

**ANTIDIARRHOEAL EFFECT OF METHANOL STEM BARK EXTRACT OF
HYMENOCARDIA ACIDA (EUPHOBIAEAE) IN LABORATORY ANIMALS**

By

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NIGERIA**

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FACULTY OF PHARMACEUTICAL SCIENCES,
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA**

JULY, 2018

Declaration

I declare that the work in this dissertation entitled “Antidiarrhoeal effect of methanol stem bark extract of *Hymenocardia acida* (Euphobiaceae) in laboratory animals” has been performed by me in the Department of Pharmacology and Therapeutics. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another Degree or Diploma in this or any other Institution.

Mus’ab Usman, ABBA
Name of Student

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Date

Certification

This dissertation entitled “Antidiarrhoeal Effect of Methanol Stem Bark Extract of *Hymenocardia Acida* (*Euphobiaceae*) in Laboratory Animals” by Mus’ab Usman ABBA meets the regulations governing the award of the degree of Master of Science in Pharmacology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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Acknowledgment

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Abstract

The objective of the present study was to evaluate the antidiarrheal activity of the methanol stem bark extract of *Hymenocardia acida* in some laboratory animals. The methanol stem bark extract of *Hymenocardia acida* (MEHA) was prepared from the freshly collected stem bark of the plant. The stem bark was collected from Galadimawa village, Giwa local government area of Kaduna State. The plant material was identified and authenticated by a botanist in the herbarium section of the Department of Botany Faculty of Life Sciences Ahmadu Bello University Zaria Nigeria and a voucher specimen (1275) was deposited for future reference. The plant material was washed, shade dried, pulverized and sieved to obtain fine powder. The powdered plant material was extracted with 70% v/v methanol by cold maceration for three days with occasional shaking and agitation. The mixture was then filtered using Whatman No 1 filter paper and the filtrate was later concentrated over a water bath at temperature of 40 to 45°C to obtain dried extract. The dried extract was kept in a bottle until needed for use. Preliminary phytochemical screening was carried out using thin layer chromatography analysis (TLC). Acute toxicity study was carried out using the method described in Organization for Economic Co-operation and Development (OECD) guidelines 423. *In vivo* antidiarrheal screening of the extract at the doses of 600, 300 and 150 mg/kg body weight was conducted using castor oil induced diarrhea model, castor oil induced enteropooling and gastric transit in mice model. *In vitro* study was carried out on isolated rabbit jejunum and guinea pig ileum using Ugo Basile microdynamometer, at extract concentration of (8 x 10⁻² to 640 x 10⁻² mg/ml) and thereafter interacted with spasmogens; acetylcholine and histamine at concentration of (2 x 10⁻⁵ to 16 x 10⁻⁵). Alkaloids, glycoside, saponin, tanins, triterpenes, flavonoids, and

steroids were detected from the extract. The result of the acute toxicity study revealed the LD₅₀ value to be in excess of 2000 mg/kg. The methanol stem bark extract of *Hymenocardia acida* at all doses investigated significantly ($p \leq 0.05$) and dose dependently delayed the onset of diarrhea in all the investigated groups in castor oil induced diarrhea model compared to the control group. The extract at the doses of 300 and 600 mg/kg also reduced the frequency of diarrheal feces compared to the negative control group. Significant reduction ($p \leq 0.05$) in the propulsive movement and transit of charcoal meal was observed at 600 mg/kg dose of the extract in gastric transit time in mice model. All extract treated groups produced significant reduction ($p \leq 0.05$) in the volume of fluid accumulation compared to the deionized water treated group in fluid accumulation test. The methanol stem bark extract (8×10^{-2} to 640×10^{-2} mg/ml) produced significant reduction in the tone and rate of spontaneous contraction of rabbit jejunum and relaxes guinea pig ileum. The extract upon interaction with the spasmogens; acetylcholine and histamine (2×10^{-5} to 16×10^{-5}) inhibited contraction induced by both Acetylcholine and histamine in a concentration dependent manner. The result of this study suggests that the methanol stem bark extract of *Hymenocardia acida* possess antidiarrheal activity that justified its ethnomedical use in the treatment of diarrhea by herbal practitioners.

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List of Abbreviations, Glossary and Symbols

ANOVA	analysis of variance
cm	centimeter
Dw	deionized water
EPEC	enteropathogenic <i>Escherichia coli</i>
g	gram
GIT	gastrointestinal tract
h	hour
HIV	human immunodeficiency virus
IBS	irritable bowel syndrome
kg	kilogram
L	liter
LD ₅₀	median lethal dose
Lop	loperamide
mg	milligram
ml	milliliter
MEHA	methanol stem bark extract of <i>Hymenocardia acida</i>
n	number
ORS	oral rehydration salt
ORT	oral rehydration therapy
<i>p.o</i>	oral administration
SEM	standard error of mean
Sp	specie
SPSS	statistical package for social sciences
UNICEF	United Nation Children Education Fund

w/v	weight per volume
WGO	World Gastroenterology Organization
WHO	World Health Organization
wt	weight
OECD	Organization for Economic Co-operation and Development
%	Percentage

CHAPTER ONE

1.0: INTRODUCTION

In spite of the advent of various modern drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs (Damodar *et al.*, 2011).

Diarrhea is the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individuals). It is an alteration in normal bowel movement characterized by an increase in the water content/volume, frequency of stools and/or abdominal pain. It involves both an increase in motility along with increased secretions and a decrease in absorption (absorptive capacity of the intestine exceeded) of fluid and thus loss of electrolytes particularly, sodium and water (WHO, 2013).

Diarrheal diseases are the second leading cause of death in children under five years old, and is responsible for killing around 760 000 children every year (Ahmed *et al.*, 2014). Diarrhea can last several days, and can leave the body without the water and salts that are necessary for survival. Children who are malnourished or have impaired immunity as well as people living with HIV are most at risk of life-threatening diarrhea (Ahmed *et al.*, 2014)

About 1.7 – 5 billion cases of diarrhea occur yearly with majority of the cases in developing countries (WHO, 2013). In 2012, it is the second most common cause of death in children younger than fives (WHO, 2013). In 2015 it accounted for 9 percent of all death among children under five years old, this translated to over 1400 young children dying each day or about 526,000 a year (UNICEF, 2016).

In many rural communities in the developing world, herbal medicines are almost always the only readily accessible and affordable therapies for the control of many diseases including diarrhea (Njume *et al.*, 2009; Green *et al.*, 2010). Up to 80% of developing world rely on medicinal plants for primary health care needs (Payyappallimana, 2010).

1.1 Statement of Research Problem

Diarrhoea has long been recognized as one of the important health problems all over the world, especially in developing countries (Rajamanickam *et al.*, 2010). It is one of the most common causes of morbidity and mortality, affecting mainly infants and children (WHO 2010; Nicholaset *al.*, 2016).

Although diarrheal disease is preventable and treatable it remains the second cause of death in children under age of five. Globally, there are nearly 1.7 billion cases of diarrheal disease every year (Ahmed *et al.*, 2014). In 2012 it is the number one cause of death in children under five accounting for the death of about 2195 everyday, more than AIDs, malaria and measles combined (UNICEF, 2012). The disease is responsible for over a quarter of death of children in the world in 2012 (Yilgwan and Okolo 2012). Most of these deaths occur in developing countries where an estimated 25% of under-fives mortality is directly attributed to diarrhea disease (Yilgwan and Okolo, 2012). In Nigeria over two hundred thousand children died from pneumonia and diarrhea in 2015 (Emeka, 2015)

The drugs used for treatments of diarrhea are not completely free from side effects ranging from mild to severe including, dryness of the mouth, drowsiness, dizziness, severe pain in stomach, abdominal bloating and vomiting, constipation, anxiety, confusion, depression, severe headache and muscle spasm (Brunner, 2010).

Plant extract can be important source of natural drugs for treatment of diarrhea. Medicinal plants have been used in treatment of diarrhea and have demonstrated conventional properties that need to be further investigated (Sarin *et al.*, 2013).

Hymenocardia acida stem bark is widely used in traditional medicine for treatment of diarrhea without any scientific validation.

1.2 Justification of study

Despite advancement in modern medicine and the various drugs available for treatment of diarrhea including loperamide, bismuth subsalicylate and oral rehydration salt (ORS), diarrheal diseases remains a major cause of mortality especially in developing countries (WHO, 2013).

The use of herbal drugs in treatment of diarrhea is a common practice in many African countries; in fact more than 80% of people in rural African communities still rely on indigenous medicine as a primary source of health care (Agunu *et al.*, 2005). This is partly due to the high cost, difficult accessibility associated with modern health care system, and sometimes conservative's attachment to culture and tradition (Maroyi, 2011).

WHO encourages inclusion of herbal medicines of proven safety and efficacy in the healthcare programmed of developing countries (WHO, 2002), in addition WHO also encourages studies for the treatment and prevention of diarrheal disease depending on traditional medical practice (Atta and Mouneir, 2004).

Generally, it is known that anti-diarrhea plant extracts have antispasmodic properties, delay gastrointestinal transit, suppress gut motility, stimulate water absorption and/or reduce electrolyte secretion (de Wet *et al.*, 2010).

Scientific research is therefore needed to provide evidence of the safety and efficacy of beneficial medicinal plants. The plant *Hymenocardia acida* stem bark is often used in Nigeria for treatment of diarrhea (Amom *et al.*, 2013). However, scientific basis for its use has not been proven. Thus the present study was prompted to evaluate the anti-diarrhea effect of the plant.

1.3 Aim and Objectives

1.3.1 Aim

The aim of the study was to evaluate the antidiarrheal activity of the methanol stem bark extract of *Hymenocardia acida* in laboratory animals.

1.3.2 Specific objectives

The specific objectives of the present work are to:

- i. Determine the acute toxicity profile of the methanol stem bark extract of *Hymenocardia acida*
- ii. Evaluate the effects of the extract on castor oil induced diarrhea
- iii. Evaluate effects of extract on castor oil induced enteropooling in mice.
- iv. Evaluate the effects of the extract on gastric transit time in mice.
- v. Evaluate the effect of the extract on perfused isolated rabbit jejunum and guinea pig ileum.

1.4 Hypothesis

The methanol stem bark extract of *Hymenocardia acida* possesses antidiarrhoeal activity

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diarrhea

2.1.1 Definition

Diarrhea is defined as the passage of liquid or watery stools at least 3 times in a 24-hours period or more frequent passage than is normal for the individual (WHO, 2013). However, frequent passage of formed stool is not diarrhea (WHO, 2013). Diarrhea can be a symptom of several diseases. It is one of the most common clinical sign of gastrointestinal disease but also can reflect primary disorder outside the digestive system (Sarin and Bafina, 2013).

2.1.2 Aetiology

Minimal change in normal intestinal fluid and electrolyte balance cause by infectious agents, toxins and other noxious substances present in the gut usually result in disruption of normal fluid secretion and stimulating the gut to expel it content thus resulting in very large change in stool consistency and volume (Njume and Goduka, 2012). Although this response is protective against acute irritation of the gut, however it becomes a problem when chronically present and no longer serving a physiological role (Payne *et al.*, 2006)

Some diarrheal diseases are due to chemical irritation of the gastrointestinal tract, while vast majority are caused by infections, most commonly associated with viruses, bacteria or protozoa (WHO, 2013).

