.94×10^{-6} M by AoME. (Table 4.5)

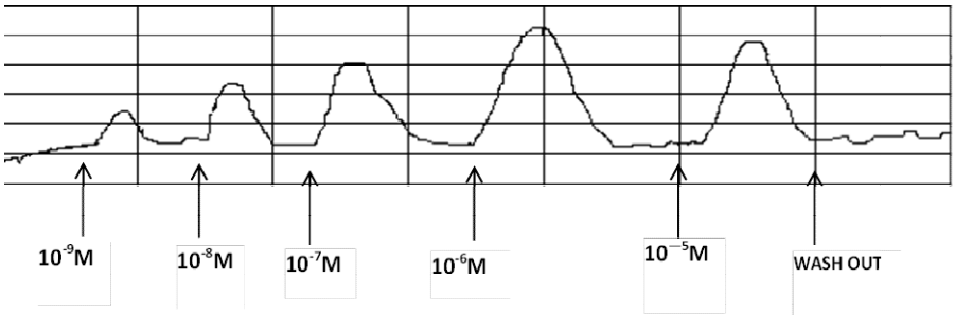


Fig. 4.7 (a). Typical tracing showing the effect of graded concentrations of acetylcholine without pre-incubation with AoME on rat stomach strip (SSP). The first five arrows represent point of cumulative addition of acetylcholine (10^{-9}, 10^{-8}, 10^{-7}, $10^{-6}$and 10^{-9}M). The last arrow represent the washed out. The successive increase in the height of the waves raised from the baseline is indicative of the contractile effect of acetylcholine on stomach strip preparation.

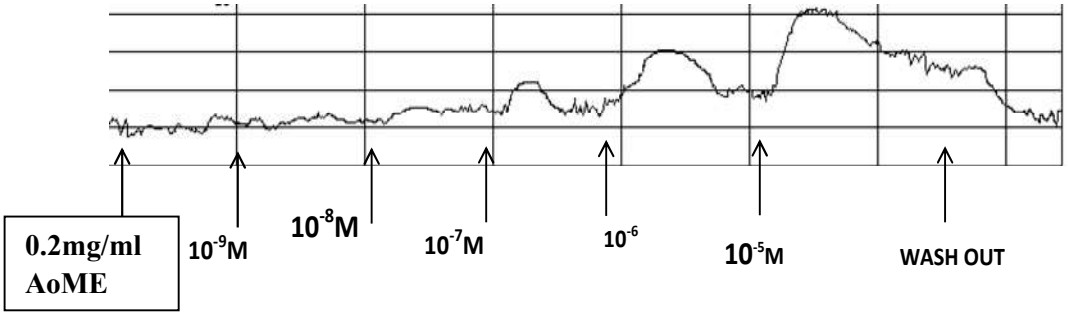


Fig. 4.7 (b). Typical tracing showing the effect of graded concentrations of acetylcholine after pre-incubation with AoME (0.2mg/ml) on rat stomach strip (SSP). The first five arrows represent point of cumulative addition of acetylcholine (10^{-9}, 10^{-8}, 10^{-7}, $10^{-6}$and 10^{-9}M). The last arrow represent the washed out. The successive increase in the height of the waves raised from the baseline by addition of Acetylcholine in the presence of AoME was reduced when compared with its initial effect alone (Fig 4.7b) This could suggest the inhibition of the contractile effect of acetylcholine on stomach strip preparation by AoME.

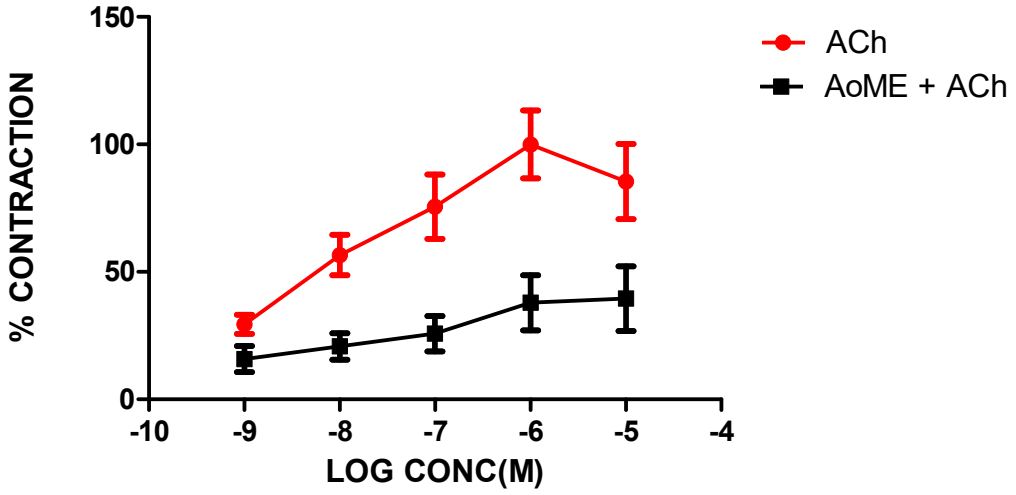


Fig. 4.7 (c). Log concentration-response curve of isolated rat stomach strips to cumulative addition of acetylcholine in the absence (Red circle) and presence of AoME (0.2mg/ml, Black circle). Each point represents Mean±SEM of six independent experiments. Log- concentration curve of acetylcholine is shifted to the right by AoME

Table 4.5. EC_{50} values of Acetylcholine Alone and in the presence of AoME in SSP

	Acetylcholine	AoME + Acetylcholine
EC_{50} (M)	6.76×10^{-9}	9.94×10^{-6}
Log EC_{50}	-8.174	-5.002

4.11. Effect of Histamine alone and in the presence of the methanol stem bark extract of *Anacardium occidentale* (AoME) on stomach strip preparations (SSP)

The results of this study are represented as shown in 4.8(a-c). Contraction of isolated stomach strip preparation by graded concentration of histamine (Histaminergic agonist) (Fig 4.8a) added cumulatively was inhibited by AoME pre-incubated at a concentration of 0.2 mg/ml (Fig 4.8b). This observation is demonstrated on the log concentration – response curves (Figures 4.8c). AoME shifted the log concentration histamine to the right. The EC_{50} value of histamine ($7.66x10^{-7}$ M) was increased to 9.94×10^{-6} M and 1.05×10^{-5} M by AoME. (Table 4.6)

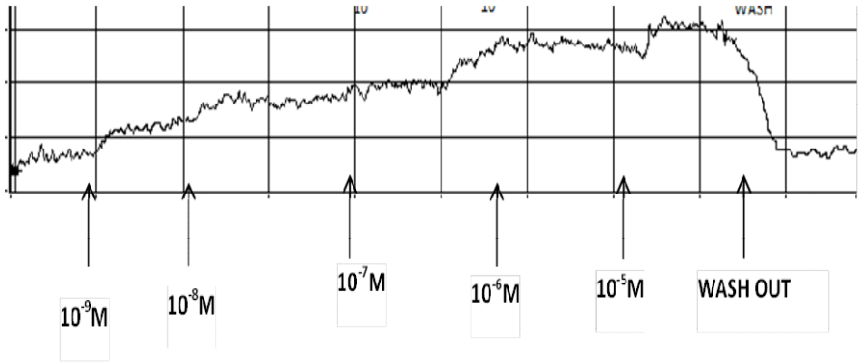


Fig. 4.8 (a). Typical tracing showing the effect of graded concentrations of histamine without pre-incubation with AoME on rat stomach strip (SSP). The first five arrows represent point of cumulative addition of acetylcholine (10^{-9}, 10^{-8}, 10^{-7}, $10^{-6}$and 10^{-9}M). The last arrow represent the washed out. The successive increase in the height of the waves raised from the baseline is indicative of the contractile effect of histamine on stomach strip preparation.

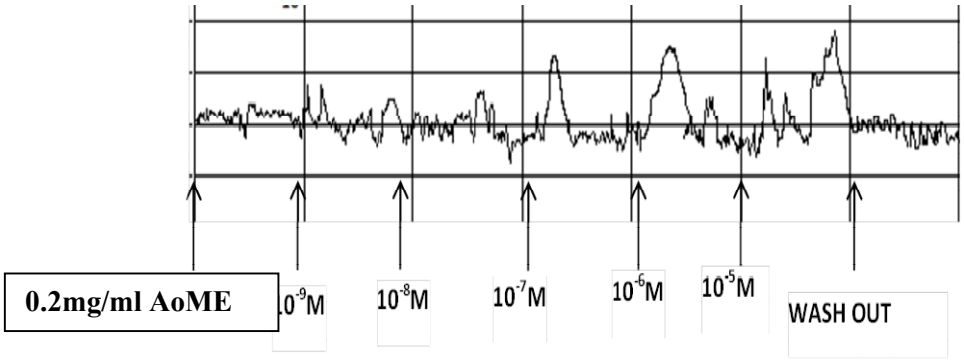


Fig. 4.8 (b). Typical tracing showing the effect of graded concentrations of histamine after pre-incubation with AoME (0.2mg/ml) on rat stomach strip (SSP). The first five arrows represent point of cumulative addition of histamine (10^{-9}, 10^{-8}, 10^{-7}, $10^{-6}$and 10^{-9}M). The last arrow represent the washed out. The successive increase in the height of the waves raised from the baseline by addition of histamine in the presence of AoME was reduced when compared with its initial effect alone (Fig 4.7b). The baseline of the tonic waves were dropping with increasing concentration of histamine in the presence of AoME. These observations suggest the inhibition of the contractile effect of histamine on stomach strip preparation by AoME.

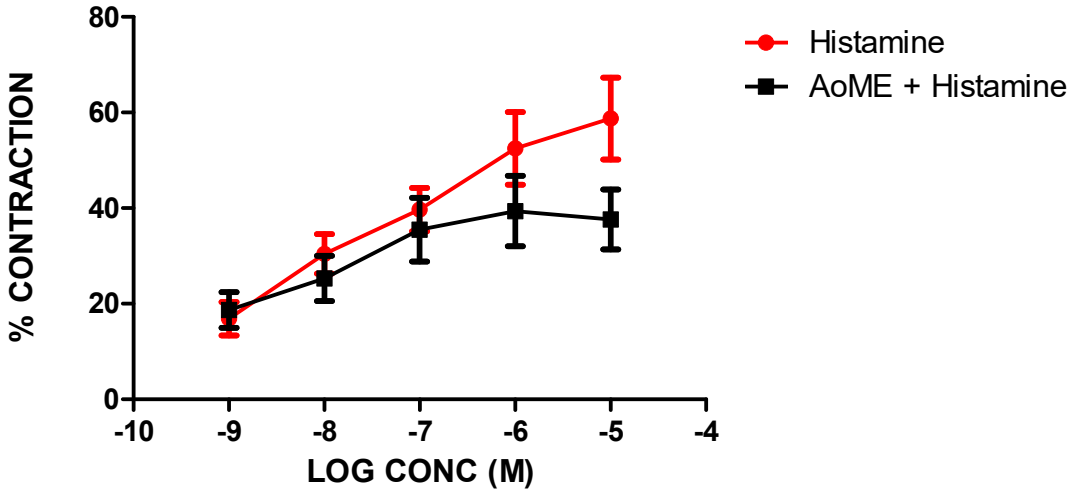


Fig. 4.8 (c). Concentration response of isolated rat stomach strips to cumulative addition of histamine in the absence (Red circle) and presence of AoME (0.2mg/ml, Black square). Each point represents Mean±SEM of six independent experiments. Log-concentration curve of Histamine is shifted to the right by AoME

Table 4.6. EC_{50} values of histamine in the presence of AoME in SSP

	Histamine	AoME +Histamine
EC_{50} (M)	7.66×10^{-7}	1.05×10^{-5}
Log EC_{50}	-6.116	-4.978

4.12. Effect of the methanol stem bark extract of *Anacardium occidentale* (AoME) on guinea pig ileum strip preparations (GPI)

Figure 4.9(a) shows a representative portion of the various recordings obtained for the effect of AoME on tonus activity guinea pig ileum strip preparation when added cumulatively. The AoME added cumulatively relaxed the ileal strips. This is shown as the decreased in the height of the spike potentials following addition of the extract from 0.1mg/ml to 0.3mg/ml .The Log concentration- response curve for the relaxant effect of AoME on the guinea pig ileum strip is shown in figure 4.9(b). The EC_{50} value of AoME determined from the curve is 0.54mg/ml.

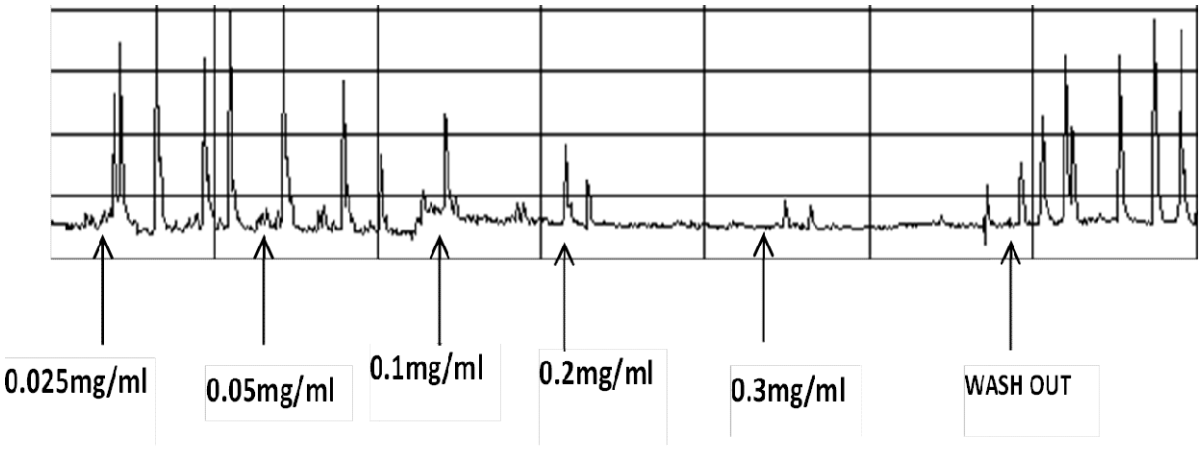


Fig. 4.9 (a). Typical tracing showing effects of graded concentrations of the methanol extract of AoME on guinea pig ileum strip preparations. AoME decreased the height of the spike potentials following addition of the extract from 0.1mg/ml to 0.3 mg/ml. This indicates the relaxant effect of the extract on the guinea pig ileum strip.

