EVALUATION OF ANTIDIARRHEAL ACTIVITY OF AQUEOUS EXTRACT OF THE PULP AND SEEDS OF THE FRUIT OF *OPUNTIA FICUS-INDICA (CACTACEAE)*

BELACHEW BORANTO (B. Pharm)

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This is to certify that the thesis prepared by Belachew Boranto, entitled: Evaluation of antidiarrheal activity of aqueous extract of the pulp and seeds of the fruit of *Opuntia ficus-indica (cactaceae)* in rats and submitted in partial fulfillment of the requirements for the Degree of Master of Sciences in Pharmacology complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Name	Signature	Date
Examiner (internal): Wondmagegn Tamiru (MSc)	_____	_____
Examiner (external): Tadesse Eguale (PhD)	_____	_____
Adviser: Teshome Nedi (PhD)	_____	_____
Adviser: Workineh Shibeshi (PhD)	_____	_____

_____

Chair of Department or Graduate Program Coordinator

Abstract

Evaluation of antidiarrheal activity of aqueous extract of the Pulp and Seeds of the fruit of *Opuntia ficus-indica (Cactaceae) in Rats.*

Belachew Boranto

Addis Ababa University, 2018

The fruits of *Opuntia ficus-indica* (L) Mill is one of medicinal plants claimed to have antidiarrheal potential, but with no scientific evidence. This study is aimed at investigating the antidiarrheal activity of aqueous extract of the pulp and seeds of the fruit of *Opuntia ficus-indica* in rats. The antidiarrheal activity was evaluated using castor oil induced diarrheal model, anti-enteropooling and charcoal meal test in rats of either sex. In this work, the test groups received a graded dose (100, 200 and 400 mg/kg) of aqueous extract of the pulp and seeds of the fruit of *Opuntia ficus-indica*, whereas positive controls received either Loperamide (2.5 mg/kg) or Atropine (3 mg/kg) and negative controls received vehicle (10 ml/kg). Pretreatment of rats at the doses of 100, 200 and 400 mg/kg of the pulp and seed of *Opuntia ficus-indica* caused a significant reduction in the frequency of total number of stools and wet stooling as well as in delaying the onset of diarrhea as compared to the controls. Both extracts showed a dose-dependent inhibition (R^2=0.908 for pulp and R^2 =0.994 for seed) on the castor oil induced intestinal fluid accumulation. The extracts also showed significant ($p<0.001$) inhibition in intestinal motility in both normal and castor oil induced models. In conclusion, the results obtained in this study suggest that both pulp and seed extracts of the fruits of *Opuntia ficus-inidca* have beneficial effect in controlling diarrhea in experimental rats and this provides a support for the traditional use of the plant as an antidiarrheal remedy.

Key words: Antidiarrheal activity, Castor oil induced diarrhea, Gastrointestinal transit, Anti-enteropooling, *Opuntia ficus-inidca*

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Acronyms and Abbreviations

CaCCs	Calcium activated Chloride Channel
cAMP	Cyclic Adenosine Monophosphate
CCBs	Calcium Channel Blockers
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
cGMP	Cyclic Guanosine Monophosphate
EAEC	Enteroaggregative Escherichia Coli
EHEC	Enterohemorrhagic Escherichia Coli
ETEC	Enterotoxigenic Escherichia Coli
FAO	Food and Agriculture Organization of the United Nation
IBDs	Inflammatory Bowel Diseases
IBS	Irritable Bowel Syndrome
IL	Interleukin
NO	Nitric Oxide
OECD	Organization for Economic Cooperation Development
ORS	Oral Rehydration Solution
SNNPR	Southern Nations Nationalities Peoples Regional State
WHO	World Health Organization

1. Introduction

1.1 Definition of Diarrhea

Diarrhea is the passage of loose or watery stools at least 3 times in a 24- hour period (Mekonnen *et al.,* 2018). It is not a disease by itself; but one of most common clinical signs of gastrointestinal tract disease and involves both an increase in the motility of the gastrointestinal tract along with increased secretions and decrease in the absorption of fluid and thus a loss of electrolytes and water results in severe dehydration and death (Mehesare *et al.,* 2017).

1.2 Classification of Diarrhea

To help guide treatment recommendations, diarrhea can be classified by suspected or proven etiology, duration, and pathophysiologic mechanism (Carlson *et al.,* 2016). Diarrhea can be associated with viral, bacterial, fungal infection, food poisoning and other disease conditions (Mehesare *et al.,* 2017). Based on the duration, diarrhea can be classified into three categories including acute diarrhea with presence of three or more watery stools within 24-hours, dysentery with bloody diarrhea and mucous presence, persistent diarrhea when episodes of diarrhea last more than 14 days, while chronic diarrhea when the episode lasts for more than a month (Caramia *et al.,* 2015; Mekonnen *et al.,* 2018). Based on the pathophysiologic mechanism diarrhea may fall into one or more of the following clinical groups: secretory, osmotic, inflammation, or motor (Carlson *et al.,* 2016). The details of each mechanism are described under the pathogenesis of diarrheal disease.

1.3 Epidemiology of Diarrhea

Diarrheal diseases have been a major health problem throughout history. In the past, diarrheal diseases were often fatal and disease outbreaks spread quickly, affecting large populations (Thiagarajah *et al.*, 2015). Today, despite the success of interventions such as oral and intravenous rehydration therapy, diarrheal diseases remains one of the leading causes of morbidity and mortality in the world and are responsible for the death of millions of people each year. According to the latest Global Burden of Disease Study, about 2.39 billion of diarrheal cases occurred globally and an estimated 1.31 million deaths annually (Troeger *et al.*, 2017), with higher incidence and case-fatality ratios in lower and middle income countries such as Africa and south-east Asia (Mokomane *et al.*, 2018). Diarrhea remains the second leading cause of infant mortality after pneumonia in children under the age of five (Alambo, 2015) and is responsible for an estimated death of 0.53 million children every year (Vos *et al*., 2016; Liu *et al.*, 2016), most deaths being occurred among children less than 2 years of age (Carvajal-Vélez *et al.*, 2016; Mokomane *et al.*, 2018). In African countries including Ethiopia, each child on average suffers from five episodes of diarrhea per year while the two weeks prevalence ranges from 10 to 40% in different parts of Ethiopia (Alambo, 2015).

1.4 Etiology of Diarrhea

Diarrheal disease can be either infectious or non-infectious in nature with infection pathogenesis responsible for the major total episode worldwide. In infectious diarrhea, the potential causative pathogens include enterotoxin-producing bacteria, such as *Vibrio Cholerae* and *Enterotoxigenic Escherichia coli*; viruses, such as *rotavirus*; enteroinvasive bacteria, such as *Shigella* and

Salmonella; and parasites, such as *Giardia*, *Entamoeba histolytica* and *Cryptosporidium parvum* (Navaneethan & Giannella, 2011; Thiagarajah *et al.,* 2015).

Diarrhea is classified as noninfectious when symptoms worsen or become chronic in the absence of an identifiable infectious organism (virus, bacterium, protozoan) and can occur acutely due to medication and food intolerance or chronically due to primary gastrointestinal (GI) disease, such as inflammatory bowel disease (Carlson *et al.*, 2016). Severe secretory diarrhea is also caused by rare congenital disorders, such as microvillus inclusion disease, familial diarrhea syndrome and tufting enteropathy, as well as by peptide-secreting neuroendocrine tumors (Thiagarajah *et al.*, 2015). Many noninfectious causes of diarrhea are prominent in developed countries; however, infectious causes of diarrhea still represent a large proportion of the disease burden in developed countries (Thiagarajah *et al*., 2015).

1.5 Pathogenesis of Diarrhea

Fluid secretion in the intestine is a normal physiologic process that serves to flush mucus into the lumen from the crypts and provides an aqueous luminal environment required for digestive process and gastrointestinal transit (Moeser & Blikslager, 2007). Under normal physiological conditions, approximately 8 liters of fluids (2 liter of ingested fluids and 6 liter from salivary, gastric, biliary, and pancreatic secretions) reach the upper small bowel and most of this fluid is reabsorbed before reaching the distal small bowel so that only about 1 liter of fluid enters the colon, which in turn reabsorbs almost all of this fluid leaving usually less than 200 ml to be excreted in the stool. The colon has the capacity to reabsorb up to a maximum of 3–4 liters of fluid and thus to salvage much of the fluid that might be lost in small intestinal malabsorptive conditions (Navaneethan & Giannella, 2011).

The movement of fluid between the intestinal lumen and blood is driven by the active transport of ions, mainly Na^+, Cl^-, HCO_3^-, and K^+, and solutes, mainly glucose (Thiagarajah *et al.,* 2015). Fluid absorption or secretion involves the coordinated activity of membrane transporters located on the apical (lumen-facing) and basolateral (circulation-facing) epithelial membranes (Thiagarajah & Verkman, 2012).