2.1.2.1 *Viral causes of diarrhea*

Rotavirus is responsible for the cause of most severe diarrhea, it account for up to 40% and 25% of cases of diarrhea in developed and developing countries respectively (Aremu *et al.*, 2011,WHO 2013), other viruses associated with diarrhea in human includes,

Norwalk virus, enteric adenovirus and norovirus (Cooke, 2010). Most viral diarrhea in adult is caused by norovirus (Navaneethan and Giannella, 2008)

2.1.2.2 *Bacterial causes of diarrhea*

Diarrhea caused by enteric bacteria is very common in tropical and developing countries and a serious problem among older children and adults as well as infants and young children. *Escherichia coli* (E.coli) is recognized as the most common cause of gastroenteritis (WHO, 2013). The *enteropathogenic E.coli* (EPEC) is an important category which is a leading cause of infant diarrhea in developing countries (Nguyen, 2005). Other bacteria include *salmonella* (non-typhoid sp), *shigella*, *campylobacter*, *clostridium difficile*, *yersinia*, *clostridium perfringes* (Haque, 2007).

2.1.2.3 *Parasitic causes of diarrhea*

Parasites can enter the body through food or water and settle in the digestive system, among the parasites associated with diarrhea include, *Entamoeba histolytica*, *giardia lamblia*, *cryptosporidium parvum*, *cyclospora cayetanensis* (Haque, 2007). Diarrhea caused by parasitic agents is uncommon in the developed world and is usually restricted to travelers (WGO, 2012).

2.1.2.4 *Non Infectious causes of diarrhea*

Noninfectious causes of diarrhea include intolerance to certain compounds in food such as lactose (milk sugar) or gluten found in wheat and barley, intestinal disorders including irritable bowel disease, celiac disease, fecal incontinence, carcinoid syndrome, acute alcohol ingestion. Treatment-related diarrhea such as during chemotherapy, hormone therapy, radiation therapy (high dose) surgery especially removal of the gall bladder (Stockmann *et*

al., 2017). Reaction to medication is the most common cause of diarrhea in the elderly probably because of age, decline in function of major organs of drug clearance (Triantafyllou *et al.*, 2010). Drugs especially antibiotics causes disruption of the Gut micro flora and result in pseudomembraneous enterocolitis, some magnesium containing antacid cause mal-digestion or mal-absorption and stimulate expulsion of the gut content (Njume and Goduka, 2012). Other drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), antihypertensive, antibiotics including sulfonamides and cephalosporins can have side effect leading to diarrhea (Makinsand Ballinger., 2003).

2.1.2.5 Others

Certain disease conditions like diabetes mellitus may be associated with diarrhea due to nerve damages and bacterial overgrowth. This occurs mainly in patients with long standing poorly controlled diabetes (Krishnan, *et al.*, 2013)

2.1.3 Epidemiology

1 out of 10 childhood deaths were due to diarrhea in 2015. This translate to 526,000 children death in the year, 1400 children per day 60 children death per hour and one child per 60 seconds (UNICEF, 2016). Diarrhea kills nearly 1.8 to 1.9 million children under five in developing countries; however this figure is an improvement from 4.5 million 20 years ago. It is estimated that annual incidence of diarrheal disease episodes in children less than five years old in developing countries stands at two billion globally with a median incidence rate of 3.2 episodes per child (WHO, 2009).

According to W.H.O 2010 diarrhea is responsible for the death of about 2.6 million people mostly among African children under the age of five years. It also accounted for over 700,000 deaths in children under-five years worldwide in 2012 making it the second

leading cause of child mortality after pneumonia (WHO, 2013). It kills more young children than AIDS, malaria and measles combined (UNICEF, 2009). In Nigeria diarrhea kills about 194,000 children under the age of five in 2012 (UNICEF, 2012).

Women are more susceptible to traveler's diarrhea than men (Schlagenhauf *et al.*, 2010). There is high prevalence of diarrhea among children whose mothers did not attend school and those children living in poorest environment particularly sub-Saharan Africa and South Asia (UNICEF, 2009). A community base study report that the ratio between male child under five to female child under five suffering from acute diarrhea is 1.2 and 1.4 respectively (UNICEF, 2012). In another report about 2 billion cases of diarrhea disease occur worldwide every year and 1.9 million children younger than five years of age die from diarrhea each year with majority of the cases in developing countries (UNICEF, 2016). This translates to 18% of all death among children under five and death of over 5000 children every day as a result of diarrheal diseases. (WGO, 2012)

Most death from diarrhea disease occur in South Asia and sub-Saharan Africa where health facilities are inadequate and majority of the people lack access to clean and safe water, a major vehicle for transmission of diarrheal disease (Kakulu, 2012). Recent mortality estimate among children less than five years old show that 5 countries account for approximately 50% of global mortalities, of these five Nigeria account for 11% of all death (Charyeva *et al.*, 2015).

Despite decrease in childhood mortality from approximately 4.6 million in the 1980's through 3.4 million in the 1990's to nearly 0.6-0.7 in 2015, the highest burden of the

disease vis avis number of death from diarrhea remains unacceptable high (Charyeva,*et al.*, 2015).

2.1.5 Risk Factors for Diarrhea

According to WHO, diarrheal related pathogens get into contact with human through fecal oral route that includes, ingestion of fecal contaminated water or food, person to person contact and direct contact with fecal matter (Walker *et al.*, 2012). According to Elizabeth *et al.*, (2014), poor handling of drinking water, clogged drainage around or near the house, breeding places for flies/insects near or in the house, total hygiene practice level, lack of hand washing with soap after defecation and before food preparation are the risk factors associated with diarrhea others includes previous international travel to endemic areas, chemotherapy, occupation like work at a day care center and presence of known immunosuppressive disorders (DuPont, 2014).

2.1.6 Types of Diarrhea

Base on clinical syndrome and duration there are basically four types of diarrhea, acute watery diarrhea, acute bloody diarrhea (dysentery), persistent diarrhea and chronic diarrhea.

Acute diarrhea is characterized by abrupt onset of frequent, watery loose stool usually without visible blood which lasts less than 14 days in duration, (Most episodes subside within 7 days of onset). Although it is often mild, acute diarrhea can lead to severe dehydration as a result of large fluid and electrolyte loss. Ninety percent of acute diarrhea result from infection causes usually virus. Medications such as antibiotic and drugs that contain magnesium products are also common offenders. Recent dietary changes can also

cause acute diarrhea. This includes coffee, tea, dietetic foods, gums that contain poorly absorbable sugars. Traveling to developing areas of the world can result in exposure to bacterial pathogens common in certain areas, contaminated food (Fletcher *et al.*, 2012).

Acute bloody diarrhea or dysentery involved diarrhea with visible blood and mucus in feces, symptoms include, anorexia, rapid weight loss and damage to the intestinal mucosa by invasive bacteria, sepsis and malnutrition may be present. Most important cause is *Shigella* (S). *Shigella dysenteriae* causes both epidemic and endemic shigellosis, however it is *Shigella Flexneri* that is the chief cause of endemic shigellosis in developing countries. (Other strain includes *S Sonnei*, *S Boydii*). Other causes of dysentery include *Campylobacter jejuni* and infrequently *enteroinvasive Escherichia coli* or *salmonella* (non-typhoid specie). In young adult dysentery is usually related to *Entamoeba histolytica*.

Persistent diarrhea refers to diarrhea that begins acutely but is of unusually long duration (at least 14 days), it may begin as watery diarrhea or dysentery, main danger is malnutrition, serious non intestinal infection, marked weight loss is frequent with a risk of dehydration

Chronic diarrhea refers to diarrhea which is recurrent or long lasting due mainly to noninfectious causes. It may be cause by gastrointestinal disease, may be secondary to systemic disease, and may be psychogenic in nature (Biu, 2006). Pathophysiologically, chronic diarrhea may be categorized as inflammatory diarrhea (cause by regional enteritis, ulcerative colitis, Crohn's disease and less commonly colon cancer, radiation therapy, infection and ischemia of the gut), osmotic or malabsorptive diarrhea (resulted from lactose intolerance, celiac disease, Whipple's disease, chronic pancreatitis, bile duct

obstruction, cholestatic liver disease, bacterial overgrowth, Zollinger-Ellison syndrome) secretory diarrhea which resulted from medication, small bowel resection, mucosal diseases thus increase release of water from the body into the bowel, and dysmotility diarrhea where foods moves too quickly through the intestine and does not allow enough time for sufficient nutrients and water to be absorbed by the gut. It is caused by condition such as diabetic neuropathy or irritable bowel syndrome (Camilleri, 2004, Nasser, 2014)

On the basis of the mechanism by which diarrhea occur (pathophysiology), diarrhea is classified into the following.

Osmotic diarrhea: this form of diarrhea involves retention of water in the bowel which results from accumulation of non-absorbable substances. This creates an osmotic pressure which pulls water and ion into the intestinal lumen leading to diarrhea, For example sugar substitute like mannitol, sorbitol slow down absorption while causing rapid motility of the small intestine. This type of diarrhea also occurs when individual with congenital lactase deficiency consumed lactose rich diet.

Secretory diarrhea: when electrolyte absorption is affected, the body releases water into the small intestine causing loose bowel movement. This type of diarrhea often occurs as a result of infection or intake of certain drugs. In this type of diarrhea there is usually an increase in active secretion or an inhibition of absorption mostly due to enterotoxins, bile acid or an over secretion of gastrointestinal hormones. There is little to no structural damage of the gastrointestinal tract and mostly associated with cholera toxins (Micheal, *et al.*, 2017).

Inflammatory (exudative) diarrhea: this occurs when the lining of the colon becomes inflamed which typically causes bloody diarrhea. This is common in people with ulcerative

colitis and chron's disease. Other cause of exudative diarrhea is infection especially with shigella, salmonella and entero invasive *Escherichia coli*. These organisms invade the epithelium and multiply, damaging the surface epithelium and causes inflammation. This type of diarrhea is due to epithelial damage (exudation and decrease absorptive capacity) as well as action of inflammatory mediators (Micheal, *et al.*, 2017)

2.1.7 Complication of diarrhea

2.1.7.1 *Dehydration*

During diarrheal episode, water and electrolytes (mostly sodium, chloride, potassium and bicarbonate) are lost, when these loses are not replace dehydration occurs. Mild to moderate dehydration characterize by thirst, restless, decrease skin elasticity and sunken eyes while in severe dehydration, shock with diminished consciousness occur, lack of urine output, cool extremities, pale skin, low or undetectable blood pressure (WHO, 2013). Dehydration and electrolyte losses associated with untreated diarrhea are the main cause of morbidity and mortality of childhood gastroenteritis (AMMCOP, 2011)

2.1.7.2 *Malnutrition*

Repeated attacks of diarrhea contribute to malnutrition, and diarrheal disease are more likely to cause death in children who are malnourished. Undernourished children in turn have compromised immune system and are at higher risk of developing other disease (Charyeva *et al.*, 2015). Continues feeding during illness tends to lessened or prevent adverse effects of diarrhea on a child nutritional status (WHO, 2013).