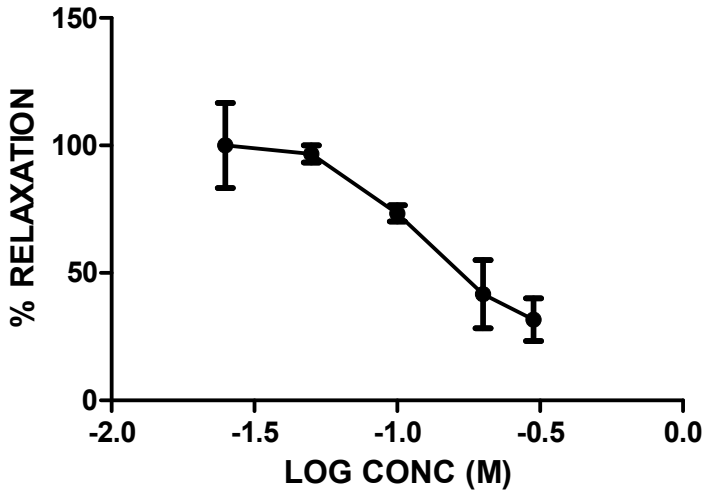


Fig. 4.9(b). Log concentration response of isolated guinea pig ileum strips to cumulative addition of AoME. Each point represents Mean±SEM of six independent experiments.

4.13. Effect of Acetylcholine alone and in the presence of methanol stem bark of *Anacardium occidentale* (AoME) on guinea pig ileum strip preparations (GPI)

The findings are represented as shown in figures 4.10(a-c). Contraction of isolated guinea pig ileum by cumulative addition of graded concentration of acetylcholine (cholinergic agonist) (Fig 4.10a) was inhibited by AoME pre-incubation at a concentration of 0.2 mg/ml (Fig 4.10b). This observation is demonstrated on the log concentration – response curve (Fig 4.10c). AoME shift the log concentration of acetylcholine to the right. The EC_{50} value of acetylcholine (3.22×10^{-9} M) was raised to 2.44×10^{-8} M by AoME (Table 4.7).

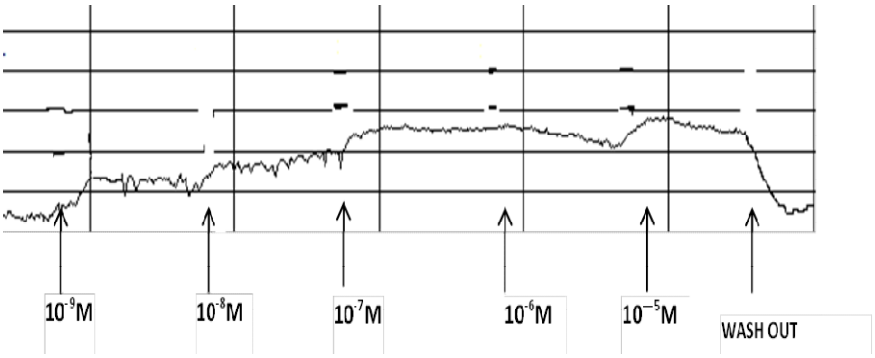


Fig 4.10 (a). Typical tracing showing the effect of graded concentrations of acetylcholine without pre-incubation with AoME on guinea pig ileum strip. The first five arrows represent point of cumulative addition of acetylcholine (10^{-9}, 10^{-8}, 10^{-7}, $10^{-6}$and 10^{-9}M). The last arrow represent the washed out. The successive increase in the height of the waves raised from the baseline is indicative of the contractile effect of acetylcholine on stomach strip preparation.

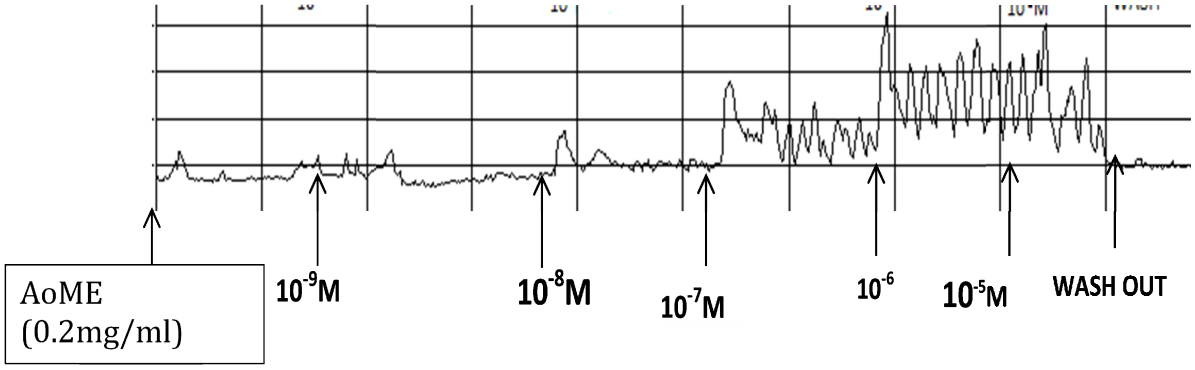


Fig. 4.10 (b) Typical tracing shows the effect of graded concentrations of acetylcholine after pre-incubation with AoME (0.2mg/ml) on guinea pig ileum strip. The first five arrows represent point of cumulative addition of acetylcholine (10^{-9}, 10^{-8}, 10^{-7}, $10^{-6}$and 10^{-9}M). The last arrow represent the washed out. The successive increase in the height of the waves raised from the baseline by addition of Acetylcholine in the presence of AoME was reduced when compared with its initial effect alone (Fig 4.10a). The baseline of the tonic waves were dropping with increasing concentration of acetylcholine in the presence of AoME. These observations suggest the inhibition of the contractile effect of acetylcholine on guinea pig ileum strip preparation by AoME.

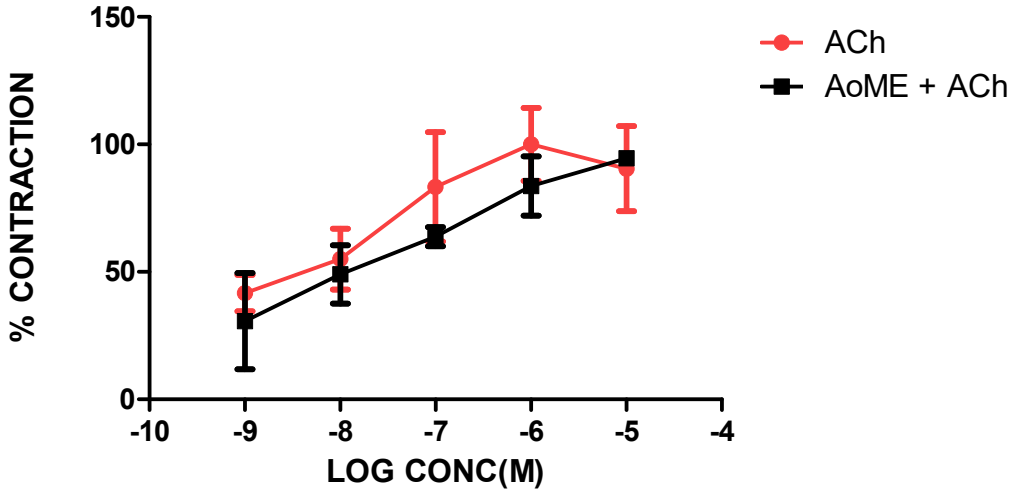


Fig.4.10(c). Log concentration response of isolated guinea pig ileum strips to cumulative addition of acetylcholine in the absence (Red circle) and presence of AoME (0.2mg/ml, Black square). Each point represents Mean±SEM of six independent experiments. Log-concentration curve of acetylcholine is shifted to the right by AoME

Table 4.7. EC_{50} values of Acetylcholine in the presence of AoME in Guinea pig ileum strip

	ACh	**AoME+ACh**
EC_{50}(M)	3.22×10^{-9}	1.21×10^{-8}
Log EC_{50}	-8.493	-7.919

4.14. Effect of Histamine alone and in the presence of methanol stem bark of *Anacardium occidentale* (AoME) on guinea pig ileum strip preparations (GPI)

Contraction of isolated guinea pig ileum by graded concentration of histamine (Fig 4.11a) added cumulatively was inhibited (Fig 4.11b) by AoME pre-incubation at a concentration of 0.2 mg/ml. The successive increase in the height of waves from the baseline with each observed when histamine alone was added to the tissue (Fig 4.11a) was leveled out when it was added in the presence of AoME (Fig 4.11b). This observation is demonstrated on the log concentration – response curve (Fig 4.11c). AoME shift the log concentration of histamine to the right. The EC_{50} value of histamine (9.88×10^{-9} M) was raised to 3.52×10^{-8} M by AoME (Table 4.8).

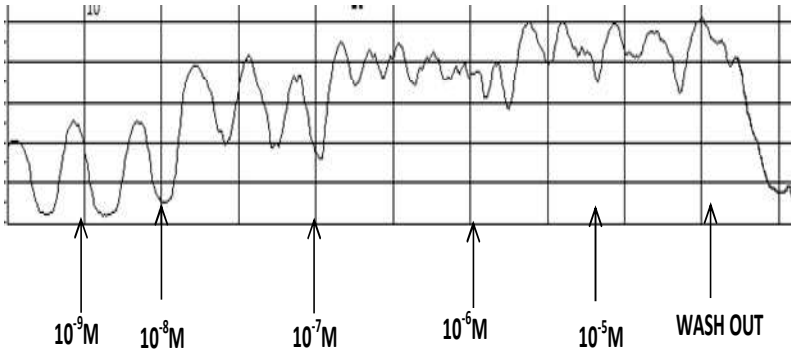


Fig 4.11(a). Typical tracing showing the effect of graded concentrations of histamine without pre-incubation with AoME on guinea pig ileum strip. The first five arrows represent point of cumulative addition of histamine (10^{-9}, 10^{-8}, 10^{-7}, $10^{-6}$and 10^{-9}M). The last arrow represent the washed out. The successive increase in the height of the waves raised from the baseline is indicative of the contractile effect of histamine on Guinea pig ileum strip preparation.

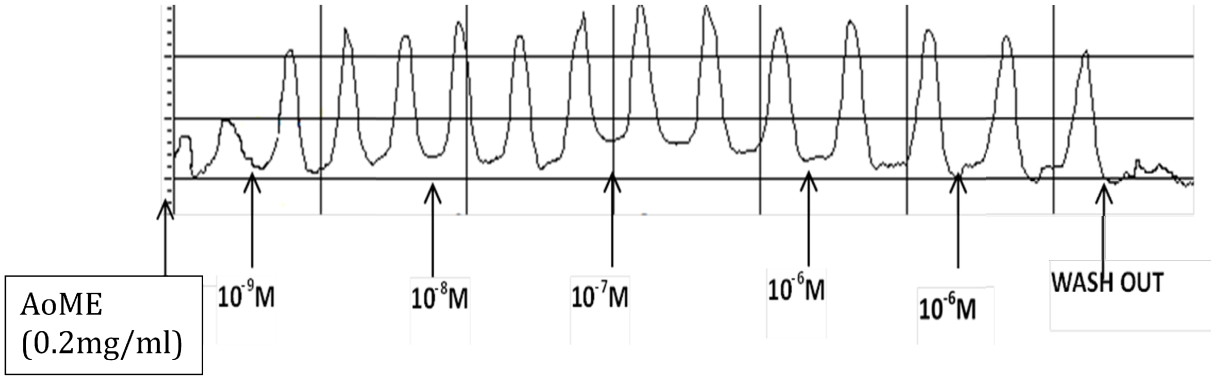


Fig 4.11(b) Typical tracing shows the effect of graded concentrations of histamine after pre-incubation with AoME (0.2 mg/ml) on guinea pig ileum strip. The first five arrows represent point of cumulative addition of histamine (10^{-9}, 10^{-8}, 10^{-7}, $10^{-6}$and 10^{-9}M). The last arrow represent the washed out. The successive increase in the height of the waves raised from the baseline by addition histamine of in the presence of AoME was leveled out when compared with its initial effect alone (Fig 4.11a). This is a clear manifestation of weak contraction. These observations suggest the inhibition of the contractile effect of Histamine on guinea pig ileum strip preparation by AoME.

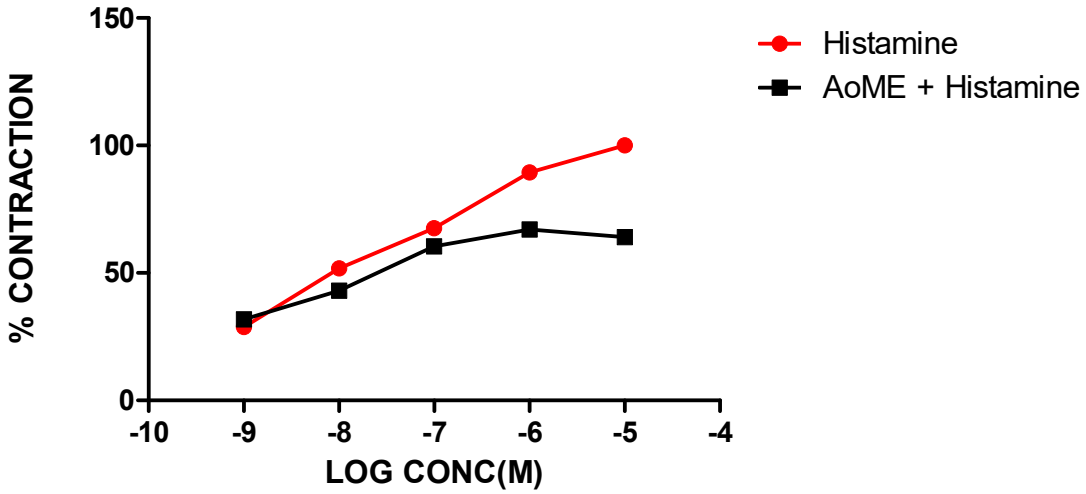


Fig. 4.11(c). Log concentration response of isolated guinea pig ileum strips to cumulative addition histamine alone (Red circle) and in the presence of AoME (0.2mg/ml, Black square). Each point represents Mean±SEM of six independent experiments. Log-concentration curve of histamine is shifted to the right by AoME

Table 4.8. EC 50 values of histamine in the presence of AoME in GPI

	Histamine	**AoME + Histamine**
EC50 (M)	9.88×10^{-9}	2.43×10^{-8}
Log EC50	-8.005	-7.613

4.15. Effect of the methanol stem bark extract of *Anacardium occidentale* (AoME) on rat uterus strip preparations (IRUS)

Figure 4.12(a) shows a representative portion of the various recordings obtained for the effect of AoME on rat uterus strip preparation when added cumulatively. The AoME slightly raised the tonic waves of the uterus strip from the baseline with increasing concentration of the extract added cumulatively. This is an indication of mild contractile effect of the extract on the uterus strip. The log concentration response curve is for this effect is shown in Fig 4.12(b) with an EC_{50} of 5.01mg/kg.

