

**ANTIDIARRHOEAL AND TOXICITY STUDIES ON METHANOL LEAF
EXTRACT OF *COMBRETUM HYPOPILINUM* DIELS (COMBRETACEAE) IN
MICE AND RATS**

BY

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MAY, 2021

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BY

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ZARIA-NIGERIA**

MAY, 2021

DECLARATION

I declare that the work in this dissertation entitled: “Antidiarrhoeal and Toxicity Studies on Methanol Leaf Extract of *Combretum hypopilinum* Diels (Combretaceae) in Mice and Rats” 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 dissertation was previously presented for another degree or diploma at this or any other Institution.

Mubarak Hussaini AHMAD

Name of the student

Signature

Date

CERTIFICATION

This Dissertation entitled “ANTIDIARRHOEAL AND TOXICITY STUDIES ON METHANOL LEAF EXTRACT OF *COMBRETUM HYPOPILINUM* DIELS (COMBRETACEAE) IN MICE AND RATS” by MUBARAK HUSSAINI AHMAD 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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ABSTRACT

The plant *Combretum hypopilinum* has been used in traditional medicine to treat diarrhoea and other gastrointestinal diseases. This study aimed to investigate the antidiarrhoeal activity and toxicity profile of the methanol leaf extract of *Combretum hypopilinum* (MECH) in mice and rats. The phytochemical screening was conducted and the oral median lethal dose (LD₅₀) was determined using standard methods. Antidiarrhoeal activity of MECH (250, 500 and 1,000 mg/kg) was evaluated using castor oil-induced diarrhoea, castor oil-induced enteropooling and intestinal motility tests. The probable mechanisms of antidiarrhoeal activity of MECH were investigated following pretreatment with naloxone, prazosin, yohimbine, propranolol, pilocarpine and isosorbide dinitrate. For the sub-acute toxicity study, the extract was orally administered to rats daily for 28-days at the doses of 250, 500, and 1,000 mg/kg. Mortality and weekly body weight were observed. The animals were sacrificed on the 29th day and blood samples were collected into ethylenediaminetetraacetic acid (EDTA) and plain bottles to analyse haematological and biochemical parameters. The liver, kidney, heart, lung, small intestine, and stomach were removed and their relative organ weight was determined. The organs were examined for histopathological changes. Phytochemical screening revealed the presence of flavonoids, cardiac glycosides, saponins, tannins, steroids, triterpenes and alkaloids. The oral LD₅₀ was higher than 5,000 mg/kg in mice and rats. The extract at the dose of 500 and 1,000 mg/kg significantly ($p<0.05$) reduced the number of diarrhoea stools, intestinal fluid volume ($p<0.05$ and $p<0.01$) and charcoal movement ($p<0.05$ and $p<0.001$) respectively. The pretreatment of the animals with naloxone, prazosin and propranolol each significantly ($p<0.01$) reduced the antidiarrhoeal activity of the extract by increasing the charcoal movement. However, pretreatment of the animals with yohimbine, pilocarpine and isosorbide dinitrate did not significantly ($p>0.05$) change the antidiarrhoeal activity of the

MECH. The extract at the dose of 500 mg/kg significantly ($p<0.05$) reduced the body weights of the animals in the first and second week. There was significant ($p<0.05$ and $p<0.01$) reduction in relative kidney weight at the dose of 500 and 1,000 mg/kg. The extract significantly reduced the alkaline phosphatase (ALP) $p<0.01$ (500mg/kg) and $p<0.001$ (1,000 mg/kg), glucose $p<0.01$ (1,000 mg/kg), potassium ($p<0.01$, $p<0.001$ and $p<0.001$), and low density-lipoprotein (LDL) $p<0.01$ (250 mg/kg), $p<0.001$ (500 mg/kg) and $p<0.05$ (1,000 mg/kg). There was no significant ($p>0.05$) difference in white blood cells, lymphocytes, red blood cells, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. There was a significant ($p<0.05$) increase in the percentage of eosinophils, basophils and monocytes and an increase in granulocyte ($p<0.01$) at the dose of 1,000 mg/kg. The extract significantly ($p<0.01$) increased the platelet levels at a dose of 500 mg/kg. There were histopathological abnormalities on the kidney, lung, stomach, and small intestine. The MECH possesses antidiarrhoeal activity possibly through its interaction with opioidergic and (α_1 and β)-adrenergic systems. The extract is relatively safe on acute exposure but moderately toxic at higher doses on sub-acute administration particularly to the kidney.

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List of Abbreviations

AC	Alveoli congestion
AIDS	Acquired immune deficiency syndrome
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AN	Alveoli necrosis
ANOVA	Analysis of variance
AST	Aspartate transaminase
cAMP	Cyclic adenosine monophosphate
cm	Centimeter
CNS	Central nervous system
CTG	Intestinal content in test groups
DW	Distilled water
EDTA	Ethylenediaminetetraacetic acid
fL	Femtoliter
g/dL	gram per liter
GIT	Gastrointestinal tract
GRAN	Granulocytes
H & E	Hematoxylin and eosin
HCl	Hydrochloric acid
HCT	Haematocrit
HDL	High-density lipoprotein
HGB	Haemoglobin
HIV	Human immune deficiency virus
<i>i,p</i>	Intraperitoneally

IVF	Intravenous fluid
Kg	Kilogram
KH	Kupffer cells hyperplasia
L	Liter
LD ₅₀	Median lethal dose
LDL	Low-density lipoprotein
LOP	Loperamide
LYM	Lymphocytes
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MECH	Methanol leaf extract of <i>Combretum hypopilinum</i>
mg	Milligram
mg/kg	Milligram per kilogram
mL	Milliliter
mmol/L	Millimole per liter
MOR	Morphine
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
n	Number of animals per group
NAL	Naloxone
NF	Normal features
NG	Normal glomerulus
NH	Normal hepatocytes
NM	Normal myocardium
NT	Normal renal tubules

OECD	Organization of Economic Co-operation and Development
ORS	Oral rehydration salt
<i>p.o</i>	Per oral
PIL	Pilocarpine
PLT	Platelet
PRA	Prazosin
PRO	Propranolol
RBC	Red blood cells
ROW	Relative organ weight
rpm	Revolution per minute
sc	Subcutaneous
SEM	Standard error of mean
SHN	Slight hepatic necrosis
SMN	Slight mucosa necrosis
SPSS	Statistical package for the social sciences
V	Normal villi
v/v	Volume/volume
VA	Moderate villi atrophy
VNC	Volume of intestinal content in the negative control group
VRE	Vancomycin-resistant <i>Enterococcus faecalis</i>
VTG	Volume of intestinal content in test groups
WBC	White blood cells
WFC	Wet faeces in the negative control group
WFT	Wet faeces in test groups
CNC	Intestinal content in the negative control group

WHO World Health Organization

YOH Yohimbine

CHAPTER ONE

1.0 INTRODUCTION

Knowledge of traditional uses of medicinal plants by indigenous people plays a significant role and contribution to the search for new bioactive compounds for treating various diseases (Easmin *et al.*, 2015). Despite the emergence of modern and advanced scientific techniques of drug discovery, medicinal plants have been one of the leading sources of drugs (Jimenez-Estrada *et al.*, 2013). Medicinal plants are commonly used for the treatment of diarrhoea in many developing countries. However, the therapeutic potentials and safety profile of some of these medicinal plants have not been scientifically investigated. Therefore, it is essential to investigate their safety and therapeutic potentials to discover novel bioactive compounds (Yuet Ping *et al.*, 2013).

Diarrhoea is a gastrointestinal tract (GIT) disorder that causes the rapid passage of gastrointestinal contents through the intestine characterized by high fluidity and frequency of semisolid or watery faeces three or more times daily (Evi *et al.*, 2018). It happens due to abnormality between the absorptive and secretory process in the GIT associated with hypermotility, leading to excessive loss of body fluids and electrolytes in faeces (Sharma *et al.*, 2015).

Under normal physiological process, there is a continuous movement of water and electrolytes across the intestinal epithelium in a regulated manner. Impairment of fluid and electrolytes transport across the intestinal epithelium causes excess fluid in the lumen and subsequently leads to diarrhoea (Sweetser, 2012). The processes that cause diarrhoea are increased water content in the intestinal lumen by osmotic substances (osmotic diarrhoea),

decrease absorption of fluids, increased water and electrolytes secretion (secretory diarrhoea) and intestinal hypermotility (Gorkiewicz *et al.*, 2013).

Some pathogenic organisms serve as causative agents of diarrhoea that cause many deaths (Bonkougou *et al.*, 2018). Enteric pathogenic bacteria are usually the main causes of diarrhoea in human beings (Evi *et al.*, 2018). Other causes of diarrhoea include viruses, protozoans, helminths, intestinal disorders, immunological factor, poor hygiene and medications (Njuguna *et al.*, 2016).

The predisposing factors of individuals to diarrhoea include younger age, breastfeeding for the insufficient duration, inadequate education by the mother, poor water supply, poor waste and faecal disposal system (Kalakheti *et al.*, 2016). Another risk factor of diarrhoea is infectious diseases such as human immune deficiency virus (HIV) and measles (Joseph *et al.*, 2017).

The use of oral rehydration therapy (ORT) has greatly contributed to the successful management of diarrhoea in the last thirty years (Farthing *et al.*, 2013). Antidiarrhoeal drugs used for the management of diarrhoea include zinc, adsorptive agents such as charcoal and pectin, probiotics, antibacterial e.g. ciprofloxacin, gentamycin, metronidazole (Efunshile *et al.*, 2018), antiviral agents such as nitazoxanide (La Frazia *et al.*, 2013), and antimotility drugs such as loperamide (Guarino *et al.*, 2014).

1.1 Statement of Research Problem

Acute diarrhoea remains a global challenge, with approximately 2 billion episodes per year. The deaths caused by diarrhoea account for 18% of all mortalities in children less than five years (Farthing *et al.*, 2013). In 2013, diarrhoea caused greater than 500,000 deaths in children worldwide (Liu *et al.*, 2015). The disease causes the highest morbidity and mortality in developing nations (Mehesare *et al.*, 2017; Evi *et al.*, 2018).

Despite international organizations continuous effort to combat diarrhoea, still remains one of the highest killer diseases accounting for about 7.1 million deaths per year (Pandey *et al.*, 2012). After respiratory tract infections, diarrhoea is the second leading cause of death among children under five years of age globally. Also, the deaths caused by diarrhoea in children is more than a combination of deaths caused by acquired immunodeficiency syndrome (AIDS), malaria and measles (Black *et al.*, 2010).

Diarrhoea affects young children in low and middle-income nations due to improper water supply, inadequate nutrition, insufficient breastfeeding, inadequate zinc and vitamin A supplementation (Khan *et al.*, 2015). In economically developed nations, acute diarrhoea is usually a mild disease and is rarely associated with mortality but with a significant hospital admission rate and financial loss (Guarino *et al.*, 2014). Diarrhoea caused about 78% of deaths in developing countries, particularly in African and South-East Asian regions (Tadesse *et al.*, 2017). It remains the second leading cause of death in children under five years of age in sub-Saharan Africa countries (Wang *et al.*, 2015). It was projected that 4.4 million children less than five years old could die annually by 2030 because of infectious

diseases, and close to 60% of the mortalities will take place in the sub-Saharan African region (Liu *et al.*, 2015).

The prevalence of diarrhoea in Nigeria was as high as 18.8%, which was more than the average of 16% in the sub-Saharan African regions where the highest burden of the disease prevails (Yakubu *et al.*, 2015). In Nigeria, it caused close to 300,000 deaths in children less than five years (Yakubu *et al.*, 2015).

Many drugs are used for the management of diarrhoea, such as diphenoxylate, loperamide, racecadotril, diloxanide, atropine sulphate and ORT (Sahoo *et al.*, 2016). These drugs are sometimes ineffective, and they have unwanted side effects such as headaches, convulsions, abdominal discomfort, vomiting, constipation, and hallucination (Yakubu *et al.*, 2015). Some antibiotics are also used to treat infectious diarrhoea, but they are also associated with adverse effects, and pathogenic microorganisms develop resistance to them (Knecht *et al.*, 2014).

Medicinal plants including *Combretum hypopilinum* are believed by people to be safe when used for treating various diseases. Although some of these medicinal plants are relatively safe, many are toxic when taken for a short or long duration (Lahlou *et al.*, 2008). There are also inadequate clinical, toxicological and pharmacological studies on many medicinal plants (Olaya *et al.*, 2010).

1.2 Justification of the Study

Diarrhoea remains an important health concern worldwide, affecting children under the age of five years in developing countries and results in more than 5-8 million deaths annually (Zaman *et al.*, 2015). Several synthetic drugs such as diphenoxylate and loperamide are used in the treatment of diarrhoea. However, they are associated with adverse effects such as headache, convulsion, abdominal discomfort, vomiting and constipation (Pandey *et al.*, 2012). In addition, these drugs are not readily available to the people living in rural communities, which make people rely on medicinal plants that are readily available and perceived to have fewer side effects (Wansi *et al.*, 2017).

World Health Organization (WHO) has encouraged the use of medicinal plants for the treatment of many diseases (de Souza Monteiro *et al.*, 2018). In several rural communities in developing countries, it is believed that herbal remedies are the only available and affordable remedies for treating various diseases including diarrhoea (Njume and Goduka 2012).

The use of herbal drugs is a common practice in many African countries such as Nigeria in the treatment of diarrhoea (Okpara *et al.*, 2017). Therefore, there is a need to investigate the medicinal plants that possess antidiarrhoeal potential as a measure of preventing the rampant spread of diarrhoea, particularly in resources limited countries (Ndukui *et al.*, 2013).

The leaves of *Combretum hypopilinum* have been used for the treatment of diarrhoea in Nigeria and other African countries (Idoh *et al.*, 2018; Fyhrquist *et al.*, 2004; Hedberg *et*

al., 1982). However, the scientific basis for its use and safety has not been evaluated. Therefore, this study aims to investigate the antidiarrhoeal activity and safety profile of the plant.

1.3 Aim and Objectives

1.3.1 Aim

The aim of this study is to investigate the antidiarrhoeal potentials and toxicological effects of methanol leaf extract of *Combretum hypopilinum* Diels (Combretaceae) in mice and rats.

1.3.2 Specific Objectives

The objectives of the study are:

1. To determine the phytochemical constituents present in the methanol leaf extract of *Combretum hypopilinum*
2. To determine the oral median lethal dose (LD₅₀) of methanol leaf extract of *Combretum hypopilinum* in mice and rats
3. To evaluate the antidiarrhoeal activity of methanol leaf extract of *Combretum hypopilinum* in mice
4. To determine the possible mechanisms of antidiarrhoeal activity of methanol leaf extract of *Combretum hypopilinum* in mice
5. To investigate the sub-acute toxicological effects of methanol leaf extract of *Combretum hypopilinum* in rats

1.4 Research Hypothesis

The methanol leaf extract of *Combretum hypopilinum* Diels (Combretaceae) possesses antidiarrhoeal activity and it is relatively safe.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diarrhoea

Diarrhoea is defined as a change in a normal intestinal movement characterized by an increase in the passage of watery stool at least three times a day (Yakubu *et al.*, 2015). The features of diarrhoea include increased gastrointestinal motility, secretion and a decrease in the absorption of fluid and electrolytes (Ezeja *et al.*, 2012). This disease serves as a notable presentation of gastrointestinal infection due to the ingestion of bacteria, viruses, or parasites transmitted by water, food, utensils, hands, and flies (Yakubu *et al.*, 2015).

2.1.1 Aetiology of Diarrhoea

Many pathogenic organisms have been indicated to cause diarrhoea (Tate *et al.*, 2012). The important causes of diarrhoea include bacteria, viruses, protozoan, helminths, disorders affecting the bowel, such as irritable bowel syndrome, immunological factor, poor hygiene and some drugs (Evi *et al.*, 2018). The different causes of diarrhoea differ based on the geographical location and clinical symptoms (Kotloff *et al.*, 2013). Careful medical history, a physical examination for signs of dehydration, laboratory tests, macroscopic and microscopic stool examination for the presence of parasites and other pathogens, and media culture to identify the causative agents are important in the diagnosis of diarrhoea (Johargy *et al.*, 2010).

2.1.1.1 Viral Causes of Diarrhoea

Viruses such as rotavirus, norovirus, enteric adenovirus and parvovirus are the main cause of diarrhoea in Africa (Tate *et al.*, 2012; Harris *et al.*, 2017). The rotavirus is the leading

cause of diarrhoea. Viral gastroenteritis is highly transmittable and is mostly contacted by intake of contaminated food or water (Tate *et al.*, 2012).

Rotavirus is the main cause of diarrhoea-related deaths in children globally. About 95% of the deaths happen in Africa and some Asian countries (Tate *et al.*, 2012). Globally, in the year 2008, diarrhoea caused by rotavirus accounts for an estimated 453,000 mortalities in children less than five years old, which put up to 37% of all lives lost due to acute diarrhoea (Tate *et al.*, 2012; Rackoff *et al.*, 2013). In Nigeria, a high occurrence of approximately more than 160, 000 deaths in children under the age of five, with 20% as rotavirus related (Mukhtar *et al.*, 2016).

Norovirus is the second most common cause of viral diarrhoea in children less than five years old after rotavirus globally. It causes an estimated 1.1 million hospital admissions and close to 218,000 deaths annually (Patel *et al.*, 2008). In addition, the cause of diarrhoea by norovirus in children less than five years of age became prominent after the decline of the rotavirus associated diarrhoea from rotavirus vaccine emergence (Shioda *et al.*, 2015).

2.1.1.2 Bacterial Causes of Diarrhoea

Bacteria such as *Escherichia coli* are among the most common cause of diarrhoea in developing nations (Boschi-Pinto *et al.*, 2008). Other microorganisms implicated in diarrhoea include *Bacillus cereus*, *Campylobacter jejunii*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexinerii*, *Vibrio cholera*, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* (VRE) and *Clostridium difficile* (Thakkar and Agrawal, 2010). Shigellosis is the main cause of deaths and illness

due to diarrhoea, particularly in developing nations causing close to 165 million episodes and 1 million deaths (Baldi *et al.*, 2009).

2.1.1.3 Parasitic Causes of Diarrhoea

In many situations, intestinal parasitic infections can present without symptoms but manifest with diarrhoea and other signs of gastroenteritis such as abdominal cramps (Won *et al.*, 2016). The parasites that mostly cause intestinal infections include *Cryptosporidium*, *Blastocystis*, *Entamoeba* species, *Giardia lamblia* and *Dientamoeba fragilis* (Fletcher *et al.*, 2012). *Cryptosporidium* and *Giardia* is the most common cause of parasite related diarrhoea (Miyamoto and Eckmann, 2015).

Also, *Cyclospora cayetanensis* causes severe diarrhoea and has contributed to many foodborne diarrhoea (Ortega and Sanchez 2010). Other parasites such as *Entamoeba histolytica* (which is highly invasive and causes amoebic colitis and coccidian), *Cystoisospora belli*, and *Cyclospora cayetanensis* are less common causes of diarrhoea (Miyamoto and Eckmann, 2015).

2.1.1.4 Non-Infectious Causes of Diarrhoea

Diarrhoea can be caused by milk allergy, pancreatic insufficiency, cystic fibrosis, immunodeficiency, glucose and lactose intolerance (Mushtaq *et al.*, 2017). Gastroparesis is usually associated with dysfunction of the small intestine and colon in patients with persistent diabetes, which manifests as diarrhoea, constipation or uncontrolled stool evacuation as complications (Phillips *et al.*, 2006).

Other common causes of diarrhoea in diabetes mellitus include bile salt malabsorption, steatorrhea and drugs such as metformin (Krishnan *et al.*, 2013). Also, Conditions such as intestinal inflammatory and autoimmune disease, including Crohn's disease, ulcerative colitis and coeliac disease, can cause secretory diarrhoea where the immune system regulates the absorption of electrolytes by releasing cytokines and modulatory effects on the enteric nervous system (Wasilewski *et al.*, 2017).

Diarrhoea is one of the common side effects of drugs that interfere with intestinal fluid and electrolytes secretion (Moon *et al.*, 2015). Many drugs such as magnesium-containing antacids, erythromycin, sorbitol (osmotic laxative agent) and lactulose cause diarrhoea due to fluid retention in the small intestine (Fernandez-Banares *et al.*, 2016). Other therapeutic drugs, such as anticancer and antiretroviral drugs also cause diarrhoea (Clay and Crutchley, 2014). About 28 % of patients receiving antiretroviral therapy with protease inhibitors present with more than four watery stools in 24 hours (Pessi *et al.*, 2014). Patients treated with anticancer drugs such as 5-fluorouracil (5-FU) can suffer from serious diarrhoea as an adverse drug reaction which can negatively affect the treatment (Perez *et al.*, 2015).

2.1.2 Transmission of Diarrhoea

According to WHO, the causative pathogens of diarrhoea such as bacteria, parasites and viruses are transmitted to humans through a faeco-oral route which includes intake of contaminated water or food, person to person contact and direct contact with faecal matter (Walker *et al.*, 2012; Zambrano *et al.*, 2014). Diarrhoea caused by these pathogens is the second leading cause of deaths in children less than five years, resulting in about 760,000 mortalities every year (WHO, 2013; Wang *et al.*, 2016).

Zoonotic widespread of diseases caused by pathogenic microorganisms is a significant reason for the occurrence of life-threatening diseases. Therefore, contact between human beings and carrier animals increases the chances of diseases transmission (Coker *et al.*, 2011). Also, improper disposal of waste products in the environment and transfer of disease-causing microorganisms from animals to humans can serve as a risk of diarrhoea (Zambrano *et al.*, 2014). The possibility of contamination from faecal matter is mostly observed in localities with the dirty source of water (Ngure *et al.*, 2013).

2.1.3 Risk Factors of Diarrhoea

Many factors contribute to the incidence of diarrhoea in children, which include socio-economic, environmental and behavioural factors (Mengistie *et al.*, 2013). Diarrhoea mostly occurs in less developing countries, including sub-Saharan Africa nations where there is a lack of clean water, poor faecal disposal method, overpopulation, and poor hygiene standards (Yaya *et al.*, 2018).

The predominant contributor to childhood diarrhoea in Nigeria is water and food contaminated by pathogenic organisms that persist in resources limited communities (Yaya *et al.*, 2018). The factors at the individual and community level also determine the chances and occurrence of diarrhoea (Azage *et al.*, 2016). The risk factors for diarrhoea include young age, male gender, inadequate breastfeeding, low level of education by mothers, inadequate clean water, improper water maintenance at home, lack of handwashing habits, poor sanitation, improper faecal and waste disposal methods (George *et al.*, 2014).

The vulnerability, severity and duration of acute diarrhoea are higher in economically constraint children (Ganguly *et al.*, 2015). Children whose home have good quality water and sanitation are still at risk of diarrhoea provided they live in a community with improper defecation practice and contaminated water (Hathi *et al.*, 2016). Children living in poverty are ten times more likely to die from diarrhoea than their more privileged counterparts (Chola *et al.*, 2015). Poor sanitation is also associated with infectious diseases such as diarrhoea, soil-transmitted helminth infection, trachoma and schistosomiasis (Clasen *et al.*, 2014).

2.1.4 Types of Diarrhoea

Based on duration, diarrhoea can be divided into acute diarrhoea when it lasts for less than 14 days, persistent when it lasts between 14 to 29 days, or chronic, which takes approximately 30 days above (Guerrant *et al.*, 2001).

2.1.4.1 Acute Diarrhoea

Acute diarrhoea can be defined as the high amount of watery faeces out of the body, which lasts less than fourteen days. Sometimes individuals suffering from acute diarrhoea present with a sudden onset of watery stool three or more times within 24 hours (Riddle *et al.*, 2016). Acute infectious diarrhoea is the main reason for hospitalization, which drastically reduces the patients' quality of life (Scallan *et al.*, 2011). The signs and symptoms of acute diarrhoea include nausea, vomiting, stomachache and cramps, bloating, flatulence, increased body temperature and bloody diarrhoea.

In low economic and industrialized nations, acute diarrhoea causes infant and childhood deaths despite ORT and vaccine utilisation (Gordon and Akobeng, 2016). There were an estimated 1.7 billion acute diarrhoea events globally, and close to 700,000 fatalities occur annually in children before their fifth birthday (Bhutta *et al.*, 2013).

Dysentery is diarrhoea that presents with blood and mucus in the stool with drastic weight loss, intestinal injury, sepsis and malnutrition. Dysentery can be categorized as amoebic and bacillary dysentery, where they manifest with similar symptoms. Bacillary and amoebic dysentery cause dehydration due to excessive loss of fluids and electrolytes (Agung Fitri *et al.*, 2017). Bacillary dysentery is caused by intestinal gram-negative *Shigella dysenteriae* that mostly affect children. The amoebic dysentery is an intestinal parasitic infection caused by *Entamoeba histolytica* which presents with increased body temperature, bloody or mucous diarrhoea, chills, and abdominal discomfort (Marie and Petri, 2013).