2.1.7.3 *Cardiovascular complication of diarrhea*

Diarrhea can lead to dehydration which decreases blood volume. Mild dehydration can cause symptoms of orthostatic hypotension such as weakness, dizziness and fatigue (Palande, 2012).

2.1.8 Prevention of diarrhea

2.1.8.1 *Exclusive breast feeding*

Exclusive breast feeding for the first 6 months of life without additional foods or liquids including water protects infants from disease and guarantees them a food source that is safe, clean, accessible and perfectly tailored to their needs. Nearly half of all diarrhea episodes could be prevented with increase breast feeding in low and middle income countries (UNICEF, 2016)

Adequate complementary feeding and continues breastfeeding is thus another factor necessary to halt diarrhea. Good nutrition support immune system and provide protection from disease. From 6 months to 2 years adequate complementary feeding provides children with adequate quantities of safe nutritious foods, it also reduce incidence of child death and recovery from illness.

2.1.8.2 *High dose vitamin A supplementation*

High dose vitamin A supplementation help maintain strong immune system and reduce all-cause mortality by about 24 % and cases of diarrhea by fifteen percent. Children between 6-59 months should be protected with 2 high dose supplements of vitamin A every year (especially in countries with high under five mortality or if vitamin A deficiency is a public health problem) (Grotto *et al.*, 2003).

2.1.8.3 *Immunization*

Use of rotavirus vaccines provides protection against one of the most common cause of childhood diarrheal related deaths.

2.1.8.4 *Safe drinking water and hygiene*

Safe drinking water, sanitation and hygiene, hand washing with soap alone cut the risk of diarrhea by almost 40%. Almost 60% of death due to diarrhea worldwide is attributed to unsafe drinking water and poor hygiene and sanitation. It is therefore vital to have a clean home environment, good hygiene, safe drinking water and proper disposal of human waste. (WHO 2013)

2.1.9 Management of diarrhea

The objectives of any antidiarrheal treatment is to replace or minimize fluid and electrolyte loss (identify and treat dehydration), reduce stool frequency and other symptoms such as abdominal pain, fecal losses and ultimately reduce duration and severity of illness (Njume and Goduka, 2012). The administration of oral rehydration salt (ORS) to replace fluid and electrolyte loss in diarrheal patients is therefore sine qua non to effective treatments (Njume and Goduka, 2012)

2.1.9.1 *Oral rehydration therapy*

ORT is the administration of appropriate solutions by mouth to prevent or correct diarrheal dehydration. Oral rehydration salts (ORS) used in oral rehydration therapy (ORT), contain specific amount of important salts that are lost in diarrhea stool. Initially WHO ORS preparation had higher sodium concentration due to its development in areas with higher incidence of cholera (which is complicated by hyponatramia. However, after multiple

modification the current WHO guidelines recommend a reduced osmolarity ORS with lower sodium concentration (75mmol/l versus 90mmol/l) and glucose 75mmol versus 111mmol/l. Advantages is that its associated less vomiting, less stool output, less chance of hypernatremia, reduced needs for intravenous infusion as well as safety and efficacy in both cholera and non-cholera diarrhea (WGO, 2012).

The observation that glucose sodium co-transport was unaffected in cholera and recognition that secretory and absorptive process in the intestine are separate, lead to the formulation of ORS. With similar concentration of sodium and glucose that optimize absorption together with potassium, chloride and bicarbonate. Therefore the mechanism lies in the fact that sodium/glucose co-transport proteins on the brush border cells of the intestinal lumen pull sodium and glucose from the gut into the cells (Forsberg *et al.*, 2007, Cooke 2010). As the cellular osmotic pressure increase, water is reabsorbed out of the gut into the body. This action reverses electrolyte imbalances and re-hydrates the patients.

In newer formulation of ORS rice base ORS is use as alternative therapy to standard ORS in cholera as it add additional substrate to the gut lumen without increase osmolality thus providing additional glucose mediated absorption (Cooke 2010).

2.1.9.2 *Supplemental zinc therapy*

Zinc deficiency is common in young children in the developing countries and is associated with impaired electrolyte and water absorption, decrease brush border enzyme activity and impaired cellular and humeral immunity. Therefore zinc supplementation as an immune buster reduces the duration and severity of acute and persistent diarrhea and reduces the risk of recurrent episodes in the next 2-3 months. The recommended dose for children

under six months is 10mg while for those over six months is 20 mg for 10 to 14 days (Scrimgeour and Lukaski, 2008).

2.1.9.3 *Probiotics in the treatments of acute diarrhea*

Probiotics are live microorganism that are believed to work by stimulating the host immune system and competing for binding site on intestinal epithelial cells. Probiotics may be an effective adjunct to the management of diarrhea but only those that have been documented to be effective. *Lactobacillus GG* (Gorbach and Golden) and *saccharomyces boulardii* especially when given in early cause of the diarrhea (Allen *et al.*, 2010) dose 10^{10} CFU/day. The advantage includes; reduction in the duration of diarrhea by approximately one day, thus reduces hospitalization (WGO, 2012), and reduces persistent diarrhea. However there are concern about use of probiotic in developing world where there is high prevalence of bacterial diarrhea thus probiotics may be less efficacious and safety issues related to immunosuppression may arise (Dupont, 2014).

2.1.10 Antidiarrheal drugs

2.1.10.1 *Antimotility and antisecretory agents*

Opioids: Most opioids has a constipating action. In fact morphine was first used in the treatment of diarrhea before it was used as an analgesic, however it is no longer use to treat diarrhea because it produce objectionable side effects like respiratory depression and habituation (dependency and addiction) (Rang and Dale, 2003), therefore only loperamide and diphenoxylate are used.

Loperamide: This is a synthetic opiate agonist that does not cross the blood brain barrier and thus has little or no analgesic (dose) property or potential for addiction (CNS effect). It

acts on the mu receptor in the myenteric plexus of large intestine. Activation of the mu receptors inhibits release of acetylcholine (Regnard *et al.*, 2011). Loperamide also produces rapid and sustained inhibition of the peristaltic reflex through depression of longitudinal and circular muscles activity. The usual adult dose is 4mg start then 2 mg after every loose stool up to 16 mg per day (Li, 2007).

Diphenoxylate and its active metabolite diphenoxin (diphenoxylate) are piperidine derivatives that are structurally related mepiridine. They produce their antidiarrheal effect by inhibiting presynaptic cholinergic nerve in submucosa and myenteric plexus via binding to peripheral mu receptors resulting in decrease motility leading to increase absorption of water. Higher dose can produce central nervous system effect thus have a potential for abuse and addiction. They are therefore marketed with subtherapeutic dose of atropine to discourage abuse and deliberate overdose. (25 ug atropine and 2.5 mg diphenoxylate, or 25 ug atropine and 1 mg diphenoxin). Usual dose is two tablet stat followed by one tablet every 3-4hours. The anticholinergic effect of atropine may contribute to anti-diarrheal effect (Kenneth, 2007).

2.1.10.2 *Alpha-2 adrenergic agonist.*

Alpha 2 agonists such as clonidine interact with specific receptors on enteric neurons and enterocytes, it leads to reduction in the release of neurotransmitters by inhibition of adenylyl cyclase thereby stimulating absorption and inhibiting secretion of fluids and electrolyte and increasing intestinal transit time. These agent have a special role in diabetics with chronic diarrhea in whom autonomic neuropathy can lead to loss of noradrenergic innervation, also useful in patients with diarrhea due to opioid withdrawal (Dharmasathaphorn, 1986; Pasricha, 2006)

2.1.10.3 *Somatostatins*

Somatostatin is a peptide released in the gastro intestinal tract and pancreatic δ cells, enteric nerves and hypothalamus. Octreotide is an octapeptide analogue of somatostatins that is effective in inhibiting severe secretory diarrhea brought about by hormone secreting tumours of the pancreas and the GI tract. Its mechanism of action involves inhibition of hormone secretion including serotonin and various other GI peptides including gastrin, vasoactive intestinal polypeptide, insulin, secretin, and glucagon (Szilagyi and Shrier 2001). Octreotide has been used with varying success in other forms of secretory diarrhea such as chemotherapy induced diarrhea, diarrhea associated with human immune deficiency virus (HIV) and diabetes associated diarrhea (Fried, 1999). The half-life of somatostatin is 2-3 minutes while that of octreotide is 1.5-2 hours (Werle and Schnürch, 2006).

2.1.10.4 *Kaolin and pectin*

Active form of kaolin is attapulgite; naturally occurring hydrated magnesium aluminium silicate, pectin is an indigestible carbohydrate derived from apple. Both act as absorbents of bacteria toxins and fluids thereby decreasing the frequency of diarrhea and stool liquidity. They are generally safe but may interfere with absorption of other drugs. Usual dose is 2 to 10g initially followed by same dose after every bowel movement.

2.1.10.5 *Bile acid sequestrant*

Cholestyramin, colestipol, and colesevalam effectively bind bile acids and some bacterial toxins. Useful in treatment of bile acid induced diarrhea. They are also useful in treating mild antibiotic associated diarrhea and mild colitis due to *Clostridium difficile*.

2.1.10.6 *Bismuth subsalicylate*

Although their mechanism of action remain poorly understood. Bismuth subsalicylate is a crystal complex consisting of trivalent bismuth and salicylate suspended in magnesium aluminium silicate clay. In the low pH of the stomach it rapidly dissociate into bismuth and subsalicylate, the bismuth component then react with HCL to form oxychloride which is not absorbed at all, and has antibacterial activity by absorbing/bind bacterial and excrete it, while the salicylic acid is rapidly absorb into the stomach and small intestine to produce anti-inflammatory effect via decreasing prostaglandin synthesis. Bismuth is also known to have antisecretory effects. It is usually used as prophylaxis to traveler's diarrhea (Pasricha, 2006).

2.2 The Plant *Hymenocardia Acida*

2.2.1 Botanic description

Hymenocardia acida is a small savannah tree or shrub about 9 m high. Branchlets become rusty brown as the bark peels. The bole is short, often flattened and usually crooked. The branches form a fairly heavy, rounded crown. Bark smooth or flaky, pinkish-brown when fresh but becoming pale brown or grey later. Leaves thin, leathery, elliptic-oblong up to 8.75 cm long and 3.75 cm broad, apex obtuse to rounded, base obtuse; petiole slender, up to 1.8 cm long. Leaves are usually pubescent when young with a dense mat of fine hairs and with golden glands beneath (Amom *et al.*, 2013).

Flowers unisexual, male flowers reddish-yellow occurring in clusters of spikes up to 6.5 cm long; calyx cupular, red, anthers creamy white. Female flowers green, placed on axils of leafy lateral branches and bearing a prominent crimson stigma spreading about 1.25 cm. Fruit compressed, obcordate and reddish-brown, 2.5 cm long and 2.5-3.75 cm broad. Fruits

are developing in pairs along one edge, each with a thin pale brown nearly square wing. Seed flattened glossy brown (Adjanohoun *et al.*, 1991; Amom *et al.*, 2013).