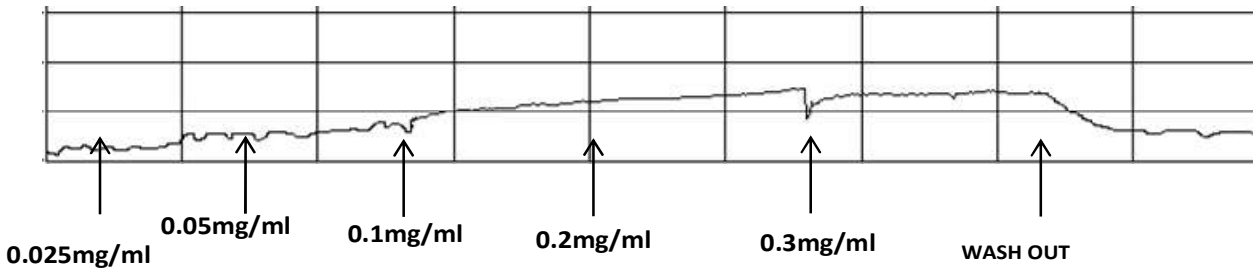


Fig. 4.12 (a). Typical tracing showing effects of graded concentrations of the methanol extract of AoME on rat uterus strip preparations. The AoME slightly raised the tonic waves of the uterus strip from the baseline with increasing concentration of the extract added cumulatively. This is indication of the mild contractile effect of the extract on the stomach strip.

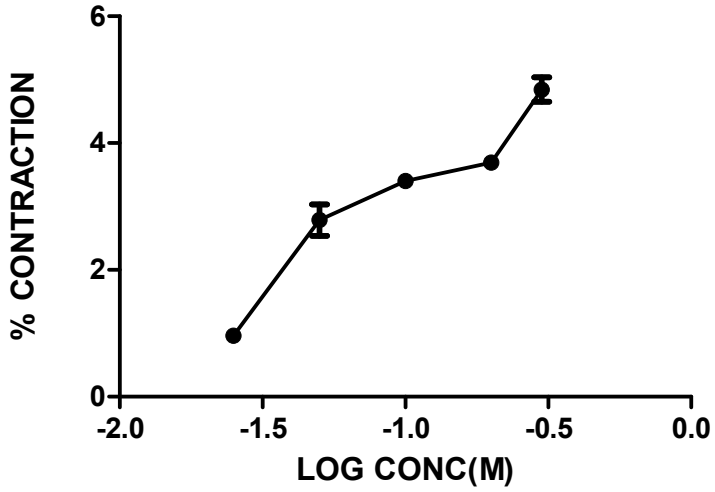


Fig. 4.12 (b).). Log Concentration response of isolated rat uterus strips to cumulative addition of AoME. Each point represents Mean±SEM of six independent experiments.

4.16. Effect of Acetylcholine alone and in the presence of methanol stem bark of *Anacardium occidentale* (AoME) on rat uterus strip preparations (IRUS)

The findings are represented as shown in figures 4.13(a-c) Contraction of isolated rat uterus strips by graded concentration of acetylcholine (cholinergic agonist) added cumulatively was almost completely blocked by AoME pre-incubation at a concentration of 0.2 mg/mL. The successive increase in the height of the waves following the addition of acetylcholine alone as shown in Fig 4.13a was transformed into unsteady waves with reduced height in the presence of AoME (Fig 4.13b). This observation is demonstrated on the log concentration – response curves (Fig. 4.13c). AoME shift the log concentration of acetylcholine to the right. The EC_{50} value of acetylcholine (5.32×10^{-9}M) was raised to 6.50×10^{-6}M by AoME (Table 4.9)

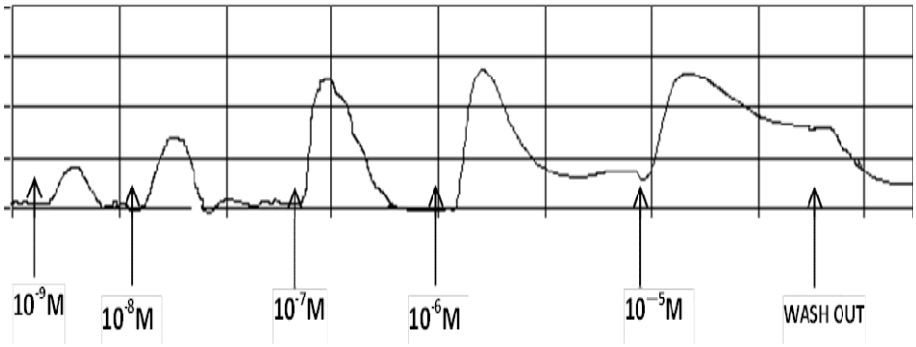


Fig. 4.13(a). Typical tracing showing the effect of graded concentrations of acetylcholine without pre-incubation with AoME on rat uterus strip. The first five arrows represent point of cumulative addition of acetylcholine (10^{-9}, 10^{-8}, 10^{-7}, $10^{-6}$and 10^{-9}M). The last arrow represent the washed out. The cumulative increase in the height of the waves raised from the baseline is indicative of the contractile effect of acetylcholine on rat uterus strip preparation.

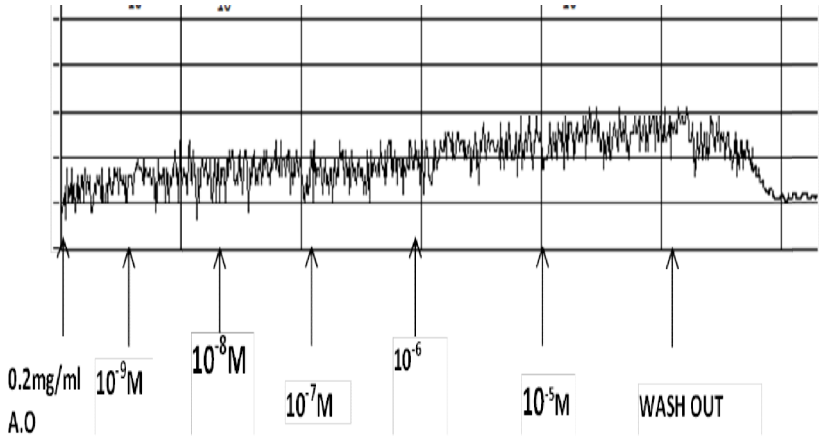


Fig 4.13(b) Typical tracing shows the effect of graded concentrations of acetylcholine after pre-incubation with AoME (0.2 mg/ml) on rat uterus strip. The first five arrows represent point of cumulative addition of acetylcholine (10^{-9}, 10^{-8}, 10^{-7}, $10^{-6}$and 10^{-9}M). The last arrow represent the washed out. The successive increase in the height of the waves raised from the baseline by addition of acetylcholine in the presence of AoME was leveled out when compared with its initial effect alone (Fig 4.13a). This is a clear manifestation of weak contraction. These observations suggest the inhibition of the contractile effect of acetylcholine on rat uterus strip preparation by AoME.

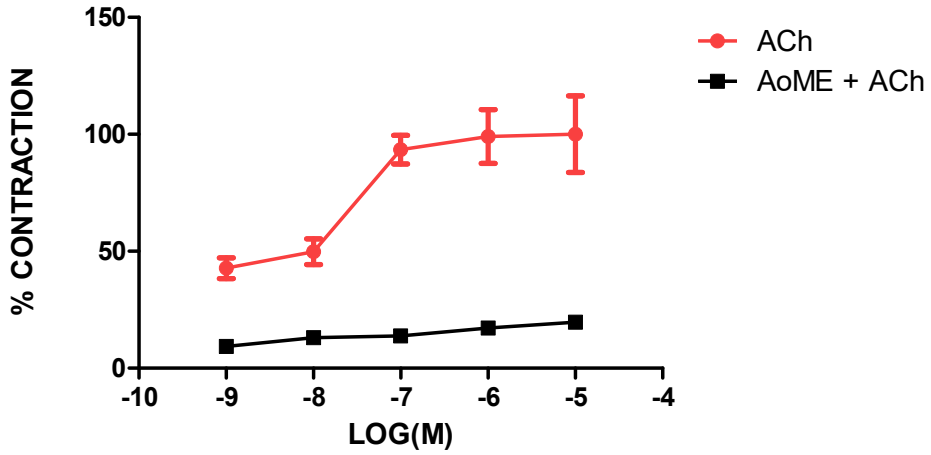


Fig 4.13(c) Log concentration response of isolated rat uterus strips to cumulative addition acetylcholine (Red circle) and in the presence of AoME (0.2 mg/ml, Black square). Each point represents Mean±SEM of six independent experiments. Log-concentration curve of acetylcholine is shifted to the right by AoME

Table 4.9. EC $_{50}$ values of Acetylcholine in the presence of AoME in IRUS

	ACh	**AoME +ACh**
EC_{50} (M)	5.32×10^{-9}	3.52×10^{-6}
Log EC_{50}	-8.724	-4.45

4.17. Effect of Oxytocin alone and in the presence of methanol stem bark of *Anacardium occidentale* (AoME) on rat uterus strip preparations (IRUS)

The findings of this study are represented in Figures 4.14(a-c) Contraction of isolated rat uterus strips by graded concentration of Oxytocin (Oxytocitic agonist) (Fig 4.16a) added cumulatively was almost completely blocked by AoME pre-incubation at a concentration of 0.2 mg/mL. This observation is demonstrated on the log concentration – response curves (Fig 4.14c). AoME shift the log concentration of Oxytocin to the right. The EC_{50} value of oxytocin (3.16×10^{-9} M) was raised to 3.52×10^{-6}M by AoME (Table 4.10)

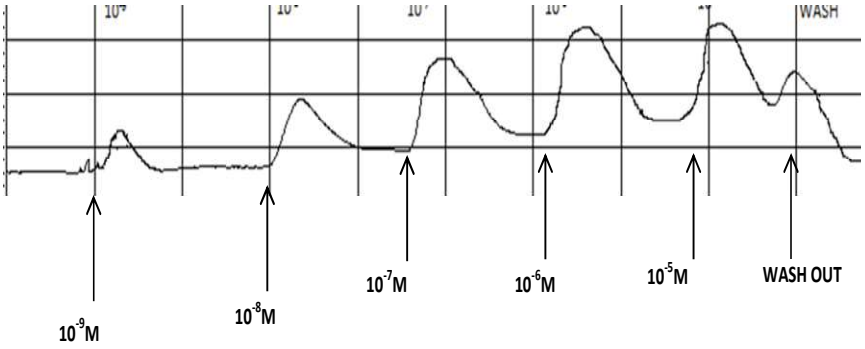


Fig 4.14(a): Typical tracing showing the effect of graded concentrations of Oxytocin without pre-incubation with AoME on rat uterus strip. The first five arrows represent point of cumulative addition of Oxytocin (10^{-9}, 10^{-8}, 10^{-7}, $10^{-6}$and 10^{-9}M). The last arrow represent the washed out. The cumulative increase in the height of the waves raised from the baseline is indicative of the contractile effect of oxytocin on rat uterus strip preparation.

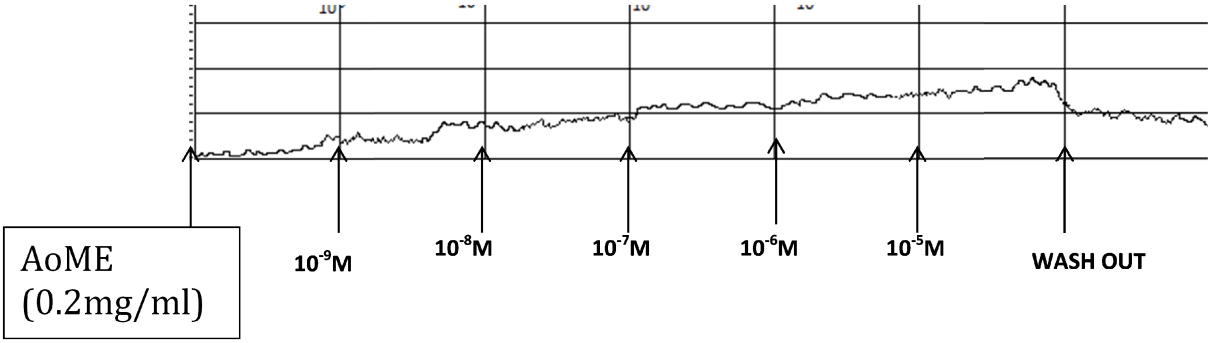


Fig 4.14(b) Typical tracing shows the effect of graded concentrations of Oxytocin after pre-incubation with AoME (0.2 mg/ml) on rat uterus strip. The first five arrows represent point of cumulative addition of Oxytocin (10^{-9}, 10^{-8}, 10^{-7}, $10^{-6}$and 10^{-9}M). The last arrow represent the washed out. The successive increase in the height of the waves raised from the baseline by addition of Oxytocin in the presence of AoME was leveled out when compared with its initial effect alone (Fig 4.13a). This is a clear manifestation of reduced contraction. These observations suggest the inhibition of the contractile effect of Oxytocin on rat uterus strip preparation by AoME.