2.1.4.2 Persistent Diarrhoea

Majority of the diarrhoeal episode that affects people is acute, which last between 3 to 7 days. However, if diarrhoea extends more than 14 days, it is termed persistent diarrhoea (WHO, 2013). Persistent diarrhoea means diarrhoea that begins within a few days but continues for a long duration between two to four weeks. This type of diarrhoea may start as watery diarrhoea or dysentery. The main complication resulting from persistent diarrhoea is excessive fluid loss, electrolytes, nutrient, weight loss and dehydration (Polman *et al.*, 2015).

2.1.4.3 Chronic Diarrhoea

Chronic diarrhoea can be defined as the removal of greater than three watery faeces in a single day, with a stool weight of more than 200 g that last for more than four weeks (Stotzer *et al.*, 2015). The factors that cause chronic diarrhoea are related to the social and economic status of the individuals and their community (Schiller, 2012).

The most common investigations in patients with chronic diarrhoea in developed nations are irritable bowel syndrome, inflammatory bowel disease, malabsorption and chronic infections. While in developing countries, a chronic infection caused by bacteria, mycobacterium and parasites is the leading cause of chronic diarrhoea, inflammatory bowel disease and malabsorption (Fragkos *et al.*, 2016).

2.1.5 Physiology of Fluid and Electrolytes Regulation in the GIT

The intestinal epithelial barrier regulates the absorption and secretion of fluids across the intestinal epithelium (Camilleri *et al.*, 2017). The movement of water across the intestinal barrier and systemic circulation is regulated by the active movements of glucose and ions such as sodium (Na^+), chloride (Cl^-), bicarbonate (HCO_3^-), and potassium (K^+). The absorption of water is due to the active movement of Na^+ across the intestine in the same direction with Cl^- or HCO_3^- driven by the basolateral Na^+/K^+ -ATPase pump (Thiagarajah *et al.*, 2014). The absorption or secretion of fluids needs an organized activity of the membrane transporters found on the apical and basolateral epithelial lining (Thiagarajah and Verkman, 2018). The intestinal epithelium structure contains villi which are a finger-like projection and crypts that are glandular distributed relatively along the length of the intestine that regulates the intestinal fluid and electrolytes transportation. The intestinal

secretory and absorptive mechanisms happen more at crypts and villi, respectively (Thiagarajah *et al.*, 2014). The large intestine reabsorbs Na^+ in exchange for K^+ by the action of the Na^+/K^+ pump (Philip *et al.*, 2017).

2.1.6 Pathophysiology of Diarrhoea

Diarrhoea develops due to dysregulation in the absorption of water, solutes and electrolytes across the intestinal epithelium (Thiagarajah *et al.*, 2015). Intestinal hypersecretion develops due to inconsistent secretion of fluids and electrolytes in the intestines caused by some disease conditions or infections (Moon *et al.*, 2015). Some pathogenic organisms such as *Vibrio cholera*, *Escherichia coli*, rotavirus, *Entamoeba histolytica* and *Cryptosporidium parvum* when ingested, release toxins into the intestine (Kotloff *et al.*, 2013). The toxins such as cholera toxin cause the production of cyclic adenosine monophosphate (cAMP) and increase calcium (Ca^{2+}) concentration which subsequently prevents Na^+ absorption at the same time activating Cl^- secretion from the intestinal cells (Moon *et al.*, 2015).

Drugs such as digoxin disrupt the intestinal secretion and absorption, thereby leading to secretory diarrhoea by attaching to specific receptors to increase cAMP and intracellular Ca^{2+} transport with overall water and electrolytes loss (Field, 2003). The digoxin also inhibits the Na^+/K^+ ATPase, which prevents the Na^+ reabsorption and causes excessive loss of water and intestinal fluid accumulation (Philip *et al.*, 2017).

2.1.7 Clinical Features of Diarrhoea

The typical clinical signs and symptoms observed in acute diarrhoea range from a slight stomach upset, usually for 1 to 2 days, accompanied by diarrhoea with little stool to severe

watery diarrhoea for more days. In addition, there may be an abdominal cramp that may stop after defaecation. Other clinical features include vomiting, increased body temperature, headache, weakness in the limbs, loss of appetite and acute weight loss (Amiebenomo and Osaghae, 2017).

Following the drastic loss of water and electrolytes, signs and symptoms of dehydration and other complications become evident, which are important in categorizing the severity of the diarrhoea. The extent of dehydration is rated as mild dehydration (no signs or symptoms), moderate dehydration (thirst, restlessness, irritable behaviour, decreased skin elasticity and sunken eyes) or severe dehydration (symptoms become more severe, shock with diminished consciousness, lack of urine output, cold extremities, a rapid pulse, hypotension and pale skin) (WHO 2013).

2.1.8 Complications of Diarrhoea

Diarrhoea causes a negative effect on patients' physical and mental development. It causes and worsens malnutrition in children, which can drastically reduce physical activities and negatively affect the work productivity rate in adults (Guerrant *et al.*, 1992). Diarrhoea can also result in electrolytes imbalance, renal impairment, dehydration and impaired immune system. It also may reduce the therapeutic effects of drugs as a result of increased intestinal motility, which limits the time for the drug to be absorbed (Moon *et al.*, 2015)

2.1.8.1 Dehydration

Acute, persistent and chronic diarrhoea may cause dehydration where there are insufficient fluids and electrolytes in the body, which affect muscle activity and other biochemical and

metabolic functions in the body. The common signs and symptoms of dehydration in adults are feeling thirsty, weakness, dry skin and tongue, dark urine, dizziness and sunken eyeballs, while in infants and young children, it is usually accompanied by high fever, absence of tears when crying, dry mouth and sunken eyes (Sarin and Bafna, 2012).

Dehydration is the main life-threatening complication that results in deaths and serious illness in children suffering from short term diarrhoea which eventually causes biochemical derangements in the body fluids such as hyponatraemia, hypokalaemia and metabolic acidosis (Okposio *et al.*, 2015). The dehydration level is usually categorized as mild, moderate or severe dehydration depending on the amount of water and electrolytes lost (Canavan and Arant, 2009).

2.1.8.2 Malnutrition

Diarrhoea as the major reasons for morbidity and mortality in children under five years of age in developing countries leads to malnutrition. In addition, inadequate nutritional intake increases the chances of diarrhoea. Diarrhoea caused by bacterial infection is frequently observed in severe and complicated malnutrition as a result of loss in the integrity of the mucosal barrier to adequately absorb nutrients along with the GIT (Singh *et al.*, 2014). The total number of diarrhoeal episodes is 5-7 times more in malnourished children than adequately nourished children. The rate of mortalities due to diarrhoea is higher among undernourished children than well-nourished ones (Bilal *et al.*, 2016).

Usually in malnutrition, there is an abnormal electrolyte and other elements in the body. This abnormality worsens during diarrhoea as a result of more loss of fluid and electrolyte,

particularly sodium, potassium and bicarbonate. The most common electrolytes imbalances in malnourished children suffering from diarrhoea are hyponatraemia and hypokalaemia, which pose a high risk of deaths (Roy *et al.*, 2011).

2.1.8.3 Cardiovascular Complications

Diarrhoea can cause serious health effects on the function of the heart, where water loss can cause dehydration with subsequent orthostatic hypotension. These adverse health effects happen because of the decreased blood volume, which eventually results in orthostatic hypotension (Bello, 2015).

2.1.9 Managements of Diarrhoea

The successful management of diarrhoea includes replacement of the lost fluid and electrolyte in order to correct the dehydration associated with it, reduce the frequent passage of the stool and minimize associated symptoms such as abdominal pain and reduce duration and severity of illness (Njume and Goduka, 2012). For effective management of the diarrhoea, evaluation of the patient's dehydration status is important in curtailing the worsening of the symptoms, which is targeted toward reduction and prevention of mortality and morbidity (Fonseca *et al.*, 2004).

Children suffering from mild to moderate dehydration improve when treated with oral rehydration salt (ORS) alone, while those with severe disease require intravenous fluids (IVF) to limit any life-threatening complications (Fonseca *et al.*, 2004). The administration of ORS in an attempt to replace fluid and electrolyte loss in diarrhoea plays a huge role in effective treatments (Njume and Goduka, 2012).

2.1.9.1 Oral Rehydration Therapy

Dehydration is the main complication with diarrhoea accompanied by weight loss, decreased skin elasticity and changes in respiration pattern. The first step in managing acute diarrhoea is rehydration, preferably using oral rehydration therapy (Horne *et al.*, 2014). The assessment of the patients' body weight presented with diarrhoea is important to evaluate the fluid lost, followed by the rehydration and fluid maintenance therapy. The ORS contains a mixture of salts, glucose, and water for proper intestinal sodium-glucose coupled cellular transport mechanism (Barr and Smith, 2014).

2.1.9.2 Supplemental Zinc Therapy

Zinc is a nutritional supplement usually given as zinc sulfate, zinc acetate, or zinc gluconate, which are all water-soluble compounds (Alam *et al.*, 2017). The use of zinc as a supplement in slowing the duration and severity of diarrhoea in children under five years is well established (Lamberti *et al.*, 2013).

Zinc supplement administered at a dose of 20 mg per day for 10 days in children older than two months may play a crucial role in the effective management of acute diarrhoea, particularly in developing countries (Farthing *et al.*, 2013). Zinc supplementation decreases dehydration, reduces the duration and severity of the diarrhoea by 20% - 40% (Liberato *et al.*, 2015). The zinc supplemented during diarrhoea enhances intestinal water and electrolytes absorption, mucosal integrity, immunity, gene expression and oxidative stress (Canani *et al.*, 2011). Zinc supplement in the diet reduces acute diarrhoea, especially in malnourished children and its inclusion in oral rehydration therapy decreases acute diarrhoea particularly in children of developing nations (Sandle, 2011).

2.1.9.3 Diet

Provision of a sufficient diet to a child is required for the regeneration of intestinal mucosa. Inadequate feeding practices halt the proper management of acute diarrhoea in children (Brandt *et al.*, 2015). The cells lining the intestinal epithelium obtain their nutrients primarily from the content of the intestinal lumen. Therefore, dietary restrictions can reduce the regeneration of cells damaged by an infectious process (Sandhu, 2001).

Adequately feeding the child with a nutritious diet must be maintained after rehydration therapy four to five times daily (Guarino *et al.*, 2014). Also, the provision of an adequate diet rich in the needed nutrients as well as other vitamins during diarrhoea decreases episodes of diarrhoea, prevents malnutrition and boost the immune system against other pathogens (Guerrant *et al.*, 2008).

2.1.9.4 Probiotics in the Treatments of Acute Diarrhoea

Probiotics can be defined as living microorganisms administered in an adequate amount to confer a health benefit to the host (Ahmadi *et al.*, 2012). It is a living microorganism administered to promote the health of the host by treating or preventing infections caused by various types of pathogenic microorganisms (Sharif *et al.*, 2016).

Probiotics, when administered form part of the host intestinal flora and can protect the GIT against disease-causing pathogens (Lau and Chamberlain, 2016). Probiotics have been used to prevent and treat GIT conditions, including diarrhoea (Lau and Chamberlain, 2016). They prevent disturbances of normal intestinal flora and decrease the chances of invasion by pathogenic bacteria (Guarner *et al.*, 2012). They are included in managing acute

diarrhoea as a supportive measure and reducing diarrhoea by 14% in developed nations (Applegate *et al.*, 2013). The European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommends using probiotics such as *Lactobacillus rhamnosus GG* or *Saccharomyces boulardii* in the management of acute diarrhoea (Guarino *et al.*, 2014). *Saccharomyces boulardii* is a non-disease causing fungi that directly inhibit the growth of pathogenic microorganisms and inhibit intestinal secretion (Sharif *et al.*, 2016).

Diarrhoea can also develop due to interruption of the protective ability of the intestinal flora by drugs such as antibiotics (Guarner *et al.*, 2012). Probiotics inhibit the growth of pathogenic microorganisms in the intestine, suppress the multiplication and invasion of pathogenic bacteria. Therefore, probiotics enhance intestinal functions and protect the intestinal barrier (Rohde *et al.*, 2009).

The processes by which probiotics cause protective effects in acute diarrhoea are not well defined but could be attributed to the augmentation of intestinal epithelial function, which helps in the regulation of immune responses and maintenance of homeostasis (Jones and Versalovic, 2009). Probiotics have therapeutic activity on rotavirus associated diarrhoea in children, thus included in the guidelines for the management of acute diarrhoea in children by the European Society for Pediatric Infectious Diseases (Passariello *et al.*, 2012).

2.1.10 Prevention of Diarrhoea

2.1.10.1 Safe Drinking Water, Sanitation and Hygiene

The supply of safe water for drinking, cooking, and personal hygiene is essential for maintaining good health (McGuinness *et al.*, 2017). Therefore, to reduce diarrhoea, there is

a need for the provision of treated drinking water, improved sanitation, and increased handwashing with soap (Luby *et al.*, 2018). Sub-Saharan African countries have a high incidence of diarrhoea because about 20% of the populations are supplied by untreated water, particularly in less privileged settlements (Dos Santos *et al.*, 2015).

The measures to be adopted to prevent faeco-oral transmission of pathogens should focus majorly on proper handwashing, improved sanitation and access to clean water supply (Aluisio *et al.*, 2015). These measures can be significantly improved by proper health awareness and campaign at the group or individual level on hygiene education and training, posters, and educational leaflets (Gebru *et al.*, 2014).

2.1.10.2 Exclusive Breastfeeding

The cases of diarrhoea-related child sickness and deaths are obvious in children from developing countries due to inadequate breastfeeding practices, poor water supply, improper sanitation, inaccessibility to vaccines, and inadequate diarrhoea treatments (Ahs *et al.*, 2010). Breast milk contains nutrients, antioxidants, hormones, and antibodies needed by the child for survival and development (Naeem and Parveen, 2016). Exclusive breastfeeding for the first six months of life contributes significantly to the prevention of diarrhoea (Jones *et al.*, 2003). WHO recommends exclusive breastfeeding for the first six months of infants' life and continued breastfeeding of the child up to 2 years of age, enhancing the immune system activity for protection against various diseases (WHO, 2014).

2.1.10.3 Immunization

Rotavirus is the most common cause of acute diarrhoea in children in developed and developing nations (Revelas, 2012). The virus causes about 39% of all hospital admissions and about 199,000 fatalities of infants annually (Bustreo *et al.*, 2015). Almost every child will likely be infected with the rotavirus before the fifth birthday, and children between 4-23 months are mostly affected (Braeckman *et al.*, 2012).

Diarrhoea caused by rotavirus has raised the alarm at an international level. Rotavirus vaccine is the first means of decreasing mortality caused by this infectious agent (Steel and Glass, 2011). Vaccines are also available to prevent diarrhoea related morbidity caused by *Vibrio cholera*, *Salmonella typhi* and measles (Apata *et al.*, 2014).

2.1.10.4 Vitamins and Micronutrients Supplementation

Several vitamins and micronutrients are important for the well functioning of innate and adaptive immunity in young children (Katona and Katona-Apte, 2008). Worldwide, approximately 2 billion people are affected by micronutrient deficiencies, including vitamins A, C, and E and essential minerals such as zinc, iron, and iodine which can result in poor growth, cognitive impairment, increased mortality and vulnerability to infectious diseases such as diarrhoea (Mokomane *et al.*, 2018).

To prevent and curtail diarrhoea, the WHO recommends improvement in child nutrition and micronutrients supplementation. Zinc and vitamin A have been shown to reduce diarrhoea incidence (Aggarwal *et al.*, 2007). Also, vitamin D deficiency can lead to impaired immunologic properties, resulting from increased susceptibility to diarrhoea

causing pathogens. Therefore, vitamin D is an additional micronutrient required to prevent childhood diarrhoea (Aluisio *et al.*, 2013).

2.1.11 Antidiarrhoeal Drugs

Antidiarrhoeal drugs are medications used to alleviate diarrhoea. The most common antidiarrhoeal drugs include antimotility agents (loperamide), antisecretory agents (racecadotril), probiotics, and adsorbents (Faure, 2013).

2.1.11.1 Antimotility and Antisecretory Agents

Opioid receptor agonists are used to treat increased intestinal motility associated with diarrhoea by relaxing the intestinal smooth muscle, thereby increasing the small intestinal residence time for effective absorption (Winkler *et al.*, 2010). Loperamide and diphenoxylate are the first-line drugs used as antimotility in diarrhoea (Kumpf, 2014).

Loperamide is a synthetic and peripherally acting mu (μ) opioid receptor agonist devoid of unwanted central nervous system (CNS) effects such as dizziness, euphoria and addiction because it hardly crosses the blood-brain barrier. It binds to intestinal μ opioid receptors in the myenteric plexus and inhibits intestinal secretion and smooth muscle contraction (Laaveri *et al.*, 2016). The loperamide also prevents acetylcholine release and decreases intestinal motility and secretion due to its inhibitory effects on muscarinic acetylcholine receptors on the secretory cells along the intestinal wall eventually reduces water and electrolytes loss, stool volume and maintain stool consistency (Lee, 2015). The drug's stated dosage regimen is 4 mg starting dose, then 2 mg after every loose stool (Laaveri *et al.*, 2016).

Diphenoxylate is weak μ opioid receptor agonist used in the treatment of diarrhoea in combination with atropine (Nemlekar *et al.*, 2018). Each tablet for oral use contains diphenoxylate hydrochloride (2.5 mg) and atropine sulfate (0.025 mg) included to limit the chances of abuse while enhancing the antidiarrhoeal action of the diphenoxylate. However, at a high dose, atropine sulfate causes unpleasant side effects such as tachycardia, mouth dryness and blurred vision (Mehra *et al.*, 2013).

The antidiarrhoeal action of diphenoxylate is by its direct action on intestinal smooth muscle where it binds to μ opioid receptor and reduces intestinal transit (Cooper, 2013). Diphenoxylate crosses the blood-brain barrier and binds to central μ opioid receptors which accounts for its abuse potential (Mehra *et al.*, 2013).

Racecadotril is another antisecretory agent that acts by selective inhibition of neutral endopeptidase enzymes, called enkephalinase, a cell membrane peptidase enzyme found mostly in the small intestinal epithelium that breaks enkephalins which have absorptive and antisecretory properties (Eberlin *et al.*, 2012). The drug promotes antisecretory properties by prolonging the presence of the enkephalins through the inhibition of the neutral endopeptidase. The inhibitory effect of racecadotril on endopeptidases reduces the hypersecretion of water and electrolytes without effects on intestinal motility and transit (Gordon and Akobeng, 2016).

2.1.11.2 Somatostatins and analogue

Somatostatin and its synthetic analogue octreotide have an intestinal antimotility effect. These drugs decrease intestinal motility and fluid and electrolyte secretion. These drugs

inactivate adenylate cyclase and inhibit G protein-coupled influx of calcium and potassium ions efflux, enhancing fluid and electrolyte absorption (Szilagyi and Shrier, 2001). Octreotide improved secretory diarrhoea management such as chemotherapy, HIV, and diabetes-associated diarrhoea (Fried, 1999).

2.1.11.3 Adsorbents

Adsorbents such as bismuth subsalicylate, kaolin, pectin, activated Attapulgit are drugs used in the treatment of diarrhoea. These drugs adsorb intestinal toxins, reduce stool fluidity, thereby enhancing fluid and electrolyte absorption. However, these drugs produce dose-dependent side effects including stools darkness and constipation (Nwachukwu and Okebe, 2008).

2.1.11.4 Bile Acid Sequestrant

The presence of excess bile in the colon usually causes intestinal hypermotility and mucus secretion, which eventually causes diarrhoea. Also, bile acid malabsorption occurs in patients suffering from ileo-caecal disease, surgical removal of ileum, radiation, cholecystectomy, bacterial overgrowth and intestinal dysmotility. In addition, bile acid malabsorption occurs in patients with functional diarrhoea or irritable bowel syndrome (Lee, 2015). Cholestyramin, colestipol, and colesevelam bind to bile acids and some bacteria toxins; these drugs are useful in treating bile acid-induced diarrhoea and *Clostridium difficile* associated-diarrhoea (Puri *et al.*, 2015).

Cholestyramine is a bile acid sequestrant, remains the first drug of choice in treating bile acid-induced diarrhoea. However, some patients poorly comply because of its unpleasant

taste (Halilbasic *et al.*, 2013). Cholestyramine has non-digestible resins which is attached to bile acids in the intestine and enhance its removal in the stool. It is used to control bile acid-induced diarrhoea, particularly in irritable bowel syndrome conditions (Lee, 2015).

2.1.11.5 Alpha-2 Adrenergic Agonist

Clonidine is an important drug used in the treatment of chronic diarrhoea (Tack, 2012). It binds to α_2 -adrenoceptors on the intestinal cells and increases water and electrolytes absorption, prevents intestinal hypersecretion and increases intestinal resident time. It also has antimotility effects by inhibiting acetylcholine release from the presynaptic nerves in the myenteric plexus (Fragkos *et al.*, 2016). This drug has been used to treat chronic secretory diarrhoea, opioids withdrawal diarrhoea, diabetic diarrhoea and chemotherapy-induced diarrhoea (Li and Vaziri, 2012).

2.1.11.6 Bismuth Subsalicylate

Bismuth subsalicylate is a drug used to treat conditions such as diarrhoea, acid reflux, gastritis and *Helicobacter pylori* infection in people of more than 12 years of age. Its probable mechanism of action is by coating the intestinal wall where it prevents intestinal hypersecretion, increases fluid and electrolytes absorption, decreases inflammation, exerts bactericidal effects and inhibits toxins from binding to the intestine (Sheele *et al.*, 2015).

2.2 Toxicity of Medicinal Plants

Several medicinal plants are used to treat various diseases for years without investigating their toxic effects. These medicinal plants are identified as safe, but some studies have identified the harmful effects of many of the herbal medicines (Nfozon *et al.*, 2019). The toxic effects of the medicinal plants in animals and humans are evaluated by analyzing the

physiological parameters such as general behaviour, body weight, biochemical and haematological parameter and histopathology (Jothy *et al.*, 2011).

2.3. The Plant *Combretum hypopilinum* Diels (Combretaceae)

The plant *Combretum hypopilinum* Diels (Combretaceae) is a synonym of *Combretum collinum* Fresen sub-specie *hypopilinum* (Diels). It is commonly known as *Jar taramniya* or *jar ganye* in Hausa, *buski daneehi* in Fulfulde, *katankara* in Kanuri, and *aro* in Yoruba language of Nigeria (Adamu *et al.*, 2005; Saganuwan *et al.*, 2010). It is a shrub average in size with many stems that annually sheds its leaves. The plant has many branches of about 12–17 m in height. It grows in various soils with semi-arid to moderate rainfall conditions (Idoh *et al.*, 2018). It is distributed in the tropical and subtropical African region (Hedberg *et al.*, 1982). Plate I shows the branch of *Combretum hypopilinum* Diels in its natural habitat showing leaves and stems.

2.3.1 Taxonomy of *Combretum hypopilinum*

The taxonomy of *Combretum hypopilinum* (Diels) is as follows:

- Kingdom: Viridiplantae
- Division: Streptophyta
- Class: Magnoliopsida
- Order: Myrtales
- Family: Combretaceae
- Genus: *Combretum*
- Species: *Combretum collinum* Diels
- Sub-species: *Combretum hypopilinum*

2.3.2 Ethnomedicinal Uses of *Combretum hypopilinum*

The plant is used in traditional medicine for treating several diseases in Africa. The infusion of the dried or fresh leaves and the decoction of the root bark are used to treat cholestasis and GIT diseases such as diarrhoea, dysentery and abdominal pain. It has also been reported to be used as a diuretic and purgative (Fyhrquist *et al.*, 2004).

The decoction of leaves and twigs is taken orally and the fresh roots are chewed to treat respiratory diseases such as cough, bronchitis and tuberculosis. It is also used to treat jaundice and snake bite. The juice from the roots is applied externally to the affected part to treat wounds, sores and toothache (Eloff, 1999). The root bark is usually macerated or taken as a decoction to treat gonorrhoea and infertility in males and females. Leaves are also crushed and applied as a poultice in the treatment of fatigue and rheumatism. Also, the leaves and roots infusion are used to enhance blood production (Abreu *et al.*, 1999).

2.3.3 Previous Studies on *Combretum hypopilinum*

Pharmacological and phytochemical investigations were carried out on *Combretum hypopilinum*. Amino acids such as aspartic acid, glycine, glutamic acid and alanine were isolated from the gum fluid *Combretum hypopilinum* (Anderson *et al.*, 1987). Additionally, combretastatin A and B, which contains stilbenoids and several phenanthrenes, were identified and isolated from the aerial parts of the plant (Rogers and Coombes, 1999).

Fyhrquist *et al.* (2004) reported the antibacterial activity of aqueous stem bark and root extract of *Combretum hypopilinum* against *Proteus mirabilis*, while the leaf extract was found to have weak antifungal activities *in vitro*. In a study conducted by Odda *et al.*

(2008), the ethanolic bark extract of *C. hypopilinum* elicited larvacidal activity against *Aedes aegypti*. Idoh *et al.* (2018) reported the liver protective effect of the ethanolic root bark extract of the plant in Wistar rats.