The generic name Hymenocardia is derived from the Greek words 'hymen' - membrane and 'kardia'- heart, in reference to the heart-shaped fruits which have a transparent covering membrane (hymen). The specific epithet acida describes the sour taste of its fruits. Some authors consider the genus under the family Hymenocardiaceae (Amom *et al.*, 2013).

2.2.2 Biology

Hymenocardia acida is dioecious, male and female flowers occurring on different trees. In Zambia and Nigeria flowering starts from September-November. Seeds mature from June-September.

2.2.3 Taxonomy

The genealogy of the plant is as follows;

Kingdom: Plantae

Order: Malpighiales

Family: Phyllanthaceae

Tribe: Hymenocardiaceae

Genus: *Hymenocardia*

Species: *Hymenocardia acida* Tul

2.2.4 Synonyms

Hymenocardia acida (Euphorbiaceae) is very popular in African Trado-medicine. It is called "Heart-fruit" in English (Amom *et al.*,2013) "ii-kwarto" in Tiv, "emela" in Etulo, "Uchuo" in Igede "enanche" in Idoma (Abu and Uchendu, 2011). It is commonly known as "jan yaro" in Hausa, "yawa satoje" in Fulani, "ikalaga" in Igbo, and "Orunpa" in Yoruba (Ibrahim *et al.*, 2007).



Plate I: *Hyemenocardia acida* in its natural habitat (Galadimawa Giwa local government, on 10th July 2018)

2.2.5 Ethno-Medicinal Uses of *Hymenocardia acida*

All parts of the plant are useful as remedies for many ailments. The powdered root and stem decoction is used for fever, diarrhea and dysentery. The root ash is used to treat mouth infections. The powdered roots are also used as depurative and for treating colds, muscular pains, headaches, jaundice, hypotension, chest pains and nephritis (Irvine, 1961).

Among the *Idoma* and *Igede people* of North Central Nigeria, the decoction of root and stem bark is used in the treatment of diabetes. In folkloric medicine of Idoma people, *Hymenocardia acida* is used as douche for female personal hygiene. The leaves, stem bark and root bark of *Hymenocardia acida* are used either in infusion or powder form to treat hypertension, diabetes, sickle cell, epilepsy, schizophrenia (Amom *et al.*, 2013).

Experimental studies have confirmed antifungal and antimycobacterial, antimicrobial, anti-sickling, anti-ulcer, antiplasmodial, and *in vitro* trypanocidal activities of *Hymenocardia acida* (Abu and Uchendu, 2011). Muanza *et al.* (1995) have also reported anti-HIV and anti-inflammatory activities of this plant.

It is clear that this plant is an important source of herbal medicine of human and perhaps of veterinary and agricultural importance too. Its ethno medicinal significance may be as a result of a wide range of secondary metabolites (Mwine and Damme, 2011) such as alkaloids, terpenoids, glycosides, flavonoids, saponins and tannins (Sofidiya *et al.*, 2009; Abu *et al.*, 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant material

The stem bark of *Hymenocardia acida* was obtained from Galadimawa, Giwa Local Government Area of Kaduna State. The plant was identified and authenticated at the Herbarium Section of the Department of Botany Faculty of life Sciences Ahmadu Bello University, Zaria where a voucher number was obtained (1275) for future reference..

3.1.2 Experimental animals

Swiss albino mice of both sex (18-24 g), three healthy New Zealand rabbit (1-2.5 kg) as well as three male guinea pigs (300-500 g) were used for the study. The animals were obtained from the Animal House of the Department of Pharmacology and Therapeutics Ahmadu Bello University Zaria. They were provided with rodent diet and water *ad libitum*.

3.1.3 Equipment

Ugo Basilemicrodynamometer (Italy), water bath (HH-S digital thermostatic water bath, China), weighing balance, cages, dissecting kits (gold cross DS Malaysia), filter paper (Whatman No 1), pipette, test tubes (Pyrex France), porcelain pestle and mortar, stop watch, syringes (1 ml, 2 ml, 5 ml).

3.1.4 Chemicals and drugs

Methanol (AR JHD UNI 230, Guangdong Guanghua, Sci-tech Co LTD, China), Castor oil (Bell, Sons and Co LTD, Southport PR9 AL, England), Loperamide (Imodium[®]) (Jansen Pharmaceuticals, Pakistan), Medicinal charcoal (ultracarbon powder –Merck KGaA Darmstadt Germany), histamine (sigma Aldrich INC, St Luis USA), Acetylcholine (Gland Pharmaceuticals, India)

3.2 Methods

3.2.1 Preparation of plant material

The stem bark was cleaned, shade dried, pulverized using mortar and pestle, it was then sieved to obtained fine powder. The powdered drug (1.2 kg) was extracted using cold maceration method with 70% methanol solvent for three days with occasional stirring and agitation. The mixture was then filtered using Whattman filter paper No 1. The filtrate was concentrated and subjected to drying in crucible under reduced pressure and controlled temperature (40-45⁰C) over a water bath. The resultant dried extract was then stored in a leveled tight container for future use.

3.2.2 Thin layer chromatography (TLC) analysis

A 100 mg/ml solution of *Hymenocardia acidia* stem bark extract was made using methanol. The solution was spotted on silica gel thin layer chromatographic plate using a capillary tube. The plates were developed in a solvent system (chloroform: ethylacetate 5:3) and each plate was sprayed with a different visualizing reagent (Wagner and Bladt, 1996) as follows:

Alkaloids – dragendorff’s reagent was used as visualizing reagent. Appearance of orange red spots indicate presence of alkaloid.

Flavonoids - Ferric chloride solution and aluminum chloride were used as visualizing reagent. Appearance of blue spot and yellow fluorescence indicates presence of flavonoids.

Tannins - vanillin hydrochloride was used as visualizing reagent. Appearance of red spot indicates presence of condense tannins.

Anthraquinones - Bontrager’s reagent (5% ethanolic potassium hydrochloride) was used as visualizing reagent. Red colour spot indicates anthraquinones while yellow spots represent anthrones and anthranols.

Cardiac glycoside – P-anisaldehyde was used as visualizing reagent. Sugar phenylhydrazone produces green-yellow spot in 3minutes, while sugars produces blue, green,violet spot in 10minutes. Bials reagent was also used for the detection of glycosides and glycolipids which produces violet spots.

Saponins: Libermann-Burchard reagent was used as visualizing reagent. Green and violet spots represent steroids and triterpenes respectively.

3.2.3 Acute toxicity studies.

The acute toxicity study was carried out based on Organization for Economic Co-operation and Development (OECD) guidelines 423 and fixed dose studies was adopted with 2000 mg/kg weight of test animal as the limit dose.

Six animals were used (three per step). Three female mice were fasted for 3-4 hours, and each mouse was administered 2000 mg/kg of methanol stem bark extract of *Hymenocardia*

acida base on the fasted body weight. Food but not water was withheld further for 1 hour post extract administration. Each mouse was observed individually for the first 30 minutes and periodically during the first 4 hours and then daily for 14 days. The same procedure was repeated for another group of three mice (female). The animals were observed for signs and symptoms of toxicity, such as tremor, convulsion, salivation, lacrimation, diarrhea, lethargy, sleep, respiratory, behavior pattern, time of onset of toxicity if any and length of recovery period as well as time of death.

3.2.5 In vivo antidiarrheal studies

3.2.5.1 *Castor oil induced diarrhea*

The experiment was performed according to the method described by Awouters *et al.*, (1978). Mice fasted overnight were randomly divided into five groups of six mice each. Animals in group i received deionized water 10 ml/kg, group ii-iv received stem bark extract of *Hymenocardia acida* (150 mg, 300 mg and 600 mg respectively, group v received loperamide (3 mg/kg). one hour after administration, all mice received 0.4 ml castor oil *p.o* and then were placed individually in cages whose floor were lined with pre-weighed filter paper. Observation was made base on the time of onset, frequency and/or number of wet feces excreted. Percentage protection against diarrhea was calculated with respect to the number of wet feces using the formula below.

$$\% \text{ inhibition} = \frac{\text{Total No of WFC} - \text{Total No of WFE}}{\text{Total No of WFC}} \times 100$$

WFC = wet of feces in negative control group

WFE = wet of feces in test group

3.2.5.2 *Effect of methanol stem bark extract of Hymenocardia acida on gastric transit time in mice*

Intestinal motility test was done according to the method of Mascolo *et al.*, (1994). The mice were fasted for 12 hours and divided into five groups of six mice each. Group i received deionised water 10 ml/kg p.o, Group ii, iii, iv received graded doses of the extract at 150 mg, 300 mg, and 400 mg respectively while group v received atropine sulphate (5mg/kg). Sixty minutes after each animal was given 0.5 ml charcoal meal (5 % activated charcoal suspended in 0.5 g acacia). All mice were sacrificed after 30 minutes of charcoal meal administration by cervical dislocation. The stomach and small intestine were then removed and extended on a clean surface. The distance moved by the charcoal meal from the pylorus was measured and then expressed as a percentage of the total distance from the pylorus to the caecum as follows

$$\% \text{ movement of charcoal meal} = \frac{\text{distance travel by charcoal meal}}{\text{distance from the pylorus to the caecum}} \times 100$$

$$\text{Percentage inhibition} = \frac{A-B}{A} \times 100$$

A = Mean Movement of Charcoal Meal in Negative Control Group

B = Mean Movement of Charcoal Meal in Test Control Group

3.2.5.3 *Enteropooling*

The method describe by Robert *et al.*, (1976) was adopted. Thirty mice were fasted overnight and then randomly divided into five groups of six animals each. Group I served as the negative control received 10 ml/kg deionised water, group ii, iii and iv received 150 mg, 300 mg and 600 mg of methanol stem bark extract of *Hymenocardia acida*, while group v pretreated with loperamide as positive control. Sixty minutes later 0.5 ml castor oil

was administered to all the animals and 30 minutes after that all the mice were sacrificed by light ether anesthesia and their small intestines removed. The intestinal content were collected by milking into a graduated syringe, and the volume measured and recorded. Volume obtained from the negative control group (group i) was used to compare with the rest of the groups. Values less than negative control group were considered as protection from diarrhea.

3.2.4 In vitro studies

3.2.4.1 Effect of Methanol Stem Bark Extract of *Hymenocardia acida* on Rabbit Jejunum

A New Zealand adult male rabbit was starved of feed for about 18 hours. It was then sacrificed by cervical dislocation. The abdomen was cut open and segments of the jejunum (about 3 cm long) removed and dissected free of adhering mesentery. The tissue was then suspended in a 25 ml organ bath containing thyroid solution and allowed to stabilize for 30 minutes. The effects of acetylcholine (2×10^{-5} – 16×10^{-5}) and the methanol stem bark extract of *Hymenocardia acida* were then tested on the jejunum, the tissue was allowed a resting period before addition of the next concentration. Responses were recorded using a microdynamometer set at 3.0 mV and speed of 24 mm/minutes. The response of atropine as an antagonist was also recorded as well as responses of the interaction between the extract and the drug (ACH).