In addition to the absorptive and secretary function of the intestine, motor functions also play a key role in facilitating digestion and absorption of fluids and nutrients (Thiagarajah *et al.,* 2015). Synchronized migrating motor complexes normally occur during fasting in the stomach and small bowel with increased contractions following feeding with the total small bowel transit time of approximately 3 hour for the food to reach the colon (Kerlin *et al.,* 1982). This normal gut physiology relies on a functioning enteric nervous system that coordinates the gut ion transport and motor activity and when any of these pathways are disrupted, diarrhea can result (Sisson, 2011).

Despite different pathophysiological changes in different types of diarrhea, there are four major mechanisms responsible for pathophysiology in electrolyte and water transport that is, osmosis, active secretion, exudation or inflammation, and altered motility (Bahekar & Kale, 2015). However, for understanding the pathophysiology of diarrhea, diarrheal syndromes are classified into secretory, osmotic, inflammatory, iatrogenic, and functional diarrhea (Navaneethan & Giannella, 2011; Thiagarajah *et al.,* 2015). Most etiologies have a complex pathophysiology involving one or more of these mentioned mechanisms as described below.

Secretory Diarrhea

Secretory diarrhea occurs when there is an increase in the amount of fluid being drawn into the lumen of the bowel such that the ability of the intestines to reabsorb is overwhelmed (Sisson, 2011). Typically, infectious agents such as *Vibrio cholerae*, *E. coli*, *Campylobacter jejuni*, *Salmonella*, *Shigella,* and *Clostridium difficile* are the cause of secretory diarrhea but any substance that causes fluid to be pulled into the bowel can be the culprit (Sisson, 2011).

The basic pathophysiology of secretory diarrhea involves either net secretion of ions (chloride or bicarbonate) or inhibition of net sodium absorption. Most causes of secretory diarrhea alter the second messenger systems through alteration in cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), or intracellular calcium-regulated ion transport pathways which lead to the cystic fibrosis transmembrane conductance regulator (CFTR) mediated Cl^- secretion and inhibition of small intestinal-coupled Na–Cl transport (Navaneethan & Giannella, 2011). Activation of epithelial inflammatory signaling pathways such as nuclear factor kappa-light chain-enhancer of activated B cells (NF-κB) results in Ca^{2+} or cyclic nucleotide signaling and stimulation of Cl^-secretion or inhibition of Na^+ absorption (Thiagarajah *et al.,* 2015).

The driving force for intestinal ion secretion can arise from the gut lumen as with infectious diarrhea (enterotoxins), from the sub epithelial space (inflammatory mediators), or from the systemic circulation (peptide hormones produced from endocrine tumors) (Navaneethan & Giannella, 2011). Infectious agents cause secretory diarrhea by adhering to the mucosa of the small intestine and secret enterotoxins that disrupts the absorptive/ secretary process of enterocyte through increasing cAMP, cGMP, or intracellular calcium concentration which inhibit Na^+–H^+ exchange and stimulate Cl^- secretion in the small intestine producing active secretion without

causing significant acute inflammation or mucosal destruction (Navaneethan & Giannella, 2011). The release of inflammatory mediators such as tumor necrosis factor (TNF) and IL-6 by activated T cells and neutrophils, which causes degranulation of mucosal mast cells and release of histamine and prostaglandins, can also stimulate Cl^- secretion leading to secretory diarrhea (Thiagarajah *et al.,* 2015).

Non-infectious secretagogues are also responsible for causing secretory diarrhea by stimulating intestinal secretion and include chemicals produced by certain types of cancer such as vasoactive intestinal peptides produced by pancreatic islet tumors, serotonin, bradykinin and substance P which are elaborated by carcinoma tumors, prostaglandins produced in patients with bowel inflammation and substances not well absorbed such as fatty acids and bile acid (Sisson, 2011; Navaneethan & Giannella, 2011).

The congenital absence or alterations in the numerous transporters that maintain the constant flux of the ions and water can also result in secretory diarrhea. According to Navaneethan & Giannella (2011), rare congenital syndromes are caused by the absence of a specific transport molecule, such as congenital chloride diarrhea; due to defect in brush border Cl/HCO_3 exchange in the ileum and the colon and hence impaired absorption of chloride, congenital sodium diarrhea; results from a defect in Na–H exchange in the small bowel and congenital bile acid diarrhea; results from a congenital defect in Na–bile acid absorption in the colon. Persons with secretory diarrhea will typically have stool volume of more than one liter daily, with neutral pH and have no change in the amount of stool produced with fasting (Sisson, 2011).

Osmotic Diarrhea

Osmotic diarrhea occurs when a poorly absorbed substance retains intestinal fluids and leads to a flux of water and electrolytes into the lumen as the gut adjusts to the osmolality of the plasma (Carlson *et al.,* 2016). The causes of this type of osmotic diarrhea are varied but can be broken down into decreased enzymatic availability (lactose intolerance), a genetic abnormality that decreases or eliminates the ability of the body to absorb certain nutrients (celiac sprue), sugars that are poorly absorbed (sorbitol, mannitol or lactose), "laxatives, magnesium containing antacids, amebiasis and antibiotic administration" as well as malabsorption of certain fats (Sisson, 2011).

Other causes have more to do with changes within the bowel that decrease the ability to reabsorb fluid and nutrients as the stool is propelled through the lumen. Protein-calorie malnutrition that causes "reversible atrophy of the villi and brush border", resection of parts of the bowel, especially the terminal ileum that mechanically decrease the body's ability to absorb due to decreased length of intestine available as well as inflammation of the bowel due to infection or disease processes (Crohn's disease) can be another cause of osmotic diarrhea (Sisson, 2011).

Inflammatory Diarrhea

Inflammation is the body's first line of defense against infection and hazardous stimuli with injury or infection in the GIT, resulting in the activation of neutrophils and macrophages (lwalewa e*t al*., 2007). Once activated, the immune cell (e.g. macrophage and neutrophils) assist with the killing of pathogenic microorganisms and or the removal of harmful and cell debris (Guton & Hall, 2006), through the release of numerous pro-inflammatory cytokines (tumour necrosis factor [TNF]-α and interleukin [IL] - β1-, IL-3, IL-6); chemokines and chemoattractants (IL-8 and monocyte chemoattractant protein [MCP]-1) (Conforti *et al.,* 2008). While inflammation process is beneficial

to the body as it removes the insulting cause, the large recruitment and activation of macrophages can induce changes in gut motility, neuronal functionality, and hydro electrolyte movement with resultant diarrhea (Gelberg, 2014). Infectious enteric pathogens such as *Clostridium difficile* (Thiagarajah *et al*., 2015) elicit inflammatory cascade and mediators to manifest diarrhea (Guttman & Finlay, 2009). Inflammatory bowel disease (IBD) is one of the most common and important causes of inflammatory diarrhea.

Functional/Motility Disorders

Under normal physiological condition of the intestines, solids and fluid are moved through the gut with peristaltic waves of the smooth muscles within the intestines. This movement is slow and may take 3-5 hours for the mass to move from the pyloric valve at the proximal point of the small intestine to the large intestine and as long as 24 hours for the mass to move from the small intestine to the rectum to be expelled during defecation (Sisson, 2011).

When the intestines are not functioning normally, motility can be either increased or decreased and both can lead to diarrhea. Increased motility can be caused by infectious agents, changes within the bowel by inflammatory bowel disease or by irritable bowel syndrome (IBS) (Sisson, 2011) and may decrease the time for the luminal contents to be in contact with the epithelium for absorption resulting in secretory diarrhea as observed in diabetes mellitus, amyloidosis, and postprandial diarrhea (Navaneethan & Giannella, 2011). Navaneethan & Giannella (2011), also proposed that disturbances in the neural control (from brain to visceral nerves) and the gut in the form of visceral nociception and abnormal motility mediated by changes in neurotransmitters like serotonin, cholecystokinin, and neurokinins contribute to diarrhea in patients with IBS.

Counter-intuitively, decreased motility can also lead to diarrhea. Typically, decreased mobility will lead to constipation, which, in its most severe form, can allow a large bolus of stool to form in the lower intestine and cause an impaction. However, the stool behind this bolus may become liquid again due to the action of bacteria on the stool and leaking around the bolus cause diarrhea (Sisson, 2011).

Iatrogenic /Drug-Induced Diarrhea

Diarrhea can also result following certain surgical procedures and usage of certain drugs. Although the exact pathophysiology remains unclear diarrhea can follow cholecystectomy in 5–10% of patients, some of which respond to treatment with bile salt-binding resins (Camilleri, 2004; Navaneethan & Giannella, 2011). There are a number of drugs that are known to cause diarrhea either as a side effect or as the desired effect of the drug. Some offending drug categories include: antibiotics, magnesium and phosphate containing antacids, osteoarthritis medications, cardiac medications, chemotherapeutic medications, Alzheimer's disease medications and oral hyperglycemic drugs (Sisson, 2011).