Plate I: A branch of *Combretum hypopilinum* in its natural habitat, showing leaves and stems (Photo credit: Umar Gallah)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Chemicals and Drugs

Analytical grade chemicals and drugs were used. These included methanol (Sigma Aldrich Chemical Co. USA), Castor oil (Bell and Sons, Southport PR9 AL, England), gum acacia, medicinal charcoal (ultracarbon powder-Merck KGaA Darmstadt Germany), distilled water, normal saline (Fidson Health Care, Nigeria), magnesium sulphate (BDH Chemical Ltd; Poole England), concentrated hydrochloric acid (BDH Limited Poole, England); Chips of magnesium metal (BDH Limited Poole, England), concentrated sulphuric acid (BDH Limited Poole, England), strong lead sub-acetate solution, chloroform (Sigma Aldrich Chemical Co. USA), ferric chloride (BDH Ltd Poole, England), glacial acetic acid (Searle Essex, England), acetic acid anhydride (BDH Ltd Poole, England), strong ammonia (BDH Ltd Poole, England), loperamide (Imodium[®]) (Jansen Pharmaceuticals, Pakistan), atropine sulphate (Ningbo Chemicals, China), naloxone hydrochloride, prazosin hydrochloride, yohimbine hydrochloride (Sigma Aldrich Chemical Co. USA), propranolol hydrochloride, pilocarpine hydrochloride (Sigma Aldrich Chemical Co. USA), isosorbide dinitrate (Isordil[®]) (MEDA Manufacturing GmbH, Germany) and morphine sulphate (Martindale Pharmaceuticals, UK).

3.2 Equipments and Apparatus

The equipment and apparatus used included water bath (HH-S digital thermostatic water bath, China), weighing balance, cages, dissecting kit (gold cross DS Malaysia), filter papers (whatman), pipette, test tubes (Pyrex France), porcelain pestle and mortar, watch, syringes (1mL, 2 mL, 5 mL), canula, centrifuge tubes, pair of scissors and hand gloves.

3.3 Experimental Animals

Adult Swiss albino mice (18-24g) and Wistar rats (120-160g) of both sexes were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Kaduna State, Nigeria. The animals were housed in well-ventilated cages, fed with a rodent diet (Vital feed, Jos, Nigeria) with access to water *ad libitum* and maintained under standard laboratory conditions in accordance with the protocols approved by the Ahmadu Bello University Ethical Committee on Animal Use and Care Research Policy (ABUCAUC) with an approval number (ABUCAUC/2020/40).

3.4 Plant Collection and Identification

The leaves of *Combretum hypopilinum* were collected from Galadimawa, Giwa Local Government Area of Kaduna State in September, 2018. It was identified and authenticated by a taxonomist, Mallam Sanusi Namadi of the Herbarium Section of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University Zaria, Nigeria, where a voucher number (012063) was obtained by comparing with an existing specimen.

3.5 Preparation and Extraction of Plant Material

The leaves of *Combretum hypopilinum* were dried in a shaded environment with intermittent weighing until a constant weight was obtained and size-reduced into a powdered form using pestle and mortar. One kilogram (1 kg) of the powdered leaves material was extracted with 70% v/v aqueous-methanol using soxhlet apparatus for 72 hours. The solvent was removed by placing the extract on a water bath set at 45 $^{\circ}\text{C}$. The extract was solid and reddish in colour. It was then stored in a tightly labelled container for

subsequent experiments. It is hence referred to as methanol leaf extract of *Combretum hypopilinum* (MECH). A solution of the extract was freshly prepared with distilled water for each study. The percentage yield of the extract was calculated using the following formula:

$$\text{Percentage yield} = \frac{\text{Weight of the extract}}{\text{Weight of the crude leaf}} \times 100$$

3.6 Preliminary Phytochemical Screening

The presence of phytochemicals such as steroids, triterpenes, flavonoids, alkaloids, saponins, tannins, glycosides and anthraquinones in MECH was determined using the method of Evans, (2002) and Sofowora, (1993) as follows:

3.6.1 Test for Flavonoids

Shinoda test: One hundred mg of the extract was dissolved in 2 mL of 50% methanol and warmed on a steam bath. Then few pieces of metallic magnesium chips and four drops of concentrated hydrochloric acid were added. The appearance of a reddish colour indicated the presence of flavonoids.

Sodium hydroxide test: Few drops of 10% sodium hydroxide were added to an aqueous solution of the methanol extract. Yellow colouration was formed which indicated the presence of flavonoids.

3.6.2 Test for Cardiac Glycosides

Keller Killiani's test: 1 mL of glacial acetic acid containing one drop of FeCl_3 was used to dissolve 20 mg of the extract. The mixture was transferred into a dry test tube, and then 1

mL of concentrated sulphuric acid was added along the side of the test tube to form a layer at the bottom. Formation of purple ring colour at the interface was observed which indicated the presence of cardiac glycosides.

3.6.3 Test for Saponins

Frothing test: 1 mL solution of the methanol leaf extract of *Combretum hypopilinum* was shaken with 3 mL of distilled water vigorously for 30 seconds and allowed to stand for 30 minutes in a vertical position. Formation of honey comb froth which persisted for more than 30 minutes was observed, indicating the presence of saponins.

3.6.4 Test for Tannins

Ferric chloride test: To 1mL of methanol leaves extract of *Combretum hypopilinum* solution, 3 drops of ferric chloride solution were added. A greenish-black precipitate was formed which indicated the presence of condensed tannins.

Lead sub-acetate test: Methanol leaves extract of *Combretum hypopilinum* (0.5g) was dissolved in 2 mL of water, and then 3 drops of lead sub-acetate solution were added and observed for the formation of a black-green coloured precipitate which indicated the presence of tannins.

3.6.5 Test for Steroids and Triterpenes

Lieberman-Burchard's test: To 1 mL of the chloroform solution of the extract, an equal volume of acetic acid anhydride was added and mixed gently. 1 mL of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. Changes

were observed immediately and over one hour. A brownish-red colour was observed immediately at interphase, which indicated the presence of triterpenes and blue-green at the upper layer indicated the presence of steroids.

3.6.6 Test for Alkaloids

The MECH (0.5 g) was stirred with 5mL of 1% aqueous HCl and then filtered. The filtrate was tested carefully with some alkaloidal reagents as follows:

Dragendoff's test: To a few mL of the filtrate, two drops of Dragendoff's reagent were added by the side of the test tube. The orange-red precipitate was observed which indicated the presence of alkaloids.

Wagner's test: To a few mL of the filtrate, two drops of Wagner's reagent were added by the side of the test tube. Reddish-brown precipitate was observed which confirmed the presence of alkaloids.

3.6.7 Test for Anthraquinones

Bontrager's test: To 500 mg of the extract in a dry test tube, 5mL of chloroform was added, and then the test tube was stoppered, shaken for at least 5 minutes and filtered. The filtrate was shaken with an equal volume of 10% ammonia solution. The upper aqueous layer was observed for bright pink colour as an indication of the presence or absence of free anthraquinones.

3.7 Acute Toxicity Study

The acute toxicity study was conducted according to the Organization of Economic Co-operation and Development (OECD) 423 guideline (OECD, 2001). The oral LD₅₀ was

determined using nulliparous and non-pregnant female mice and rats. In this method, two groups of three animals each (rats/mice) were fasted before administration of the extract (food but not water was withheld overnight for rats and 4 hours for mice).

In the first phase, a single dose of the extract was administered to each animal at the dose of 5,000 mg/kg via the oral route. Food but not water was withheld further for 1 hour after the extract administration. The animals were observed for signs and symptoms of toxicity at least once every 30 minutes for the first 4 hours and then daily for 14 days. After 14 days, the study was terminated. The signs and symptoms observed included tremor, convulsion, salivation, lacrimation, diarrhoea, lethargy, sleep, respiratory, behavioural pattern, time of onset of toxicity if any and length of recovery as well as the time of death.

3.8 Evaluation of Antidiarrhoeal Activity

3.8.1 Castor Oil-Induced Diarrhoea in Mice

The method described by Awouters *et al.* (1978) was adopted. Mice were fasted for 18 hours and randomly divided into five groups of five mice each. Group I (negative control) and group V (positive control) were administered 10 mL/kg distilled water and 5 mg/kg loperamide per oral respectively. Group II, III and IV were administered the MECH at a doses of 250, 500 and 1000 mg/kg respectively.

Sixty minutes after treatment, all the mice were orally administered 0.5 ml of castor oil and then each mouse was placed in a separate cage whose floor was lined with white filter paper for an observation period of 4 hours. Observation was made base on the time of onset of diarrhoea, number of wet faeces (diarrhoea stools) and total number of faeces.

Percentage protection against diarrhoea was calculated with respect to the number of wet faeces using the formula below:

$$\% \text{ inhibition of defaecation} = \frac{\text{No of faeces in negative control} - \text{No of faeces in test group}}{\text{Total No of faeces in the negative control group}} \times 100$$

$$\% \text{ inhibition of diarrhoea} = \frac{\text{No of WFC} - \text{No of WFT}}{\text{No of WFC}} \times 100$$

Where;

WFC = wet faeces in negative control group WFT = wet faeces in test group

3.8.2 Castor Oil-Induced Enteropooling in Mice

The method described by Robert *et al.* (1976) was adopted. The mice were fasted for 18 hours and adequately allowed free access to water *ad libitum*. They were randomly divided into five groups of five mice in each group. Group I (negative control) and group V (positive control) administered 10 mL/kg distilled water and 5 mg/kg loperamide per oral respectively. Group II, III and IV were administered the MECH at a doses of 250, 500 and 1000 mg/kg respectively.

After 60 minutes of treatment, 0.5 ml castor oil was orally administered to all the mice. Then 30 minutes following castor oil administration, all the mice were sacrificed and their small intestines from the pylorus to caecum were removed and weighed. The intestinal content of each mouse was collected into a graduated syringe and the volume was measured and recorded to determine the volume of intestinal content of each mouse. The empty intestine of each mouse was reweighed to determine the weight of the intestinal content. The weight and volume of intestinal content obtained from the negative control group were used to compare with the weight and volume of intestinal contents of the treated groups.

Percentage inhibition of fluid accumulation was determined by calculating the mean volume of intestinal content of the test groups and comparing it with the mean volume of intestinal content of the negative control group as follows:

$$\% \text{ weight decrease of intestinal content} = \frac{\text{Weight of intestinal CNC} - \text{Weight of intestinal CTG}}{\text{Weight in intestinal CNC}} \times 100$$

$$\% \text{ decrease in volume of intestinal content} = \frac{\text{mean intestinal VNC} - \text{mean intestinal VTG}}{\text{Mean intestinal VNC}} \times 100$$

Where;

CNC= Contents in negative control group

CTG= Contents in test group

VNC= Volume of the negative control group;
group

VTG= Volume of the test

3.8.3 Intestinal Motility Test in Mice

An intestinal motility test was carried out according to the method described by Di Carlo *et al.* (1993). The mice were fasted for 18 hours and divided into five groups of five mice each. Group I (negative control) and group V (positive control) were administered 10mL/kg distilled water (10ml/kg) and 5 mg/kg loperamide per oral respectively. Group II, III and IV were administered the MECH at a doses of 250, 500 and 1000 mg/kg respectively.

After 30 minutes, all the mice were orally administered castor oil (0.5 ml). Then 30 minutes later, each mouse was administered 0.5 ml charcoal meal (10% activated charcoal suspension in 5% acacia). After 30 minutes of charcoal meal administration, all the mice were sacrificed after placing them in an enclosed bowl containing cotton wool soaked with

chloroform. The abdomen of each mouse was opened, the intestine was removed and placed lengthwise on moist filter paper on a horizontal surface to measure the distance in centimetres (cm) travelled by charcoal meal down the length of the intestine using a calibrated ruler. The distance moved by the charcoal meal from the pylorus was measured and then expressed as a percentage of the total distance of the small intestine from the pylorus to caecum as follows:

$$\% \text{ Peristaltic index of charcoal meal} = \frac{\text{distance travel by charcoal meal (cm)}}{\text{Total length of small intestine (cm)}} \times 100$$

$$\% \text{ inhibition of charcol movement} = \frac{A - B}{A} \times 100$$

A = Mean movement of charcoal meal in the negative control group

B = Mean movement of charcoal meal in the test group

3.9 Mechanisms of Antidiarrhoeal Activity

The most active dose (1,000 mg/kg) of MECH was subjected to mechanistic studies. The involvement of various pathways in the antidiarrhoeal activity of the extract was investigated using an intestinal motility test in mice. The pathways investigated and the drugs used for the studies were as follows:

3.9.1 Opioidergic Pathway

Thirty mice were grouped into six groups (n=5). Group I, II and III were administered distilled water (10 ml/kg, *p.o*), naloxone (2 mg/kg, *s.c*) and MECH (1,000 mg/kg, *p.o*), respectively. Group IV were pretreated with naloxone (2 mg/kg) 30 minutes before administration of MECH (1,000 mg/kg). Group V was administered loperamide (5 mg/kg, *p.o*). Group VI was pretreated with naloxone (2 mg/kg, *s.c*) 30 minutes before the

administration of loperamide (5 mg/kg). The mice in all the groups were subjected to intestinal motility test as earlier described.

3.9.2 α_1 -adrenergic Pathway

Thirty mice were grouped into six groups (n=5). Group I, II and III were administered distilled water (10 ml/kg, *p.o*), prazosin (1 mg/kg, *s.c*) and MECH (1,000 mg/kg, *p.o*), respectively. Group IV were pretreated with prazosin (1 mg/kg, *s.c*) 30 minutes before administration of MECH (1,000 mg/kg). Group V was administered morphine (10 mg/kg *s.c*). Group VI was pretreated with prazosin (1 mg/kg, *s.c*) 30 minutes before the administration of morphine (10 mg/kg *s.c*). The mice in all the groups were subjected to intestinal motility test as earlier described.

3.9.3 α_2 -adrenergic Pathway

Thirty mice were grouped into six groups (n=5). Group I, II and III were administered distilled water (10 ml/kg, *p.o*), yohimbine (1 mg/kg, *i.p*) and MECH (1,000 mg/kg, *p.o*), respectively. Group IV were pretreated with yohimbine (1 mg/kg, *i.p*) 30 minutes before administration of MECH (1,000 mg/kg). Group V was administered morphine (10 mg/kg *s.c*). Group VI was pretreated with yohimbine (1 mg/kg, *i.p*) 30 minutes before the administration of morphine (10 mg/kg *s.c*). The mice in all the groups were subjected to intestinal motility test as earlier described.

3.9.4 β -adrenergic Pathway

Thirty mice were grouped into six groups (n=5). Group I, II and III were administered distilled water (10 ml/kg, *p.o*), propranolol (1 mg/kg, *i.p*) and MECH (1,000 mg/kg, *p.o*), respectively. Group IV were pretreated with propranolol (1 mg/kg, *i.p*) 30 minutes before

administration of MECH (1,000 mg/kg). Group V was administered morphine (10 mg/kg *s.c*). Group VI were pretreated with propranolol (1 mg/kg, *i.p*) 30 minutes before the administration morphine (10 mg/kg *s.c*). The mice in all the groups were subjected to intestinal motility test as earlier described.

3.9.5 Cholinergic Pathway

Thirty mice were grouped into six groups (n=5). Group I, II and III were administered distilled water (10 ml/kg, *p.o*), pilocarpine (1 mg/kg, *s.c*), and MECH (1,000 mg/kg, *p.o*) respectively. Group IV were pretreated with pilocarpine (1 mg/kg, *s.c*), 30 minutes before administration of MECH (1,000 mg/kg). Group V was administered atropine sulphate (5 mg/kg *p.o*). Group VI was pretreated with pilocarpine (1 mg/kg, *s.c*) 30 minutes before the administration of atropine sulphate (5 mg/kg *p.o*). The mice in all the groups were subjected to intestinal motility test as earlier described.

3.9.6 Nitric Oxide Pathway

Thirty mice were grouped into six groups (n=5). Group I, II and III were administered distilled water (10 ml/kg, *p.o*), isosorbide dinitrate (150 mg/kg, *p.o*) and MECH (1,000 mg/kg, *p.o*), respectively. Group IV was pretreated with isosorbide dinitrate (150 mg/kg, *p.o*) 30 minutes before administration of MECH (1,000 mg/kg). Group V was administered morphine (10 mg/kg *s.c*). Group VI was pretreated with isosorbide dinitrate (150 mg/kg, *p.o*) 30 minutes before the administration of morphine (10 mg/kg *s.c*). The mice in all the groups were subjected to intestinal motility test as earlier described.

3.10 Sub-Acute Toxicity Study

The 28-days repeated oral toxicity study was conducted to the Organization of Economic Co-operation and Development (OECD) 407 guidelines (OECD, 2008). Twenty four (24) adult Wistar rats of both sexes (3 males and 3 females) were grouped into four groups containing six rats in each group. Group I was administered 1 mL/kg distilled water per oral. Groups II, III and IV were administered graded oral doses of 250, 500 and 1,000 mg/kg MECH, respectively, using an orogastric cannula daily for 28-consecutive days. The weekly body weight of rats in each group was recorded and observed for signs and symptoms of toxicity. On the 29th day, all the animals were euthanized using chloroform anaesthesia, and blood samples were collected from each rat through cardiac puncture in tubes containing ethylenediaminetetraacetic acid (EDTA) for haematological analysis, while blood samples for biochemical analysis were collected in a plain tubes.

3.10.1 Biochemical Analysis

Blood for biochemical analysis was kept for an hour at room temperature and allowed for adequate clotting. The blood was centrifuged at 3000 revolutions per minute (rpm) for 10 minutes to obtain serum which was used to determine the changes in the levels of biochemical parameters such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), albumin, total bilirubin, direct bilirubin, total protein, urea, creatinine, electrolytes, cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoprotein (LDL) and glucose.

3.10.2 Haematological Analysis

The haematological analysis was carried out to determine the effect of 28-days treatment on the levels of red blood cells (RBC) count, haemoglobin (HGB), haematocrit (HCT), mean

corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet (PLT), white blood cell (WBC) count, lymphocytes (LYM), granulocytes (GRAN), monocytes (MXD), neutrophils (NEU), eosinophils and basophils.

3.10.3 Histological examination and Relative Organ Weight

At the end of 28 days, each rat was weighed, and the liver, kidney, lung, heart, stomach and small intestine of the animals were removed and weighed. The relative organ weight (ROW) was calculated by expressing the absolute organ weight as a percentage of total body weight using the following formula:

$$\text{Relative organ weight} = \frac{\text{Organ weight (g)}}{\text{Final body weight of the animal (g)}} \times 100$$

The organs were processed for embedment in paraffin wax after fixation in 10% formalin. Sections from the liver, kidney, heart, lung, stomach and small intestine were cut 4-5µm with rotary microtome, stained with hematoxylin and eosin and examined by light microscopy. The photomicrographs were observed for any histopathological changes.

3.11 Data Analysis

Data were presented as mean ± standard error of mean (SEM) in tables, figures and photomicrograph as appropriate. The data were analyzed with SPSS Version 20 using One Way Analysis of Variance (ANOVA) for castor oil-induced diarrhoea, castor oil-induced enteropooling, gastrointestinal motility tests, ROW, biochemical and haematological analysis followed by Dunnett's post hoc test. The repeated measure ANOVA was used to analyse the weekly body weight of rats followed by Dunnett's post hoc test. The results were considered statistically significant at $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage Yield of Methanol Leaf Extract of *Combretum hypopilinum*

The percentage yield of methanol leaf extract of *Combretum hypopilinum* was 12.62 %
w/w.

4.2 Phytochemical Constituents of *Combretum hypopilinum*

Preliminary phytochemical screening of MECH revealed the presence of flavonoids, cardiac glycosides, saponins, tannins, steroids, triterpenes and alkaloids. However, anthraquinones were absent (Table 4.1).

Table 4.1: Phytochemical Constituents of Methanol Leaf Extract of *Combretum hypopilinum*

Phytochemical constituents	Result
Flavonoids	+
Cardiac glycosides	+
Saponins	+
Tannins	+
Alkaloids	+
Steroids and triterpenes	+
Anthraquinones	-
Key: + = Present and - = Absent	

4.3 Acute Toxicity Study

The oral LD₅₀ of MECH was estimated to be greater than 5,000 mg/kg in both mice and rats. The physical observation of mice and rats administered with 5,000 mg/kg of MECH during the 14 days revealed that none of the animals showed signs of toxicity in their skin, fur, eyes, mucus membrane, or behavioural changes, diarrhoea, tremors, salivation, sleep and coma. Also, there was no mortality of the animals observed at the tested dose.

4.4 Antidiarrhoeal Activity of *Combretum hypopilinum*

4.4.1 Effect of Methanol Leaf Extract of *Combretum hypopilinum* on Castor Oil-induced Diarrhoea in Mice

The mice in the distilled water group showed copious diarrhoea within the first hour (54.60 ± 13.10) minutes after castor oil administration. The oral administration of MECH at doses of 250, 500 and 1,000 mg/kg non-significantly ($p > 0.05$) delayed the onset of diarrhoea to (90.20 ± 25.87), (107.80 ± 13.63) and (148.40 ± 20.59) minutes respectively when compared with distilled water group. However, significant ($p \leq 0.05$) delay in the onset of diarrhoea (158.00 ± 30.42) minutes was observed in the group treated with loperamide (5 mg/kg) compared with distilled water group (Table 4.2).

Also, the MECH inhibited the number of wet faeces (46.67%, 56.67% and 60.00%) at doses of 250, 500 and 1,000 mg/kg, respectively, which was significant ($p \leq 0.05$) at higher doses (500 and 1,000 mg/kg) compared with distilled water group. Also, a significant ($p \leq 0.01$) inhibition in the number of wet faeces was observed in the group treated with loperamide (5 mg/kg) compared with distilled water group (Table 4.2).

Table 4.2: Effect of Methanol Leaf Extract of *Combretum hypopilinum* on Castor Oil-induced Diarrhoea in Mice

Treatments (mg/kg, <i>p.o</i>)	Onset of diarrhoea (min)	Total number of wet faeces	Total number of faeces	% inhibition of diarrhoea	% inhibition of defecation
DW (10 ml/kg)	54.60 ± 13.10	6.00 ± 1.05	6.00 ± 1.05	-	-
MECH (250)	90.20 ± 25.87	3.20 ± 0.80	4.80 ± 1.39	46.67	20.00
MECH (500)	107.80 ± 13.63	2.60 ± 0.68*	3.40 ± 0.68	56.67	43.33
MECH (1,000)	148.40 ± 20.59	2.40 ± 0.68*	4.00 ± 1.14	60.00	33.33
LOP (5)	158.00 ± 30.42*	1.60 ± 0.40**	1.60 ± 0.40	73.33	73.33

Data are presented as Mean ± SEM; * $p \leq 0.05$ and ** $p \leq 0.01$ versus distilled water group (One Way ANOVA followed by Dunnett's post hoc test), DW= distilled water, MECH= Methanol leaf extract of *C. hypopilinum*, LOP= Loperamide, n=5

4.4.2 Effect of Methanol Leaf Extract of *Combretum hypopilinum* on Castor Oil-induced Enteropooling in Mice

The MECH did not significantly ($p>0.05$) change intestinal fluid accumulation volume at the lowest dose of 250 mg/kg. However, a significant ($p\leq 0.05$ and $p\leq 0.01$) reduction (40.00% and 51.67%) in the volume of intestinal fluid was observed at the higher doses (500 and 1,000 mg/kg respectively. The loperamide (5 mg/kg) significantly ($p\leq 0.001$) reduced the volume of the intestinal fluid (Figure 4.1).