3.2.4.2 Effect of methanol stem bark extract of *Hymenocardia acida* on guinea pig ileum

Similar protocol as for that of the effects of the extract on isolated rabbit jejunum was followed. The effect of histamine (2×10^{-5} – 16×10^{-5}) and the methanol stem bark extract of *Hymenocardia acida* were then tested on guinea pig ileum. The response produced by

mepyramin, was also recorded as well as response of the interaction between the extract and the drug (histamine).

3.2.6 Data analysis

The data collected were expressed as mean \pm SEM. Results were presented as graph and tables where appropriate. Data were compared using one way analysis of variance (ANOVA) followed by *Dunnett's post hoc* test using SPSS version 20 software where p values ≤ 0.05 were considered statistically significant.

CHAPTER FOUR

4.0 RESULT

4.1 Percentage Yield of Methanol Stem Bark Extract of *Hymenocardia acida*

The weight of the dried extract obtained was 107 g and the percentage yield was 8.92%

4.2 Phytochemical Constituents of Methanol Stem Bark Extract of *Hymenocardia acida*

Preliminary Phytochemical screening of the crude methanol stem bark extract revealed the presence of steroids/terpenes, flavonoids, tannins, saponins, alkaloid and flavonoids (Table 4.1).

Table 4.1 **Phytochemical Constituents of the Crude Methanol Stem Bark Extract of *Hyemenocardia acida***

Constituents	Inference
Alkaloids	+
Flavonoids	+
Saponins	+
Steroids	+
Tannins	+
Triterpenes	+
Glycosides	+
Antraquinones	-

+ = Present, - = absence

4.3 Toxicity Profile (LD₅₀) of Methanol Stem Bark Extract of *Hymenocardia acida*

The present study conducted as per Organization for Economic Co-operation and Development (OECD) guidelines 423 revealed that the extract did not produce any mortality throughout the study period of 14 days at 2000 mg/kg body weight (limit dose). The oral LD₅₀ was determined being in excess of 2000 mg/kg body weight. So according to the OECD 423 guidelines testing the extract at a higher dose may not be necessary. No significant change was observed in the body weight and wellness parameters including salivation, sleep, tremor, lacrimation, convulsion, respiration, behavior pattern and mortality. However the animals were observed to cluster together especially day one of the administration. This is summarized in the Table 4.2 below.

Table 4.2: **Acute Toxicity Profile of Methanol Stem Bark Extract of *Hymenocardia acida* (OECD 423 guidelines)**

Parameters	observations	
	Before extract administration	After extract administration
Tremor	Negative	Negative
Convulsion	Negative	Negative
Salivation	Negative	Negative
Lacrimation	Negative	Negative
Diarrhea	None	None
Lethargy	Normal	Normal
Alertness	Normal	Dull (Clustered)
Sleep	None	None
Respiratory	Normal	Normal
Behavior pattern	Normal	Normal
Mortality	None	None

4.4 Castor Oil Induced Diarrhea in Mice

An hour after oral administration of castor oil to all the animals, mice in the negative control group produced diarrhea. Pretreatment of mice with methanol stem bark extract of *Hymenocardia acida* delay the onset of diarrhea in all the groups as well as loperamide treated group. Significant reduction ($p \leq 0.05$) in the frequency of diarrheal faeces was observed at 300 and 600 mg/kg. Similarly loperamide (3 mg/kg) produced significant reduction ($p \leq 0.05$) in the frequency of diarrheal feces (Table 4.3).

Table 4.3: Effect of Methanol Stem Bark Extract of *Hymenocardia acida* on Castor Oil Induced Diarrhea in Mice

Treatments (mg/kg)	Onset of Diarrhea (Min)	Frequency of Dry Feces	Frequency of Wet Feces	Percentage Protection (%)
DW 10ml/kg	27.20± 4.71	2.60 ± 0.98	5.20 ± 0.49	
HA (150)	76.80± 7.35*	2.00 ± 0.32	4.00 ± 0.71	23.08
HA (300)	82.80± 3.06*	1.20 ± 0.80	2.40 ± 0.68*	53.85
HA (600)	83.20± 6.36*	1.00 ± 0.45	1.00 ± 0.45*	80.77
LOP (3)	93.80± 2.31*	1.00 ± 0.32	0.20 ± 0.20*	96.15

Data are presented as mean ± SEM *p ≤ 0.05 Oneway ANOVA followed by Dunnett's *Post Hoc* Test, HA: *Hymenocardia acida* Extract, DW: Deionised Water, LOP: Loperamide, Min: Minutes SEM: Standard Error of Mean, n=6

4.5: Effect of Methanol Stem Bark Extract of *Hymenocardia acida* on Gastric Transit Time in Mice

Propulsive movements of charcoal meal after oral administration along the intestinal length were delayed with increasing doses of the extract as compared to the negative control group (DW). However, *Hymenocardia acida* (600 mg/kg) produced significant ($p \leq 0.05$) reduction in the propulsive movements and transit of charcoal meal in the gastrointestinal tract. The standard antidiarrheal drug atropine (5 mg/kg) also showed significant ($p \leq 0.05$) reduction in the distance traveled by the charcoal meal when compared to the control (Table 4.4).

Table 4.4: Effect of Methanol Stem Bark Extract of *Hymenocardia acida* on Gastric Transit Time in Mice

Treatments (mg/kg)	Length of Intestine (cm)	Movement of Charcoal (cm)	Percentage Movement (%)	Percentage Protection (%)
DW 10ml/kg	46.38 ± 1.18	41.33 ± 1.08	89.11	
HA (150)	45.83 ± 1.08	38.50 ± 0.56	84.00	06.85
HA (300)	50.93 ± 1.60	38.25 ± 2.42	75.10	07.45
HA (600)	45.17 ± 1.86	27.58 ± 3.09*	58.46	33.27
LOP (3)	44.87 ± 2.30	27.91 ± 2.78*	62.22	32.47

Data are presented as mean ± SEM *p ≤ 0.05 Oneway ANOVA followed by *Dunnett's Post hoc* Test (2 sided), HA: *Hymenocardia acida* Extract, DW: Deionised Water, LOP:Loperamide, SEM: Standard Error of Mean
n=6

4.6: Effect of Methanol Stem Bark Extract of *Hymenocardia acida* on Fluid Accumulation Test (Enteropooling)

A significant ($p \leq 0.05$) reduction in volume of fluid accumulation was observed at the tested doses (150, 300 and 600 mg) in lumen, also the standard antidiarrheal drug loperamide significantly reduced the volume of intestinal content when compared to the control. This is summarized in Table 4.5.

Table 4.5: Effect of Methanol Stem Bark Extract of *Hymenocardia acida* on Enteropooling in Mice

Treatments (mg/kg)	volume of intestinal content(ml)	Percentage inhibition (%)
DW (10ml/kg)	0.30±0.02	-----
MEHA (150)	0.12±0.02*	60.00
MEHA (300)	0.15±0.02*	50.00
MEHA (600)	0.11±0.05*	63.33
LOP (3)	0.10±0.01*	66.66

Data are presented as mean ± SEM *p ≤ 0.05 Oneway ANOVA Followed by *Dunnett's Post hoc* Test, HA *Hymenocardia acida* Extract, DW: Deionised Water, LOP: Loperamide, SEM: Standard Error of Mean, Kg –Kiloogram
n= 6

4.7: *Invitro* Studies of Methanol Stem Bark Extract of *Hymenocardia acida* on Isolated Tissues

The effects of the extract alone as well as spasmogens in the presence and absence of the extract on rabbit jejunum and guinea pig ileum is graphically represented below.

4.7.1: Effect of Methanol Stem Bark Extract of *Hymenocardia acida* on Isolated Rabbit Jejunum

The methanol stem bark extract of *Hymenocardia acida* (8×10^{-2} – 640×10^{-2} mg/ml) produced significant concentration dependent reduction in the tone and rate of spontaneous contraction of rabbit jejunum Fig 4.1. The extract (8×10^{-2} – 640×10^{-2} mg/ml) of *Hymenocardia acida* when interacted with ACH (2×10^{-5} – 16×10^{-5} mg/ml) inhibited ACH induced contraction in a dose dependent manner Fig 4.3. Acetylcholine produced concentration dependent significant increase in the tone and rate of spontaneous contraction of rabbit jejunum Fig 4.2. As shown in the graph below Fig 4.3 the extract caused a non-parallel rightward shift in ach concentration response curve and the maximum response to ach was not achieved in the presence of the extract.

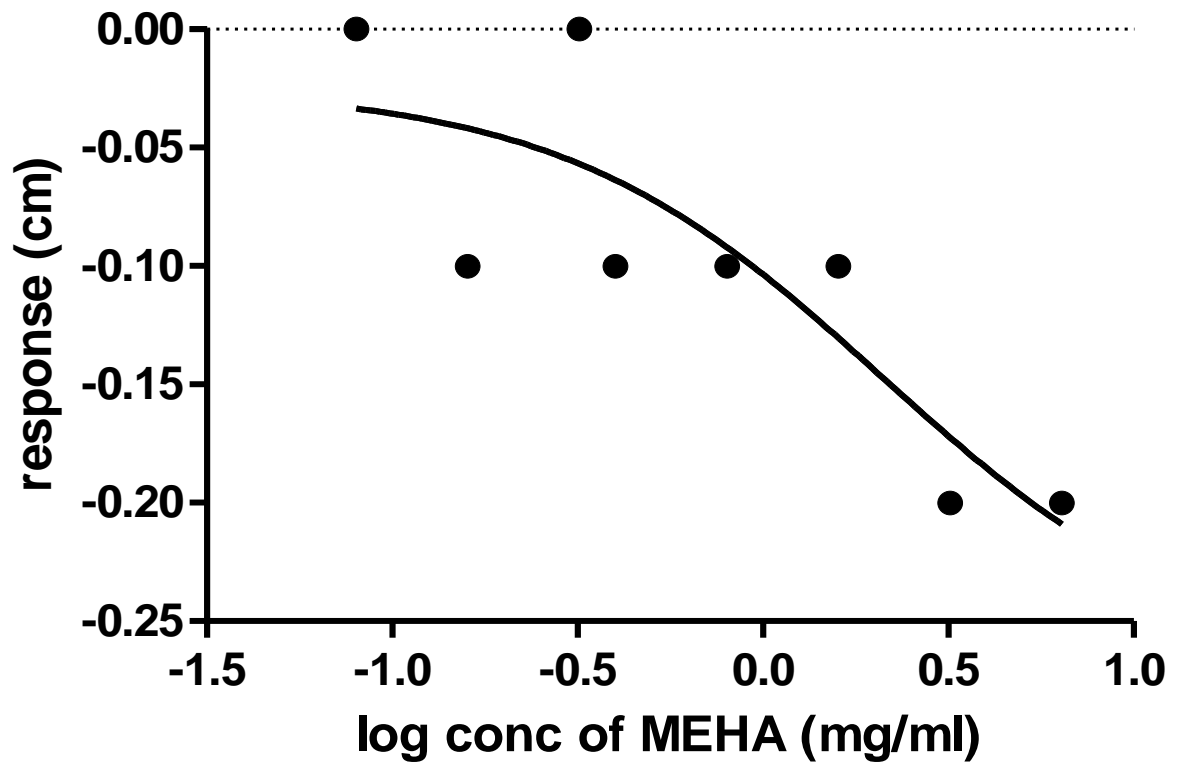


Figure 4.1: Effect of Methanol Stem Bark Extract of *Hymenocardia acida* on Isolated Rabbit Jejunum