The mechanism of causing diarrhea can vary from drug to drug and may involve one or more of the above-mentioned mechanisms. Antibiotic use may alter the bacterial flora in the colon resulting in impaired colonic salvage of malabsorbed carbohydrates resulting in diarrhea. Some of the drugs like lactulose may cause osmotic diarrhea, while others may cause secretory diarrhea. Theophylline may increase intracellular cAMP and fluid secretion, while erythromycin interacts with the motilin receptors increasing the motility to cause diarrhea. Similarly chemotherapeutic drugs may cause diarrhea because of decreased rate of proliferation of the enterocytes (Navaneethan & Giannella, 2011).

1.6 Management of diarrhea

Knowledge of the underlying causative processes in diarrhea facilitates effective treatment and every effort should be made to identify and correct the specific causes of diarrhea (Dipiro *et al.*, 2008). Physiologically, diarrhea is considered beneficial to the GIT as it provides an important mechanism of flushing away harmful luminal substances (Valeur *et al.,* 2009). However, it becomes pathological when the loss of fluids and electrolytes exceeds the body's ability to replace the losses. In general, the treatment is aimed at preventing or reversing dehydration, shortening the duration of the illness and reducing the period that a person is infectious (Grimwood & Forbes, 2009). There are three main approaches to the treatment of infectious diarrhea. These are supportive therapy, antidiarrheal therapy and specific therapy.

1.6.1 Supportive therapy

Fluid and electrolyte replacement therapy

The major concern with diarrhea is dehydration, regardless of the cause of the diarrhea (Casburn-Jones & Farthing, 2004; Sisson, 2011). Maintaining adequate hydration or restoring hydration is therefore the first step in the treatment of diarrheal diseases, and particularly of acute-onset diarrhea (Soriano & Vaziri, 2011). Oral rehydration is an important aspect in the prevention of severe dehydration (Sisson, 2011; Soriano & Vaziri, 2011) and acidosis (Casburn-Jones & Farthing, 2004). In severe dehydration in infants and young children, intravenous fluids are advisable (Casburn-Jones & Farthing, 2004). Although ORS effectively treats dehydration when administered appropriately, it does not change fluid losses, diarrheal output or duration of illness.

1.6.2 Antidiarrheal therapy

While every effort should be made to identify and correct the specific causes of diarrhea, in many cases, causes that are specific and potentially treatable are often not identifiable and symptomatic therapy alone is commonly indicated (Manatsathit *et al,* 2002). There are two classes of antidiarrheal agents useful for reducing stool frequency, abdominal cramps and possibly stool volume (Casburn-Jones & Farthing, 2004). These are antimotility agents and antisecretory agents.

Antimotility Agents

Antimotility agents provide symptomatic relief and serve as useful adjuncts to antibiotic therapy in the treatment of diarrhea. The putative mechanism of action for antimotility drugs is increasing intestinal transit time and enhancing the potential for reabsorption of fluid and electrolytes (Casburn-Jones & Farthing, 2004; Thiagarajah *et al.,* 2015). They include loperamide, diphenoxylate, codeine, and other opiates (Manatsathit *et al,* 2002). Among all antimotility drugs, Loperamide and diphenoxylate are μ-opioid agonists that are widely used for mild, nonspecific diarrhea (Thiagarajah *et al.,* 2015). However, such antimotility agents are contraindicated in diarrhea caused by invasive pathogens because the induced intestinal stasis may enhance tissue invasion by the organisms or delay their clearance from the bowel (Manatsathit *et al,* 2002; Casburn-Jones & Farthing, 2004). Hence, bloody diarrhea with high fever, immunocompromised host and septicemic prone conditions with diarrhea should not be given this group of drugs. Antimotility agents are also not recommended for children and young infants due to the potential for central nervous system side effects and the theoretical possibility of respiratory depression (Casburn-Jones & Farthing, 2004).

Apart from opiates, calcium channel blockers (CCBs) such as verapamil and nifedipine were found to have an important role in the treatment of diarrhea resulting from microscopic colitis and diarrhea-predominant IBS by their prolongation of colonic motility (Soriano & Vaziri, 2011). A centrally acting α_2-adrenergic agonist clonidine is another non opioid agent that works mainly by an alteration of gut motility with an effect on intestinal transport. It stimulates sodium and chloride absorption and inhibits chloride secretion by interacting with its receptor on enterocytes and is mainly prescribed for patients with diabetic diarrhea, although it can also be used for secretory diarrhea of unknown etiology and diarrhea associated with opiate (Soriano & Vaziri, 2011).

Antisecretory Drugs

There are several drugs that were shown to have antisecretory effects in vitro and work by a variety of different mechanisms including inhibition of prostaglandins and effects on cAMP, calmodulin inhibition, inhibition of gut hormones and encephalinase inhibition of chloride channels (Manatsathit *et al,* 2002). Such drugs are capable of stimulating absorption directly and reduce secretion of water and electrolytes in gastrointestinal tract, decrease propulsion and increase contact time of intestinal content with mucosal surface, which in turn favors absorption. They include codeine, loperamide, diphenoxylate, lidamidine, bismuth subsalicylate, racecadotril and clonidine (Velázquez *et al.,* 2012). Serotonin receptor antagonists especially antagonists of 5-HT_3 receptor were found to inhibit extrinsic sensory neuron stimulation (which can inhibit nausea, vomiting, stomach pain and bloating) and reduce peristalsis and secretory reflex. Therefore, they were found to have a pivotal role in controlling motility and secretion of the gut (Manatsathit *et al,* 2002).

1.6.3 Specific therapy

Another medication groups that are aimed at reducing duration and severity of diarrhea are antimicrobials. The issue of antimicrobial therapy for self-limiting and non-infectious diarrhea is usually not encouraged to avoid development of drug resistance microbes. In the majority of episodes of acute residential diarrhea, the cause usually remains unknown because of the self-limiting nature of the disease and the difficulty and delay in identifying the pathogen (Kotwani *et al.,* 2012). Moreover, viral pathogens such as rotavirus accounts for 70% to 80% of all diarrheal episodes. Using antimicrobial agents for diarrhea with unknown etiology can, therefore, lead to inappropriate use of antimicrobials; which in turn increases the risk of side effects, higher costs and higher rates of antimicrobial resistance in community pathogens (Kotwani *et al.,* 2012). However, in cases of established infectious diarrhea with known pathogenic agents, specific therapeutic intervention using antimicrobial drugs targeting the causative microbes may be applied.

There is a large body of evidence to show that antimicrobial agents can reduce the severity and duration of some intestinal infections, especially in those bacteria and infections that produce acute watery diarrhea (Casburn-Jones & Farthing, 2004). The effectiveness of a particular antimicrobial agent, however, depends on the etiologic agent and its antibiotic sensitivity. It is proposed in the work of Sisson (2011) that antibiotics should be used selectively only in case of traveler's diarrhea (in which *E. coli* is the likely pathogen and treatment can shorten the duration of the illness), persistent diarrhea (suggestive of giardiasis), febrile diarrheal illnesses consistent with invasive disease and *clostridium difficile* infection.

1.7 Herbal medicine

Since the time immemorial, medicinal plants are part and parcel in the attempt of humans to combat diseases including diarrhea. According to WHO, over three quarters of the world population rely on plants and their extracts for healthcare needs (Agbor & Naidoo, 2015) and many countries in African, Asia and Latin America use traditional medicine (TM) to meet some of their primary health care needs (Deribe *et al.,* 2006). Furthermore, more than 25 % of the modern pharmaceutical drugs have botanical origins (Bodeker *et al*, 2003).

Natural products have a unique chemical diversity, which results in diversity in their biological activities and drug-like properties. Across the globe there are various herbal plants that possesses anti-diarrheal activity, which could be attributed to the presence of several bioactive compounds such as tannins, alkaloids, saponins, flavonoids, steroids, and terpenoids within the plants (Otshudi *et al.,* 2000; Komal *et al.,* 2013; Umer *et al.*, 2013).

In Ethiopia wide range of medicinal plants are claimed to have antidiarrheal effect although scientific evidence of most of them is lacking. *Zehneria scabra* (Tadesse *et al.,* 2014), *Lantana camara* linn (Mengistu *et al.,* 2015), *Ajuga remota Benth* (Yaecob *et al.,* 2016), *Croton macrostachyus* Hocsht (Degu *et al.,* 2016) and *Justicia schimperiana* (Mekonnen *et al.,* 2018) are just few of the many medicinal plants which are approved for their pharmacological effect.