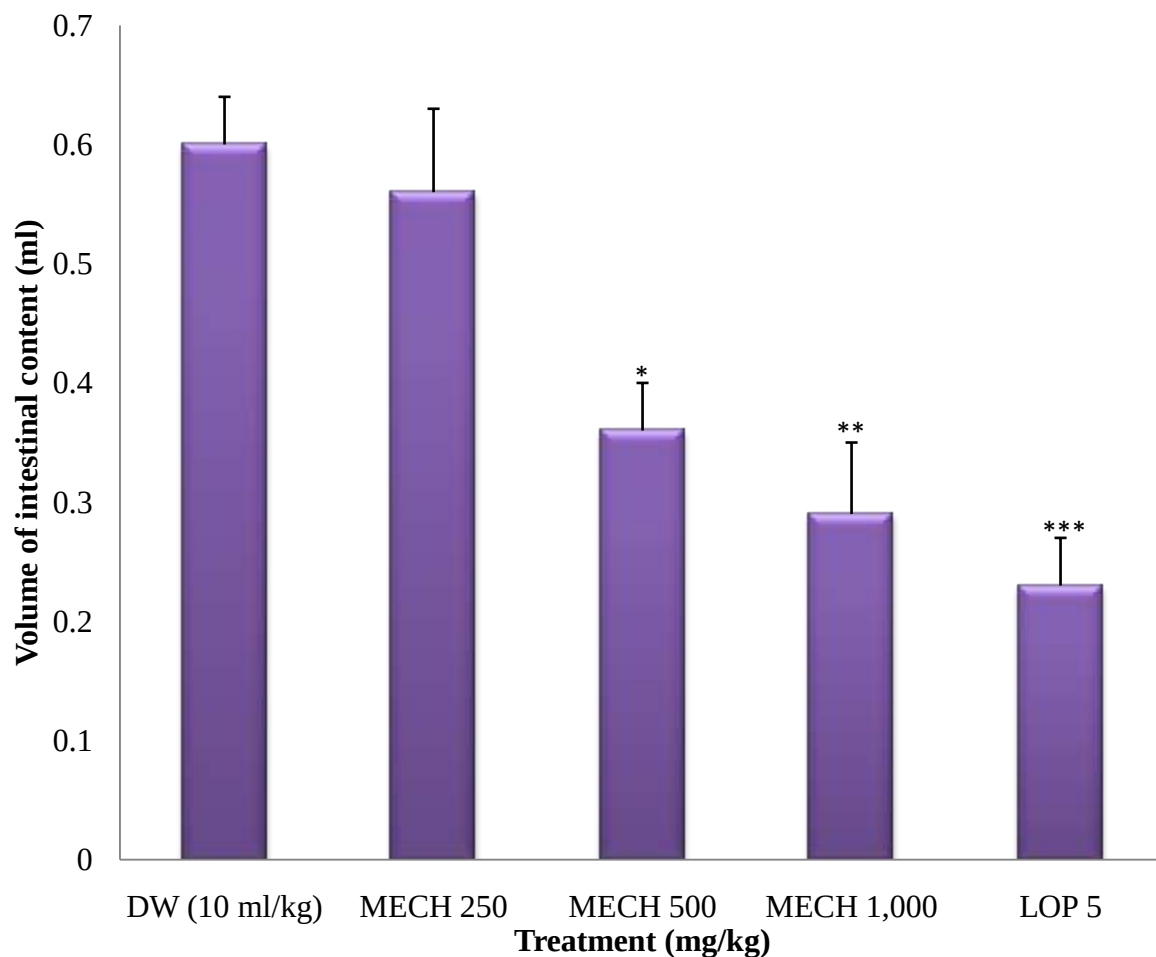


Figure 4.1: Effect of Methanol Leaf Extract of *Combretum hypopilinum* on Castor Oil-induced Enteropooling Test in Mice

Data are presented as Mean $\pm$ SEM; * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ distilled water group (One Way ANOVA followed by Dunnett's post hoc test), DW= distilled water, MECH= Methanol leaf extract of *C. hypopilinum*, LOP= Loperamide, n=5

4.4.3 Effect of Methanol Leaf Extract of *Combretum hypopilinum* on Intestinal Motility in Mice

In the distilled water group, the charcoal travelled 75.46% of the total length of the small intestine. The MECH did not show a significant ($p>0.05$) difference in the charcoal movement at the lowest dose (250 mg/kg). However, there was significant ($p\leq 0.05$ and $p\leq 0.001$) decrease (38.55% and 74.54%) in the charcoal movement at the doses of 500 and 1,000 mg/kg respectively. The loperamide (5 mg/kg) also exhibited a significant ($p\leq 0.001$) decrease in the charcoal movement with a percentage inhibition of 65.66 % (Figure 4.2).

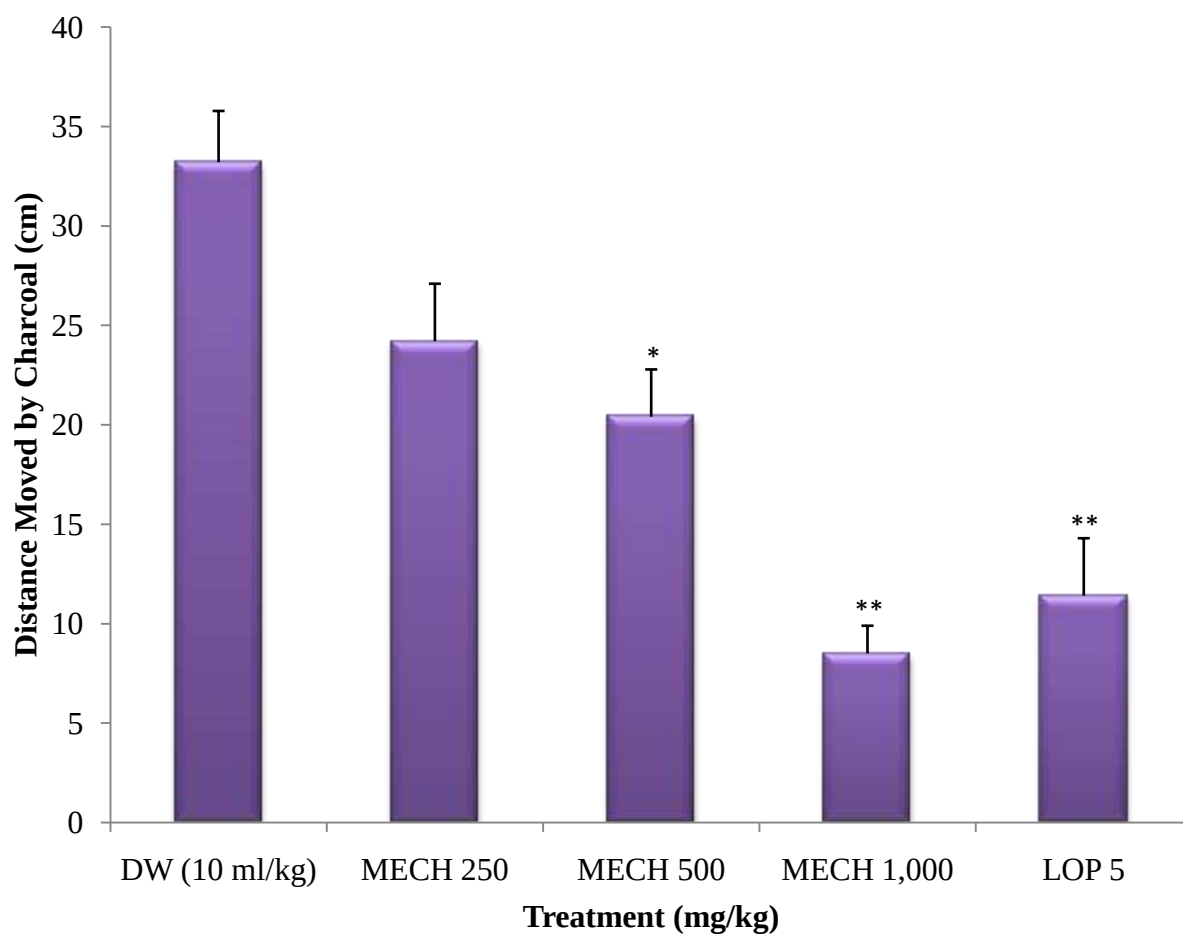


Figure 4.2: Effect of Methanol Leaf Extract of *Combretum hypopilinum* on Intestinal Transit in Mice

Data are presented as Mean $\pm$ SEM; * $p \leq 0.05$ and ** $p \leq 0.001$, versus distilled water group (One Way ANOVA followed by Dunnett's post hoc test), DW= Distilled Water, MECH= Methanol leaf extract of *C. hypopilinum*, LOP= Loperamide, n=5

4.5 Mechanisms of Antidiarrhoeal Activity of Methanol Leaf Extract of *Combretum hypopilinum*

The administration of MECH ($p \leq 0.001$), loperamide ($p \leq 0.01$), morphine ($p \leq 0.01$) or atropine sulphate ($p \leq 0.05$) each significantly reduced the charcoal movement when compared with distilled water group (Figure 4.3 - 4.8). The pretreatment of the animals with naloxone, prazosin and propranolol followed by administration of the extract did not significantly ($p > 0.05$) alter the charcoal movement compared with distilled water group (Figure 4.3, 4.4 and 4.6). Also, pretreatment of the animals with naloxone or prazosin followed by administration of the extract each significantly ($p \leq 0.01$) increased the charcoal movement produced by MECH (Figure 4.3 and 4.4). However, pretreatment of the animals with yohimbine, pilocarpine or isosorbide dinitrate each did not significantly ($p > 0.05$) change the intestinal movement of charcoal produced compared with distilled water group (Figure 4.5, 4.7 and 4.8).

The groups pretreated with yohimbine or propranolol followed by administration of morphine each showed non-significant ($p > 0.05$) change in charcoal movement when compared to distilled water group (Figure 4.5 and 4.6). However, pretreatment of the animals with prazosin or isosorbide dinitrate followed by administration of morphine, each significantly ($p \leq 0.001$) decreased the charcoal movement when compared with distilled water group (Figure 4.4 and 4.8).

The pretreatment of animals with naloxone and pilocarpine followed by administration of loperamide and atropine sulphate each revealed insignificant ($p > 0.05$) difference in a charcoal movement when compared with the negative control group (Figure 4.3 and 4.7).

The naloxone, prazosin, yohimbine, propranolol, pilocarpine or isosorbide dinitrate when administered alone, each did not significantly ($p>0.05$) change the intestinal movement of charcoal when compared with the control group. (Figures 4.3 - 4.8).

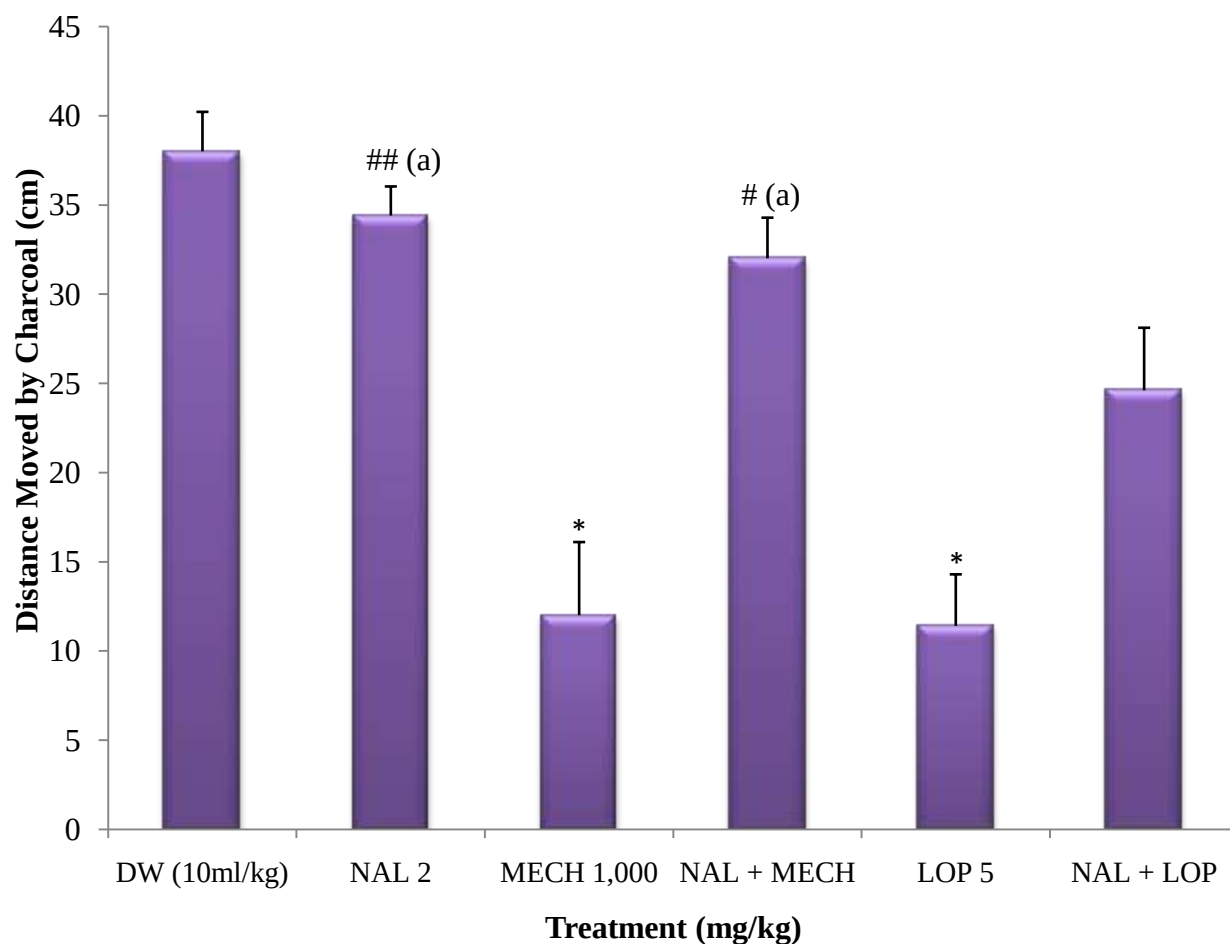


Figure: 4.3 Effect of Naloxone on the Antidiarrhoeal Activity of Methanol Leaf Extract of *Combretum hypopilinum* in the Intestinal Transit Test in Mice

Data are presented as Mean $\pm$ SEM; * $p \leq 0.001$ compared to distilled water group, # $p \leq 0.01$ and ## $p \leq 0.001$ compared to MECH, ^a $p \leq 0.001$ compared to LOP (One Way ANOVA followed by Bonferroni's post hoc test), DW= Distilled Water, MECH= Methanol leaf extract of *C. hypopilinum*, NAL= Naloxone, LOP= Loperamide, n=5

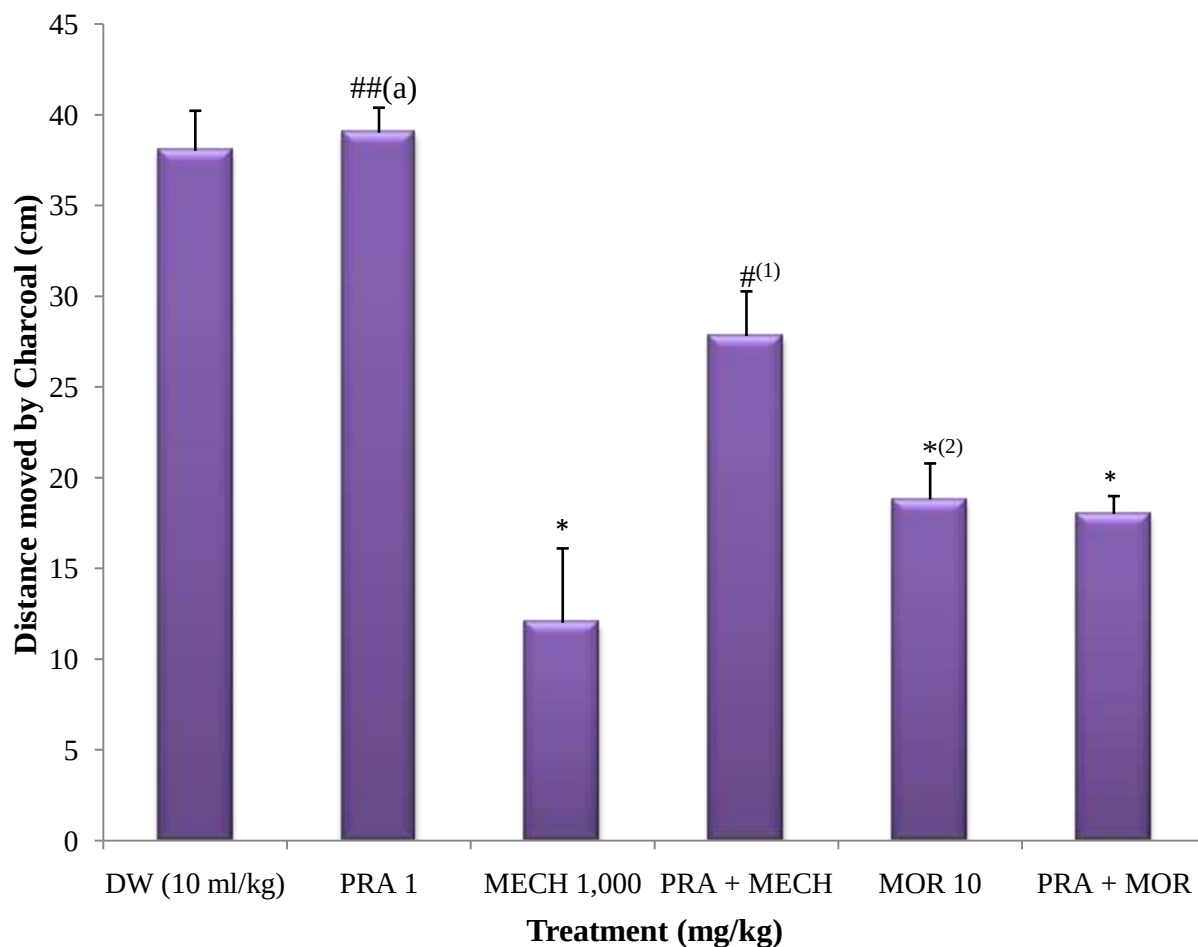


Figure 4.4: Effect of Prazosin on the Antidiarrhoeal Activity of Methanol Leaf Extract of *Combretum hypopilinum* in the Intestinal Transit Test in Mice

Data are presented as Mean $\pm$ SEM; * $p \leq 0.001$ compared to distilled water group, # $p \leq 0.01$ and ## $p \leq 0.001$ compared to MECH, ^a $p \leq 0.001$ compared to MOR, ¹ $p \leq 0.01$ and ² $p \leq 0.001$ compared to PRA (One Way ANOVA followed by Bonferroni's post hoc test), DW= Distilled Water, MECH= Methanol leaf extract of *C. hypopilinum*, PRA= Prazosin, MOR= Morphine, n=5

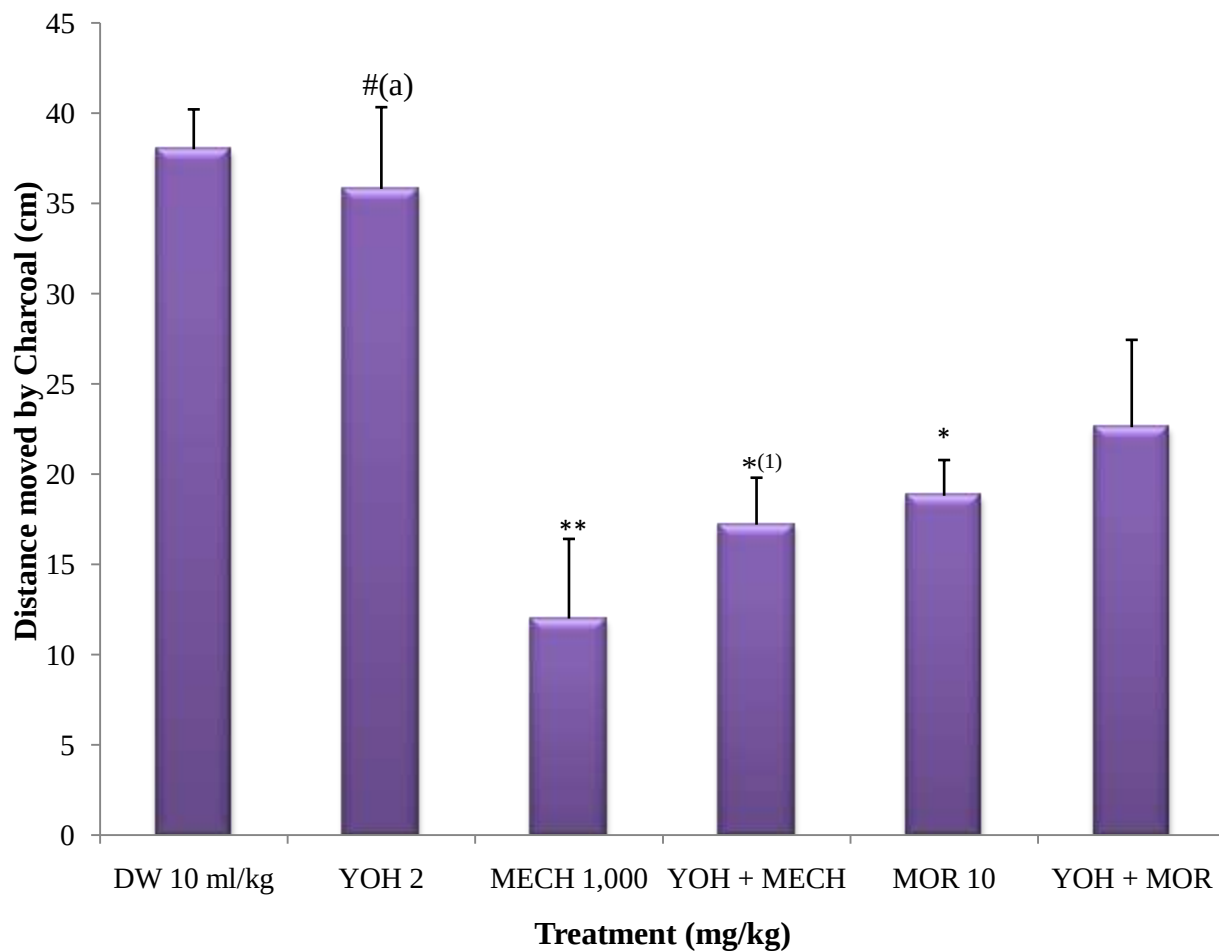


Figure 4.5: Effect of Yohimbine on the Antidiarrhoeal Activity of Methanol Leaf Extract of *Combretum hypopilinum* in the Intestinal Transit Test in Mice

Data are presented as Mean $\pm$ SEM; * $p \leq 0.01$ and ** $p \leq 0.001$ compared to distilled water group, # $p \leq 0.01$ compared to MECH, ^a $p \leq 0.05$ compared to MOR, ¹ $p \leq 0.05$ compared to YOH (One way ANOVA followed by Bonferroni's post hoc test), DW= Distilled Water, MECH= Methanol leaf extract of *C. hypopilinum*, YOH= Yohimbine, MOR= Morphine, n=5

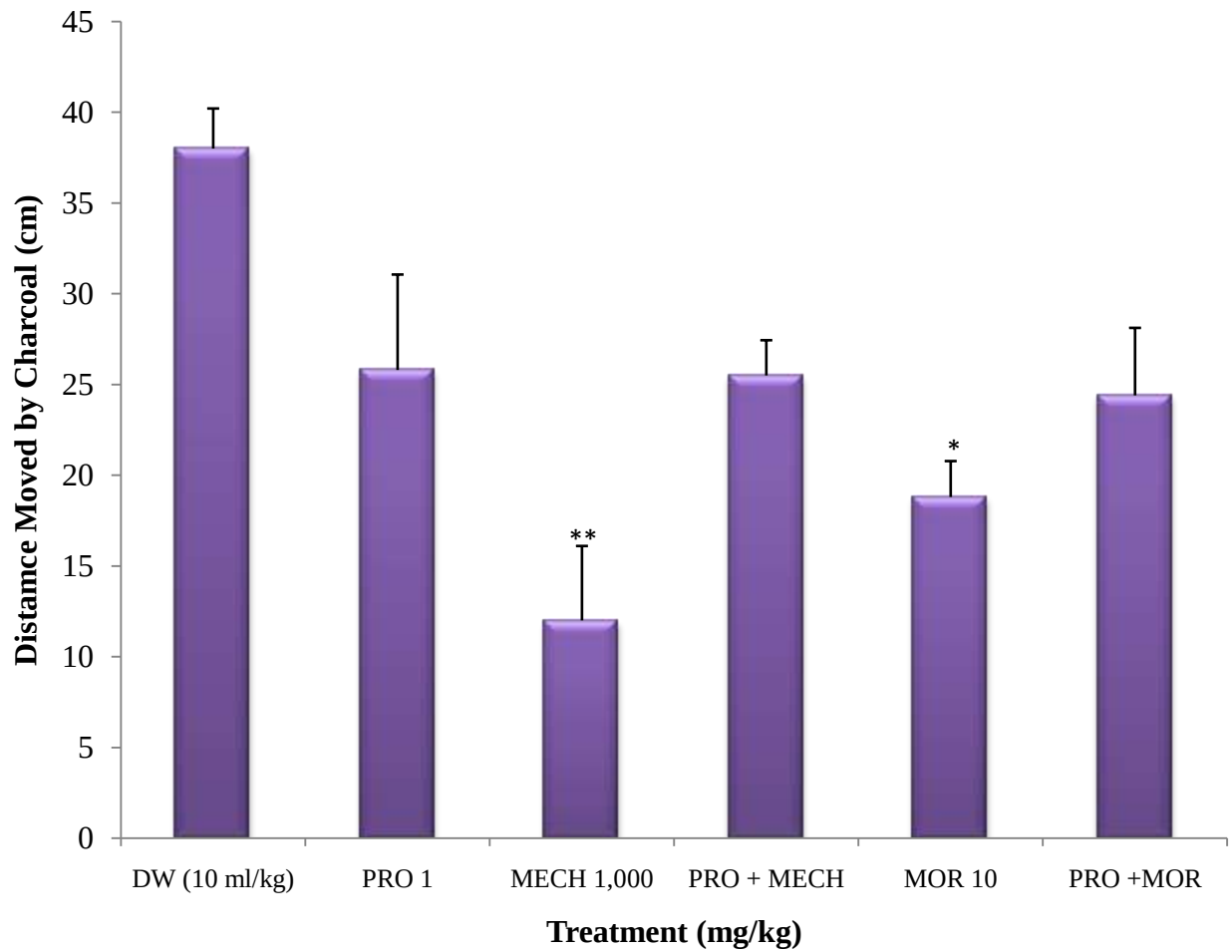


Figure 4.6: Effect of Propranolol on the Antidiarrhoeal Activity of Methanol Leaf Extract of *Combretum hypopilinum* in the Intestinal Transit Test in Mice