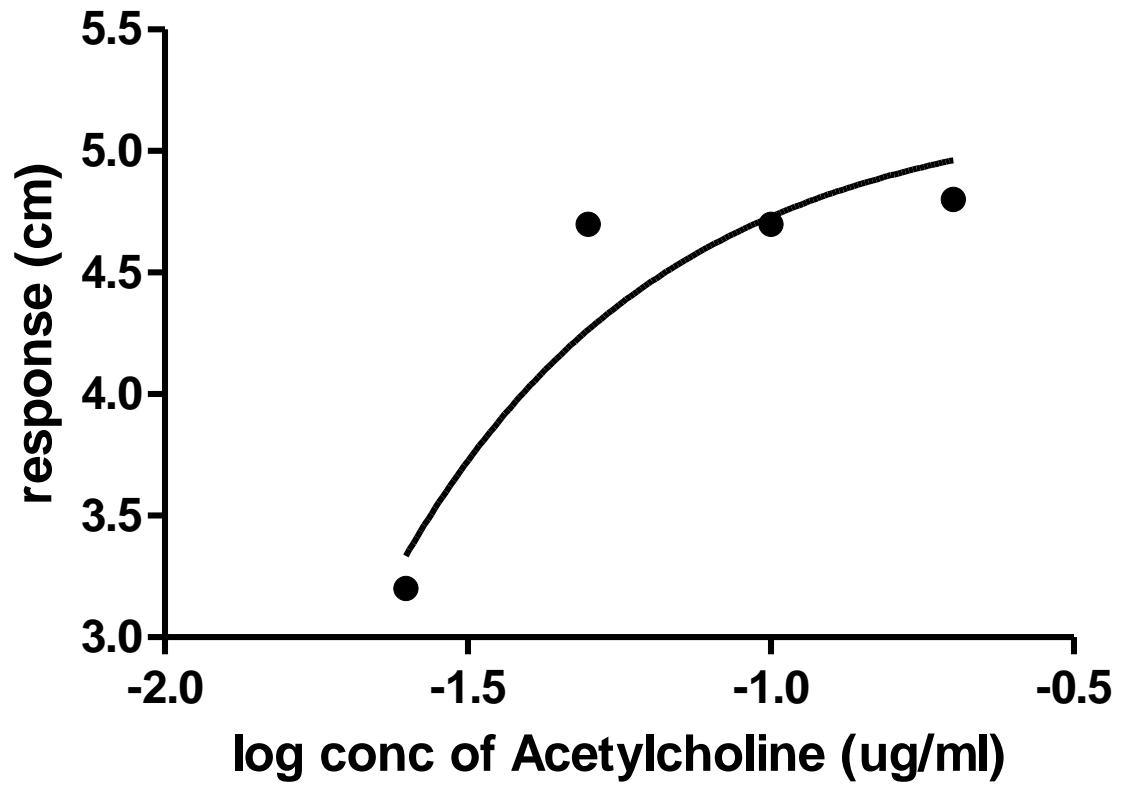
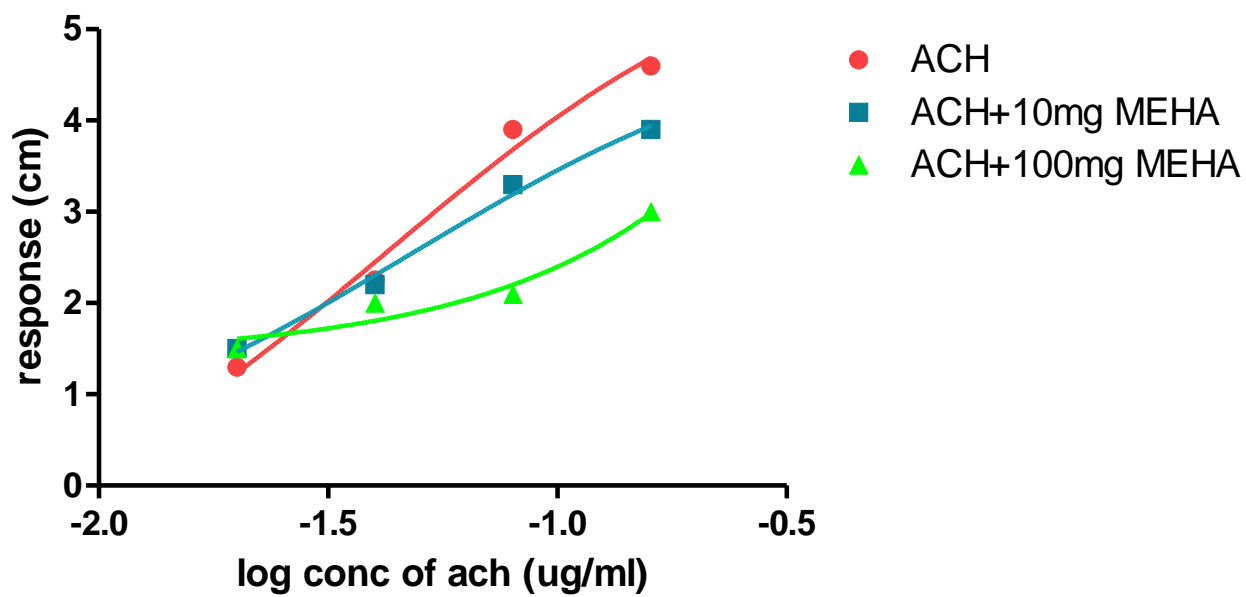


Fig 4.2: Contractile Effect of acetylcholine on Isolated rabbit jejunum n=3



**Fig 4.3 Effect of Graded Doses of Methanol Stem Bark Extract of *Hymenocardia acida* on Acetylcholine Induced Contraction on Guinea Pig Ileum.
n=3**

4.7.2: Effect of Methanol Stem Bark Extract of *Hymenocardia acida* on Isolated Guinea Pig Ileum

The extract of *Hymenocardia acida* (8×10^{-2} – 640×10^{-2} mg/ml) produced significant ($p \leq 0.05$) relaxation on guinea pig ileum in a dose dependent manner fig 4.4. The extract also significantly inhibit histamine (2×10^{-5} – 16×10^{-5} mg/ml) induced contraction on isolated guinea pig ileum Fig 4.6. Histamine produced concentration dependent significant increase in the tone and rate of spontaneous contraction of the guinea pig ileum fig 4.5. Similar to the effects of the extract on Ach CRC, the extract shifted the histamine CRC to the right in a non-parallel manner fig 4.6 with suppression of the maximum effect. This is a typical characteristic of non-competitive antagonism and/or irreversible competitive antagonist.

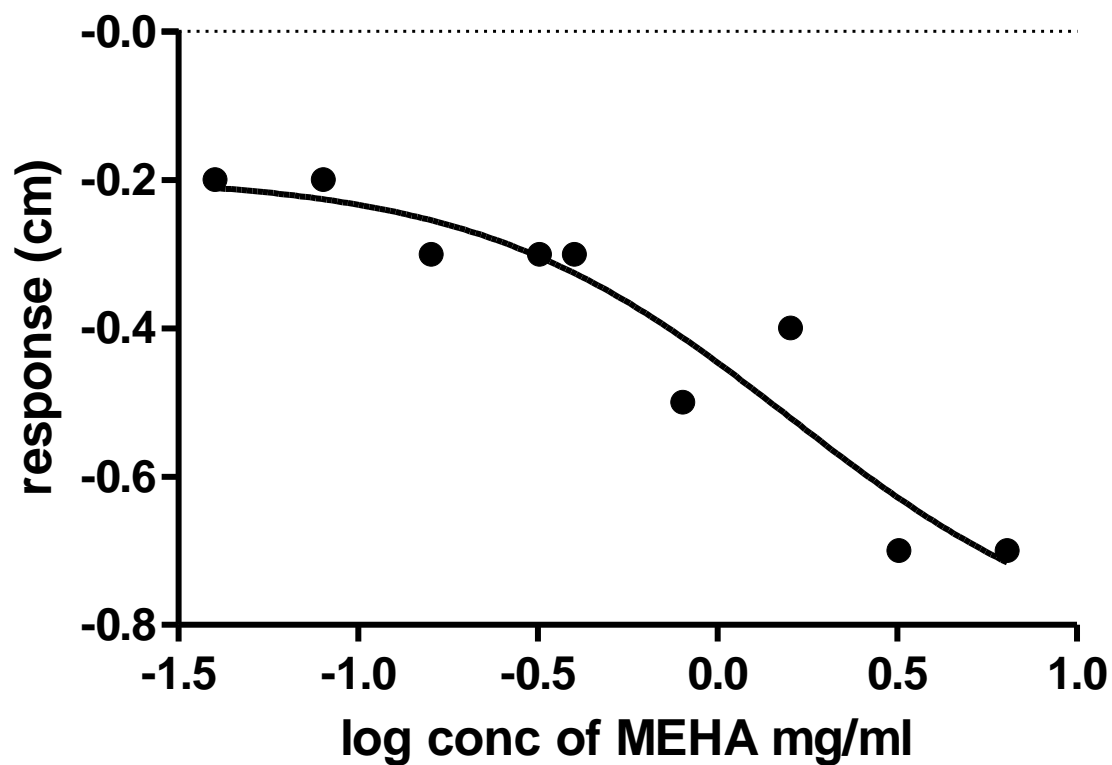


Fig 4.4: Effect of Methanol Stem Bark Extract of *Hymenocardia acida* on Isolated Guinea Pig Ileum.
n=3