1.8 Plant under Investigation

Opuntia ficus-indica (L) *Mill*, commonly known as prickly pear because of its rounded cladodes with fixed spines and small hair like prickles as shown in figure 1, belongs to the family *Cactaceae*

which contains about 130 genera and nearly 1500 species. It is widely distributed in Mexico and in all American hemispheres as well as in Africa and in the Mediterranean basin (Kaur *et al.,* 2012). In Ethiopia, one of the vernacular names of *Opuntia ficus-indica* is Papaldhotta (Konsigna), the name given by the community that uses this plant for the management of diarrhea (Addis *et al.,* 2013).

Fruits of *Opuntia ficus-indica* embraces several essential ingredients, such as taurine, amino acids, readily absorbable carbohydrates, minerals, vitamin C and soluble fibers (Ramadan & Mo¨rsel, 2003) and serves as an important source of nutrient and food. They can be consumed as fresh vegetables, added to casseroles, cooked, canned, or used in salads, syrups, alcoholic drinks, fruit juices and in cheese production (Ramadan & Mo¨rsel, 2003). The fruit, as well as cactus stem are used to prepare value-added products, such as jam, squash, wine, pickle, body lotions, shampoo, creams (Kaur *et al.,* 2012).

Apart from serving as an important source of nutrient and food, it has several medicinal and industrial uses. It has been used in traditional folk medicine because of its role in treating a number of diseases and conditions, including anti-inflammatory effects, hypoglycemic effects, inhibition of stomach ulceration, neuroprotective effects, and diuretic effects (Galati, 2001; Lee *et al.,* 2002; Kaur *et al.,* 2012). Through antioxidant actions it is also used for treating diabetes, burns, bronchial, asthma, arteriosclerosis and indigestion in many countries over the world (Galati, 2001; Lee *et al.,* 2002; Kaur *et al.,* 2012). Methanolic extract of the stem and fruits of *Opuntia ficus-indica* was shown to have better antibacterial activity against *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli* and *Vibrio Cholera (*Gnanakalai & Gopal, 2016). Moreover, the extract from *Opuntia ficus-indica* fruits on mouse ileal motility *in vitro* was able to exert a direct

antispasmodic effect on gastrointestinal motility, reducing the contractility of ileal longitudinal smooth muscle (Baldassano *et al.,* 2010).

In the sub-Saharan traditional medicine pharmacopeia, cactus fruits are given as antidiarrheal agents (Mostafa *et al.,* 2014; Seid *et al.,* 2011). In Ethiopia, using cactus fruit as an antidiarrheal agent is being practiced by the people of Konso which is located in SNNPR state (Addis *et al.,* 2013). However, the scientific basis for this usage has not been well established. The core aim of the present study is therefore, to evaluate the antidiarrheal activity of the pulp and seed of the fruit of cactus pear in rats using different models (*opuntia ficus-indica*).

Figure 1. Photograph of *Opuntia ficus-indica* (L) Mill (Captured by PI at the study site)

1.9 Rational for the Study

The need for medications that are used to treat patients with chronic gastrointestinal illnesses presenting with pain and inflammation is unmet and growing (Stark *et al.,* 2013). In spite of the overwhelming influences and the dependence on modern medicine and tremendous advances in synthetic drugs, large segments of the world population depend on drugs from plants (Agbor & Naidoo, 2015). As it is suggested in literatures; high cost of allopathic drugs, population rise, inadequate supply of drugs, side effects of several allopathic drugs, and development of resistance to currently available drugs for infectious diseases as well as cultural acceptability of the traditional system are reasons that have led to increased emphasis on the use of plants as sources of medicines for a wide variety of human ailments including diarrhea (Agbor & Naidoo, 2015; Tsobou *et al.,* 2016).

Although large population of the world are depending on traditional medicine for their primary health care, traditional medications and medical techniques are passed down verbally through generations and in most cases the effective doses and combinations proposed by traditional healers differ; as such the effective doses are not fully known, nor is the effectiveness, safety, toxicity, and variation of chemical composition between plant parts (Stark *et al.,* 2013).Therefore, proofing whether the active components contained within the medicinal plants are useful, safe and effective based on scientific evidence is the most important thing required to assure the use of the plant in the medical field. Natural products will undergo continual use toward meeting the urgent need to develop effective drugs, and they will play a leading role in the discovery of drugs for treating human diseases, especially critical diseases; medicinal plants with evidence based safety and efficacy being the most important source for developing the new lead compounds (Yuan *et al.,* 2016). Apart from acting as a baseline for the pharmaceutical industries, the scientific evidence

obtained from the research on the traditional medicine could also create a favorable condition for the development of safe and effective drugs to be used by the community with significant cost reductions.

Moreover, antimicrobial resistant strains have been spreading widely; posing a global threat to the currently available anti-infective agents including antidiarrheal agents. According to WHO (2014) antimicrobial resistance global report, there was decreased susceptibility of diarrhea causing pathogens such as *E.coli* (to 3rd generation cephalosporins, Fluoroquinolones), *Neisseria gonorrhoeae* (to 3rd generation cephalosporins) and *Shigella* species (to fluoroquinolones). Apart from these, majority of the existing drugs suffer from adverse effects like the induction of bronchospasm, vomiting (racecadotril), intestinal obstruction, constipation (Loperamide) (Pankaj, 2006) and dependency (Diphenoxylate) (Mehra *et al.*, 2013). In view of this, there is a necessity of strengthening research into culturally preserved medicinal plants to investigate alternative drugs from natural products.

2. Objectives

2.1 General Objective

- To investigate the antidiarrheal effect of crude aqueous extracts of pulp and seeds of the fruits of *Opuntia ficus-indica* (L) Mill.

2.2 Specific Objectives

- To test the acute oral toxicity of pulp and seeds of the fruits of *Opuntia ficu- indica* (L) Mill.
- To investigate the effect of aqueous extracts of pulp and seeds of the fruits of *Opuntia ficus-indica* (L) Mill on castor oil induced diarrhea in rats.
- To assess anti-enteropooling effect of aqueous extracts of pulp and seeds of the fruits of *Opuntia ficus-indica* (L) Mill on castor oil induced enteropooling in rats.
- To investigate the effect of aqueous extracts of pulp and seeds of the fruits of *Opuntia ficus-indica* (L) Mill on normal/castor oil induced gastrointestinal motility using charcoal meal test
- To screen the phytochemical constituents of pulp and seeds of the fruits of *Opuntia ficus-indica* (L) Mill.

3. Materials and Methods

3.1 Drugs and Chemicals

Castor oil (Remkaln General Trading P.LC., Amman Pharmaceutical Industries Co., Jordan) and activated charcoal (Lab. Reagent, India) were used during the experiment for induction of diarrhea and as a marker respectively. Standard drugs such as Loperamide (Remedica Cyprus, Limasol industrial estate) and Atropine sulfate injection (0.1 %) (Jeil Pharm.Co.,Ltd., Korea) were purchased from local medical stores and kept in appropriate storage conditions for use during the experiment. Chemical reagents such as Glacial acetic acid (Fisher Scientific, UK), Chloroform (Finkem Laboratory Reagent, India), Ammonia solution (Lobe chemi, India), Hydrochloric acid (HCl) and Sulphuric acid (H_2SO_4) (Park scientific Ltd, UK) were obtained from department of Pharmacology whereas Ferric chloride ($FeCl_3$), Mayer's reagent and Wagner's reagent were obtained from Department of Pharmacognosy, Addis Ababa University, Addis Ababa, Ethiopia and used in the study. Surgical hand gloves, distilled water (Ethiopian Pharmaceutical Manufacturing, Ethiopia) and Tween 80 (Atlas Chemical Industries Inc., UK) were also used during the experimentation.

3.2 Plant Materials

The ripe fruits of *Opuntia ficus-indica* (L) Mill (*Cactaceae*) was collected from Konso woreda, Segen Area peoples Zone of Southern Nations Nationalities Peoples Regional State (SNNPR) in the month of February, 2017. The identity of the plant was ascertained morphologically by a taxonomist at Addis Ababa University. A voucher specimen (AAUBB-2017001) was then deposited at the National Herbarium, College of Natural and Computational Sciences.

3.3 Experimental Animals

Adult normal Sprague Dawley rats of either sex (150-200g) were obtained from animal house of the department of Pharmacology and Clinical Pharmacy of the School of Pharmacy of Addis Ababa University. The animals were housed in polypropylene cages under standard environmental conditions on a 12h light–dark cycle with free access to pellet food and water ad libitum. Maintaining the laboratory under standard conditions, the animals were acclimatized for a week before beginning the actual experiment. All the protocols were conducted according to the guideline for the care and use of laboratory animals (Herling, 2016).

3.4 Extraction and Preparation of the Plant

After collecting the edible prickly pear fruits, they were peeled manually. Seeds were isolated from the pulp by pressing the whole edible pulp. Having washed with distilled water, the seeds were dried at room temperature, while the pulp sample was concentrated in a rotary evaporator (R3000; Buchi) under low pressure at 40^0C and then dried using a Lypholizer (Martin Christ, Germany). Dry matter of each fraction (pulp and seeds) were ground separately and passed through a 100-mesh sieve before analysis.