Data are presented as Mean $\pm$ SEM; * $p \leq 0.01$ and ** $p \leq 0.001$, compared to distilled water group, (One Way ANOVA followed by Bonferroni's post hoc test), DW= Distilled Water, MECH= Methanol leaf extract of *C. hypopilinum*, PRO= Propranolol, MOR= Morphine, n=5

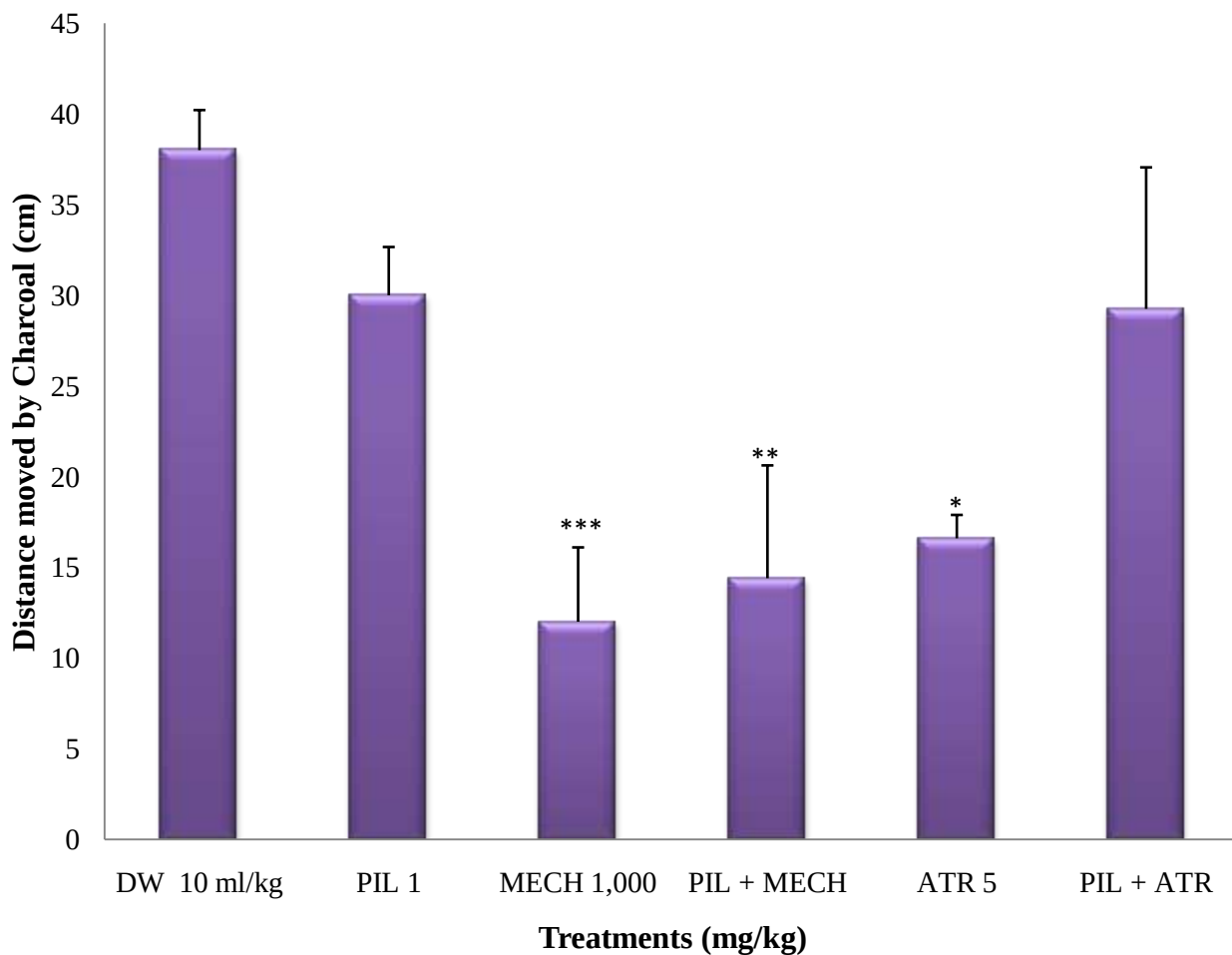


Figure 4.7: Effect of Pilocarpine on the Antidiarrhoeal Activity of Methanol Leaf Extract of *Combretum hypopilinum* in the Intestinal Transit Test in Mice

Data are presented as Mean $\pm$ SEM. * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ compared to distilled water group (One Way ANOVA followed by Bonferroni's post hoc test), DW= Distilled Water, MECH= Methanol leaf extract of *C. hypopilinum*, PIL= Pilocarpine, ATR= Atropine Sulphate, n=5

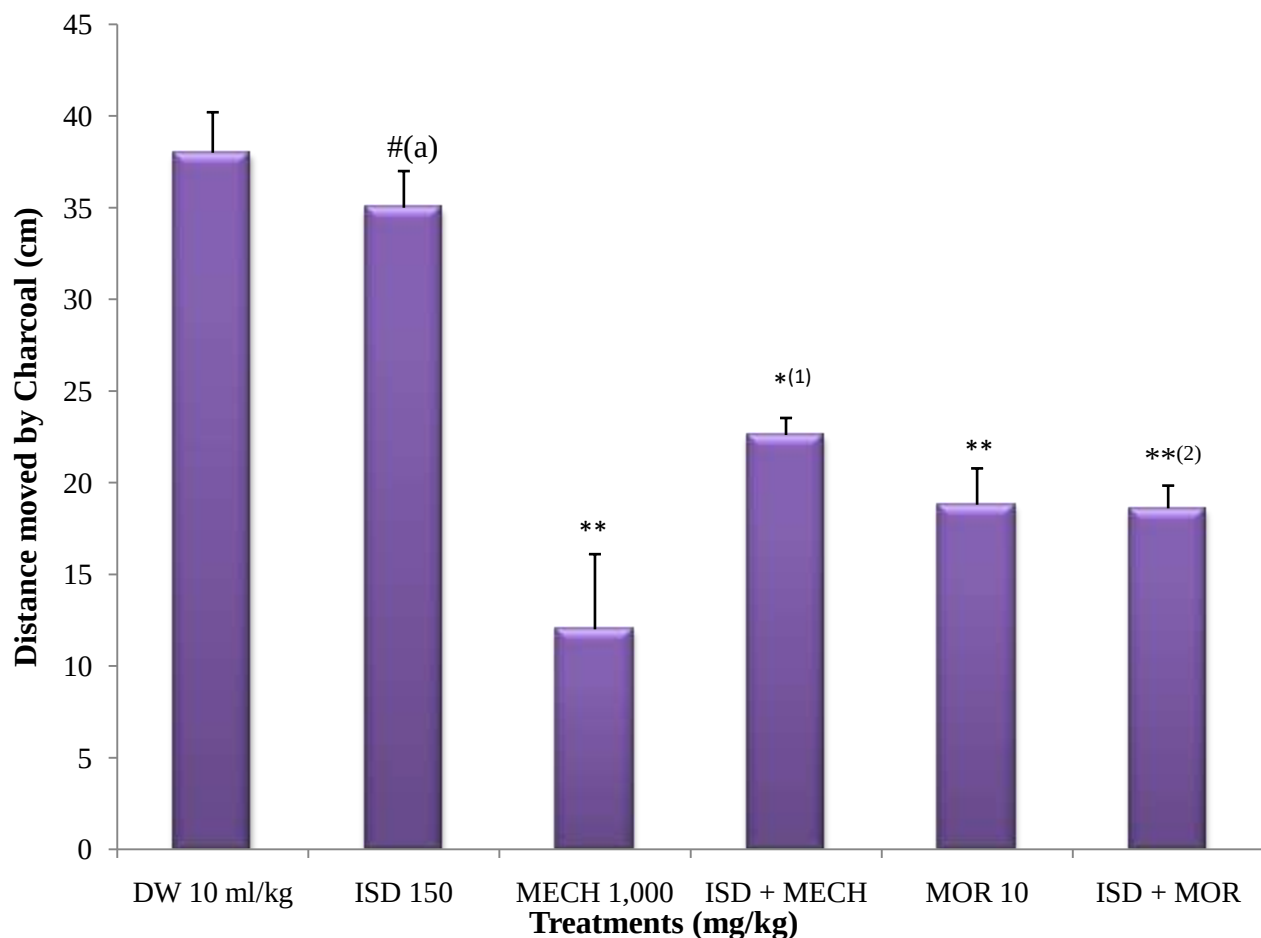


Figure 4.8: Effect of Isosorbide Dinitrate on the Antidiarrhoeal Activity of Methanol Leaf Extract of *Combretum hypopilinum* in the Intestinal Transit Test in Mice

Data are presented as Mean $\pm$ SEM; * $p \leq 0.01$ and ** $p \leq 0.001$, compared to distilled water, # $p \leq 0.001$ compared to MECH, ^a $p \leq 0.05$ compared to MOR, ¹ $p \leq 0.05$ and ² $p \leq 0.01$ compared to ISD (One Way ANOVA followed by Bonferroni's post hoc test), DW= Distilled Water, MECH= Methanol leaf extract of *C. hypopilinum*, ISD= Isosorbide Dinitrate, MOR= Morphine, n=5

4.6 Sub-Acute Toxicity Study

4.6.1 Effect of 28-Days Oral Administration of Methanol Leaf Extract of *Combretum hypopilinum* on Body Weights of Rats

The administration of MECH did not significantly ($p>0.05$) change the body weight in the groups treated with 250 and 1,000 mg/kg of the extract. However, there was a significant ($p\leq 0.05$) decrease in body weight in the group treated with 500 mg/kg of the extract in the first and second week compared with distilled water group (Figure 4.9).

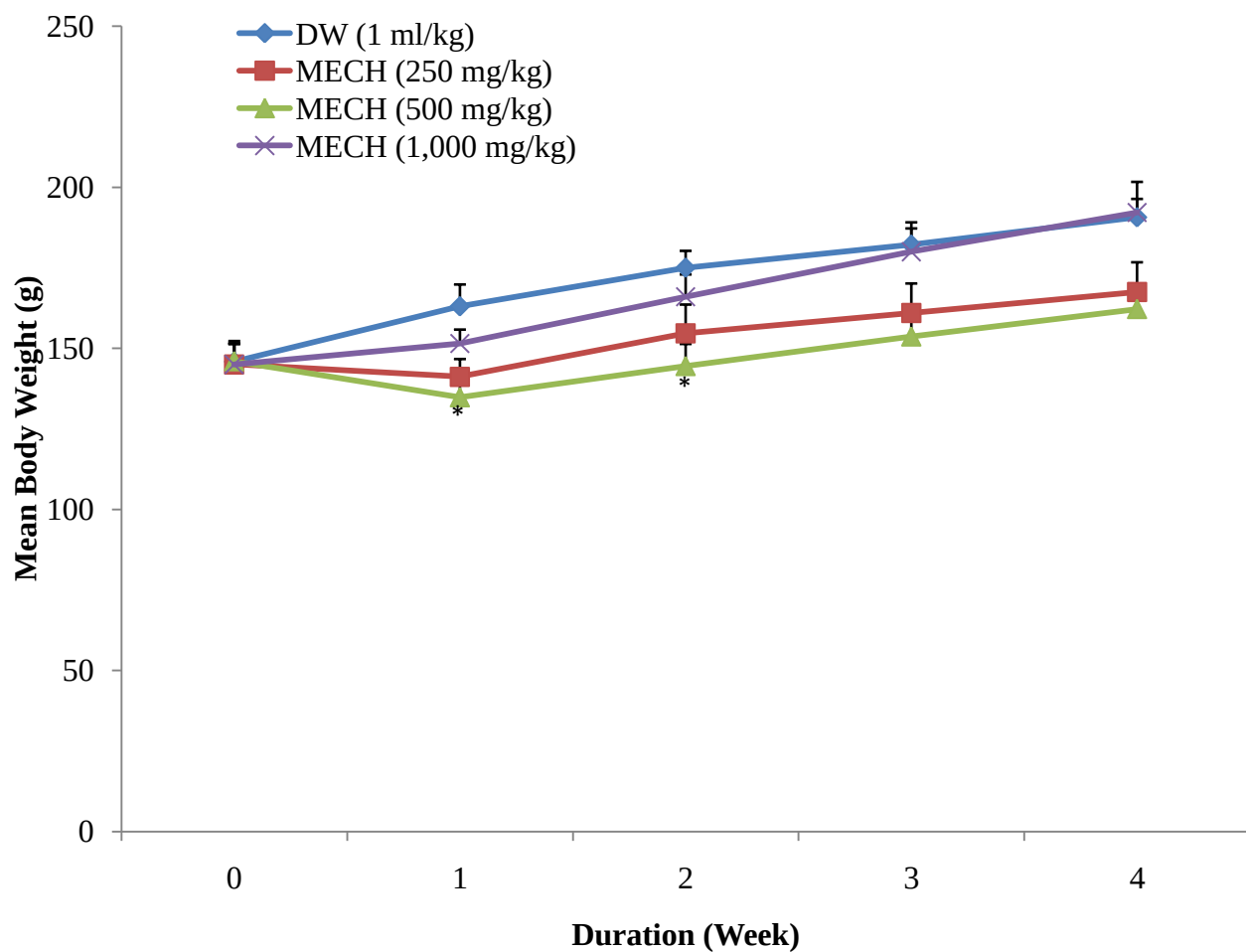


Figure 4.9: Effect of 28-Days Oral Administration of Methanol Leaf Extract of *Combretum hypopilinum* on Body Weights of Rats

Data are presented as Mean $\pm$ SEM; * $p \leq 0.05$ compared to control group (Repeated measure ANOVA followed by Dunnett's post hoc test), DW= Distilled Water, MECH= Methanol leaf extract of *C. hypopilinum*, n=6

4.6.2 Effect of 28-Days Oral Administration of Methanol Leaf Extract of *Combretum hypopilinum* on Relative Organ Weights of Rats

The repeated oral administration of MECH did not show significant ($p > 0.05$) changes in relative (liver, heart, lung, stomach and small intestine) weight. However, there was a significant ($p \leq 0.05$ and $p \leq 0.01$) decrease in relative kidney weight in groups treated with 500 and 1,000 mg/kg of MECH, respectively, compared with the control group (Figure 4.10).

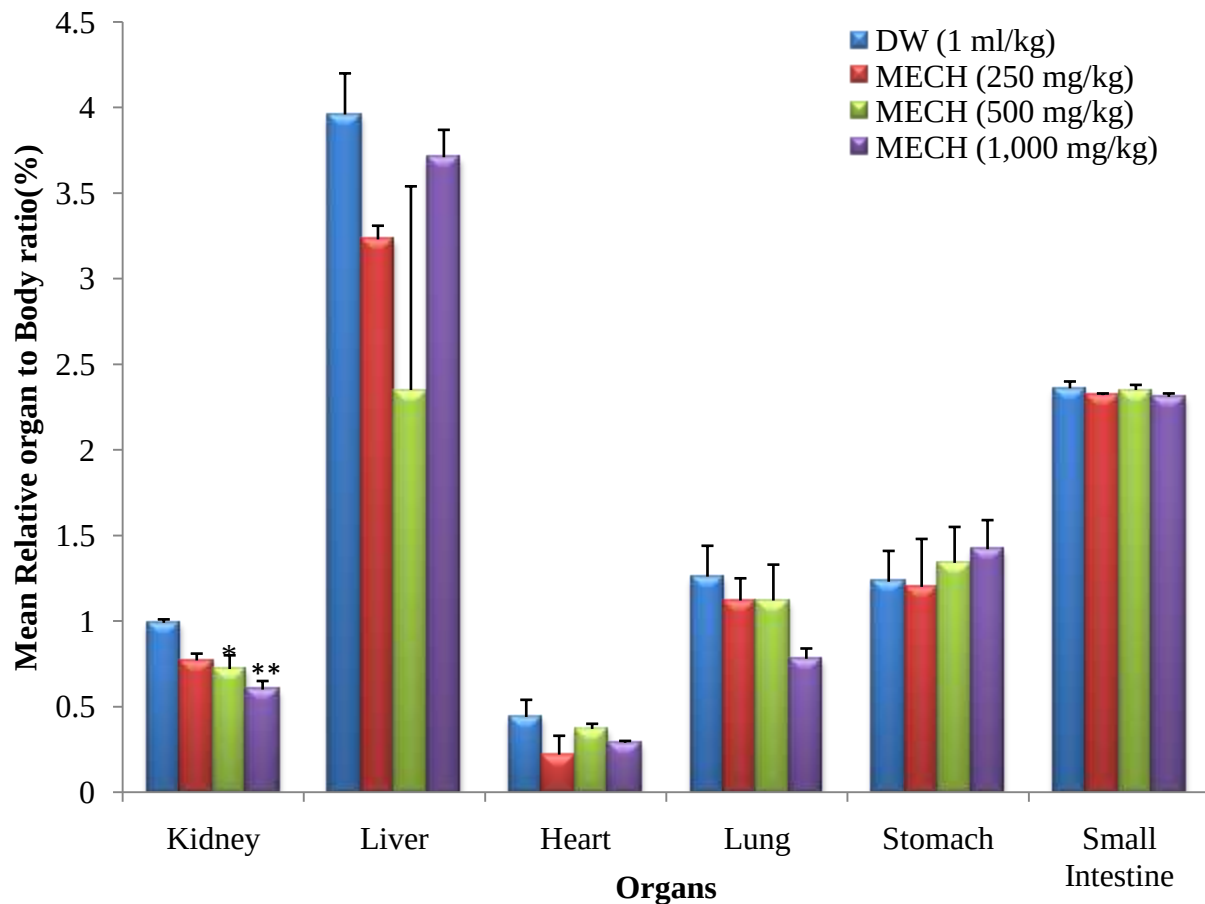


Figure 4.10: Effect of 28-Days Oral Administration of Methanol Leaf Extract of *Combretum hypopilinum* on Relative Organ Weights of Rats

Data are presented as Mean $\pm$ SEM; * $p \leq 0.05$ and ** $p \leq 0.01$ compared to control group (One Way ANOVA followed by Dunnett's post hoc test), DW= distilled water, MECH= Methanol leaf extract of *C. hypopilinum*, n=3

4.6.3 Effect of 28-Days Oral Administration of Methanol Leaf Extract of *Combretum hypopilinum* on Hepatic Parameters of Rats

The repeated oral administration of MECH did not show a significant ($p>0.05$) difference in hepatic biomarkers such as ALT, AST, total protein, albumin, total bilirubin and direct bilirubin. However, there was a significant ($p\leq 0.01$ and $p\leq 0.001$) decrease in ALP in groups treated with 500 and 1,000 mg/kg of the extract, respectively. Also, a significant ($p\leq 0.01$) reduction in glucose was observed in the group treated with the highest dose (1,000 mg/kg) of the extract compared with the control group (Table 4.3).

Table 4.3: Effect of 28-Days Oral Administration of Methanol Leaf Extract of *Combretum hypopilinum* on Hepatic

Parameters of Rats

Treatments (mg/kg)	ALT (I.U/L)	AST (I.U/L)	ALP (I.U/L)	TP (mg/dL)	ALB (mg/dL)	TB (mg/dL)	DB (mg/dL)	GLU (mg/dL)
DW (1ml/kg)	61.17±0.98	106.33±5.16	49.82±2.94	11.73±1.14	2.98±0.60	11.48±0.46	4.88±0.34	90.67±5.58
MECH 250	54.00±2.38	105.00±4.68	50.55±2.47	12.30±0.51	3.12±0.87	11.38±0.66	5.68±0.19	78.33±4.71
MECH 500	62.00±3.29	89.67±11.02	31.67±4.01*	14.47±0.78	2.90±0.93	10.58±0.61	12.25±6.98	72.00±6.33
MECH 1,000	54.50±3.23	87.00±15.39	26.22±3.03**	12.28±0.70	3.03±0.61	11.62±0.55	10.28±6.54	51.83±9.04*

Data are presented as Mean ± SEM; * $p \leq 0.01$ and ** $p \leq 0.001$ compared to control group (One Way ANOVA followed by Dunnett's post hoc test), DW= distilled water, MECH= Methanol leaf extract of *C. hypopilinum*, ALT= Alanine transaminase, AST= Aspartate transaminase, ALP= Alkaline Phosphatase, TP= Total Protein, ALB= Albumin, TB= Total Bilirubin, DB= Direct Bilirubin, GLU= Glucose, I.U= International Unit, n=6

4.6.4 Effect of 28-Days Oral Administration of Methanol Leaf Extract of *Combretum hypopilinum* on Renal Parameters of Rats

The administration of MECH did not show a significant ($p>0.05$) difference in renal parameters such as urea, creatinine, sodium, chlorine and bicarbonate. However, there was a significant ($p\leq 0.01$ and $p\leq 0.001$) and dose-dependent decrease in potassium compared with the control group (Table 4.4).

Table 4.4: Effect of 28-Days Oral Administration of Methanol Leaf Extract of *Combretum hypopilinum* on Renal Parameters of Rats

Treatments (mg/kg)	Urea (mmol/L)	Creatinine (mmol/L)	Sodium (mmol/L)	Potassium (mmol/L)	Chlorine (mmol/L)	Bicarbonate (mmol/L)
DW (1ml/kg)	37.02 ± 1.74	0.78 ± 0.05	137.32 ± 1.91	27.93 ± 1.32	26.17 ± 1.96	89.75 ± 3.21
MECH (250)	33.27 ± 4.02	0.80 ± 0.05	148.69 ± 6.04	22.92 ± 0.67*	24.17 ± 1.82	82.50 ± 2.06
MECH (500)	33.95 ± 1.16	0.78 ± 0.05	157.53 ± 2.59	20.00 ± 0.57**	22.17 ± 1.45	76.67 ± 2.38
MECH (1,000)	36.07 ± 1.92	0.85 ± 0.06	160.57 ± 1.20	19.85 ± 1.01**	23.33 ± 1.05	87.00 ± 2.84

Data are presented as Mean ± SEM; * $p \leq 0.01$ and ** $p \leq 0.001$ compared to control group (One Way ANOVA followed by Dunnett's post hoc test), DW= distilled water, MECH= Methanol leaf extract of *C. hypopilinum*, n=6

4.6.5 Effect of 28-Days Oral Administration of Methanol Leaf Extract of *Combretum hypopilinum* on Haematological Parameters of Rats

The administration of MECH did not show a significant ($p>0.05$) difference in WBC count, percentage of lymphocytes, RBC, HGB, HCT, MCV, MCH and MCHC. However, a significant ($p\leq 0.05$) increase in the percentage of eosinophils, basophils and monocytes and a significant ($p\leq 0.01$) increase in granulocytes were observed in the group treated with the highest dose of the extract (1,000 mg/kg). Also, a significant ($p\leq 0.01$) increase in platelet count was observed in the animals treated with 500 mg/kg of the extract compared with the control group (Table 4.5).