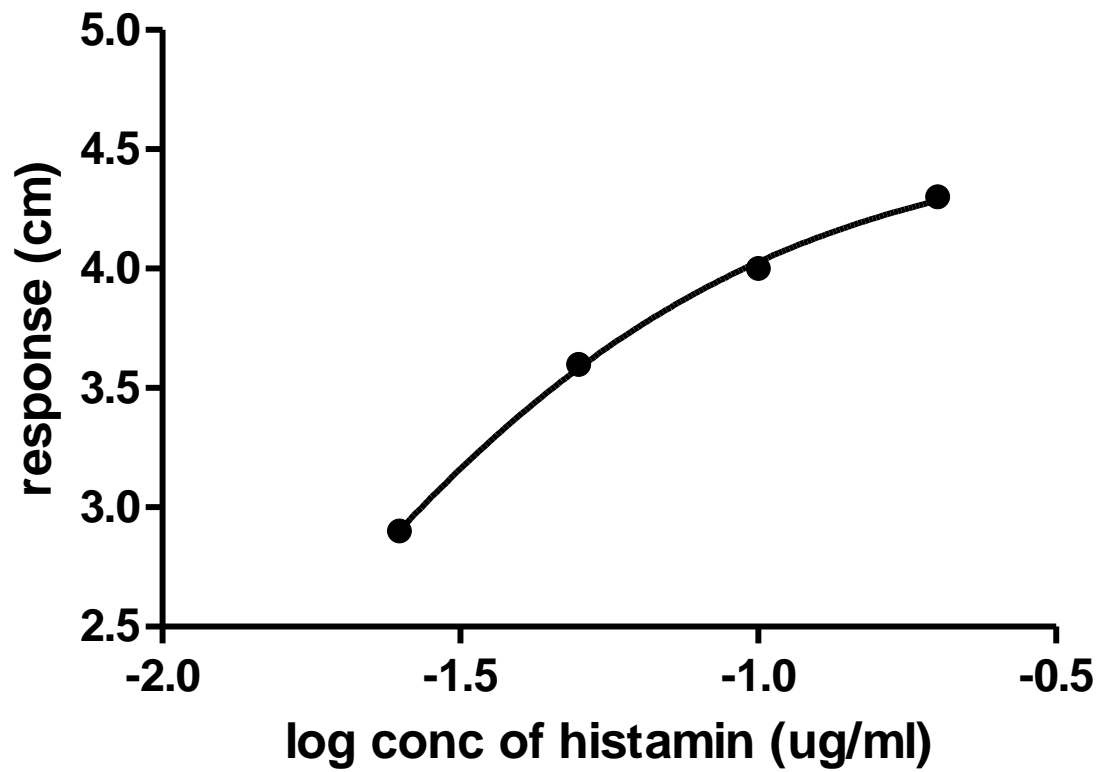


Fig4.5: Contractile Effect of Histamine on Isolated Guinea Pig Ileum

n=3

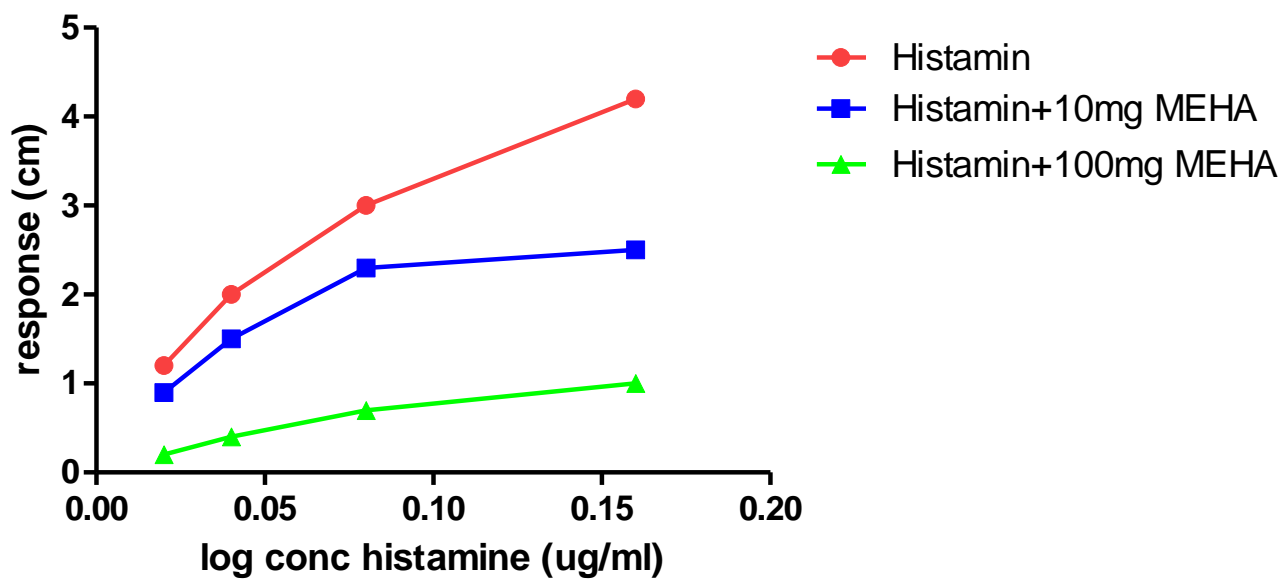


Fig4.6: Effect of Graded Doses of Methanol Stem Bark Extract of *Hymenocardia acida* on Histamine Induced Contraction on Guinea Pig Ileum, n= 3

CHAPTER FIVE

5.0 DISCUSSION

The most common test of acute toxicity is the median lethal dose (LD₅₀) test (Akhila, 2007). From the result the oral LD₅₀ of methanol stem bark extract of *Hymenocardia acida* was found to be greater than 2000 mg per Kg body weight of the animals as per OECD guidelines 423. (LD₅₀) provides information regarding margin of safety of the plants or substance in question. This suggests that the plant is non-toxic orally (OECD, 2001). Determination of (LD₅₀) is usually an initial step in the assessment and evaluation of the toxic characteristic of a substance, and the larger the (LD₅₀) value the lower the toxicity (safer) and vice versa (Rhandawa, 2009). Although (LD₅₀) is usually not regarded as a biological constant since different results are usually obtained on repetition or when determinations are carried out in different laboratories (Hunter *et al.*, 1979), however it gives a guide on subsequent doses to be used for experimental procedure. In our study doses of less than or equal to 30% of the (LD₅₀) were used, which is the range of dose demonstrated to be relatively safe for ethno pharmacological research (Vongtau *et al.*, 2004). Ethanol extract of the leaves of *Hymenocardia acida* has been reported to be safe in both acute and chronic toxicity tests (Obidike *et al.*, 2011a).

The four major mechanisms responsible for the pathophysiology of diarrhea are increased luminal osmolarity (osmotic diarrhea), increased electrolyte secretion (secretory diarrhea), decrease electrolyte absorption and deranged intestinal motility causing a decreased transit time (Umer *et al.*, 2013). The antidiarrheal properties of methanol stem bark extract of *Hymenocardia acida* was evaluated by employing castor oil induced diarrhea and

enteropooling, gastrointestinal motility test as well as *in vitro* test using isolated rabbit jejunum and guinea pig ileum.

The effects of castor oil are mediated by ricinoleic acid, a hydroxylated fatty acid released from castor oil by intestinal lipases. The triglyceride present in castor oil is hydrolyzed in the small bowel by the action of lipases into glycerol and ricinoleic acid (Semwal *et al.*, 2014). The active agent in castor oil is ricinoleic acid which induces diarrhea by hypersecretory response (Almeida *et al.*, 1995). It is polar in nature and therefore poorly absorbed thus its presence in the small intestine results in changes in permeability of the intestinal mucosa to electrolyte and stimulates peristaltic activity in the intestines which in turn produces hypersecretion and fluid accumulation (Nasrin *et al.*, 2013).

Ricinoleic acid also produces irritation and inflammatory action on the intestinal mucosa leading to releases of autocooids and prostaglandins (Sarin *et al.*, 2013). The prostaglandins release promote vasodilatation, smooth muscles contraction and mucus secretion in the small intestine and thereby producing diarrhea. Prostaglandins of the E series are considered to be good diarrheagenic agents in experimental animals as well as in humans, inhibitors of prostaglandin biosynthesis are considered to delay castor oil induced diarrhea (Brijesh *et al.*, 2009). From the results obtained in this study methanol stem bark extract of *Hymenocardia acida* produced statistically significant reduction in severity of diarrhea induced by castor oil in a dose dependent manner. It is also noted that the extract significantly ($p \leq 0.05$) delayed the onset of diarrhea induced by castor oil. Therefore, methanol stem bark extract of *Hymenocardia acida* may also have ability to inhibit prostaglandins biosynthesis or possess anti-inflammatory activity.

Loperamide, a well-known antidiarrheal agent also produced significant ($p \leq 0.05$) protection against the castor oil induced diarrhea. Loperamide was also reported to antagonize diarrhea induced by prostaglandin and cholera toxins (Awouter, 1993). Loperamide is an analogue of morphine (opioid receptor agonist) that act on the mu (μ) receptor in the myenteric plexus of the large intestine thus decreasing the activity of the myenteric plexus which leads to decrease in the tone of longitudinal and circular muscles of the intestinal wall. It activates presynaptic opioid receptors in the enteric nervous system to inhibit acetylcholine release and thus decrease peristalsis, this lead to increase transit time allowing for more water to be absorbed from the fecal matter (Holzer, 2009). The pharmacological effect of loperamide is due to its antimotility and anti-secretory properties (Holzer, 2009). Thus from the results obtained, it is likely that the plant extract in this study mediate its effect through similar mechanism.

Hypermotility characterizes a form of diarrhea where the secretory component is not the causative factor. The test conducted on the methanol stem bark extract of *Hymenocardia acida* on gastric transit time is a method used to study the effect of drugs on the motility of the intestine (peristalsis). Anti-cholinergic agents are known to inhibit gastrointestinal hypermotility (Saralaya *et al.*, 2010). Similarly, activated charcoal is a non-absorbable agent that prevent absorption of chemicals and drugs due to it adsorption properties. In this study, atropine sulphate and different doses of methanol stem bark extract of *Hymenocardia acida* decreased the propulsive movements of the charcoal meal compared to the negative control. However significant effects was observed only at 600 mg compared to atropine.

The distance travelled by charcoal meal in relation to intestinal length is called peristaltic index. In the current study, this index was found to be statistically significant ($p \leq 0.05$) at 600 mg/kg dose of the extract compared to atropine. The basis for this test is that a decrease in motility of the gastrointestinal muscles prolongs the stay of substances within the intestinal lumen thereby allowing for better absorption of water and other electrolyte; this will reduce water texture of the fecal matter. Several antidiarrheal medications are well known for reduction of intestinal contractions and reducing the intestinal transit (Mathad *et al.*, 2005). From the result it is likely that the extract inhibit gastrointestinal hypermotility in diarrhea through anticholinergic effect.

It is also noted that the extract significantly reduced the castor oil induced intestinal fluid accumulation and the volume of intestinal content in a non-dose dependent manner. Castor oil has also been reported to induce diarrhea by increasing the volume of intestinal content via prevention of reabsorption of water and other electrolytes, this causes fluid accumulation and which will lead to diarrhea. (Havagiray *et al.*, 2004). The current result shows that the methanol stem bark extract of *Hymenocardia acida* inhibit gastrointestinal hypersecretion and enteropooling by enhancing water and electrolyte reabsorption.

In the peripheral nervous system, acetylcholine activates gastrointestinal smooth muscles and is a major neurotransmitter in the autonomic nervous system (Westfall and Westfall, 2011). Acetylcholine with increasing concentration causes a dose dependent stimulation of rabbit jejunum contraction. This effect is attributed to muscarinic effect of the drug (Joan and Palmer, 2006). The ability of the extract to inhibit the spontaneous contraction of rabbit jejunum is an indication of its effect on cholinergic receptors.