3.4.1 Preparation of Aqueous Extract of the Pulp Sample

A total of two hundred grams of the dried sample of the pulp was extracted through maceration technique. During the extraction process, one hundred gram of the sample was soaked in an Erlenmeyer flask with 1000ml of distilled water and placed at room temperature for 3 days with occasional shaking using orbital shaker. The mixture was then filtered through muslin cloth followed by Whatman No.1 filter paper, after standing for a couple of minutes. Marc from the

plant was then re-macerated twice using the same volume of solvent to exhaustively extract the material from the plant. The extract obtained was then dried using a Lypholizer. The freeze-dried extract of the plant was collected, weighed and percentage yield was calculated which was found to be 18.75%. The obtained powder was stored in a desiccator until it was reconstituted with distilled water for oral administration.

3.4.2 Preparation of Aqueous Extract of the Seed Sample

A total of two hundred grams of the coarse powder of the seed of the fruit of *Opuntia ficus-indica* was extracted through maceration technique. During the extraction process, one hundred gram of the coarse powder was soaked in an Erlenmeyer flask with 1000ml of distilled water and placed at room temperature for 3 days with occasional shaking using orbital shaker. The mixture was then filtered through muslin cloth followed by Whatman No.1 filter paper, after standing for a couple of minutes. Marc from the plant was then re-macerated twice using the same volume of solvent to exhaustively extract the material from the plant. The extract obtained was then dried using a Lyophilizer (freeze dryer). The freeze-dried extract of the plant was collected, weighed and percentage yield was calculated which was found to be 10.25%. The obtained powder was stored in a desiccator until it was reconstituted with distilled water for oral administration.

3.5 Acute Toxicity Test

Acute toxicity test was done based on the limit test recommendations of Organization for Economic Cooperation Development (OECD) 425 Guideline (OECD, 2008). First, two Sprague Dawley female rats were fasted for 4 hours and then loaded with 2000 mg/kg of the either extracts, orally. The rats were then observed for physical or behavioral changes within 24 hours strictly, with special attention during the first 4 hours. Since no death was observed for both samples within

24 hours, additional four rats for each samples were fasted for 4 hours and administered the same doses of the extract. The animals were observed continuously for 4 hours with 30 min interval and then for 14 consecutive days with an interval of 24 hours for the general signs and symptoms of toxicity such as food, water intake and mortality.

3.6 Grouping and Dosing

In all models, adult normal Sprague Dawley rats (150-200g) were weighed, labeled and randomly allotted into eight groups and were fasted for 18 hours but with free access to water before the test for antidiarrheal effect. Group I were used as negative control and were treated with 10 ml/kg distilled water. Group II, III and IV were given 100, 200 and 400mg/kg of aqueous extract of the pulp of the fruit of *Opuntia ficus-indica* orally respectively. Group V, VI and VII were given 100, 200 and 400mg/kg of aqueous extract of the seed of the fruit of *Ountia ficus-indica* orally respectively. Group VIII, was used as a positive control and treated with either Loperamide (2.5 mg/kg p.o) in case of castor oil induced diarrhea and anti-enteropooling models or Atropine sulphate (3mg/kg i.p) in case of normal or castor oil induced gastrointestinal transit models.

3.7 Determination of Antidiarrheal Activity

3.7.1 Effect of the aqueous extracts on castor oil-induced diarrhea

Anti-diarrheal activity of the preparation was evaluated using the castor oil-induced diarrheal model in rats (Yacob *et al.,* 2016; Oghenesuvwe *et al., 2018).* Fourty eight rats of either sex were weighed, labeled and randomly allotted into eight groups of six rats each and fasted for 18 hours prior to the test, but was allowed free access to water. After 1 hour of treatment with graded doses of either pulp or seed extract of *Opuntia ficus-indica*, 10 ml/kg normal saline or 2.5 mg/kg Loperamide, diarrhea was induced by administration of 1ml of castor oil orally to each rats. The rats were then housed individually in transparent metabolic cages, the bottom of which was lined with white sheet of paper for observation of the number and consistency of fecal droppings. The papers were changed every hour to make the fecal droppings visible for counting and to check stool consistency (were assigned as: normal stool = 1, semi-solid stool = 2 & watery stool = 3). The animals were observed for period of 6 hours, during which the onset of diarrhea, the number of both dry and wet stools excreted by the animals were recorded and compared with the control for assessing the antidiarrheal activity. The onset was measured as the time interval in minutes between the administration of castor oil and the appearance of the first diarrheal stool. The total number of diarrheal feces of the control group was considered 100 % and percent inhibition (PI) was calculated as follows;

$$\text{PI}= \frac{\text{Mean number of wet stools of (control group}-\text{treated group)}}{\text{Mean number of wet stools of control group}} \times 100$$

3.7.2 Effect of the aqueous extracts on castor oil-induced enteropooling

Intraluminal fluid accumulation was determined using the method used by Yacob *et al* (2016). Fourty eight rats of either sex were weighed, labeled and randomly allotted into eight groups of six rats each and fasted for 18 hours prior to the test, but was allowed free access to water. After 1 hour of treatment with graded doses of either pulp or seed extract of *Opuntia ficus-indica*, 10 ml/kg distilled water or 2.5 mg/kg Loperamide, diarrhea was induced by administration of 1ml of castor oil orally to each rat. One hour after treatment with castor oil, all rats were sacrificed by cervical dislocation, and the small intestine accumulated with fluid was ligated both at the pyloric sphincter and at the ileocecal junctions and was dissected out. The small intestine was then weighed. The intestinal contents were collected by milking the whole length of the small intestine with the fingers into a pre-weighed graduated tube (m_0) and the tube with the intestinal content was weight (m_1). The volume of the intestinal content was read directly from the graduation while the mass was obtained as (m_1-m_0) g.

3.7.3 Effect of the aqueous extracts on normal gastrointestinal transit

Normal gastrointestinal transit was investigated in rats according to the method described by Yacob *et al* (2016). Fourty eight rats of either sex were weighed, labeled and randomly allotted into eight groups of six rats each and fasted for 18 hours prior to the test, but were allowed free access to water. After 1 hour of treatment with graded doses of either pulp or seed extract of *Opuntia ficus-indica*, 10 ml/kg distilled water or 3 mg/kg atropine sulphate (standard drug), each animal was administered 1ml of freshly prepared 10% active charcoal suspension in 2 % tween 80 orally. One hour after charcoal administration, animals were sacrificed. The abdomens were opened and small intestine from the pylorus to caecum was isolated. The peristaltic index (PI) for each rat was calculated and expressed as percentage of the distance traveled by the charcoal meal relative to the total length of the small intestine. The percentage inhibition relative to the control was also calculated as:

$$\text{Peristaltic index (PI)} = \frac{\text{LM}}{\text{LSI}} \times 100$$

Where, LM = Length of distance travelled by Charcoal Meal

LSI = Length of Small Intestine

$$\%\ \text{Inhibition} = \frac{\text{Mean of distance travelled by marker of (Control} - \text{Test) group}}{\text{Mean of distance travelled by marker of control group}} \times 100$$

3.7.4 Effect of the aqueous extracts on Castor oil induced gastrointestinal transit

The effect of the fruit extract of *Opuntia ficus-indica* on gastrointestinal motility was evaluated as described by Yacob *et al*. (2016). Fourty eight rats were weighed, labeled and randomly allotted into eight groups of six rats each and fasted for 18 hours prior to the test but had free access to water. After 1 hour of treatment with graded doses of either pulp or seed extract of *Opuntia ficus-indica*, 10 ml/kg distilled water or 3 mg/kg Atropine (standard drug), diarrhea was induced by administration of 1ml of castor oil orally to each rat. After 1 hour of castor oil administration, all animals received 1 ml of charcoal meal marker (10 % charcoal suspension in 2 % tween 80) orally and all of them were sacrificed after 1 h of marker administration. The small intestine was dissected out and the distance travelled by charcoal meal plug from the pylorus to caecum was measured and expressed as a percentage of the total distance of the small intestine.