Table 4.5: Effect of 28-Days Oral Administration of Methanol Leaf Extract of *Combretum hypopilinum* on Haematological Parameters of Rats

Haematological parameters	Treatments (mg/kg)			
	DW (1 ml/kg)	MECH (250)	MECH (500)	MECH (1,000)
WBC($\times 10^9$ /L)	5.70 $\pm$ 0.58	5.82 $\pm$ 1.07	6.10 $\pm$ 0.74	6.88 $\pm$ 0.75
LYM (%)	31.88 $\pm$ 2.17	33.68 $\pm$ 1.94	36.92 $\pm$ 2.51	32.90 $\pm$ 1.86
MID (%)	5.22 $\pm$ 0.59	7.23 $\pm$ 1.66	9.13 $\pm$ 1.24	10.92 $\pm$ 0.78*
GRAN (%)	44.03 $\pm$ 1.86	47.22 $\pm$ 3.16	53.15 $\pm$ 2.36	56.13 $\pm$ 2.04**
RBC ($\times 10^{12}$ /L)	4.80 $\pm$ 0.06	5.08 $\pm$ 0.31	4.65 $\pm$ 0.15	4.96 $\pm$ 0.12
HGB (g/dL)	15.14 $\pm$ 0.81	14.73 $\pm$ 0.45	13.77 $\pm$ 0.32	14.58 $\pm$ 0.47
HCT (%)	45.33 $\pm$ 2.40	42.83 $\pm$ 1.99	42.00 $\pm$ 1.24	46.17 $\pm$ 2.12
PLT ($\times 10^9$ /L)	222.67 $\pm$ 24.17	336.80 $\pm$ 36.20	400.67 $\pm$ 25.72**	311.67 $\pm$ 25.92
MCV (fL)	85.11 $\pm$ 1.75	87.48 $\pm$ 1.55	86.88 $\pm$ 1.48	88.08 $\pm$ 1.75
MCH (pg)	32.48 $\pm$ 1.66	29.05 $\pm$ 0.91	30.27 $\pm$ 0.48	31.12 $\pm$ 0.52
MCHC (g/dL)	34.96 $\pm$ 0.64	33.62 $\pm$ 0.47	33.83 $\pm$ 0.35	34.22 $\pm$ 0.27

Data are presented as Mean $\pm$ SEM; * $p \leq 0.05$ and ** $p \leq 0.01$ compared to control group (One Way ANOVA followed by Dunnett's post hoc test), DW= Distilled water, MECH= Methanol leaf extract of *C. hypopilinum*, WBC= White blood cell count, LYM %= Percentage of lymphocytes, MID %= Percentage of eosinophils, basophils and monocytes, GRA %= Percentage of granulocytes, RBC= Red blood cell count, HGB= Haemoglobin, HCT= Haematocrit, PLT= Platelet, MCV= Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration, n=6

4.6.6 Effect of 28-Days Oral Administration of Methanol Leaf Extract of *Combretum hypopilinum* on Lipid Profile of Rats

The administration of MECH did not significantly ($p>0.05$) change the level of cholesterol, triglycerides and HDL. However, there was a significant ($p\leq 0.01$, $p\leq 0.001$ and $p\leq 0.05$) and non-dose dependent decrease in LDL, with the highest effect observed at 500 mg/kg compared with the control group (Figure 4.11).

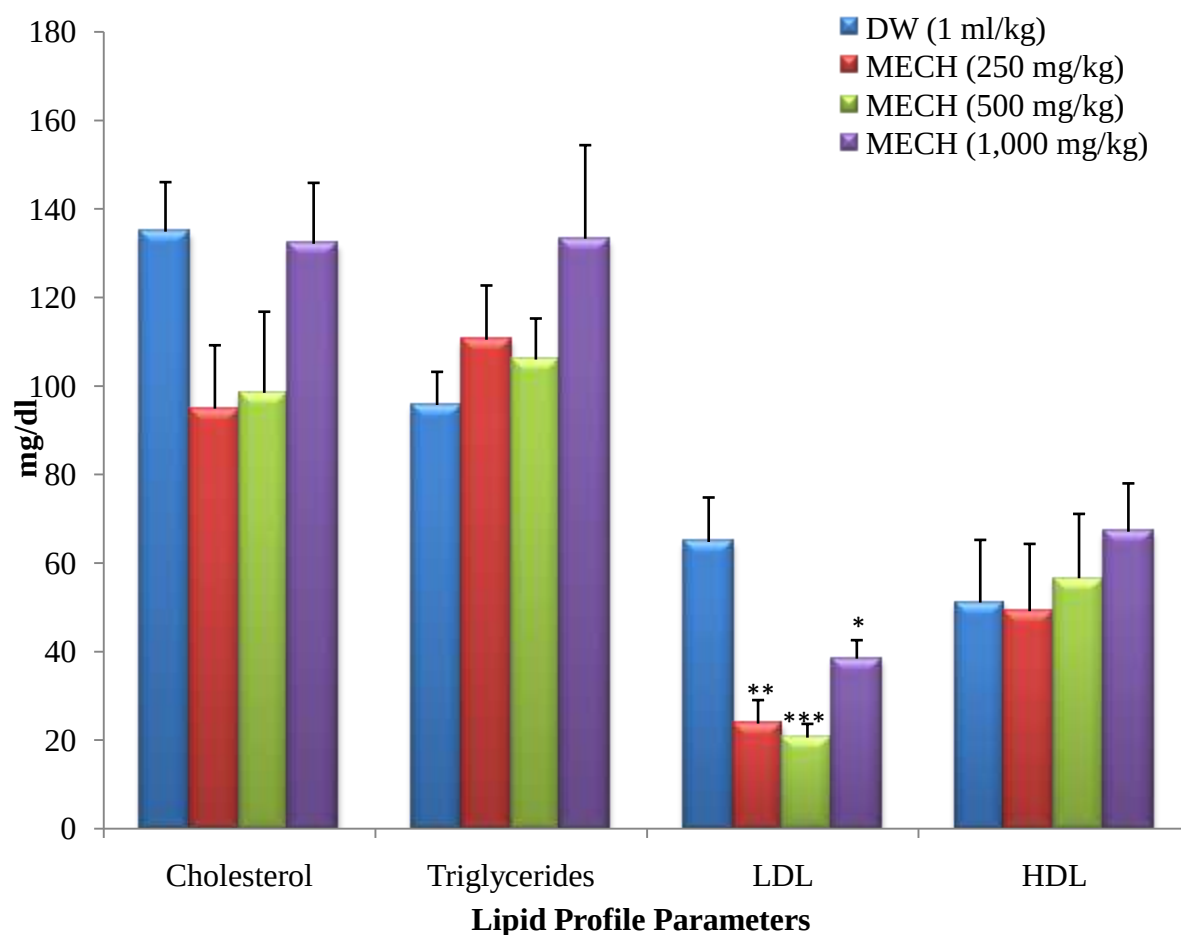


Figure 4.11: Effect of 28-Days Oral Administration of Methanol Leaf Extract of *Combretum hypopilinum* on Lipid Profile of Rats

Data are presented as Mean $\pm$ SEM; * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ compared to control group (One way ANOVA followed by Dunnett's post hoc test), DW= distilled water, MECH= Methanol leaf extract of *C. hypopilinum*, LDL= Low-Density Lipoprotein, HDL= High-Density Lipoprotein, n=6

4.6.7 Effect of 28-Days Oral Administration of Methanol Leaf Extract of *Combretum hypopilinum* on Histology of Some Organs of Rats

There were no pathological changes on the liver of rats treated with 250 mg/kg of MECH. However, the group treated with 500 mg/kg of the extract showed kupffer cell hyperplasia, while the group treated with the highest dose (1,000 mg/kg) of the extract showed slight hepatic necrosis and kupffer cell hyperplasia (Plate II). There were no pathological changes in the kidney of the group treated with 250 mg/kg of the extract, while the groups treated with 500 and 1,000 mg/kg both showed moderate tubular necrosis (Plate III). Also, there were no pathological changes in the heart muscles of rats in all treated groups (Plate IV). The groups treated with 250 and 500 mg/kg of the extract showed normal lung features, while the group treated with the highest dose of the extract (1,000 mg/kg) revealed moderate alveoli congestion and slight alveoli necrosis (Plate V). The group treated with the lowest dose of the extract showed normal gastric mucosa, while the groups treated with 500 and 1,000 mg/kg showed slight necrosis of gastric mucosa (Plate VI). The group treated with 500 mg/kg revealed normal intestinal villi, while the group treated with 250 mg/kg and 1,000 mg/kg revealed slight small intestinal atrophy and moderate villi atrophy, respectively (Plate VII). There were no pathological changes in the liver, kidney, heart, lung, stomach and small intestine of all the groups treated with distilled water (Plates II to VII).

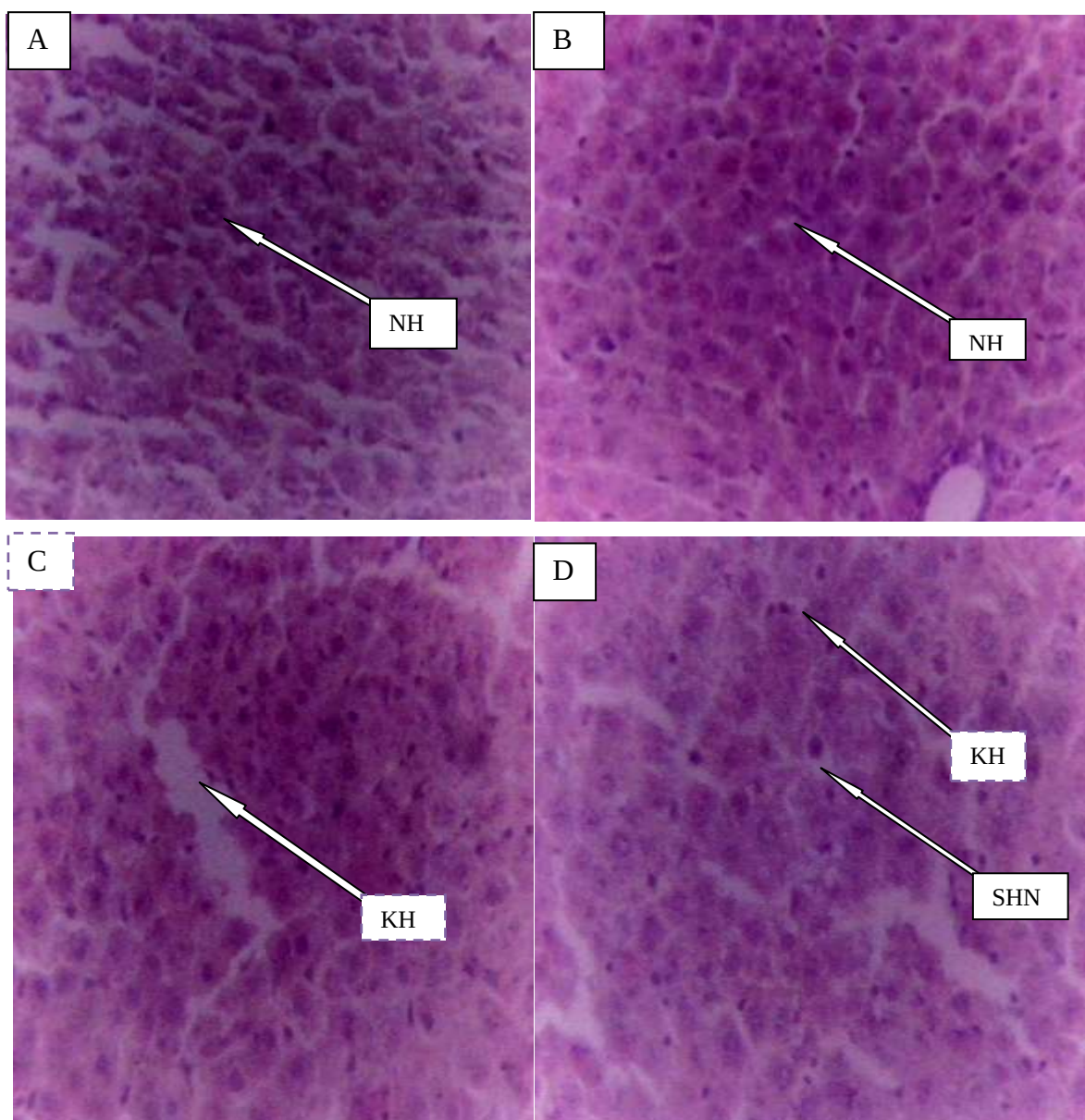


Plate II: Photomicrographs of Liver Sections from Rats Orally Treated with Methanol Leaf Extract of *Combretum hypopilinum* for 28-Days (H and E Stained At ×250)

A) Control (Distilled water, 1 ml/kg), B) MECH (250 mg/kg), C) MECH (500 mg/kg), D) MECH (1,000 mg/kg), NH= Normal hepatocytes, SHN= Slight hepatic necrosis, KH= Kupffer cell hyperplasia, MECH= Methanol leaf extract of *Combretum hypopilinum*

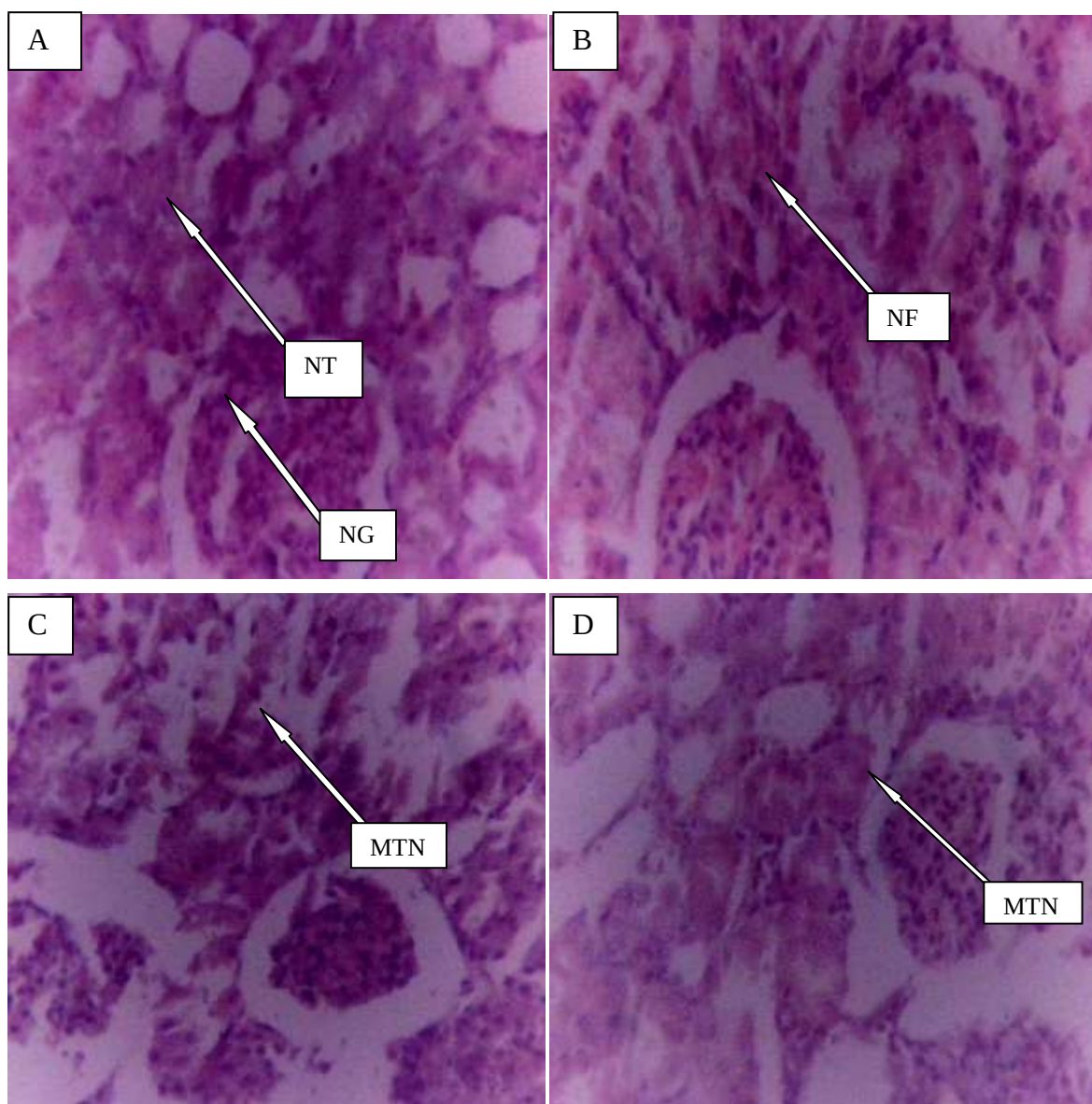


Plate III: Photomicrographs of Kidney Sections from Rats Orally Treated with Methanol Leaf Extract of *Combretum hypopilinum* for 28-Days (H and E stained at $\times 250$)

A) Control (Distilled water, 1 ml/kg), B) MECH (250 mg/kg), C) MECH (500 mg/kg), D) MECH (1,000 mg/kg), NG= Normal glomerulus, NT= Normal tubules, NF= Normal renal features, MTN= Moderate tubular necrosis, MECH= Methanol leaf extract of *Combretum hypopilinum*

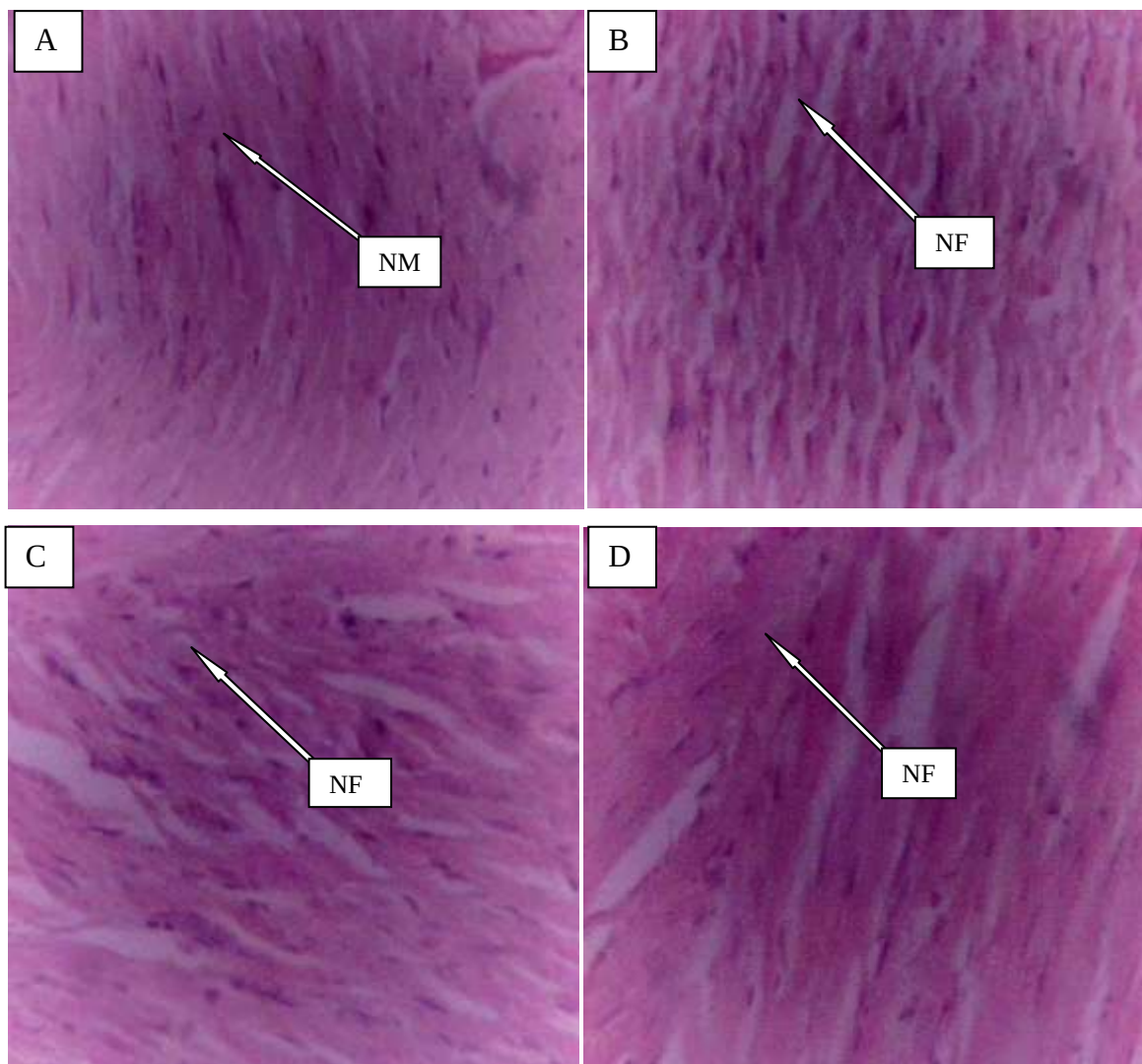


Plate IV: Photomicrographs of Heart Sections from Rats Orally Treated with Methanol Leaf Extract of *Combretum hypopilinum* for 28-Days (H and E stained at $\times 250$)

A) Control (Distilled water, 1 ml/kg), B) MECH (250 mg/kg), C) MECH (500 mg/kg), D) MECH (1,000 mg/kg), NM= Normal myocardium, NF= Normal feature, MECH= Methanol leaf extract of *Combretum hypopilinum*

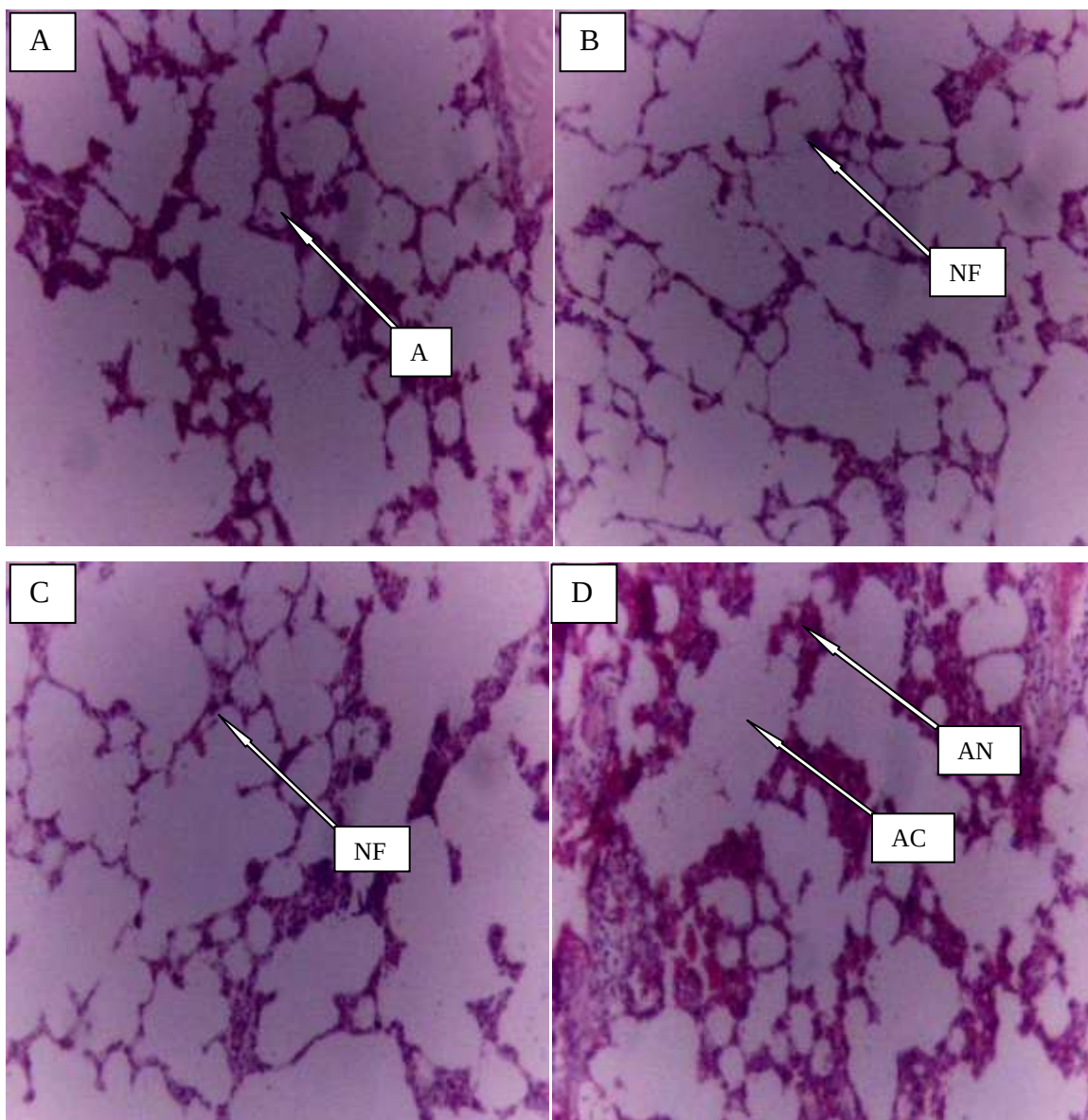


Plate V: Photomicrographs of Lung Sections from Rats Orally Treated with Methanol Leaf Extract of *Combretum hypopilinum* for 28-Days (H and E stained at $\times 250$)

A) Control (Distilled water, 1 ml/kg), B) MECH (250 mg/kg), C) MECH (500 mg/kg), D) MECH (1,000 mg/kg), A= Normal alveoli, NF= Normal feature, AC= Moderate alveoli congestion, AN= Slight alveoli necrosis, MECH= Methanol leaf extract of *Combretum hypopilinum*