Acetylcholine in the gastrointestinal tract produced contraction by activating muscarinic receptors, specifically M₃. Activation of M₃ receptor causes stimulation of phospholipase C (PLC) resulting to formation of inositol triphosphate (IP₃) and diacylglycerol (DAG). The IP₃ causes release of calcium ion (Ca²⁺) from intracellular stores and can also mobilize Ca²⁺ secondarily through Ca²⁺ sensitive channels or store dependent mechanism. DAG phosphorylates various proteins via activation of protein kinase C to directly activate non selective cationic channels which is known to stimulate voltage dependent calcium channel (Sanders, 2008). It may be possible that *Hymenocardia acida* stem bark extract binds on muscarinic receptors or affects at least one of these mechanisms. The contractile activity of Ach on ileum was significantly inhibited by the extract suggesting the presence of anticholinergic component(s) (Saikia *et al.*, 2017). This is evident from the graph of concentration response curve (CRC) of the extract on isolated rabbit jejunum since its contraction is believed to be mediated by Ach. This is further confirmed when the plot of the extract on the Ach-induced contraction shifted the CRC of the Ach to the right with suppression of the maximum response, behaving like a non-competitive antagonist and/or irreversible competitive antagonist. The effects of non-competitive antagonist on dose response curve for an agonist is the same as the effect of irreversible competitive antagonist (Gupta and Henthorn, 2009). The practical difference between non-competitive antagonist and irreversible competitive antagonist is specificity, the former antagonizes agonists acting through more than one receptor system where as the latter antagonizes only agonist acting through one receptor system (Gupta and Henthorn, 2009).

Another possible mechanism for spasmolytic activity of the extract could be through inhibition of histamine receptors. Histamine receptors are found in the lumen or mucosal

lining of the gastrointestinal tract, activation of histamine receptors of smooth muscles causes concentration dependent membrane depolarization and excitability (Coruzzi *et al.*, 2000). This effect is mediated through H₁ receptor (Bertaccini *et al.*, 1979). From the present result the extract exerted a dose dependent relaxatory effect on guinea pig ileum, as is evident in the concentration response curve and equally reduced the contractile effect produced by histamine. From the graph, the extract shifted the histamine CRC to the right with suppression of the maximum effect of histamine. This is a typical characteristic of non-competitive antagonist and/or irreversible competitive antagonist (Boxton, 2006). Both non-competitive antagonist and irreversible competitive antagonist attenuate the effect of an agonist. The distinction between the two is that non-competitive antagonist bind to an allosteric site (non-agonist site) on the receptor to prevent activation of the receptor by the agonist while irreversible antagonist bind covalently to the same site as the agonist and cannot be easily displaced by competing ligands (Boxton, 2006). The primary effect of non-competitive antagonist and irreversible competitive inhibitor is shift to the right of the CRC and reduction in the maximal effect produced by the agonist.

Therefore, the effect of the extract is said to be non-specific since it affects muscarinic as well as histaminergic receptors. Interestingly, most of the H₁ receptor antagonists are reported to inhibit acetylcholine responses, mediated by muscarinic receptors. It could be possible that one component of the extract is responsible for both antihistamine and anticholinergic effect of the extract and/or perhaps more than one component from the extract can inhibit acetylcholine and histamine evoked responses (Saikia *et al.*, 2017).

Ammon *et al.*, (2006) in their study on the spasmolytic and atonic effects of SWT5 (iberogast[®]), (is a fixed combination of nine medicinal plants extracts effective in the

treatments of functional dyspepsia and irritable bowel syndrome) reported that SWT5 (iberogast[®]) elicit its antispasmodic effects on serotonin, histamine and muscarinic receptors (i.e. the effect is non-specific on single receptor type). They therefore suggested that SWT5 may possibly elicit its antispasmodic effect via inhibition of cyclic nucleotide phosphodiesterase (similar to papaverin) thus increase cyclic AMP which relaxes increasing contractility of smooth muscles. The extract may as well be said to elicit its spasmolytic action like papaverine.

The spasmolytic activity may be attributed to the phytochemicals found in the extract such as flavonoids, saponin, tannins and alkaloids. These phytoconstituents have been reported to have relaxant activity on smooth muscles (Niaz and Shah, 2011). Studies have shown that some of the phytoconstituents such as alkaloid, glycoside, tannins and essential oil inhibit release of autocooid and prostaglandins (Tiwari *et al.*, 2011). Flavonoids inhibit gastrointestinal release of acetylcholine (Tiwari *et al.*, 2011) as well as the ability to inhibit intestinal motility, water and electrolyte secretion and thus antidiarrheal activity (Dosso *et al.*, 2012). Moreover *invivo* and *invitro* tests have shown that flavonoids are able to inhibit prostaglandin E2 induced intestinal secretion and spasmogen induce contraction (Dosso *et al.*, 2012). Ezeigbo *et al.*, (2013), reported flavonoid and saponin to be responsible for antidiarrheal activity of *Acacia occidentalis*.

Tannins denature protein by forming a complex (protein tannate), which coats the intestinal mucosa and make it more resistant while simultaneously diminishing gastric secretions i.e. prevents prostaglandin E2 induced enteropooling (Mahesh *et al.*, 2013). Saponin inhibits *in vitro* release of histamine (Tiwari *et al.*, 2011).

A number of plants exhibit their antidiarrheal property through their antimicrobial activities (Carlo *et al.*, 1993). Methanol stem bark extract of *Hymenocardia acida* have been shown in previous studies to possess activity against *Escherichia coli*, *proteous mirabilis*, *pseudomonas erogenousa* and *staphylococcus aureus* (Obidike *et al.*, 2011b) which are among the microorganism that are implicated in diarrhea. Antioxidant and antibacterial activities of *Hymenocardia acida* leaf extract was also reported by (Sofidiya *et al.*, 2009). Tannin, saponins and flavonoids are secondary metabolite of plants which are known to have antibacterial activities (Lewis and Ausubel, 2006). Since the methanol stem bark extract of *Hymenocardia acida* in the present study also contains tannin, saponins and flavonoids, it may also possess antimicrobial activity. This may therefore contribute to its antidiarrheal effects.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

Phytochemical screening of methanol stem bark extract of *Hymenocardia acida* using thin layer chromatographic analysis revealed the presence of secondary metabolites including alkaloid, saponin, flavonoids, tannin, triterpenes and steroids.

Acute toxicity studies to determine the LD₅₀ value confirmed the extract to be relatively safe at 2000 mg/kg as per OECD guideline 423.

The methanol stem bark extract of *Hymenocardia acida* was capable of inhibiting castor oil induced diarrhea by delaying the onset as well as severity of diarrhea feces in the mice.

In the test for gastric transit time in mice, the methanol stem bark extract of *Hymenocardia acida* suppressed the propulsion of charcoal meal, however only at a dose of (600 mg/kg) shows statistically significant in a manner similar to atropine sulphate (3 mg/kg). The increase in gastric transit time will thereby cause an increase in the time for absorption of water and electrolyte into the system.

Effect of the extract on castor oil induced enteropooling significantly decrease the volume of intestinal content across all the doses tested (150 mg, 300 mg and 600 mg/kg body weight respectively) when compared to the negative control group.

Isolated tissue studies on both rabbit jejunum and guinea pig ileum show relaxatory activity. The extract was able to block contractile effects of the spasmogens including

acetylcholine and histamine on the tissues indicating the probable anti-muscarinic and antihistaminergic activity of the extract.

6.2 Conclusion

The methanol stem bark extract of *Hymenocardia acida* elicited antidiarrheal activity by delaying the onset as well as severity of diarrheal feces in mice, delay the propulsive movement of charcoal meal (antimotility), decrease the volume of intestinal content (increasing absorption and decrease secretion), inhibit acetylcholine and histamine induced contraction on isolated tissues.

This study has shown that the methanol stem bark extract of *Hymenocardia acida* contains pharmacologically active substances with antidiarrheal properties thus justifying the popular use in folk medicine of this plant as remedy for diarrhea.

6.3 Recommendation

- i. Further studies should be carried out to fractionate, isolate and characterized the phytoconstituents of the extract responsible for the observed pharmacological activities.
- ii. In-depth studies should be carried out to establish the precise mechanism of action of the extract.

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APPENDICES

Appendice I: Effect of *Hymenocardia acida* stem bark extract (Plate I= 10mg/ml; Plate II= 100mg/ml) on spontaneous contraction of isolated rabbit jejunum

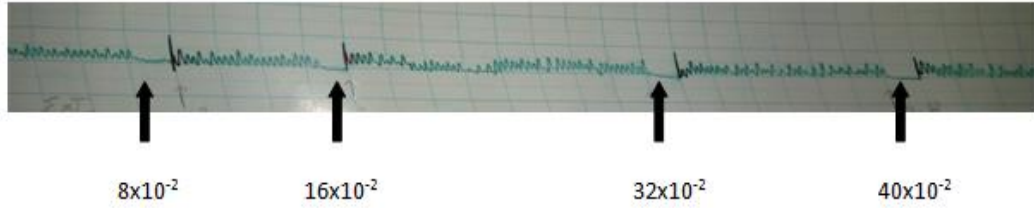


PLATE: I

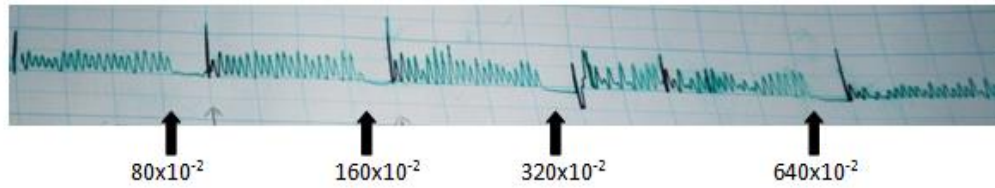


PLATE: II

Appendice II: Relaxatory effect of Hymenocardia acida stem bark extract on isolated guinea pig ileum



PLATE III: Relaxatory effect of 10mg extract on isolated guinea pig ileum



PLATE IV: Relaxatory effect of 10mg extract on isolated guinea pig ileum

Appendice III: Effect of Hymenocardia acida stem bark extract on isolated guinea pig ileum in the presence and absence of histamine

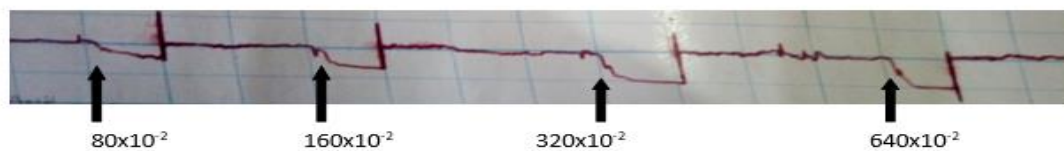


PLATE V: Nothing

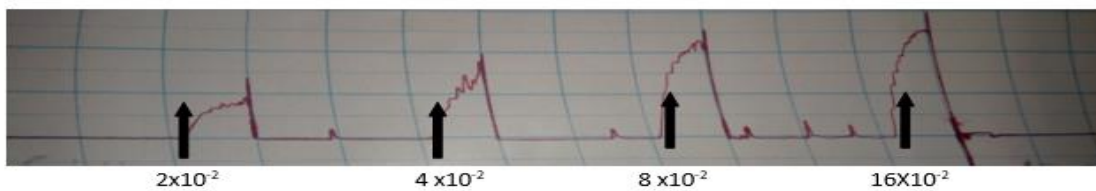


PLATE VI: Effect of 10mg extract in presence of histamine