In vivo antidiarrheal index (ADI)

In vivo antidiarrheal index (ADI) of treated groups was determined using data from castor oil-induced diarrhea, enteropooling, and gastrointestinal motility tests using the formula developed by Aye-Than as described below (Mekonnen *et al.*, 2018).

$$ADI\ in\ vivo = \sqrt[3]{DDT \times GMT \times NFS}$$

Where; **DDT** is the delay in defecation time or diarrheal onset (as % of control)

GMT is the gastrointestinal motility by charcoal travel reduction (as % of control)

NFS is the reduction in the number (frequency) of stools (as % of control).

$$\text{DDT} = \frac{\text{Onset of diarrhea in minute of the (test } - \text{negative control) group}}{\text{Onset of diarrhea in minute of the negative control group}} \times 100$$

$$\text{GMT} = \frac{\text{Distance travelled by the charcoal marker of the (negative control } - \text{test) group}}{\text{Distance travelled by the charcoal marker in the negative control group}} \times 100$$

$$\text{NFS} = \frac{\text{Mean number of wet stools of (negative control } - \text{treated)group}}{\text{Mean number of wet stools of negative control group}} \times 100$$

3.8 Phytochemical Screening

The aqueous extracts of both pulps and seeds of the plant was tested for the presence of secondary metabolites such as alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides, phenols and steroids.

Detection of Alkaloids

0.5 gram of crude extracts of each sample was mixed with 2ml of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixtures. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids (Iqbal, 2012).

Detection of Saponins (Frothing test)

50 mg of extracts of each sample were diluted with distilled water and made up to 20 ml. Each suspension was then shaken for 15 min in a graduated cylinder. A formation of 2-cm layer of foam was taken as an indicator of the presence of saponins (Thangaraj, 2016).

Test for tannins

Crude extract of 0.5gram of each sample was mixed with 2ml of 2% solution of FeCl3. A blue-green or black coloration was an indicator of the presence of tannins (Yadav & Agarwala, 2011).

Test for terpenoids (Salkowski test)

2 ml of chloroform was added to 0.5 g of each sample of the plant. Then, 3 ml concentrated sulfuric acid was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids (Degu *et al.*, 2016).

Test for flavonoids

About 10 ml of ethyl acetate was added to 0.2 g of each samples and heated on water bath for 3 min. The mixture was cooled and filtered. Then, about 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and the yellow color in the ammoniacal layer indicated the presence of Flavonoids (Degu *et al.*, 2016).

Test for glycosides (Keller-kilani test)

Solvent extract of 0.5 gram of each sample was dissolved in 2.0 ml of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution. Each mixture was then under laid with 1.0 ml of concentrated sulfuric acid. A brown ring obtained at the interface indicated the presence of glycosides (Iqbal, 2012).

Test for phenols

0.5gram of both pulp and seed extracts were treated with few drops of neutral ferric chloride solution 5%, intense colour developed indicates the presence of phenols (Iqbal, 2012; Trease & Evans, 1996).

Test for steroids

0.5gram of crude extracts of both pulp and seed were mixed with 2ml of chloroform and concentrated H_2SO4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids (Yadav & Agarwala, 2011).

3.9 Statistical Analysis

The obtained data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 22.0 software. Results were expressed as a mean ± standard error of the mean (SEM), and statistical analyses were carried out by employing one-way analysis of variance (ANOVA), followed by Tukey post Hoc test to compare results with controls and among groups. In all cases, $p < 0.05$ was considered as statistically significant. Dose-dependent effect was confirmed using linear regression analysis.

4. Results

4.1 Acute Oral Toxicity Test

Oral administration of the pulp and seed extracts of the *Opuntia ficus-indica* produced no visible signs of toxicity in the animals at the limit dose of 2000 mg/kg in rat. No mortalities were recorded during the 24 hrs. In addition, no toxic symptoms were observed and neither food nor water intake was found to be reduced during the period of 14 days.

4.2 Effect of the aqueous extracts on castor oil-induced diarrhea

All rats in the control group produced either wet stools or watery diarrhea starting from half hour after castor oil administration. Pretreatment of rats at the doses of 100, 200, and 400 mg/kg of the pulp and seed of *Opuntia ficus-indica* caused a significant ($p < 0.001$) decrease in the frequency of wet and total number of stools. It also caused a dose dependent delay in the onset of diarrhea as compared to the negative controls (R^2=0.984 for pulp and R^2=0.933 for seed). The percentage inhibition of purging frequency (number of wet stools) relative to negative controls at different doses (100, 200,and 400 mg/kg) of the fruits of *Opuntia ficus-indica* was 65.89%, 70.40%, 75.03 % ($p < 0.001$) for the pulp and 52.25%, 61.39%, 63.57% for the seed extracts as shown in Table 1. The percentage inhibition of purging frequency of the standard drug loperamide at 2.5 mg/kg, on the other hand, was found to be 90.86% ($p < 0.001$). The pulp extract at the dose of 100mg/kg and seed extract at 100mg/kg and 200mg/kg had no significant effect on the onset of diarrhea as compared to the negative controls. The superior effect on both delay of the onset of diarrhea and frequency of the wet stools was observed with 400mg/kg of the pulp and seed of the *Opuntia ficus-indica.*

Table 1. Effect of the aqueous extract of pulp and seeds of *Opuntia ficus-indica* on castor oil diarrhea model in rats

Groups	Onset of diarrhea (min)	Total stool frequency in 6 hr	Frequency of wet stool	Inhibition of wet stool (%)
DW10	47.17±4.15	8.67±0.61	7.33±0.76	-
L2.5	267.50±21.13^{a3c3f3g3h3}	1.83±0.17$^{a3\ f1g1h1}$	0.67±0.42^{a3f2}	90.86
P100	119.00±19.86^{b3e1}	3.17±0.54 ^{a3}	2.50±0.43^{a3}	65.89
P200	175.67±20.23^{a3b1}	3.00±0.68 ^{a3}	2.17±0.60^{a3}	70.40
P400	211.83±23.21^{a3}	3.17±0.60 ^{a3}	1.83±0.48^{a3}	75.03
S100	57.33±5.55^{b3h3}	4.50±0.56 ^{a3b1}	3.50±0.43^{a3b3}	52.25
S200	87.50±7.37^{b3h2}	4.17±0.48 ^{a3b1}	2.83±0.31^{a3}	61.39
S400	169.67±18.90^{a3b3f3g2}	4.33±0.67 ^{a3b1}	2.67± 0.49 ^{a3}	63.57

Values are expressed as Mean ± S.E.M (n = 6), ^{a} compared to control,^{b} to standard drug,^{c} to 100 mg/kg pulp,^{d} to 200 mg/kg pulp,^{e} to 400 mg/kg pulp, ^{f} to 100mg/kg seed,^{g} to 200 mg/kg seed,^{h} to 400 mg/kg seed; $^{1}p < 0.05$, $^{2}p < 0.01$, $^{3}p < 0.001$. DW= distilled water, L= loperamide, P=pulp & S=seed.

4.3 Effect of the aqueous extracts on castor oil induced enteropooling

Percentage inhibition in intestinal fluid accumulation was 34.37% ($p < 0.05$), 40.25% ($p < 0.01$) and 48.30% ($p < 0.001$) for the pulp and 24.77%, 35.60% ($p < 0.05$), and 43.96% ($p < 0.01$) for the seeds of the fruit of *Opuntia ficus-indica* at the doses of 100, 200 and 400 mg/kg, respectively, compared to the negative controls as shown in Table 2. The standard drug, loperamide HCl (2.5mg/kg, p.o), was found to have a percentage inhibitory effect of 70% ($p < 0.001$). Pretreatment with the graded doses (100, 200 and 400 mg/kg) of both extracts showed a dose-dependent inhibition (R^2=0.908 for pulp and R^2 =0.994 for seed) on the castor oil induced intestinal fluid accumulation. Both pulp ad seed extracts of *Opuntia ficus-indica* also showed significant weight reduction ($p < 0.001$) in the small intestinal contents as compared to the negative controls at all dose levels tested. A seed extract of 100mg/kg of the plant was found to have no significant effect on intestinal fluid accumulation, compared to the negative controls, whereas the pulp extract of 400mg/kg dose produced maximal inhibitory effect followed by the corresponding equivalent dose of the seed extract.

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Table 2. Effect of the aqueous extract of pulp and seeds of *Opuntia ficus-indica* on castor oil induced enteropooling in rats

Groups	Mean weight of small intestinal content (gm)	% inhibition	Mean volume of small intestinal content(ml)	% inhibition
DW10	4.66±0.29	-	3.23±0.28	-
L 2.5	1.68±0.12^{a3c2d1f2}	63.95	0.95±0.16^{a3c2f2g1}	70.00
P100	2.86±0.14^{a3b2}	38.63	2.12±0.20 ^{a1b2}	34.37
P200	2.17±0.14^{a3}	53.43	1.93±0.21^{a2b1}	40.25
P400	1.96±0.17^{a3}	57.94	1.67±0.22^{a3}	48.30
S100	2.73±2.27^{a3b2}	41.42	2.43±0.20^{b2}	24.77
S200	2.39±1.87^{a3}	48.71	2.08±0.28^{a1b1}	35.60
S400	2.02±1.65^{a3}	56.65	1.81±0.26^{a2}	43.96

Values are expressed as Mean ± S.E.M (n = 6), [a] compared to control,[b] to standard drug,[c] to 100 mg/kg pulp,[d] to 200 mg/kg pulp,[e] to 400 mg/kg pulp, [f] to 100 mg/kg seed,[g] to 200 mg/kg seed,[h] to 400 mg/kg seed; [1]p <0.05, [2]p <0.01, [3]p<0.001. DW= distilled water, L= loperamide, P=pulp & S=seed.