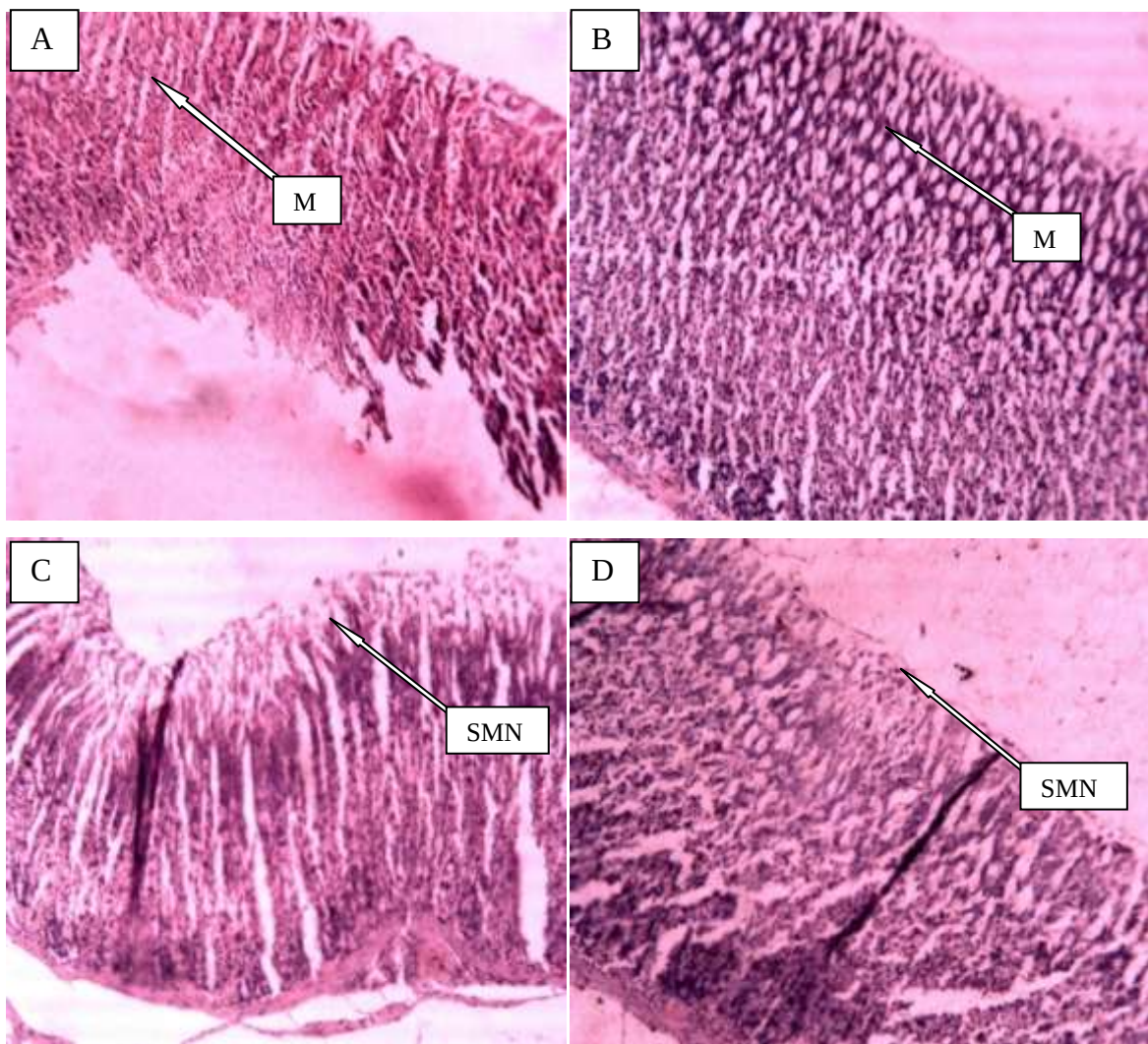


Plate VI: Photomicrographs of Stomach Sections from Rats Orally Treated with Methanol Leaf Extract of *Combretum hypopilinum* for 28-Days (H and E stained at $\times 250$)

A) Control (Distilled water, 1 ml/kg), B) MECH (250 mg/kg), C) MECH (500 mg/kg), D) MECH (1,000 mg/kg), M= Normal gastric mucosa, SMN= slight mucosa necrosis, MECH= Methanol leaf extract of *Combretum hypopilinum*

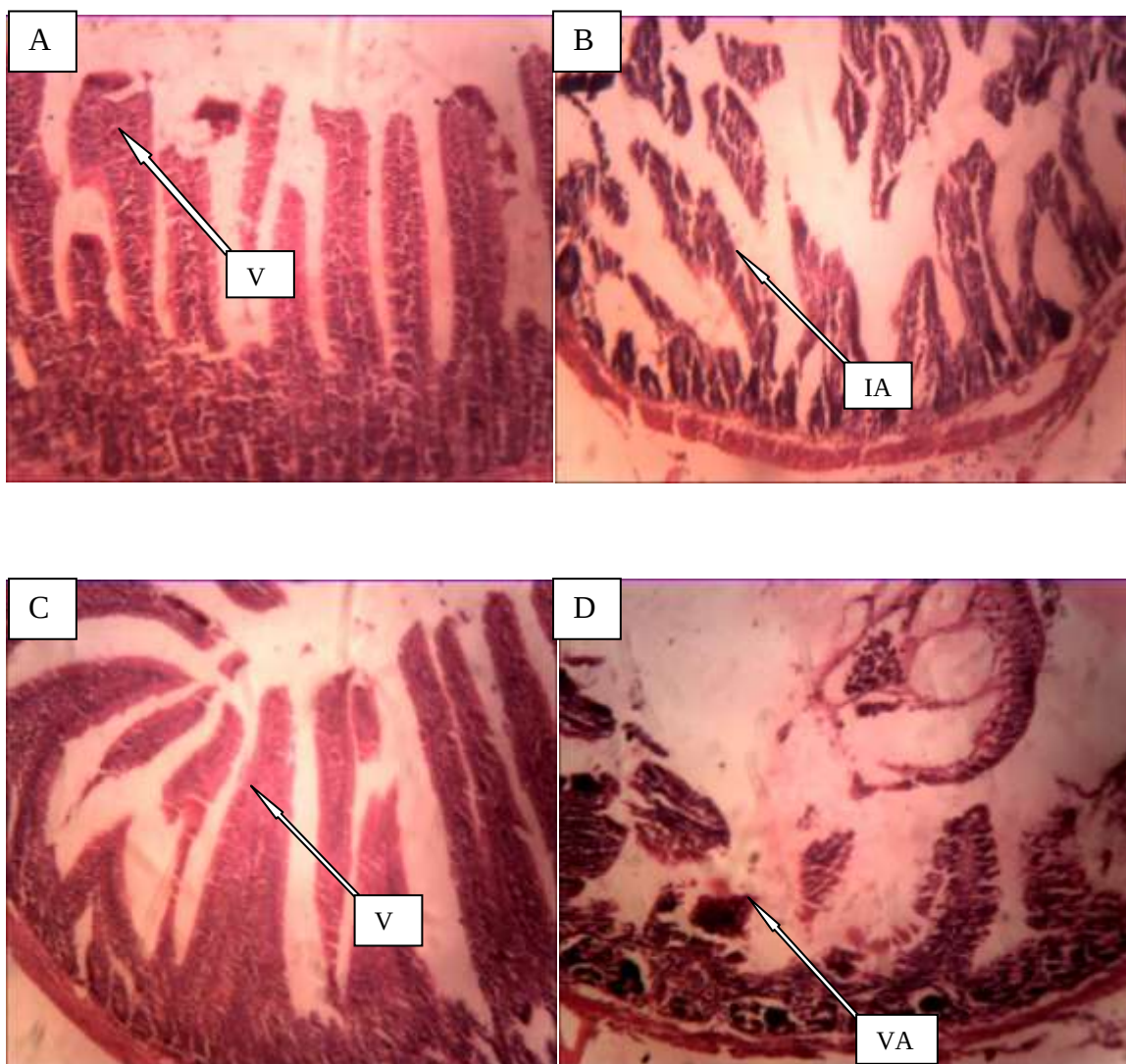


Plate VII: Photomicrographs of Small Intestine Sections from Rats Orally Treated with Methanol Leaf Extract of *Combretum hypopilinum* for 28-Days (H and E stained at $\times 250$)

A) Control (Distilled water, 1 ml/kg), B) MECH (250 mg/kg), C) MECH (500 mg/kg), D) MECH (1,000 mg/kg), V= Normal villi, IA= slight small intestinal atrophy, VA= moderate villi atrophy MECH= Methanol leaf extract of *Combretum hypopilinum*

CHAPTER FIVE

5.0 DISCUSSION

Several medicinal plants used in traditional medicine to treat diarrhoea have been scientifically investigated by evaluating their effects on intestinal motility and hypersecretion (Mekonnen *et al.*, 2018). Based on this assertion, the present study investigated the antidiarrhoeal activity of MECH and its sub-acute toxicity effects. The possible involvement of opioidergic, adrenergic (α_1 , α_2 and β), cholinergic, and nitric oxide pathways in the extract's antidiarrhoeal activity were investigated.

Acute toxicity study of a chemical substance is used to evaluate the likely clinical signs that can result from administration of the chemical substance and gives a range of doses to be used in subsequent experiments. It is also important in estimating a therapeutic index of a drug (Mbiri *et al.*, 2017). From the acute toxicity test conducted in this study, there was no death, and no physical signs of toxicity in both mice and rats treated orally with 5,000 mg/kg of MECH throughout the observation period of 14 days. These findings suggested that the LD₅₀ of the extract is greater than 5,000 mg/kg and may be relatively safe after acute oral administration in mice and rats. After acute oral administration of a chemical substance for 14 days, if there is no death and any signs of toxicity at a dose of 5,000 mg/kg, the substance could be considered safe (OECD, 2001).

Diarrhoea is a common disease that has been causing illness and deaths in children and infants, particularly in developing nations. This disease is caused by four pathophysiological mechanisms: increased intestinal osmolarity, increased water and electrolytes secretion, decreased water and electrolytes absorption, and intestinal

hypermotility that cause decreased intestinal residence time. In diarrhoea treatment, drugs that possess antimotility and antisecretory activities are mainly used to alleviate the disease (Mekonnen *et al.*, 2018).

Going by the safety of *C. hypopilinum* on acute administration, it is therefore important to investigate its antidiarrhoeal potential in an attempt to discover novel compounds to treat diarrhoea. In this study, the antidiarrhoeal activity of MECH was evaluated using castor oil-induced diarrhoea, castor oil-induced enteropooling and gastrointestinal motility test in mice. The choice of these models results from the evidence that there is a predominantly increased fluid secretion in some forms of diarrhoea, while another type of diarrhoea is characterized by gastrointestinal hypermotility (Ezeja *et al.*, 2012).

Castor oil-induced diarrhoea is an effective model for evaluating both secretory diarrhoea and intestinal motility because it reduces the absorption of water, increases fluid and electrolytes secretion and causes intestinal hypermotility (Taqvi *et al.*, 2018). Castor oil is metabolized to ricinoleic acid in the duodenum by intestinal lipases. The ricinoleic acid is poorly absorbed in the small intestine. It causes irritation and inflammation of the intestinal mucosa, which subsequently results in the production and release of autocooids and prostaglandins that stimulate intestinal motility, changes electrolyte permeability of the intestinal mucosa and increased fluid and electrolytes secretion (Mehesare *et al.*, 2019). In this study, the MECH showed an insignificant and dose-dependent delay in onset of diarrhoea and significant inhibition of diarrhoea stools, thereby inhibiting the castor oil-induced diarrhoea. This inhibitory effect suggested that the extract could possess its antidiarrhoeal activity by stimulating the re-absorption of water and electrolytes from the

intestinal lumen and antagonizing prostaglandin, which plays a role in the pathophysiology of diarrhoea. Nwabunike *et al.* (2018) suggested that the antidiarrhoeal activity of medicinal plants in castor oil-induced diarrhoea could be due to antisecretory activity.

The intraluminal fluid accumulation was evaluated by castor oil-induced enteropooling. Castor oil and its active metabolite ricinoleic acid alter electrolytes and water movement across the intestinal mucosa, thereby enhance secretion and fluid accumulation in the intestine (Saheed and Tom, 2016). The fluid accumulation results from the release of platelet-activating factor, nitric oxide, tachykinins, and cAMP (de Oliveira *et al.*, 2017).

This study also showed that the MECH at the doses of 500 and 1,000 mg/kg significantly reduced intestinal fluid volume in mice. The reduction in intestinal fluid volume could be due to prostaglandin inhibition, platelet-activating factor, nitric oxide, tachykinins, and cAMP biosynthesis that eventually inhibit fluid hypersecretion, reduced intestinal motility, enhanced intestinal fluid and electrolyte absorption, reduced intraluminal fluid accumulation and subsequently inhibit diarrhoea (Saheed and Tom, 2016). This study further revealed that the extract might possess its antidiarrhoeal activity, possibly via antisecretory activity.

Diarrhoea also develops due to change in the intestinal motility and fluid accumulation (Rahman *et al.*, 2013). The intestinal motility test model is used to investigate the possibility of a compound to retard the intestinal smooth muscle movement (Shoba and Thomas, 2014). Going by the present finding, the MECH reduced the intestinal movement of the charcoal meal along the length of the small intestine. This decrease in the movement of the charcoal meal by the extract suggested its antimotility effect, which may prolong small intestinal residence time and promote water and electrolyte absorption.

Loperamide is an antidiarrhoeal drug that decreases intestinal motility by acting at the myenteric plexus and increases small intestinal residence time, allowing adequate time for water absorption (Emudainohwo *et al.*, 2015). In this study, loperamide also significantly inhibited the castor oil-induced diarrhoea, castor oil-induced enteropooling and intestinal movement, showing that it possesses antisecretory and antimotility effects. Similarly, the MECH produced antisecretory and antimotility effects. Therefore, the extract could possess its antidiarrhoeal activity through similar actions to loperamide.

The regulation of gastrointestinal movement is complex and involves many signalling pathways, including gastrin, 5-hydroxytryptamine, dopamine, opioids, catecholamines, acetylcholine and nitric oxide (de Oliveira *et al.*, 2017). Therefore, in the present work; the mechanistic studies targeting μ opioidergic, (α_1 , α_2 and β)-adrenergic, cholinergic and nitric oxide pathways likely to be involved in the antidiarrhoeal activity of the extract were investigated. For the mechanistic studies, drugs with established mechanisms for blocking these pathways were employed to elucidate the mechanism of action of MECH, namely; naloxone (a non-selective opioid receptor antagonist), prazosin (a selective α_1 -adrenoceptor antagonist), yohimbine (a selective α_2 -adrenoceptor antagonist), propranolol (a non-selective β -adrenoceptor antagonist), pilocarpine (a non-selective muscarinic receptor agonist) and isosorbide dinitrate (a nitric oxide donor).

The pretreatment of animals with naloxone, prazosin and propranolol each attenuated the observed antidiarrhoeal activity of MECH compared with the negative control group. The attenuation of the activity of the extract by these antagonists suggested the involvement of

the μ opioidergic and adrenergic (α_1 and β) systems in the antidiarrhoeal activity of the extract. In contrast, pretreatment of mice with yohimbine, pilocarpine, and isosorbide failed to reverse the antidiarrhoeal activity produced by the extract compared with the distilled water group. The lack of reversal of the activity of the extract by these antagonists suggests the non-involvement of the α_2 -adrenergic, cholinergic and nitric oxide pathways in the antidiarrhoeal activity of the extract.

The finding in this study suggested the involvement of the μ opioidergic system in the antidiarrhoeal action of MECH as revealed by the fact that pretreatment of the animals with naloxone reversed the observed antidiarrhoeal activity of the extract and loperamide. Morphine and other opiates agonist such as loperamide have been found to decrease intestinal movement *in vivo* and have antispasmodic effect *in vitro* by interfering with neurotransmitters' release. Additionally, opiates inhibit intestinal movement through central and peripheral mechanisms to attenuate the pathophysiological mechanism of diarrhoea (Paredes *et al.*, 2016). The ability of naloxone to reverse the antidiarrhoeal activity produced by loperamide has been reported (Besra *et al.*, 2003). The findings in this work have established that the extract reduced small intestinal transit and enhanced intestinal residence time for effective absorption of fluids and electrolytes similar to loperamide, possibly interacting with opioid receptors found in the gastrointestinal wall.

Interestingly, the finding of the present study also suggested that (α_1 and β)-adrenergic pathways are also likely to be involved in the antidiarrhoeal activity of MECH by stimulating (α_1 and β)-adrenergic receptor. The possible participation of these pathways was derived from the fact that pretreatment of animals with prazosin and propranolol

abolished the antidiarrhoeal action produced by MECH compared with the negative control group. Also, pretreatment of animals with propranolol in this work attenuated the antidiarrhoeal activity of morphine. Activation or inhibition of intestinal receptors accounts for different biological effects in the intestine, such as secretion and peristaltic movement. Stimulation of intestinal (α and β)-adrenoceptors attenuates the rate and force of intestinal contraction and inhibits intestinal fluid secretion (Adeniyi *et al.*, 2017).

Another interesting finding in this work showed that the antidiarrhoeal activity of morphine was found to be related to stimulation of (α_2 and β)-adrenergic receptors because yohimbine and propranolol each reversed its antidiarrhoeal activity. However, the antidiarrhoeal activity of morphine was independent of α_1 -adrenergic and nitric oxide pathway due to the fact that pretreatment of the animals with prazosin and isosorbide dinitrate each did not reverse its antidiarrhoeal activity. Wong, (1984) reported that the antimotility effect of morphine was abolished when the animals were pretreated with yohimbine which preferentially blocks α_2 -adrenergic receptors. Conversely, in the same research, prazosin failed to attenuate the effect of morphine, as clearly confirmed in this study. Stimulation of α_2 -adrenoceptors delay intestinal movement and elicit absorption of water and electrolytes. Therefore, α_2 -adrenergic antagonists such as yohimbine can promote diarrhoea (Adeyemi and Akindele, 2008; Mbagwu and Adeyemi, 2008).

Also, atropine sulphate showed an intestinal spasmolytic effect in the present work, where it significantly reduced intestinal propulsive movement, which was diminished by pretreatment with pilocarpine. Atropine prevents intestinal propulsive movement, decreases

water and electrolytes secretion, and retard gastric emptying, leading to the antidiarrhoeal effect (Adeyemi and Akindele, 2008).

Phytochemical screening is conducted to determine the presence of the secondary metabolites in a plant. The secondary metabolites are responsible for the potential biological and harmful effects of the plants (Pandey *et al.*, 2013). Therefore, it is important to test the presence of phytochemicals responsible for biological activities in medicinal plants (Pandey *et al.*, 2013). The present study revealed the presence of secondary metabolites such as flavonoids, saponins, tannins, alkaloids, steroids and triterpenes in MECH. Therefore, it can be suggested that the antidiarrhoeal activity of the extract observed in this study may be attributed to the presence of these phytochemical compounds. Tannins have spasmolytic and smooth muscle relaxant effect, flavonoids prevent intestinal secretion caused by prostaglandin E₂, saponins inhibit histamine release, and terpenoids inhibit prostaglandin release and phenols inhibit intestinal secretion and motility. These secondary metabolites' overall effects lead to decreased intestinal hypersecretion, antimotility, and diarrhoea inhibition (Mekonnen *et al.*, 2018).

A change in the body weight of an animal indicates the animals' general health condition (Yuet Ping *et al.*, 2013). There are usually changes in body weight gain and internal organ weights in animals after exposure to potentially toxic substances, which indicate toxicity (Prasanth *et al.*, 2015). However, scientific evaluations revealed that changes in body weight of animals might occur due to accumulation of fat and physiological response to the plant extract instead of an indication of toxic effects of the chemical substances that cause a reduction in appetite and low energy consumption by the animal (Nfozon *et al.*, 2019). The

decrease in body weight in groups treated with 500 mg/kg of MECH in the first and second week may not indicate the extract's toxic effect. This decrease in body weight could be due to the animals' physiological adjustment in response to the extract, which caused decreased appetite and, hence, low caloric intake as the animals regained their normal body weight in the subsequent weeks.

The organ weight of an animal is useful to reveal the healthy and diseased condition of the animals. It is also important to ascertain the exposure of the organ to injury. The liver and kidney weights are vital in evaluating toxic substances due to their sensitivity in predicting toxicity which corroborates with histopathological changes (Amna *et al.*, 2013). Based on the present findings, there was a significant decrease in relative kidney weight in groups treated with 500 and 1,000 mg/kg of MECH, which indicated that the kidney might be adversely exposed to the extract's toxic effect. The decrease in relative kidney weight correlates with moderate tubular necrosis observed in groups treated with 500 and 1,000 mg/kg of the extract. The renal tubular necrosis could indicate damage to renal tubular cells, impairing renal reabsorption and secretion of substances, including electrolytes. Therefore, renal function may have to be monitored on long term administration of the extract. However, there were no significant differences in relative weights of liver, heart, lung, stomach and small intestine in all treated groups.

Analysis of biochemical parameters on experimental animals helps evaluate toxic effects on different tissues, especially the liver and kidney (Traesel *et al.*, 2016). The liver's normal metabolic function is determined by serum hepatic biomarkers (El Kabbaoui *et al.*, 2017). ALT and AST are important markers of hepatic function. ALT is an enzyme present in the

hepatic cytoplasm which significantly increases during hepatocellular toxicity. AST is an extracellular enzyme in various tissues such as the heart, skeletal muscles, liver, kidneys, pancreas and red blood cells released from these tissues after cellular damage or alteration in cell membrane permeability (Li *et al.*, 2019). ALT and AST are proportionately released into the systemic circulation after cellular injury (Mbiri *et al.*, 2017). ALP is present in the cells lining the liver's biliary duct and is important in evaluating the biliary duct's disease (El Kabbaoui *et al.*, 2017). Based on liver function in this study, the MECH did not significantly change the serum level of ALT and AST in all treated groups. The non-significant differences in serum level of ALT and AST suggested that the extract may not be hepatotoxic. However, a significant reduction in ALP was observed in groups treated with 500 and 1,000 mg/kg of the extract. Previous researches have shown that reduction in the ALP level is associated with zinc deficiency, hypothyroidism, vitamin (C and B₁₂) deficiency, protein-energy malnutrition and magnesium deficiency (Cho *et al.*, 2007; Ray *et al.*, 2017). Therefore, the finding of the present study suggested that the extract could cause malnutrition and interfere with metabolic activities in the body.

Bilirubin is a by-product of haemoglobin degradation associated with liver diseases such as jaundice, defective erythropoiesis and cholestasis (Reena *et al.*, 2012). Total plasma protein is used to assess the changes in renal and hepatic functions. Abnormal level of total proteins is related to liver infections or chronic hepatic inflammation (El Kabbaoui *et al.*, 2017). Albumin is a protein synthesized in the liver, which when decreased, may be an indication of reduced liver synthetic ability while a high level is associated with dehydration (Donkor *et al.*, 2014). From the result of this study, the extract did not significantly alter total and direct bilirubin, serum total protein and albumin in all treated

groups. These results further suggested that the extract may not have adverse effects on the liver and red blood cell production, which corroborate its possible lack of toxic effects on erythropoiesis and the liver observed in this study.

Several medicinal plants affect glucose metabolism (Mbiri *et al.*, 2017). Studies reported that polyphenols and flavonoids containing substances elicit hypoglycemic effect by inhibiting α -amylase and α -glucosidase, increasing glucose uptake by peripheral tissues and stimulating the release of insulin from pancreatic β cell (El Kabbaoui *et al.*, 2017). In this study, the MECH caused a significant decrease in plasma glucose level in the group treated with the highest dose of the extract (1,000 mg/kg), which showed that the extract may possess a hypoglycemic effect.

The kidney plays an important function in maintaining homeostatic balance by reabsorbing essential substances and excretion of waste products (Donkor *et al.*, 2014). Evaluation of urea and creatinine in the blood is used to assess renal function. In acute and chronic toxicity, the urea and creatinine significantly increase four to five times higher than in the control group (Arsad *et al.*, 2013). There was no significant difference in urea and creatinine level in all the groups treated with MECH in the present study.

Additionally, the serum levels of electrolytes such as sodium, chloride and bicarbonates, were not significantly altered. However, a remarkable reduction in potassium was observed in a dose-dependent manner. Yang *et al.* (2019) suggested that severe damage to glomeruli and renal tubules leads to deterioration of renal tubular reabsorption and glomerular filtration mechanisms or high secretion of aldosterone impair absorption of ions. From the

present study, the significant decrease in potassium may indicate renal tubular damage, which is consistent with the reduction in relative organ weight of kidney and renal tubular necrosis in the groups treated with 500 and 1,000 mg/kg of the extract.

The hematopoietic system is among the sensitive targets of toxic substances and an important indicator of the normal physiological and disease state in animals (El Kabbaoui *et al.*, 2017). Reduction in the level of blood parameters such as RBC, MCV, MCH, MCHC and HGB can cause anaemia (Olorunnisola *et al.*, 2012). The results of the present study did not show significant changes in haematological parameters in all groups treated with MECH, which suggested that the extract may not interfere with the normal production of RBC and may not induce anaemia.

WBC plays an important role in cellular defensive mechanisms in the body's response to infectious agents, tissue damage and inflammatory mechanism (Yuet Ping *et al.*, 2013). Reduction in RBC, WBC and platelets count if associated with signs of acute toxicity are mostly related to bone marrow suppression which cause anaemia, leucopenia and thrombocytopenia, respectively (Arsad *et al.*, 2013). Based on the results of this study, total WBC and lymphocytes in all treated groups showed no significant difference with the control group. These findings indicated that the MECH might not cause leucopenia, bone marrow and immune system suppression.