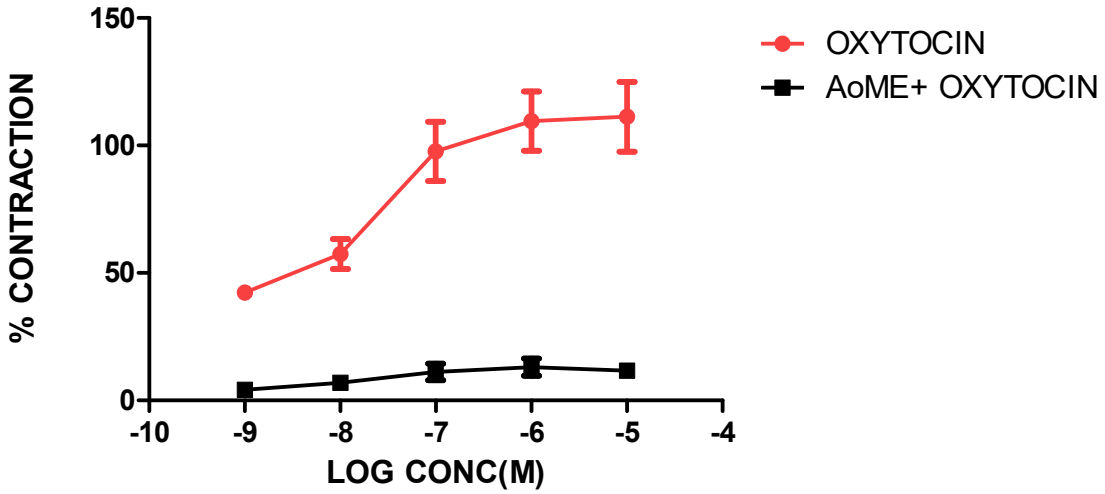


Fig. 4.14(c) Log concentration response of isolated rat uterus strips to cumulative addition Oxytocin (Red circle) and in the presence of AoME (0.2mg/ml, Black square). Each point represents Mean±SEM of six independent experiments. Log- concentration curve of Oxytocin is shifted to the right by AoME

Table 4.10. EC $_{50}$ values of oxytocin in the presence of AoME in IRUS

	Oxytocin	AoME + Oxytocin
EC_{50} (M)	3.16×10^{-9}	6.50×10^{-5}
Log EC_{50}	-8.500	-4.187

4.18. Effects of the fractions of AoME on isolated guinea pig ileum preparations

The effects of graded concentration of fractions of AoME were tested on normal tonus activity of isolated guinea pig ileum non-cumulatively on microdyanomometer. The fractions of AoME that was investigated are: Hexane fraction (AoHF), Ethylacetate fraction and (Methanol fraction). The effect of AoMe was also evaluated non-cumulatively in this study. The representation of the observed tracings showing the relaxant effect of the each concentration of the fractions and AoMe are as shown in Figures 4.15, 4.16, 4.17 and 4.18. The AoHF and AoEF had no activity at the low concentrations (0.0067-0.053 mg/mL). The Height of downward deflection of the normal tonus of isolated GPI was measured in millimeters and regarded as height of relaxation. The log concentration of the fractions were plotted against the height of relaxation to generate the log concentration-response curve as shown in Figures 4.19,4.20, 4.21 and 4.22. The IC_{50} value for the AoEF was the least among the three fractions. This value was lower than that of stem bark extract (AoME) (Table 4.11)

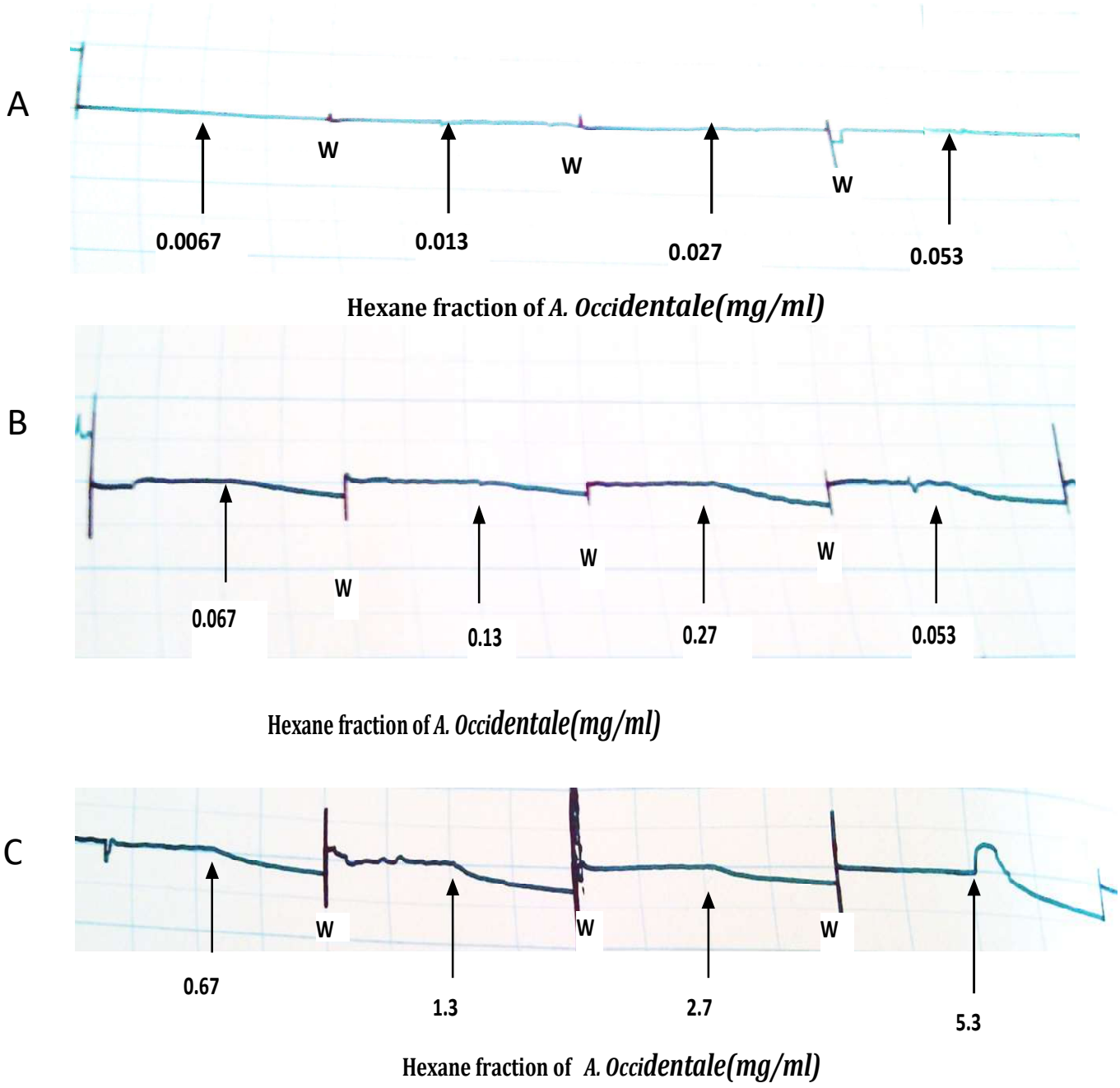
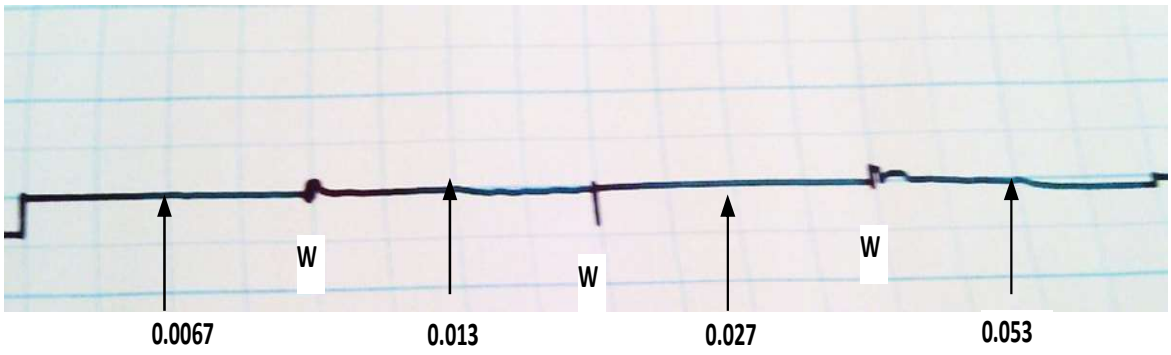


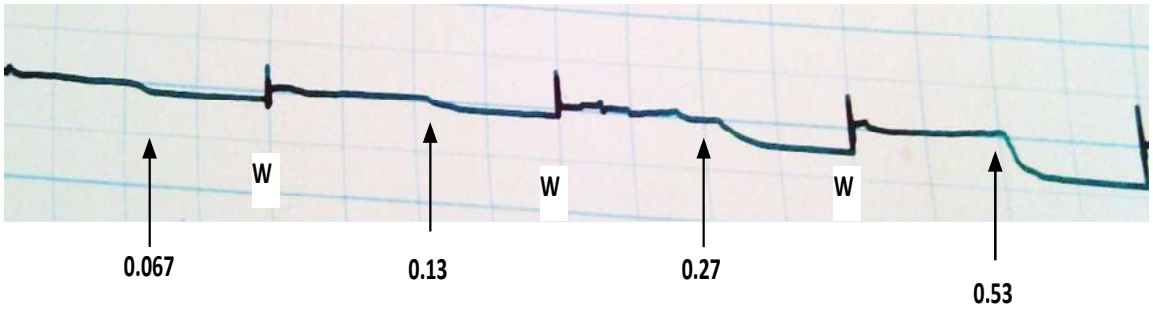
Fig. 4.15. Concentration –dependent relaxation effect of AoHF *(0.067mg/ml-5.3mg/ml) on guinea pig ileum strip*

A

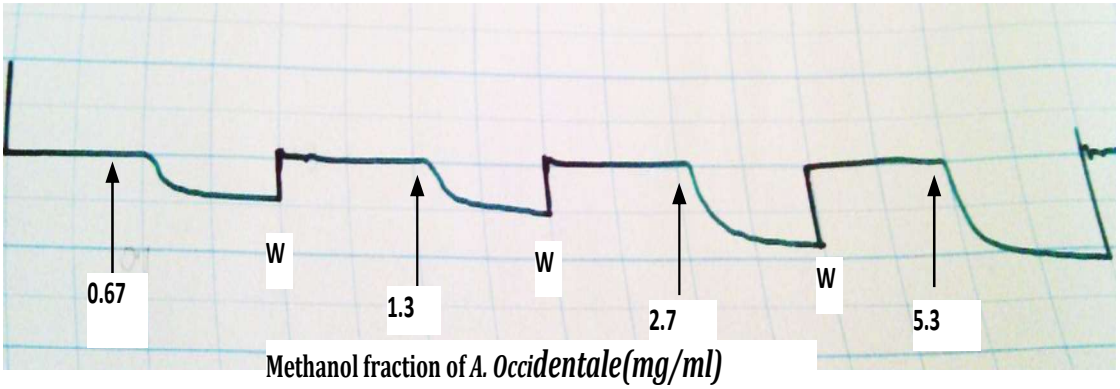


Methanol fraction of *A. Occidentale(mg/ml)*

B



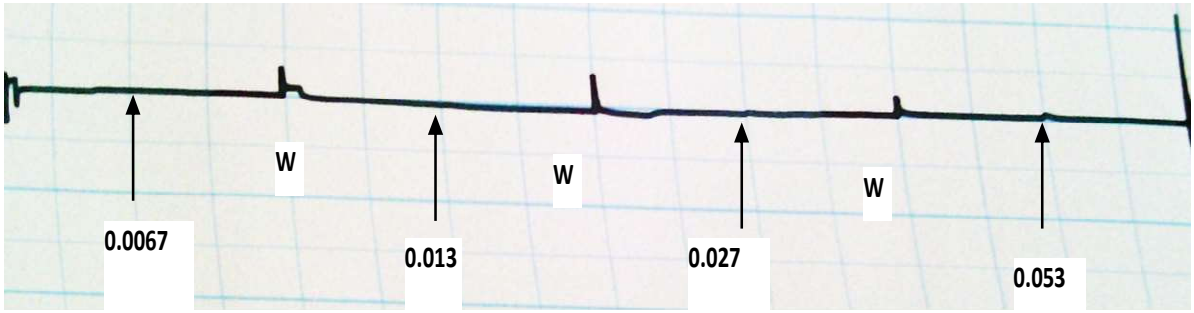
Methanol fraction of *A. Occidentale(mg/ml)*



Methanol fraction of *A. Occidentale(mg/ml)*

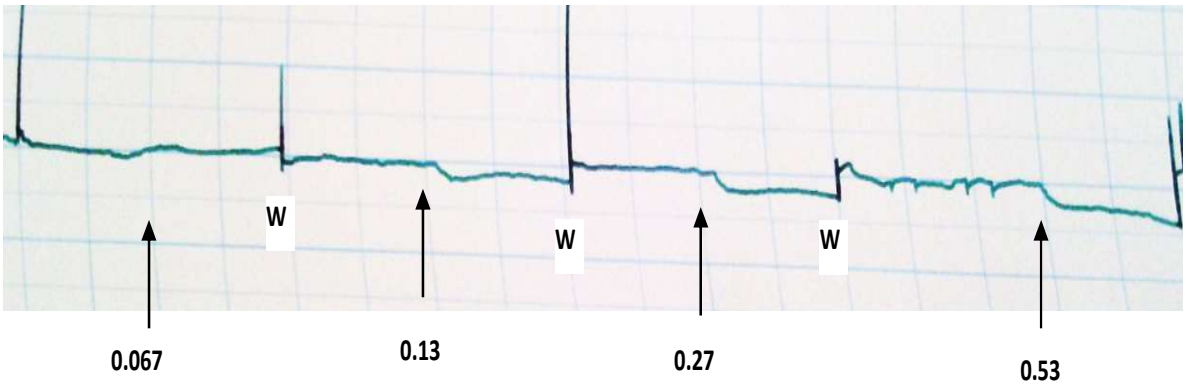
Figure 4.16 : concentration –dependent relaxation effect of AoMF *as shown in plate B and* C *on guinea pig ileum strips*