4.4 Effect of the aqueous extracts on normal gastrointestinal propulsion

The aqueous extract of the plant decreased propulsion and consequently the percentage of intestinal transit of the charcoal meal through the gastrointestinal tract during normal gut transit as compared with control group. The pulp extract of the O*puntia ficus-indica* inhibited the normal intestinal transit of charcoal meal plug by 19.23 %, 38.73%, and 39.19%*(p<0.01)* at doses of 100mg/kg, 200mg/kg and 400mg/kg, while the same graded doses of the seed extract of the plant inhibited the normal intestinal transit of charcoal meal plug by 17.34%, 32.22%, and 27.30% *(p<0.01)* respectively. Atropine sulphate at the dose of 3mg/kg showed 59.60% (*p<0.001)* inhibition as shown in Table 3. Both the pulp and seed extracts of the plant inhibited the normal intestinal propulsion significantly ($p<0.01$). The pulp extract at the dose of 400mg/kg produced maximal inhibition in intestinal propulsion in the normal intestinal transit, while the dose of 100mg/kg showed no significant effect compared to the control.

Table 3. Effect of the aqueous extract of pulp and seeds of *Opuntia ficus-indica* on normal gastro intestinal transit in rats

Groups	Total length of small intestine (cm)	Distance moved by the charcoal meal (cm)	Peristalsis index (%)	%Inhibition
DW10	98.17±2.26	71.00±3.56	72.32	-
A 3	98.67±2.26	28.83±1.90 [a3c3f3g2h2]	29.22	59.60
P100	96.17±2.15	56.17± 3.65[b3]	58.41	19.23
P200	101.17±1.58	44.83±1.96[a2]	44.31	38.73
P400	102.33±2.19	45.00±7.36[a2]	43.98	39.19
S100	100.66±3.70	60.17±2.73[b2]	59.78	17.34
S200	102.00±4.43	50.00±2.90[a2b2]	49.02	32.22
S400	99.67±3.27	50.83±5.16[a2b2]	52.58	27.30

Values are expressed as Mean ± S.E.M (n = 6), [a] compared to control,[b] to standard drug,[c] to 100 mg/kg pulp,[d] to 200 mg/kg pulp,[e] to 400 mg/kg pulp, [f] to 100 mg/kg seed,[g] to 200 mg/kg seed,[h] to 400 mg/kg seed; [1]p <0.05, [2]p <0.01, [3]p<0.001. DW= distilled water, A= Atropine, P=pulp & S=seed.

4.5 Effect of the aqueous extracts on Castor oil induced gastrointestinal transit

The charcoal meal moved farther in the castor oil induced intestinal transit compared to the normal intestinal transit. The pulp extract of the *Opuntia ficus-indica* inhibited the intestinal transit of charcoal meal induced by castor oil by 46.49%, 52.37 % and 63.74% ($p<0.001$) at doses of 100mg/kg, 200mg/kg and 400mg/kg, respectively in a dose dependent manner ($R^2= 0.967$). The same graded dose of the seed of the plant produced the percentage inhibition of 56.01%, 66.78% and 63.60 ($p<0.001$), while atropine sulphate at the dose of 3mg/kg showed 74.02% ($p<0.001$) inhibition as shown in Figure 2. Although significant effect is observed in both cases, the inhibitory effect of the extracts in castor oil-induced intestinal transit was greater as compared to that of the normal intestinal transit. The pulp extract at the dose of 400mg/kg produced an inhibitory effect - comparable to that of 200mg/kg and 400mg/kg seed extracts in the castor oil-induced intestinal transit.

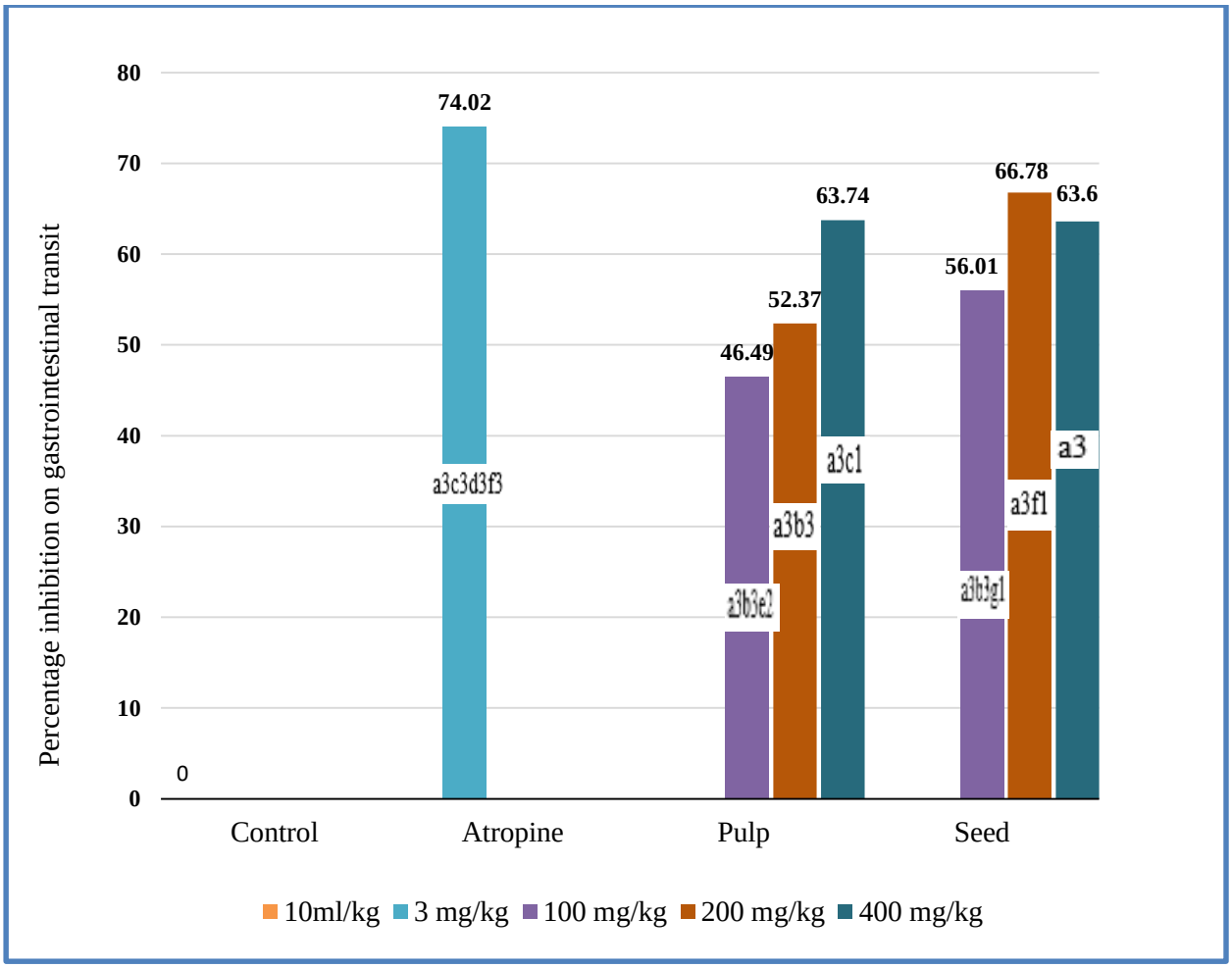

Figure 2. Effect of the aqueous extracts of the fruits of the plant on castor oil induced gastrointestinal transit. Values are expressed as Mean ± S.E.M (n = 6), [a] compared to control,[b] to standard drug (Atropine),[c] to 100 mg/kg pulp,[d] to 200 mg/kg pulp,[e] to400 mg/kg pulp, [f] to 100 mg/kg seed,[g] to 200 mg/kg seed,[h] to 400 mg/kg seed; [1]p <0.05, [2]p <0.01, [3]p<0.001.

4.6 The Antidiarrheal Index

The antidiarrheal index (ADI) is a measure of the combined effects of the different components of diarrhea such as purging frequency, onset of diarrheal stools, and intestinal fluid accumulation. Results for the *in vivo* antidiarrheal indices were 77.04, 101.58, and 118.45 at the doses of 100, 200 and 400 mg/kg for the pulp extract and 39.37, 70.65 and 101.47 at the same graded doses for the seed extract of the *Opuntia ficus-indica* respectively. The pulp extract at 400mg/kg gave a maximum index of 118.45 as shown in Figure 3. Both extracts produced antidiarrheal activity as shown by the antidiarrheal index (ADI) in a dose dependent manner (R^2=0.989 and 1.000 respectively for pulp and seed extracts).