In addition, Nfozon *et al.* (2019) suggested that when the haematological investigation of animals revealed a significant increase in granulocyte, the plant may be involved in innate immune response, especially phagocytosis. From the present study, the significant increase

in granulocytes in the group treated with the highest dose (1,000 mg/kg) of MECH showed that it could boost immune response. Furthermore, a significant increase in the percentage of eosinophils, basophils and monocytes was also observed in the group treated with the highest dose (1,000 mg/kg) of the extract.

The administration of some therapeutic substances stimulates platelet antibodies that destroy platelet, which could cause thrombocytopenia (Nfozon *et al.*, 2019). In the present study, the MECH produced a non-dose dependent increase in platelet level, which was significant at the dose of 500 mg/kg. The significant elevation in the platelet showed that the extract could stimulate thrombopoietin production and prevent haemorrhages.

Hyperlipidemia is considered among the main predisposing factors of atherosclerosis which causes coronary artery diseases. A high level of cholesterol and triglycerides in the blood is related to heart and vascular diseases such as atherosclerosis (Reena *et al.*, 2012). During the circulation of LDL in the blood, it is deposited within the arterial walls, causing arterial plaques, resulting in atherosclerosis. However, HDL takes cholesterol away from the arteries to the liver. Therefore, an elevation of HDL prevents atherosclerosis and cardiovascular diseases, while increase LDL level is a risk factor of cardiovascular diseases (Jatsa *et al.*, 2018). Based on this study, the MECH did not show significant alteration in serum level of cholesterol, triglycerides, and HDL but revealed a significant and non-dose-dependent decrease in LDL, suggesting that the extract may not adversely affect lipid metabolism. The non-toxic effects of the extract on lipid metabolism correlate with histopathological examination of the heart, which revealed normal cardiac muscles devoid of any histopathological abnormality in all treated groups.

The safety investigation of substance involves macroscopic and microscopic evaluation of pathological changes of the treated animals' organs (Traesel *et al.*, 2016). Kupffer cells are situated in liver sinusoids and have an essential role in host defence mechanisms. They prevent pathogens derived from the portal and systemic circulation from getting into the liver by phagocytosis. These cells also play an essential role in the specialization of hepatocytes in new liver cells regeneration (Dixon *et al.*, 2013). Stimulation of kupffer cells is critical in the liver protective response against infection or injury by activating the inflammatory response, protecting the organisms against the infection and restraining hepatocellular and liver damage (Helmy *et al.*, 2006). The results of histopathological examination of the liver revealed kupffer cells hyperplasia in groups treated with 500 and 1,000 mg/kg of MECH. Besides, there was mild hepatic necrosis in the highest dose group. These results further suggested the non-toxic effects of the extract on the liver. The extract revealed slight hepatic necrosis, which may not be considered toxic because it did not cause any malfunction associated with hepatic biomarkers and the liver's synthetic ability. The liver can form new cells and clear necrotic cells and restore normal hepatic structure and functions, making it tolerant to moderate zonal and extended necrosis (Olaniyan *et al.*, 2016).

The kidney is an organ majorly exposed to toxic substances due to its vascularity (El Kabbaoui *et al.*, 2017). The histopathological analysis of the kidney observed in the present work showed moderate tubular necrosis resulting from delivery of toxic substances to the kidney from systemic circulation and may cause loss of integrity of the renal tubular system. This histopathological change concurs with the reduction in relative kidney weight, which reveals possible nephrotoxic effects of the extract. There was no morphological

alteration on the myocardium in all treated groups, which suggested the extract's non-toxic effect on the heart.

The alveoli are tiny air sacs present in the lungs where the exchange of oxygen and carbon dioxide takes place. Alveoli congestion and mild necrosis were observed in the group treated with the highest dose of the extract (1,000 mg/kg), which may interfere with the diffusion of oxygen and other gaseous substances across the alveoli epithelium into the pulmonary circulation and impair physiological functions of the body.

The stomach plays critical functions in the digestion of food and secretion of gastric juice. There is also secretion of mucus which inhibits gastric erosion caused by gastric juices and gastric hormones. In this study, the histopathological examination demonstrated a mild gastric mucosal necrosis in the group treated with 500 and 1,000 mg/kg of the extract, which indicated the extract's possible gastro-toxic effect.

The intestinal mucosa is essential for survival and biological roles such as absorption of nutrients, protection against injury and immunological functions. The atrophy of intestinal mucosa shows intestinal malfunction and structural alterations such as the reduced height of intestinal villi, crypt depth and surface area (Shaw *et al.*, 2002). There were intestinal atrophy and villi atrophy in groups treated with 250 and 1,000 mg/kg, respectively, showing that the extract may interfere with the normal absorption of nutrients. However, the GIT effects observed were not dose-dependent.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The powdered leaves of *Combretum hypopilinum* yielded 12.62 % of MECH. The extract possesses phytochemical constituents such as flavonoids, cardiac glycosides, saponins, tannins, steroids, triterpenes and alkaloids. The acute toxicity study showed that the oral median lethal dose (LD₅₀) of MECH was greater than 5,000 mg/kg in mice and rats. Therefore, the extract could be relatively safe for acute oral consumption. The study also demonstrated that the MECH possesses antidiarrhoeal activity at doses of 500 and 1,000 mg/kg due to its inhibitory effects on fluid secretion and intestinal propulsion, supporting its traditional use in the treatment of diarrhoea. The investigation of the mechanisms of antidiarrhoeal activity of MECH revealed the involvement of opioidergic and (α_1 and β)-adrenergic pathways in its antidiarrhoeal activity.

The extract has no toxic effects on body weight, relative organ weight of liver, heart, lung, stomach and intestine; liver and heart functions; blood formation and lipid metabolism on sub-acute oral administration. Nevertheless, the extract caused a nephrotoxic effect demonstrated by the significant reduction in relative kidney weight and serum potassium level and renal tubular damage associated with moderate tubular necrosis. The extract also revealed histopathological abnormalities on the lung, stomach and small intestine.

6.2 Conclusion

It may be concluded from the results of this study that the MECH possesses antidiarrhoeal activity. The results suggested that the extract is relatively safe on acute exposure and at a lower dose on sub-acute administration but moderately toxic at higher doses on sub-acute administration, especially on the kidney function.

6.3 Recommendations

- i. Further studies should be carried out to fractionate, isolate, characterize and elucidate the structure of the bioactive compounds responsible for the observed pharmacological activities.
- ii. Further studies should also be done to evaluate the molecular basis of its opioidergic and adrenergic actions.
- iii. Chronic toxicity studies should be carried out to investigate the toxicological profile of *Combretum hypopilinum* further.

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APPENDICES

Appendix 1: Calculation of Percentage Yield of Methanol Leaf Extract of *Combretum hypopilinum*

One thousand grams of the powdered leaf was extracted using 7.5 litres of methanol.

Weight of the powdered crude leaf = 1,000 g

Weight of the extract obtained = 126.24 g

Percentage yield = $\frac{\text{Weight of the extract}}{\text{Weight of the crude leaf}} \times 100$

$$= \frac{126.24}{1,000} \times 100$$

$$= 12.62 \%$$

Appendix 2: Effect of Methanol Leaf Extract of *Combretum hypopilinum* on Castor Oil-Induced Enteropooling in Mice

Treatments (mg/kg, p.o)	volume of intestinal content (ml)	Weight of intestinal content (g)	% reduction in volume of intestinal content	% reduction in weight of intestinal content
DW (10ml/kg)	0.60 ± 0.04	0.76 ± 0.07	-	-
MECH (250)	0.56 ± 0.07	0.72 ± 0.07	6.67	5.26
MECH (500)	0.36 ± 0.04*	0.62 ± 0.04	40.00	18.42
MECH (1,000)	0.29 ± 0.06**	0.52 ± 0.09	51.67	31.58
LOP (5)	0.23 ± 0.04***	0.42 ± 0.09*	61.67	44.74

Data are presented as Mean ± SEM; * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ versus distilled water group (One Way ANOVA followed by Dunnett's post hoc test), DW= distilled water, MECH= Methanol leaf extract of *C. hypopilinum*, LOP= Loperamide, n=5

Appendix 3: Effect of Methanol Leaf Extract of *Combretum hypopilinum* on Intestinal Transit Test in Mice

Treatments (mg/kg, p.o)	Length of small Intestine (cm)	Distance travelled by charcoal (cm)	Peristaltic index (%)	% inhibition of charcoal movement
DW 10ml/kg	44.00 ± 1.70	33.20 ± 2.58	75.46	-
MECH (250)	40.60 ± 0.69	24.20 ± 2.89	59.61	27.11
MECH (500)	43.80 ± 1.36	20.40 ± 2.38*	46.58	38.55
MECH (1,000)	42.00 ± 0.84	08.50 ± 1.39**	20.24	74.40
LOP (5)	39.20 ± 1.16	11.40 ± 2.89**	29.08	65.66

Data are presented as Mean ± SEM; * $p \leq 0.05$ and ** $p \leq 0.001$, versus distilled water group (One Way ANOVA followed by Dunnett's post hoc test), DW= Distilled Water, MECH= Methanol leaf extract of *C. hypopilinum*, LOP= Loperamide, n=5

Appendix 4: Effect of Naloxone on the Antidiarrhoeal Activity of Methanol Leaf Extract of *Combretum hypopilinum* in the Intestinal Transit in Mice

Pretreatments (mg/kg, <i>p.o</i>)	Treatments (mg/kg, <i>p.o</i>)	Mean length of small Intestine (cm)	Distance travelled by charcoal (cm)	Peristaltic index (%)	% inhibition of charcoal movement
DW 10ml/kg	DW 10ml/kg	45.20 ± 1.66	38.00 ± 2.21	84.07	-
DW 10ml/kg	MECH 1,000	43.60 ± 1.29	12.00 ± 4.10*	27.52	68.42
DW 10ml/kg	NAL 2 (<i>s.c</i>)	40.60 ± 1.12	34.40 ± 1.63 ^{##a}	84.72	9.47
DW 10ml/kg	LOP 5	40.00 ± 0.84	11.40 ± 2.89*	28.50	70.00
NAL 2 (<i>s.c</i>)	MECH (1,000)	40.20 ± 0.66	32.00 ± 2.28 ^{#a}	79.60	15.79
NAL 2 (<i>s.c</i>)	LOP 5	41.00 ± 0.55	24.60 ± 3.51	60.00	35.26

Data are presented as Mean ± SEM; * $p \leq 0.001$ compared to distilled water group, [#] $p \leq 0.01$ and ^{##} $p \leq 0.001$ compared to MECH, ^a $p \leq 0.001$ compared to LOP (One Way ANOVA followed by Bonferroni's post hoc test), DW= Distilled Water, MECH= Methanol leaf extract of *C. hypopilinum*, NAL= Naloxone, LOP= Loperamide, n=5

Appendix 5: Effect of Prazosin on the Antidiarrhoeal Activity of Methanol Leaf Extract of *Combretum hypopilinum* in the Intestinal Transit in Mice

Pretreatments (mg/kg, p.o)	Treatments (mg/kg, p.o)	Length of small Intestine (cm)	Distance travelled by charcoal (cm)	Peristaltic index (%)	% inhibition of charcoal movement
DW 10ml/kg	DW 10ml/kg	45.20 ± 1.66	38.00 ± 2.21	84.07	-
DW 10ml/kg	MECH 1,000	43.60 ± 1.29	12.00 ± 4.10*	27.52	68.42
DW 10ml/kg	PRA 1 (s.c)	46.00 ± 1.79	39.00 ± 1.38 ^{##} ^a	84.78	-2.63
DW 10ml/kg	MOR 10 (s.c)	43.60 ± 0.51	18.80 ± 1.98*	43.12	50.53
PRA 1 (s.c)	MECH 1,000	45.80 ± 1.11	27.80 ± 2.46 [#]	60.70	26.84
PRA 1 (s.c)	MOR 10 (s.c)	44.40 ± 1.21	18.00 ± 0.89*	40.54	52.63

Data are presented as Mean ± SEM; * $p \leq 0.001$ compared to distilled water group, [#] $p \leq 0.01$ and ^{##} $p \leq 0.001$ compared to MECH, ^a $p \leq 0.001$ compared to MOR, ¹ $p \leq 0.01$ and ² $p \leq 0.001$ compared to PRA (One Way ANOVA followed by Bonferroni's post hoc test), DW= Distilled Water, MECH= Methanol leaf extract of *C. hypopilinum*, PRA= Prazosin, MOR= Morphine, n=5

Appendix 6: Effect of Yohimbine on the Antidiarrhoeal Activity of Methanol Leaf Extract of *Combretum hypopilinum* in the Intestinal Transit in Mice

Pretreatments (mg/kg, p.o)	Treatments (mg/kg, p.o)	Length of small Intestine (cm)	Distance travelled by charcoal (cm)	Peristaltic index (%)	% inhibition of charcoal movement
DW 10ml/kg	DW 10ml/kg	45.20 ± 1.66	38.00 ± 2.21	84.07	-
DW 10ml/kg	MECH 1,000	43.60 ± 1.29	12.00 ± 4.10***	27.52	68.42
DW 10ml/kg	YOH 2 (s.c)	47.60 ± 0.98	35.80 ± 4.53 ^{#a}	75.21	5.79
DW 10ml/kg	MOR 10 (s.c)	43.60 ± 0.51	18.80 ± 1.98*	43.11	50.53
YOH 2 (s.c)	MECH 1,000	49.40 ± 0.81	17.20 ± 2.60** ¹	34.82	54.74
YOH 2 (s.c)	MOR 10 (s.c)	45.80 ± 1.11	22.60 ± 4.84	49.34	40.53

Data are presented as Mean ± SEM; * $p \leq 0.01$ and ** $p \leq 0.001$ compared to distilled water group, [#] $p \leq 0.01$ compared to MECH, ^a $p \leq 0.05$ compared to MOR, ¹ $p \leq 0.05$ compared to YOH (One Way ANOVA followed by Bonferroni's post hoc test), DW= Distilled Water, MECH= Methanol leaf extract of *C. hypopilinum*, YOH= Yohimbine, MOR= Morphine, n=5

Appendix 7: Effect of Propranolol on the Antidiarrhoeal Activity of Methanol Leaf Extract of *Combretum hypopilinum* in the Intestinal Transit in Mice

Pretreatments (mg/kg, <i>p.o</i>)	Treatments (mg/kg, <i>p.o</i>)	Mean length of small Intestine (cm)	Distance travelled by charcoal (cm)	Peristaltic index (%)	% inhibition of charcoal movement
DW 10ml/kg	DW 10ml/kg	45.20 ± 1.66	38.00 ± 2.21	84.07	-
DW 10ml/kg	MECH 1,000	43.60 ± 1.29	12.00 ± 4.10**	27.52	68.42
DW 10ml/kg	PRO 1 (<i>i.p</i>)	44.20 ± 1.16	25.80 ± 5.26	58.37	32.11
DW 10ml/kg	MOR 10 (<i>s.c</i>)	43.60 ± 1.14	18.80 ± 1.98*	43.12	50.53
PRO 1 (<i>i.p</i>)	MECH 1,000	47.80 ± 1.28	25.40 ± 1.94	53.14	33.16
PRO 1 (<i>i.p</i>)	MOR 10 (<i>s.c</i>)	44.20 ± 0.59	24.40 ± 3.72	55.20	35.79

Data are presented as Mean ± SEM; * $p \leq 0.01$ and ** $p \leq 0.001$, compared to distilled water group, (One way ANOVA followed by Bonferroni's Post Hoc test), DW= Distilled Water, MECH= Methanol leaf extract of *C. hypopilinum*, PRO= Propranolol, MOR= Morphine, n=5

Appendix 8: Effect of Pilocarpine on the Antidiarrhoeal Activity of Methanol Leaf Extract of *Combretum hypopilinum* in the Intestinal Transit in Mice

Pretreatments (mg/kg, <i>p.o</i>)	Treatments (mg/kg, <i>p.o</i>)	Length of small Intestine (cm)	Distance travelled by charcoal (cm)	Peristaltic index (%)	% inhibition of charcoal movement
DW 10ml/kg	DW 10ml/kg	45.20 ± 1.66	38.00 ± 2.21	84.07	-
DW 10ml/kg	MECH 1,000	43.60 ± 1.29	12.00 ± 4.10***	27.52	68.42
DW 10ml/kg	PIL 1 (<i>s.c</i>)	43.20 ± 1.83	30.00 ± 2.66	69.44	21.05
DW 10ml/kg	ATR 5	47.20 ± 1.80	16.6 ± 1.29*	35.17	56.32
PIL 1 (<i>s.c</i>)	MECH 1,000	43.80 ± 1.66	14.40 ± 6.22**	32.88	62.11
PIL 1 (<i>s.c</i>)	ATR 5	43.50 ± 1.19	29.25 ± 7.80	67.24	23.03

Data are presented as Mean ± SEM. * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ compared to distilled water group (One Way ANOVA followed by Bonferroni's post hoc test), DW= Distilled Water, MECH= Methanol leaf extract of *C. hypopilinum*, PIL= Pilocarpine, ATR= Atropine Sulphate, n=5

Appendix 9: Effect of Isosorbide Dinitrate on the Antidiarrhoeal Activity of Methanol Leaf Extract of *Combretum hypopilinum* in the Intestinal Transit in Mice

Pretreatments (mg/kg, p.o)	Treatments (mg/kg, p.o)	Mean length of small Intestine (cm)	Distance travelled by charcoal (cm)	Peristaltic index (%)	% inhibition of charcoal movement
DW 10ml/kg	DW 10ml/kg	45.20 ± 1.66	38.00 ± 2.21	84.07	-
DW 10ml/kg	MECH 1,000	43.60 ± 1.29	12.00 ± 4.10**	27.52	68.42
DW 10ml/kg	ISD 150	40.80 ± 0.86	35.00 ± 2.00	85.78	7.89
DW 10ml/kg	MOR 10 (s.c)	43.60 ± 0.51	18.80 ± 1.98**	41.28	52.63
ISD 150	MECH 1,000	43.60 ± 1.78	22.60 ± 0.93* ¹	51.83	40.52
ISD 150	MOR 10 (s.c)	40.00 ± 1.86	18.60 ± 1.24** ²	46.50	51.05

Data are presented as Mean ± SEM; * $p \leq 0.01$ and ** $p \leq 0.001$, compared to distilled water group, # $p \leq 0.001$ compared to MECH, ^a $p \leq 0.05$ compared to MOR, ¹ $p \leq 0.05$ and ² $p \leq 0.01$ compared to ISD (One Way ANOVA followed by Bonferroni's post hoc test), DW= Distilled Water, MECH= Methanol leaf extract of *C. hypopilinum*, ISD= Isosorbide Dinitrate, MOR= Morphine, n=5

Appendix 10: Effect of 28-Days Daily Oral Administration of Methanol Leaf Extract of *Combretum hypopilinum* on Weekly Body Weights of Rats

Treatments (mg/kg, p.o)	Mean body weights (g)				
	Week 0	Week 1	Week 2	Week 3	Week 4
DW (1 ml/kg)	145.83±6.54	163.00±6.79	175.17±5.22	182.17±5.01	190.67±5.67
MECH 250	145.00±6.28	141.17±5.47	154.67±8.88	161.00±9.10	167.50±9.17
MECH 500	146.00±5.54	134.83±6.47*	144.50±6.82*	153.67±7.50	162.17±7.66
MECH 1,000	145.00±6.40	151.50±4.28	166.00±6.90	180.00±9.07	192.17±9.46

Data are presented as Mean ± SEM; * $p \leq 0.05$ compared to control group (Repeated measure ANOVA followed by Dunnet's Post Hoc test), DW= Distilled Water, MECH= Methanol leaf extract of *C. hypopilinum*, n=6

Appendix 11: Effect of 28-Days Daily Oral Administration of Methanol Leaf Extract of *Combretum hypopilinum* on Relative Organ Weights of Rats

Treatments (mg/kg)	Mean relative organ to body weight ratio (%)					
	Kidney	Liver	Heart	Lung	Stomach	Small Intestine
DW (1 ml/kg)	0.99 ± 0.02	3.96 ± 0.24	0.44 ± 0.10	1.26 ± 0.18	1.23 ± 0.18	2.36±0.04
MECH (250)	0.77 ± 0.04	3.23 ± 0.08	0.22 ± 0.11	1.12 ± 0.13	1.20 ± 0.28	2.32±0.01
MECH (500)	0.72 ± 0.08*	2.35 ± 1.19	0.37 ± 0.03	1.12 ± 0.21	1.34 ± 0.21	2.35±0.03
MECH (1000)	0.6 ± 0.05**	3.71 ± 0.16	0.29 ± 0.01	0.78 ± 0.06	1.42 ± 0.17	2.31±0.02

Data are presented as Mean ± SEM; * $p \leq 0.05$ and ** $p \leq 0.01$ compared to control group (One way ANOVA followed by Dunnet's Post Hoc test), DW= distilled water, MECH= Methanol leaf extract of *C. hypopilinum*, n=3

Appendix 12: Effect of 28 Days Daily Oral Administration of Methanol Leaf Extract of *Combretum hypopilinum* on Lipid Profile of Rats

Treatment (mg/kg)	CHOL (mg/dl)	TRIG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
DW (1 ml/kg)	134.87 ± 11.19	95.70 ± 7.49	64.75 ± 10.05	51.05 ± 14.16
MECH (250)	94.88 ± 14.32	110.45 ± 12.26	23.70 ± 5.31**	49.10 ± 15.21
MECH (500)	98.45 ± 18.33	105.97 ± 9.28	20.53 ± 3.10***	56.55 ± 14.54
MECH (1,000)	132.13 ± 13.77	133.28 ± 21.14	38.38 ± 4.17*	67.07 ± 10.91

Data are presented as Mean ± SEM; * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ compared to control group (One way ANOVA followed by Dunnet's Post Hoc test), DW= distilled water, MECH= Methanol leaf extract of *C. hypopilinum*, LDL= Low Density Lipoprotein, HDL= High Density Lipoprotein, n=6

Appendix 13: Journal publications

Ahmad, M. H., Zezi, A. U., Anafi, S. B., Alhassan, Z., Mohammed, M., and Danraka, R. N. (2020). Mechanisms of Antidiarrhoeal Activity of Methanol Leaf Extract of *Combretum hypopilinum* Diels (Combretaceae): Involvement of Opioidergic and (α 1 and β)-Adrenergic Pathways. *Journal of Ethnopharmacology*, 113750. <https://doi.org/10.1016/j.jep.2020.113750>

Ahmad, M. H., Zezi, A. U., Anafi, S. B., Alhassan, Z., Mohammed, M., and Danraka, R. N. (2021). Data on the Mechanisms of Antidiarrhoeal Activity of Methanol Leaf Extract of *Combretum hypopilinum* Diels (Combretaceae): Involvement of Opioidergic and (α 1 and β)-Adrenergic Pathways. *Data in Brief*. 107155. <https://doi.org/10.1016/j.dib.2021.107155>

Ahmad, M. H., Zezi, A. U., Anafi, S. B., Mohammed, M., Danraka, R. N., and Alhassan, Z. (2020). Evaluation of antidiarrhoeal activity of methanol extract of *Combretum hypopilinum* Diels (Combretaceae) leaves in mice. *Advance Pharmaceutical Journal*, (5)2: 54-61.

Ahmad, M. H., Zezi, A. U., Anafi, S. B., Mohammed, M. Acute and Sub-acute Oral Toxicity Assessment of Hydromethanolic Leaves Extract of *Combretum hypopilinum* Diels (Combretaceae) in Wistar Rats. **Under review** in Toxicological research.

Appendix 14: Conference papers

Ahmad, M. H., Zezi, A. U., Anafi, S. B., Muhammad, S., and Mohammed, M. (2020). Evaluation of Antidiarrhoeal Activity of Methanol Extract of *Combretum hypopilinum* Diels (Combretaceae) Leaves in Mice. Presented at 18th NAPA National Scientific Conference: Virtual Edition, University of Nigeria Nsukka, Enugu, Nigeria, 24th to 26th August, 2020, Paper presentation. Page, 227.

Ahmad, M. H., Zezi, A. U., Anafi, S. B. (2020). Sub-acute Toxicity Study of Methanol Leaf Extract of *Combretum hypopilinum* Diels (Combretaceae) in Rats, presented at 9th International Conference/AGM of West African Society of Toxicology, Lagos, Nigeria, 12th to 15th February, 2020, Paper presentation. Page 38

Ahmad, M. H., Zezi, A. U., Anafi, S. B., and Mohammed, M. (2021). Elucidation of the Possible Mechanisms of Antidiarrhoeal Activity of Methanol Leaf Extract of *Combretum hypopilinum* Diels (Combretaceae), presented at the University of Lagos, Faculty of Pharmacy 1st Annual Scientific Conference, 10th to 11th March, 2021, Paper presentation. Page 41.