A



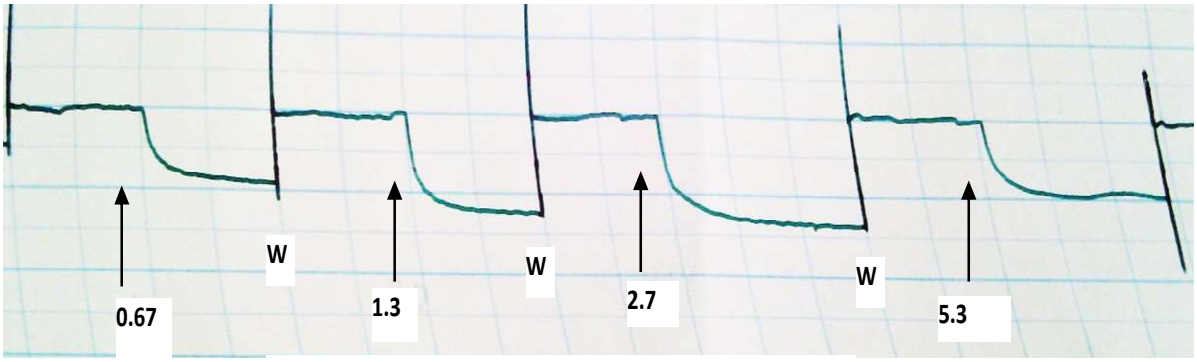
ethylacetate fraction of *A. Occidentale(mg/ml)*

B



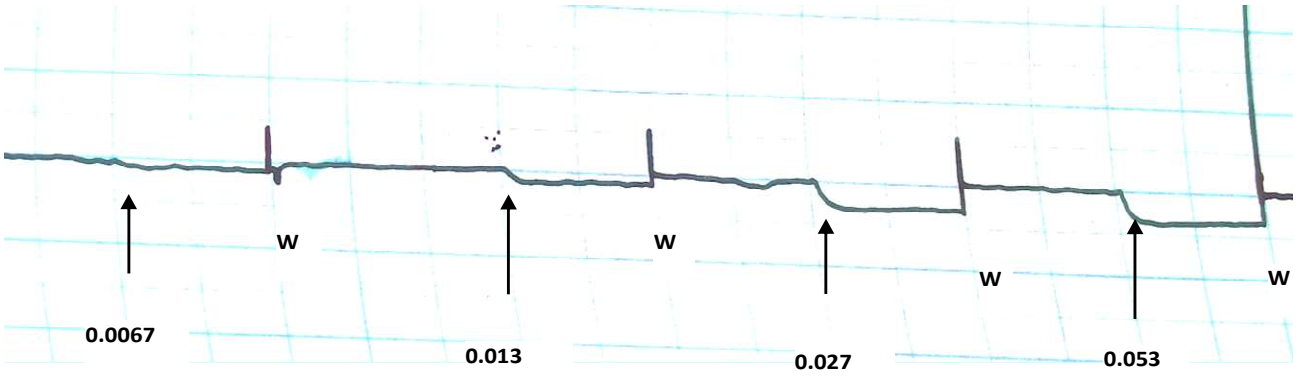
ethylacetate fraction of *A. Occidentale(mg/ml)*

C

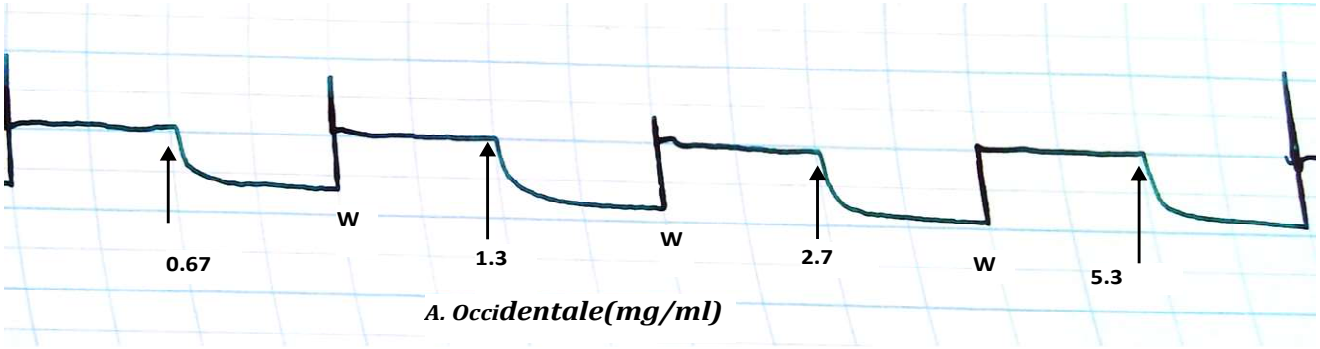


ethylacetate fraction of *A. Occidentale(mg/ml)*

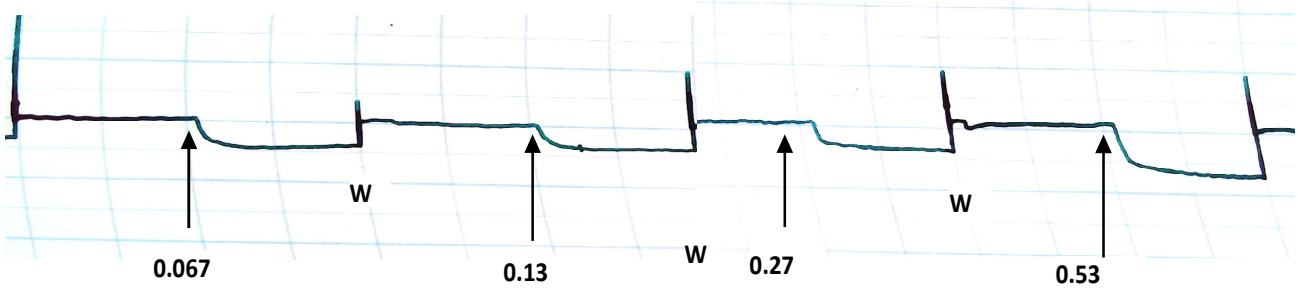
Fig. 4.17. Concentration –dependent relaxation effect of AoEF on guinea pig ileum strip(0.067-5.3mg/ml) *as shown in plate B an*d C



A. Occidentale(mg/ml)



A. Occidentale(mg/ml)



A. Occidentale(mg/ml)

Fig. 4.18. Concentration –dependent relaxation of the stem bark of AoME *(0.0067mg/ml-5.3mg/ml) on guinea pig ileum strip*

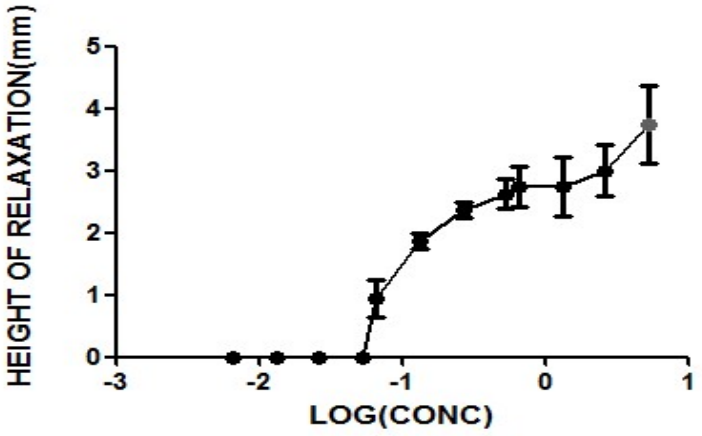


Fig. 4.19. Log concentration- response curve showing relaxation of guinea pig ileum preparation by graded concentration of AoHF

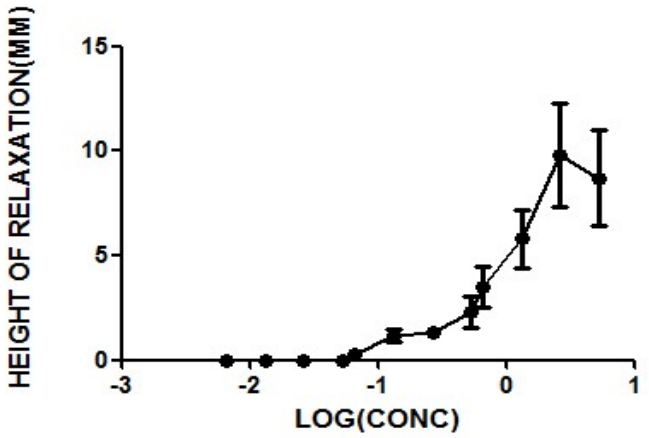


Fig. 4.20. Log concentration- response curve showing relaxation of guinea pig ileum preparation by graded concentration of AoEF

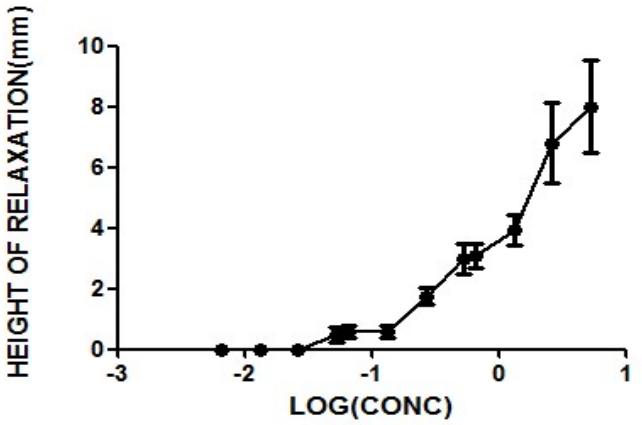


Fig.4.21. Log concentration-response curve showing relaxation of guinea pig ileum preparation by graded concentration of the AoMF

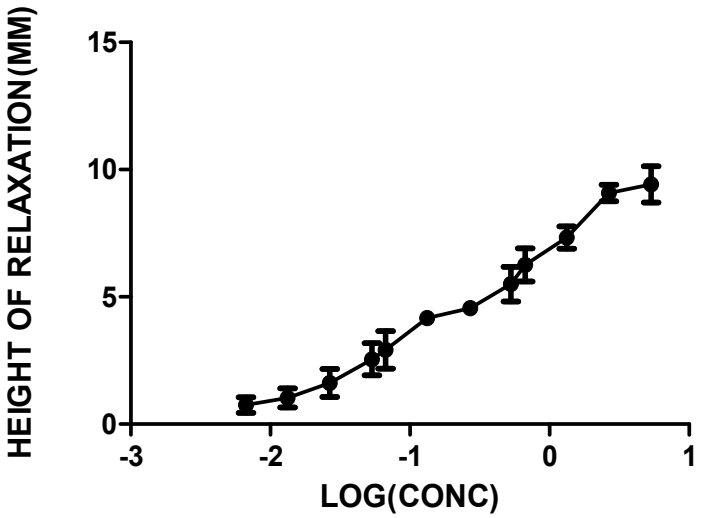


Fig. 4.22. Log concentration- response curve showing relaxation of guinea pig ileum preparation by graded concentration of AoME

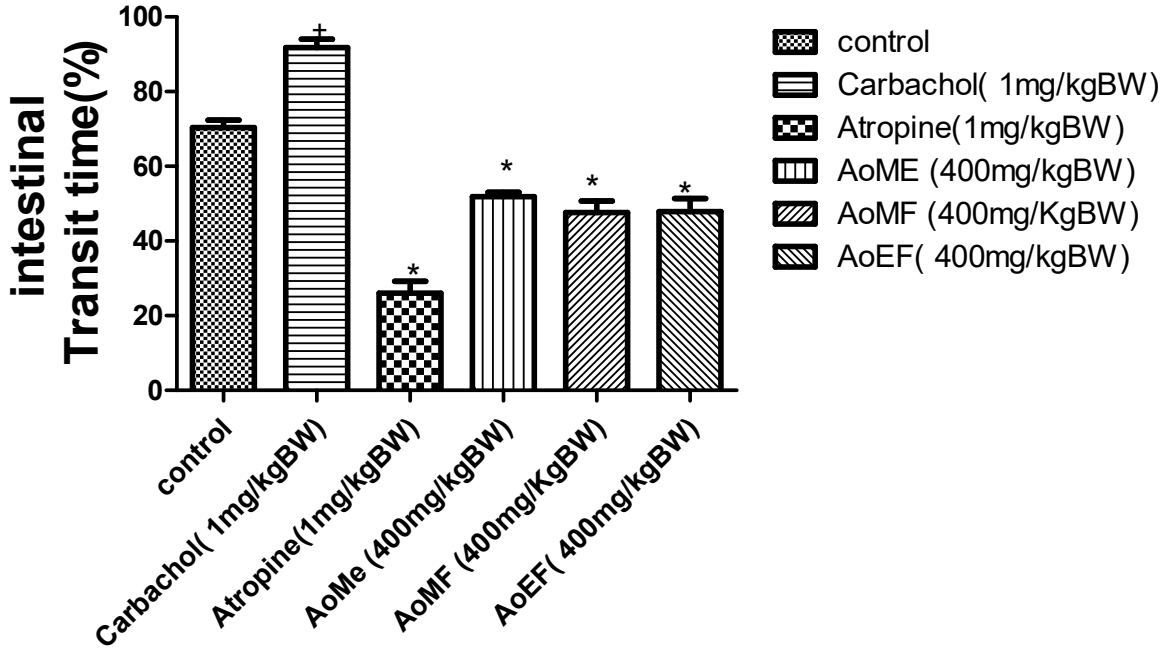
Table 4.11: IC_{50} values of different fractions of the stem bark of *Anacardium occidentale*

	LOG $1C_{50}$	IC_{50} (mg/ml)
AoHF	**-4.775**	**1.68×10^{-5}**
AoEF	**-4.900**	**1.26×10^{-5}**
AoMF	**-4.810**	**1.55×10^{-5}**
AoME	**-4.838**	**1.45×10^{-4}**

4.19. Effect of fractions of AoME on gastrointestinal motility in rats (*in-vivo*)

The obtained result is represented on a graph as shown in Fig. 4.23. The percentage of the total length travelled by charcoal meal in the proportion to the entire length of the gastrointestinal tract from pyloro-duodenal junction to the ileoceacal junction is expressed as propulsive movement of the intestine or intestinal transit of charcoal meal in mice (in-vivo). The fractions of AoME that were investigated are that of the methanol (AoMF) and ethylacetate (AoEF).

The AoMF and AoEF at doses of 400 mg/kg produced a significant decreased in the propulsive movement of the intestine when compared to the control animals which received 1 mg/100 g normalsaline ($P<0.05$). The reduction produced by both fractions was not significantly different from each other and AoME at the dose of 400 mg/mL ($P<0.05$).



significantly different from control p<0.05

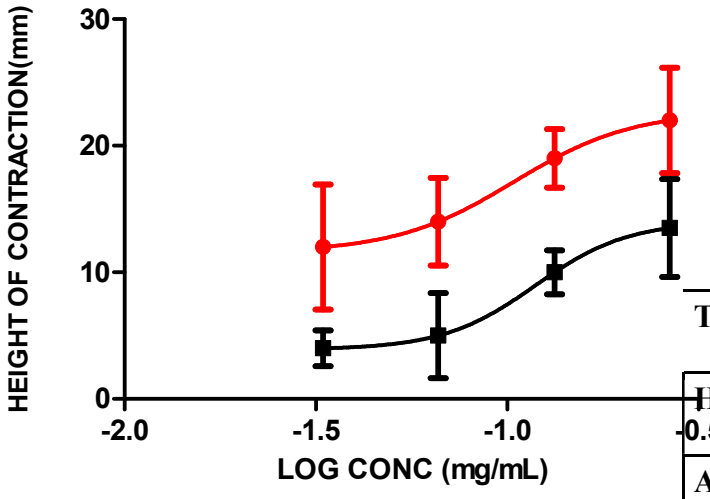
Fig. 4:23. Gastrointestinal transit time in control and fractions-treated animals

4.20. Study of the possible mechanism(s) of the relaxant effects of AoEF on guinea pig ileum strip (*in vitro*)

The possible mechanism(s) of the relaxant effect of AoEF was investigated on guinea ileum strip. AoEF was interacted with standard agonist of smooth muscle contraction in the intestine. The findings are represented on a log concentration-response curve as shown in figures 4.24, 4.25 and 4.26). AoEF at a concentration of 2.7 mg/ml shifted the log concentration curve of Histamine (H1 agonist), and Serotonin (5-HT agonist), acetylcholine (muscarinic agonist), to the right. The EC_{50} values of the three agonists were raised by AoEF.

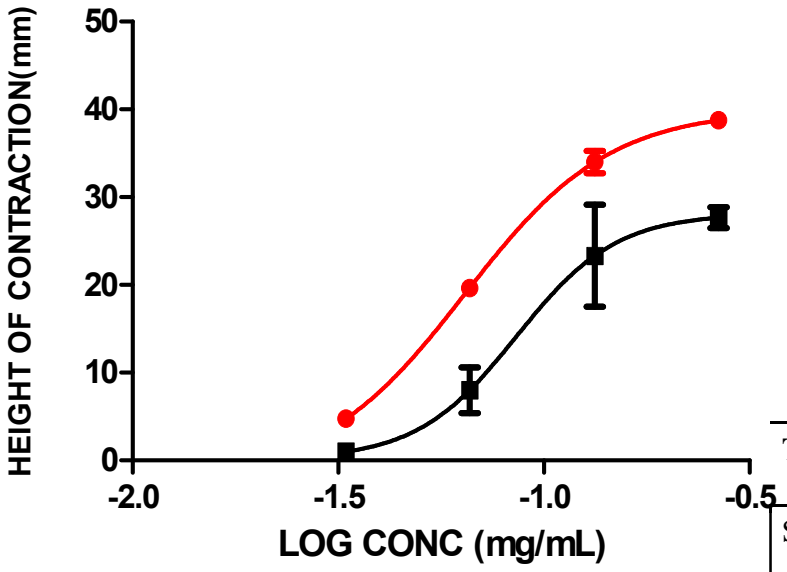
In the second phase of the study, AoEF (2.7 mg/ml) was interacted with L-NAME (NO inhibitor), hexamethonium (Nicotinic receptor antagonist) and Atropine (Muscarinic receptor antagonist)

L-NAME, hexamethonium and atropine were used in this study to assess the involvement of non-adrenergic-non-cholinergic, ganglionic and cholinergic mechanisms in the relaxant effect of AoEF. The results of the study are as shown in figures 4.27, 4.28, 4.29. The relaxant effect of AoEF was dose-dependently increased by L-NAME and hexamethonium. The observed increases were however, not significant ($P<0.05$). The AoEF relaxant effect on guinea pig ileum strip was significantly increased by atropine in a dose dependent manner ($P<0.05$)



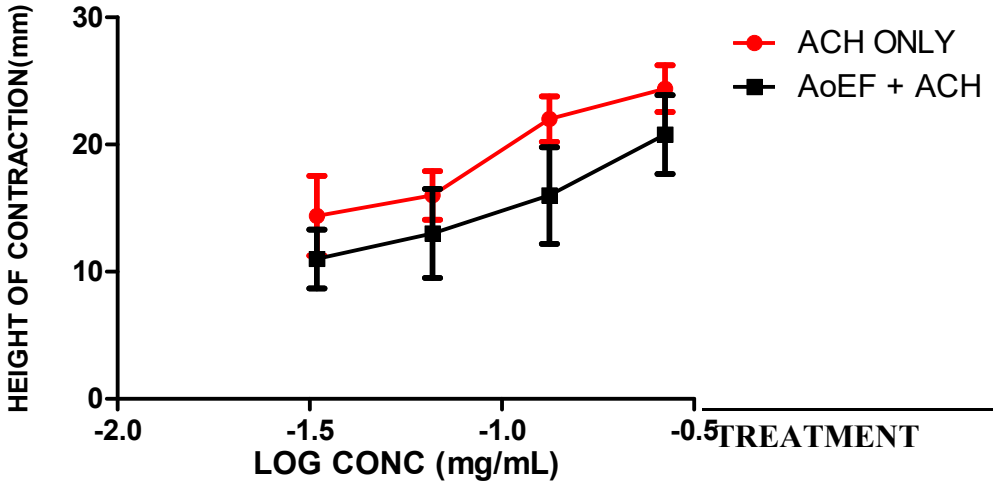
TREATMENT	EC_{50} (mg/mL)
Histamine	0.14
AoEF +Histamine	0.26

Fig. 4.24. Non cumulative Log concentration- response curve showing relaxation of histamine induced contraction of guinea pig ileum preparation by AoEF



TREATMENT	EC_{50} (mg/mL)
Serotonin	0.06
AOEF+ Serotonin	0.08

Fig. 4.25. Non cumulative Log concentration- response curve showing relaxation of serotonin induced contraction of guinea pig ileum preparation by AOEF



TREATMENT	EC_{50} (mg/mL)
Acetylcholine	0.12
AOEF +Acetylcholine	0.53

Fig. 4.26: Non Log concentration- response curve showing relaxation of acetylcholine induced contraction of guinea pig ileum preparation by AOEF

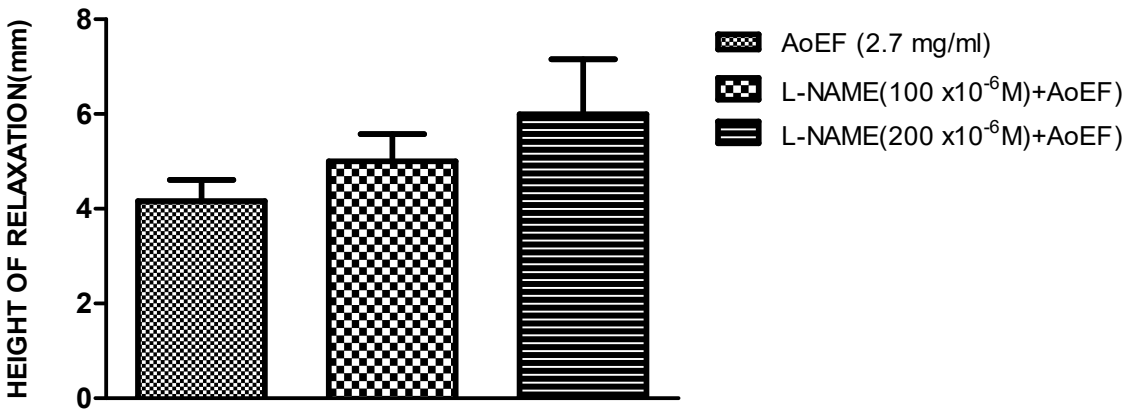


Fig. 4.27. Interaction of L-NAME with AoEF on guinea pig ileum strip

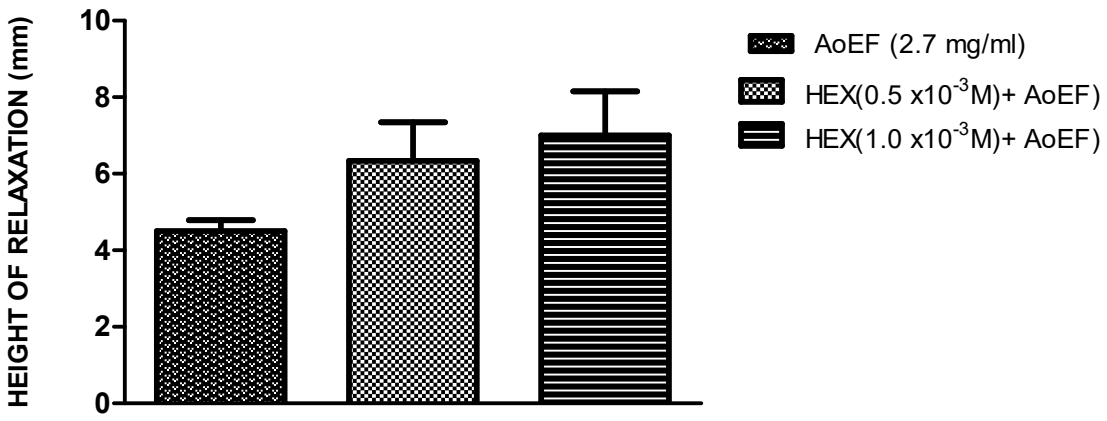
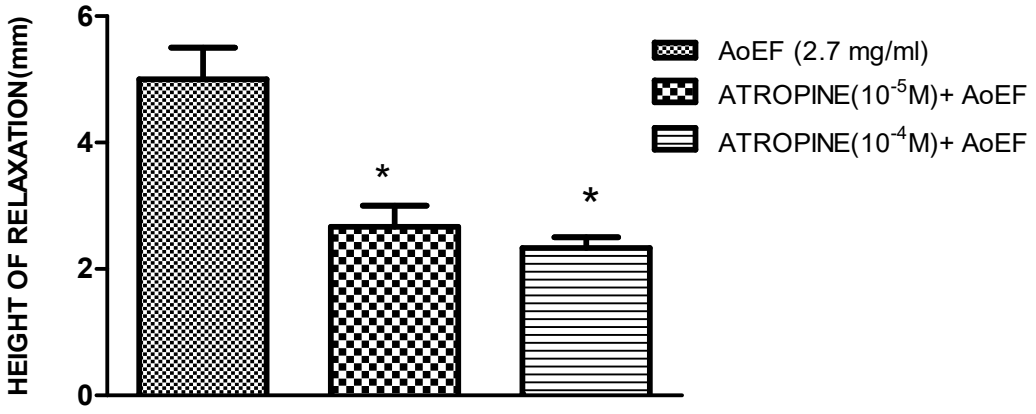


Fig. 4.28. Interaction of hexamethonium with AoEF on guinea pig ileum strip



**** Significantly different from* AoEF *p<0.05***

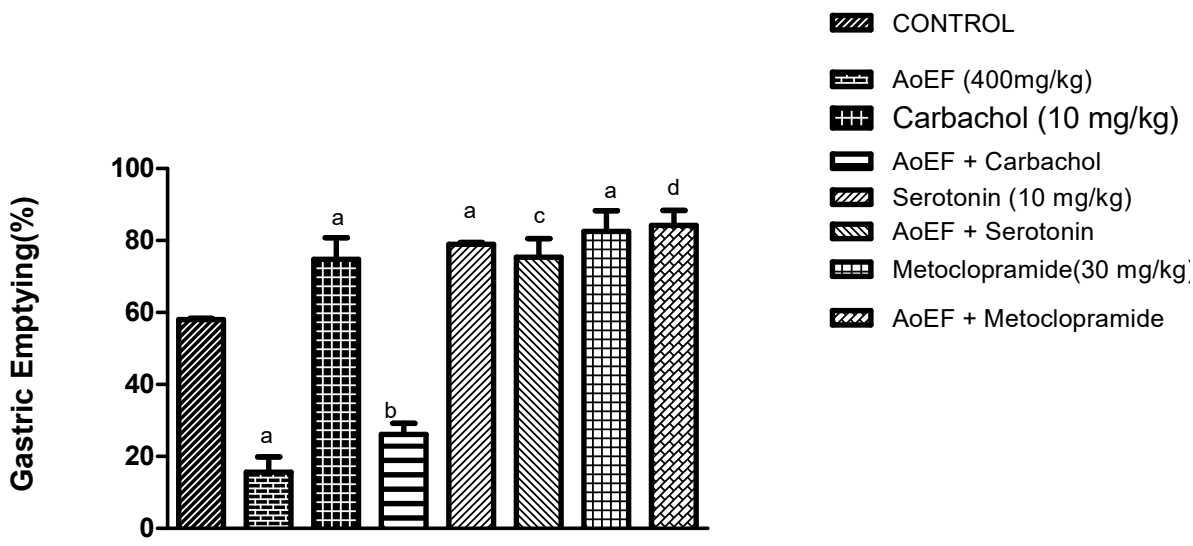
Fig. 4.29. Interaction of atropine with AoEF on guinea pig ileum strip

4.21. Study of the possible mechanism(s) of the relaxant effects of AoEF in mice (*in vitro)*

The possible mechanism of AoEF was investigated through the measurement of gastric emptying and gastrointestinal transit activated by agonists of specific pathways in the presence of AoEF. The agonists that were employed are carbachol (cholinergic pathway), Serotonin (Serotonergic pathway) and metoclopramide (dopaminergic pathway). The findings are represented on the graph as shown in figures 4.30 and 4.31. Gastric emptying was significantly ($P<0.05$) increased by carbachol (74.8±5.8%), serotonin (78.9±0.6%) and metoclopramide (82.5±5.7%) when compared with values in control animals (58.1±0.3%) Pretreatment with AoEF significantly blocked out the action of carbachol on gastric emptying (74.8±5.8 vs 26.1±3.1%) AoEF produced no significant effect on the action of serotonin and metoclopramide on gastric emptying.

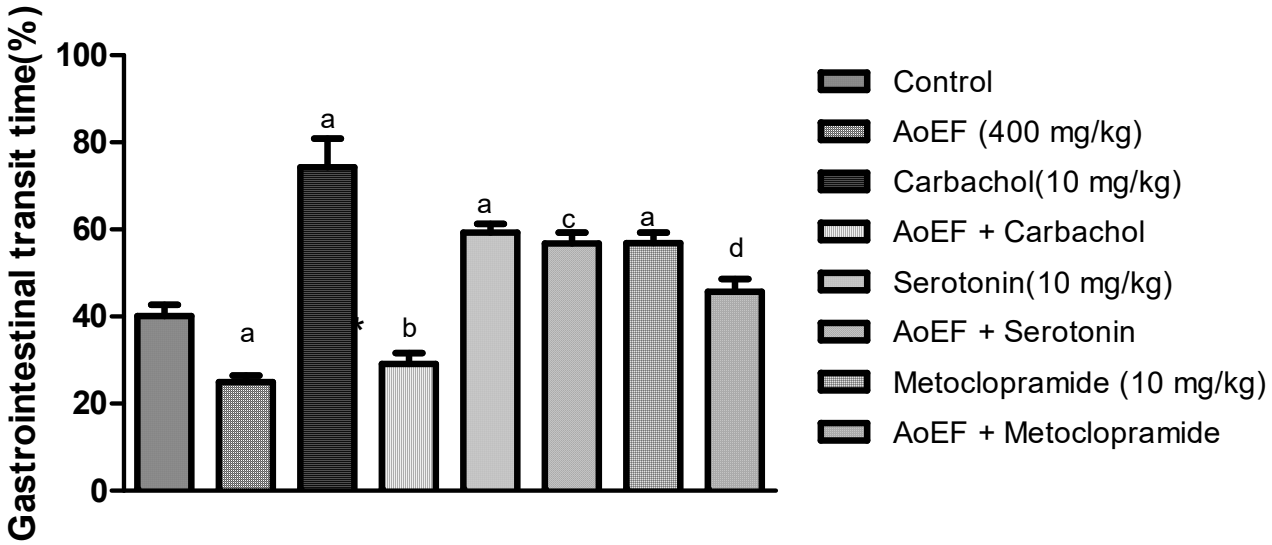
Gastrointestinal transit was significantly ($P<0.05$) increased by carbachol (10 mg/kg), serotonin (10 mg/kg) and Metoclopramide (30 mg/kg) (74.3±2.6; 59.3±2.5; 56.9±5.5%, respectively) when compared with values in control animals (40.16±1.5%) (Fig 4.31)

Pretreatment with AoEF (400 mg/kg) significantly ($P<0.05$) inhibited gastrointestinal transit induced by carbachol.



"a" is significantly different from control, "b" is significantly different from carbachol, "c" and "d" are not significant when compared to serotonin and metocloppramide respectively $p<0.05$

Fig. 4.30. Selective blockade of agonist effect on gastric emptying by AoEF



"a" is significantly different from control, "b" is significantly different from carbachol, "c" and "d" are not significant compared to Serotonin and Metocloppramide respectively $p<0.05$

Fig. 4.31. Selective blockade of agonists effect on gastrointestinal motility by AoEF

4.22. Gas chromatography mass spectrophotometry composition of AoME and AoEF

GCMS analysis of the AoME revealed the presence of 15 components (Table 4.12) However, the predominant components are the oleic-acid (45.51 %) and hexadecanoic acid (20.57 %). GCMS analysis of the AoEF reveals the presence of 24 components with the predominant components as the oleic-acid ethyl ester and 11-Octadecenoic acid methyl ester with percentage composition of 13.90 % and 10.18 % respectively. These results are also demonstrated on the chromatogram which shows the peaks of the compounds. (Fig 4.31 and 4.32)

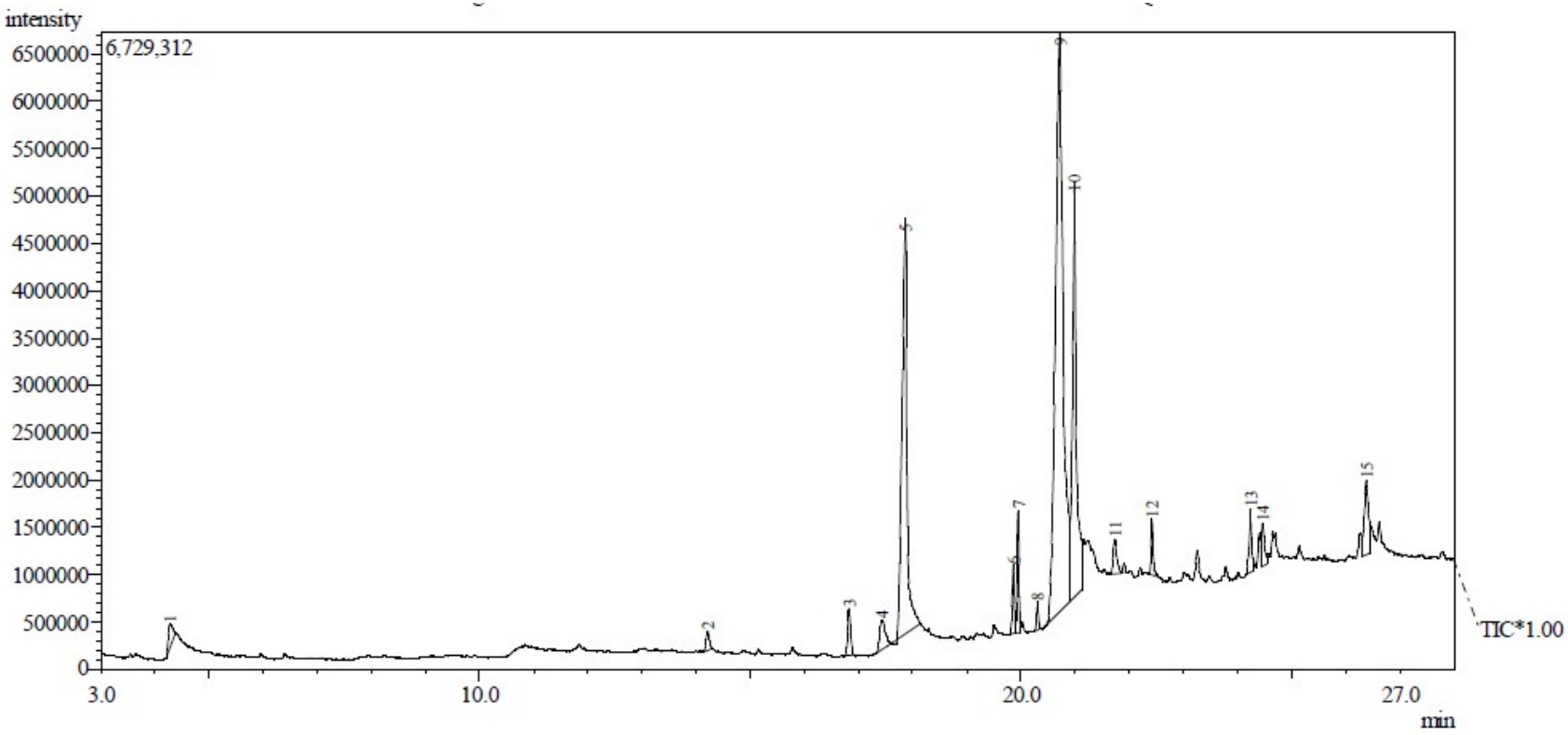


Fig. 4.32. Chromatogram showing the peak of compounds in methanol stem bark extract of *Anacardium occidentale* (AoME)

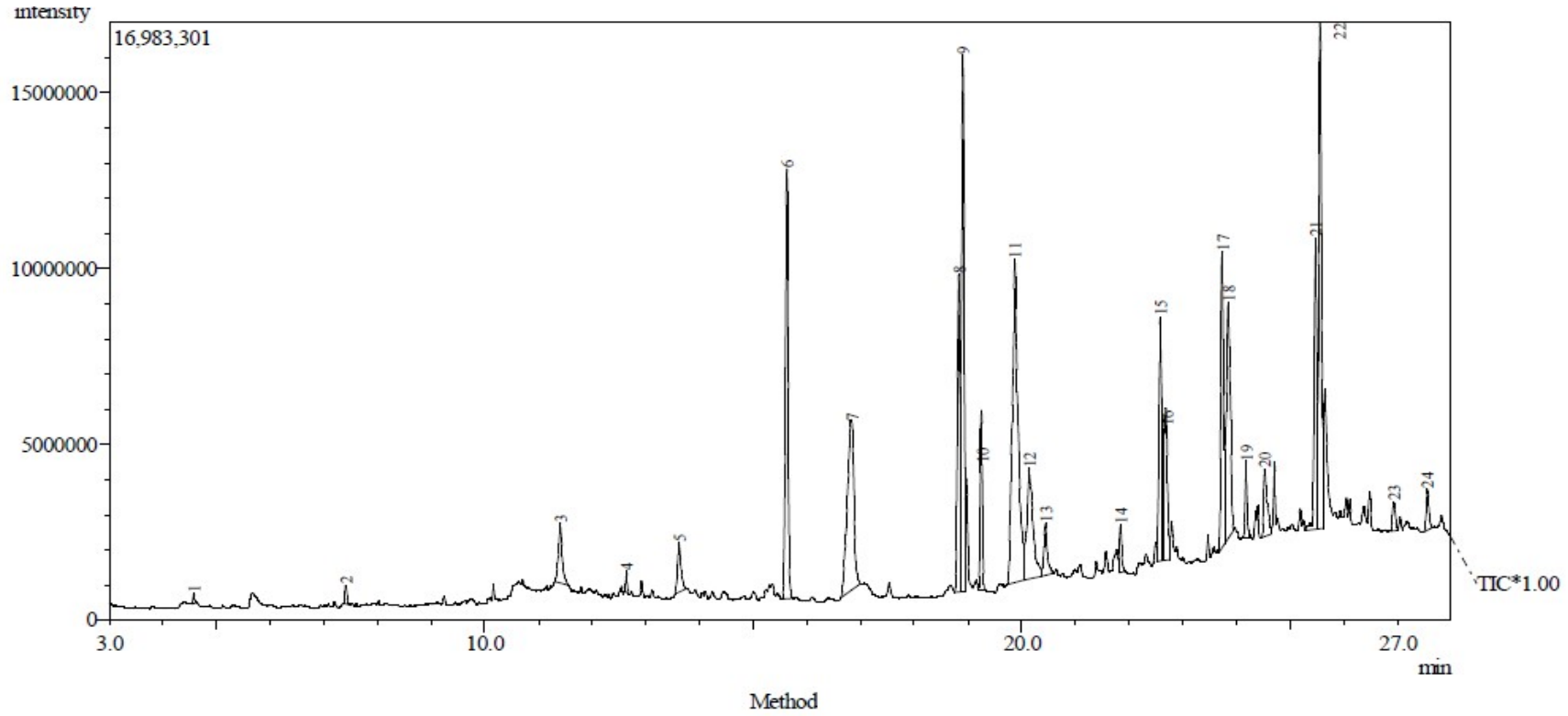


Fig. 4.33. Chromatogram showing the peak of compounds in the ethylacetate fraction of *Anacardium occidentale* (AOEF)

Table 4.12 GC-MS analysis of methanolic extract of the stem bark *anacardium occidentale* (AoME)

S/N	COMPONENTS	RT(mins)	%	SI
1.	Glyceritol	4.28	1.14	93
2.	Myristic acid	14.21	0.63	91
3.	Pentadecanoic acid 14 methyl ester	16.83	1.34	92
4.	Tetradecanoic acd	17.44	1.59	85
5.	Stearic acid	20.99	16.14	94
6.	9,12 octadecadien-1-ol	19.86	1.45	91
7.	11-Octadecanoic cid ME	19.95	2.65	92
8.	Octadecanoic acid ME	20.31	0.53	89
9.	Oleic acid	20.72	45.51*	94
10.	Hexadecanoic acid	17.87	20.57*	92
11.	1,E-11,Z-13-0ctadecatriene	21.73	1.33	87
12.	Octadecanoic acid,2-hydroxyl-1,3-propanediyl ester	22.42	1.17	84
13.	2-methyl-Z,Z-3,13-octadecadienol	24.24	1.58	87
14.	Octadecanoic acid, 2-hydroxyl 1,3-propanediyl ester	24.46	1.18	82
15.	1,2-Epoxycycyclooct-3-ene,5,5-dimethyl-8-methylene	26.38	3.18	80

Table 4.13 GC-MS analysis of Ethyl acetate fraction of the stem bark *anacardium occidentale* (AoEF)

S/N	COMPONENTS	RT(mins)	%	SI
1.	n-decane	4.580	0.12	92
2.	Decane,2,9-dimethyl	7.413	0.24	94
3.	Undecanoic acid	11.407	1.59	86
4.	Decane 3,7,dimethyl	12.642	0.22	93
5.	Tetradecanoic acid	13.617	1.25	91
6.	Pentadecanoic acid	16.626	7.95	90
7.	Octadecanoic acid 2(2-hydroxylethoxy) ethyl ester	16.826	8.10	87
8.	7,10-Hexadecadieonic acid	18.829	5.91	92
9.	11,Octadecenoic acid Methyl ester	18.907	10.18	94
10.	Octadecenoic acid Methyl ester	19.251	2.89	89
11.	(E)-9-Octadecenoic acid ethyl ester(oleic acid ethyl ester)	19.872	13.9	89
12.	n-Octadecenoic acid (stearic acid)	20.140	4.97	88
13.	Tetradecanamide	20.441	1.26	87
14.	Eicosanoic acid methylester	21.840	0.78	86
15.	9-Octadecenamide	22.581	5.27	85
16.	dodecanol	22.687	3.03	71
17.	5-Chloropentanoic acid, 2-methylphenyl ester	23.731	5.77	78
18.	Docosanoic acid, methyl ester	23.847	6.61	88
19.	1-methylheptylbromide	24.178	1.09	83
20.	9-Octadecenamide (oleic acid amide)	24.525	1.70	83
21.	2,2-dimethyl-3-vinylbicylo(2.2.1) heptane	25.475	6.00	82
22.	Phenol, 3-pentadecyl(Anacardol)	25.559	9.87	79
23.	Octadecanoic acid,2-hydroxyl methyl ester	26.933	0.56	86
24.	Hexacosanoic acid	27.561	0.77	73

CHAPTER FIVE

5.0 DISCUSSION

In Africa, there appears to be an increasing dependency of people on herbs and their products in common gastrointestinal disorders management such as diarrhoea (Agunu *et al*., 2005) *Anacardium occidentale* is one of the most common herbs use in managing diarrhoea in local communities of Nigeria and Africa (Etuk *et al*, 2009) In addition, it has been described in the literature to have antidiarrhoeal property (Omoboyowa *et al*, 2013). Motility of the gut among many other factors is crucial to the pathogenesis of diarrhea. The aim of this work is to assess the effect of the stem bark extract of *Anacardium occidentale (AoMe)* on smooth muscle preparations using rat stomach strip preparation (SSP), Guinea pig ileum strip preparation (GPI) and isolated rat uterus strip preparation (IRUS) and to also examine the extract effect on *in-vivo* activity in rats and mice.

According to Lorke (1983), LD50 that is more than 5000 mg/kg b.w. is suggested to be safe. Therefore, AoME can be considered as non-toxic and relatively safe. The high level of safety is also in agreement with its general use in folk medicine. However, this result was at variance with the work of Okonkwo *et al.,* (2010) that previously reported that the ethanol extract of the inner stem bark of *Anacardium occidentale* had LD_{50} of 2.154 g/kg *p.o*. in mice.

The Phytochemical evaluation showed the existence of high amount of flavonoids, steroids and phenols in AoME while the level of akaloids, saponins and tannins were moderate .This present result is similar to that of Francis *et al.,* (2010). In their study, *Anacardium occidentale* extract was found to have high amount of polyterpenes and phenol and flavonoids, tannins and sterols. Flavonoids and alkaloids are compounds that exhibit remarkable physiological and pharmacological activities that affect the motility of the gastrointestinal tract (Sammy and Gopalakrishakone, 2008). Flavonoids have being demonstrated to relax gut smooth muscle in a manner that depends on concentration (Marcelly, *et al* 2012). Alkaloids have also been shown to have antidiarrhoeal effect through decreasing intestinal transit time (Cowan, 1999). Tannin salt has been known to interfere with secretory process of the gut by making it's mucosa more resistant (Tripathi, 1994).

From the antidiarrhoeal study in mice, oral administration of castor oil caused diarrhoea in all animals. Evaluation of the effect of methanol stem bark extract of *Anacardium occidentale* on diarrhoea induced by castor oil in mice showed that it delayed the onset of diarrhoea, number of stool, number of loose stool, fresh weight of stool and the water content of stool when compared with the untreated animals. These effects of the methanol extract of *anacardium occidentale* are similar to that obtained for loperamide. Loperamide acts on opiod receptors to slow down GI motility and increase the intestinal fluid reabsorption (Holzer, 2009).

These findings are indication of antidiarrhoeal action and could justify the basis for its folkloric usage. The observed result is consistent with the work of Arauojo (2015) where the gum exudates of the stem bark was reported to possess significant antidiarrhoea property.

Castor oil induces diarrhoea in experimental animals and humans through its active metabolite, ricinoleic acid. Ricinoleic acid causes diarrhoea through a series of events which include stimulating the propulsive movement of the intestine and inhibiting the activity of Na^{+}-K^{+} ATPase (Musa, 2015). The latter event leads to accumulation of fluid in the intestine while, stimulation of the propulsive movement of the intestine will prevent re-absorption of fluid in the intestine which eventually leads to its loss in the feace (Vitalis *et al*., 2016).The antidarrhoeal effect of methanol stem bark extract of *Anacardium occidentale* may be via any of these mechanisms.

Transit time of charcoal in acacia meal provides an assessment of gastrointestinal motility in rats. The mixture of charcoal and acacia is stable throughout its transit in the gastrointestinal tract and give quantitative information on transit along the intestine by visualized interpretation (Peddireddy, 2010). All the doses of AoME significantly decreased the propulsive movement of the intestine when compared to the control group. This observed ability of the extract to reduce propulsive movement of the intestine explains the delay in onset of diarrhoeal and decrease in the frequency of stool and total number of faeces by the extract in this study. Decreasing the movement of the intestine will increased fluid reabsorption thereby minimizing the loss of water in the faeces resulting in the decreased in number of loose faeces and water content. The antidiarrheal activity of the methanol extract of *Anacardium occidentale* may be considered to be more of gastrointestinal motility mechanism

rather than secretory. Atropine at a dose of 1 mg/mL significantly reduced the transit of charcoal meal in the intestine while Carbachol at 1 mg/kg dose increased charcoal meal transit when compared with normal saline (1 mg/100g p.o) ($p<0.05$). This is expected because of interaction of atropine and carbachol with muscarinic receptors. Atropine blocks the effects of acetylcholine at muscarinic receptors while carbachol is an agonist at muscarinic receptor.

The study of motility activities of the gut in whole animal is usually confronted with many confounding variables due to the complexity nature of its neuronal and secretory processes. (Alan and Brian, 1966) In experiment carried on isolated strips of the gut such variables are removed thereby providing opportunity to make precise observations. The effect of AoME was carried out on isolated rat stomach and guinea pig ileum preparations both being smooth muscle of the gastrointestinal tract origin. The effect of AoME was also evaluated on rat uterus strip preparation. This was necessary in other to establish the possible effect(s) of this plant on other systems of the body aside the gut. A drug that is useful and prescribed for the treatment of a specific disease yet may be causing good and negative side effects on other systems of the body such as reproductive system (Nwinyi and Kwanashie, 2013).

The extract added cumulatively modifies the spontaneous tonus of isolated guinea pig ileum, rat stomach strip and rat uterus strip. These findings show that the extract possesses bioactive substance(s) that could interfere with smooth muscle mechanism(s) and gastrointestinal motility. Also, from the result, the AoME relaxed the guinea pig ileal smooth muscle while it contracted the isolated stomach and uterus smooth muscle preparations. It is not surprising to see such variation in the effect of herbal extracts. *Sorghum bicolor* leaf base extract was also found to relax guinea pig ileum while moderately contracting both the rat stomach and uterus strips (Nwinyi and Kwanashie, 2013).The difference in the species of the animals, physiochemical environment of the strips and the crude nature of the extract may account for this variation (Ghosh, 2005).

This present report of the relaxant effect of AoME is in conflict with the work of Ajibola *et al* (2009). In their work, the extract increases contraction of the rabbit intestine. The observed relaxant effect of the extract on the ileal strip is in line with extract effect on propulsive movement of the intestine and consequently justifies its anti-diarrhoeal activity. On the

contrary, considering its contractile effect on the rat stomach strip, it does give an impression that the extract will increase gastric emptying and in turn accelerate propulsive movement of the intestine. Under normal physiological state, the rate of gastric emptying has been shown to be the same with intestinal transit time in the upper two third of the small intestine (Linquist, & Nylander, 1969). However, *in vitro* activity in stomach strip preparation may not translate to same event in whole animal. The stomach possesses two functional areas (fundus and the corpus) with motility patterns that are quite different (Hansen, 2003). In this study the fundus was used in preparation of rat stomach strip.

Further work was carried out on AoME interaction with standard agonists. The extract inhibited contraction induced by acetylcholine on SSP, GPI and IRUS, histamine on SSP and GPI and oxytocin on IRUS. Acetylcholine and histamine are standard agonists of smooth muscle contraction in the GI tract acting at muscarinic and histaminergic receptors respectively, while oxytocin is uterine stimulant acting at oxytocin receptors. This observed result suggests that the extract may have many bioactive substances acting at different sites through which they can antagonize smooth muscle contraction. The behaviour of AoME on the SSP and IRUS in the presence of histamine, acetylcholine and oxytocin projected AoME as an antagonist. The extract almost completely blocked contraction induced by graded doses of oxytocin in IRUS.

Due to the fact that plant extracts usually occur as a combination of various types of bioactive compounds or phytochemicals with different polarities, polarity guided fractionation are usually carried out in other to eliminate variability of effects and obtain the positive bioactive substance. Fractionation of AoME yielded hexane (AoHF), ethylacetate (AOEF) and methanol (AoMF) fractions.

From the *in vitro* study on the fractions, AoEF, AoMF and AoHF added non-cumulatively reversibly relax the guinea pig ileal muscle spontaneous activity in a dose dependent manner. In the guinea pig small intestine, inhibitory transmission to the smooth muscle depends on slow inhibitory junction potentials that are regulated by nitric oxide (NO) (Mehdi *et al*., 2009). It could follow that the observed relaxant effect of AoME and its fractions may be via the activation of the release of NO.

Log-concentration curve obtained for the relaxant effect of the fractions shows the IC_{50} value of AoEF to be the least. IC_{50} measures substance effectiveness in inhibiting specific biological process. The lower the IC_{50} of a drug, the less is needed to achieve the desired effect, and the drug will most likely have less off-target effect. (Senem and Erik 2016) It therefore implies that AoEF may be the most potent among the three fractions.

Possible mechanisms of action of a drug which seems to relax the smooth muscle or reduce motility are carried out by interaction of the drug with standard agonists. Acetylcholine, serotonin and histamine are agonists of smooth muscle contraction used in this study. The action of acetylcholine is via M2 and M3 receptors. 5-HT binds to specific receptors to such as the $5\text{-}HT_3$ and $5\text{-}HT_4$ receptors to initiate gut motility. The dominant effect of histamine on GI motility is contraction and it is mediated via H_1 receptors (Byung *et al.,* 2013).

From the *in vitro* study to probe the mechanisms of action of the observed AoME relaxant effect, acetylcholine, histamine and serotonin contracted the guinea pig ileum in a concentration dependent manner. It is well known that when these agonists are dropped into a bathing medium containing guinea pig ileal muscle, they evoke contractile responses (Hurwitz and Weissinger, 1980).

Further experiments were performed in isolated guinea-pig ileum, in which the modifications produced by a pretreatment with the AoEF in the response evoked by acetylcholine, histamine and serotonin were studied. AoEF inhibited the mechanical response evoked by acetylcholine, histamine and serotonin. This finding is further supported by the shift of the log-concentration response curves of the agonists to the right. Drugs which shift the log response curve of a standard drug are regarded as an antagonist. It therefore implies that the observed relaxant effect of the extract may be via antagonizing of the muscarinic, histaminergic and serotonin receptors in the ileum. However, considering the fact that AoEF shows no selectivity between contractile agents being able to reduce contraction by different agonists, there is possibility that AoEF mechanism of action may be beyond the receptor through mechanisms that could be either indirect (transmitter release from nerve terminal) and non-specific (No release). Nitric oxide (NO) has been confirmed as a messenger in the gastrointestinal tract (Konturek and Konturek, 1995). Inhibitory component of the gut

peristalsis has been thought to be mediated by nitric oxide (NO) either singly or along with other co-transmitters. There is convincing report that the action of certain biological agents that relaxes the gut are carried out by relaxant through interactions with nitric oxide (NO) release (Tanovic *et al.*, 2000). The use of L-NAME (inhibitor of the nitric oxide synthase) to demonstrate for the involvement NO in the mediation of the inhibitory and relaxant effect of drugs is an established protocol (Ekblad and Sundel, 1998) The possibility of an indirect mechanism involving transmitter release from nerve terminal is carried out with the use of neuronal ganglion blocker hexamethonium. Therefore, the effect of pre-incubated concentration of L-NAME, and hexamethonium on AoEF-induced relaxation of the ileum was evaluated. In addition, atropine, an anti-muscarinic receptor blocker was also interacted with AoEF in a similar manner. From the finding, L-NAME and hexamethonium did not reduce the relaxant effect of AoEF on the guinea pig ileum. This is a demonstration that the relaxant effect of AoEF is independent of neural and nitrergic pathways. However, the possibility of the involvement of muscarinic receptors is likely since atropine significantly reduced the relaxant effect of AoEF.

In-vivo mechanistic studies focussing dopaminergic, muscarinic and serotonergic pathways were carried out in mice by testing the AoEF action on gastric emptying and intestinal transit induced by metoclopramide, carbachol and serotonin. Metoclopramide (Purdon & Bass, 1973) and carbachol (Ruwart *et al*., 1978) has been shown to accelerate gastric emptying while serotonin increases propulsive movement of the intestine. The present results agree with these reports.

The extract inability to alter this effects of metoclopramide and serotonin is an indication that dopaminargic and 5-HT pathway is not targeted by AoEF. However, the ability of AoEF to block both the gastric emptying and propulsive movement activated by carbachol (a muscarinic agonist like acetylcholine) suggest that the extract antagonize the action of carbachol on the muscarinic receptors.

Oleic acid being the most predominant constituent of the AoME and AoEF on GCMS analysis may be responsible for the relaxant and inhibitory effect of the extract. It has been demonstrated in humans) and in animals that oleic acid slows down gastrointestinal transit and reduced diarrhea. (Henry *et al.*, 2001)

5.1 CONCLUSION

Anarcardium occidentale stem bark extract has an antidairrhoeal effect. This is demonstrated by prolonged onset of diarrhea and decreased in total number and water content of faeces.

The mechanisms of antidiarrhoeal effect are the relaxation of tonus activity of the ileum, the inhibition of gastric emptying and propulsive movement of the intestine.

Both relaxant and the inhibitory effect are mediated through the blockage of muscarinic receptors on the stomach and the ileum.

5.2 CONTRIBUTION TO KNOWLEDGE

1. The work provided an elaborate study of the activity of the stem bark extract of *Anacardium occidentale* on smooth muscle activity in laboratory rodents.

2. It demonstrates for the first time the relaxant, anti-motility of the stem bark of extract of *Ancardium occidentale* and its mechanism in the gastrointestinal tract using functional *in vivo* and *in vitro* methods.

3. The present study confirmed that the stem bark of *Anacardium occidentale* had antidiarrheal activity

4. The mechanism of antidiarrheal activity of *Anacardium occidentale* appeared to be through relaxation of the tonus activity of the ileus thus inhibiting gastric emptying and the propulsive movements in the intestines leading to anti-motility effect

5. The antidiarheal activity of *Anacardium occidentale* is also mediated through blockade of muscarinic receptors in the gut.

6. The stem bark of *Anacardium occidentale* contracted the isolated stomach and uterus muscle of the rat, which might lead to side effects

5.4. RECOMMENDATION

Further research should be carried out to fully isolate and characterize the active constituent which could serve as template for a new antidiarrhoeal drugs. Again, molecular based mechanistic studies on the plant could further corroborate on the present findings.

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