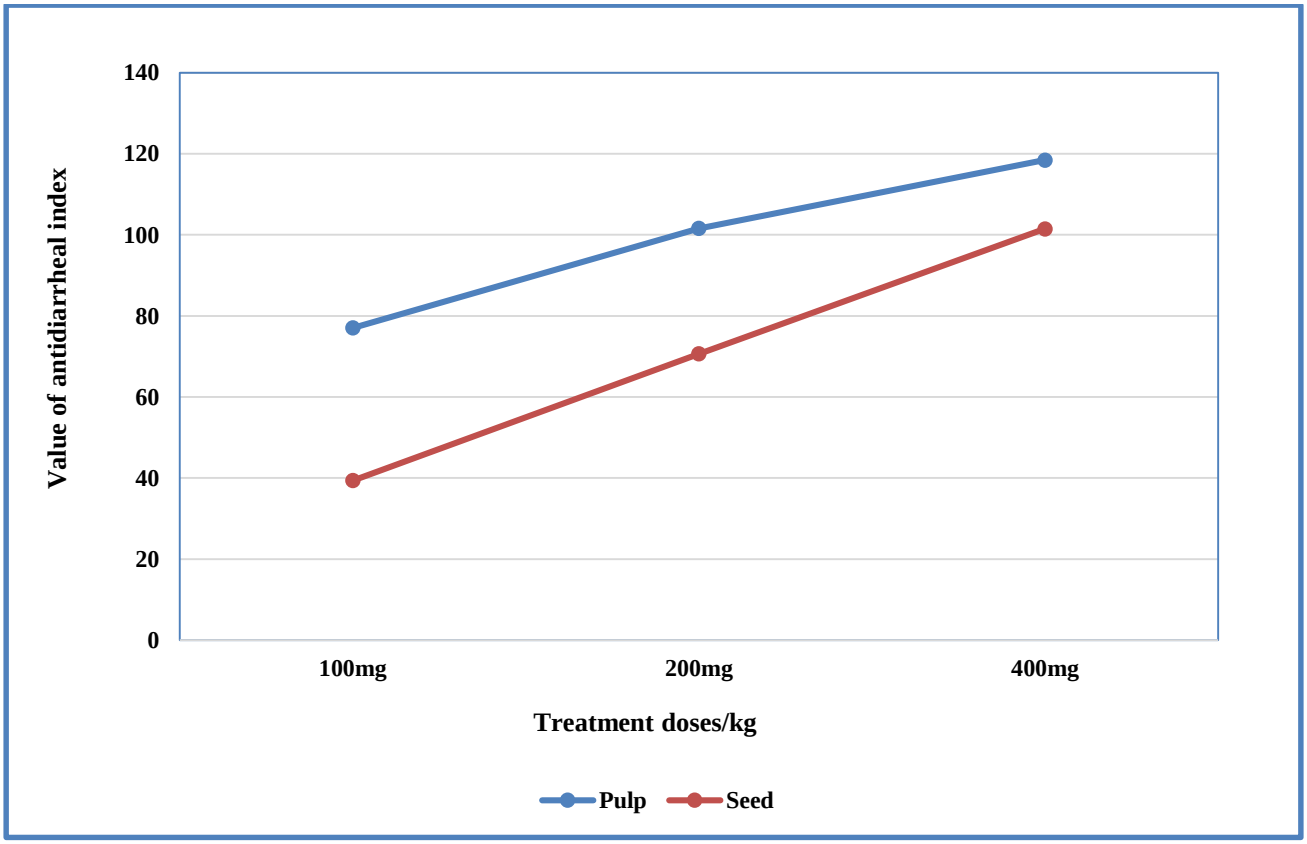

Figure 3. Antidiarrheal index of aqueous extract of pulp and seeds of the fruits of *Opuntia ficus-indica*

4.7 Phytochemical Analysis

Preliminary phytochemical analysis of the aqueous extracts of both pulp and seeds of the fruits of *Opuntia ficus-indica* showed positive results for tannins, alkaloids, saponins, flavonoids, steroids, terpenoids, glycosides, and phenolic compounds as shown in Table 4.

Table 4. Preliminary phytochemical analysis of aqueous extracts of pulp and seeds of the fruits of *Opuntia ficus-indica.*

Aqueous extracts of *Opuntia ficus-indica*	**Secondary metabolites**	**Alkaloids**	**Flavonoids**	**Glycosides**	**Phenols**	**Saponins**	**Steroids**	**Tannins**	**Terpenoids**
	Pulp	+	+	+	+	+	+	+	+
	Seed	+	+	+	+	+	+	+	+

5. Discussion

This study was conducted to evaluate the antidiarrheal activity of pulp and seeds of the plant extract in experimental animals, and the possible underlying mechanism. The results obtained from the study showed that the plant possesses the antidiarrheal activity in all antidiarrheal models used in the present study. Antidiarrheal activity of aqueous extract of pulp and seeds of *Opuntia ficus-indica* was evaluated by using castor oil induced diarrhea, castor oil induced enteropooling and charcoal meal test models.

The use of castor oil-induced diarrhea in experimental animals is a common model because of its ability to increase intestinal content by preventing the re-absorption of water and the liberation of ricinoleic acid from the oil (Saheed and Tom, 2016). The liberated ricinoleic acid results in irritation and inflammation of the intestinal mucosal leading to release of prostaglandins, which enhances motility and secretion as well as prevention of NaCl and water re-absorption (Ezeja *et al.,* 2012; Kumar *et al.,* 2014; Sharma *et al.,* 2015; Wansi *et al*., 2017). The consequential effect of this is increase in the secretion of water and electrolytes as well as corresponding increases in the number of wet faeces and intestinal transit time. Since diarrhea induced by castor oil was found to be delayed by prostaglandin biosynthesis inhibitors, it is reasonable to suggest that the extracts may have the capacity of inhibiting the effect of ricinoleic acid on the muscosa of the intestine. Therefore, the antidiarrheal effect of the pulp and seeds of the *fruits of the Opuntia ficus-indica* could result from inhibition of prostaglandin synthesis or by installing an anti- secretory mechanism (Wansi *et al*., 2017).

In the present study, aqueous extracts of both pulp and seeds of the plant exhibited significant antidiarrheal activity by significantly reducing the castor oil-induced diarrhea in rats, delaying the

onset of diarrhea, decreased the frequency of purging, reduced the number of wet stools and inhibited the severity of diarrhea generally. Regarding the effect on the onset of diarrhea the aqueous pulp extract of the plant exhibited better antidiarrheal activity than the seed extract. This is probably because of the difference in the level of bioactive compounds as it was indicated in the literatures (Mostafa *et al.,* 2014; Rtibi *et al.,* 2018). Both flavonoids and phenolic compounds present antioxidant properties (Mostafa *et al.,* 2014), which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism, thus, reducing prostaglandin induced fluid secretion (Yaecob *et al.,* 2016).

Studies on enetropooling showed that the pulp and seed extracts of the plant reduced both the weight and volume of intraluminal contents. These effects are believed to occur as the direct consequences of reduced water and electrolytes secretion into the small intestine, suggesting that the plant extracts may enhance electrolyte absorption from the intestinal lumen consistent with inhibition of hypersecretion produced by the released prostaglandins and nitric oxides (Ezenwali *et al.,* 2010; Yaecob *et al.,* 2016). Secondary metabolites such as phenolics, alkaloids, terpenoids (Yaecob *et al.,* 2016), flavonoids and tannins (Kumar *et al.,* 2014; Taddese *et al.,* 2017; Wansi *et al.,* 2017) were reported for reducing gastrointestinal motility allowing more time for better absorption of water and electrolytes (Ihekwereme *et al.,* 2016). Since electrolyte absorption determines the efficiency of nutrient absorption, it is more likely that the enhanced electrolyte absorption by the extracts may have encouraged the absorption of other intestinal solutes, which is a function of the rate of water uptake in that region (Ezenwali *et al.,* 2010). Thus, the extract-enhanced solute absorption may have created an osmotic gradient across enterocytes which stimulated water absorption. These observations reasonably suggest that the extract might have

inhibited gastrointestinal hyper-secretion and enteropooling by enhancing electrolytes, solutes and water absorption from the intestinal lumen.

In castor oil induced enteropooling, the pulp extract of the plant significantly inhibited the castor-oil induced intestinal fluid accumulation and weight of intestinal content at all levels of the tested doses. However, only 200 mg/kg and 400 mg/kg of the seed extract of the plant showed significant effect as compared to the negative controls. The anti-enteropooling activity of the plant could be attributed to the presence of secondary metabolites that have previously been described in both extracts of the plant including terpenoids, steroids, flavonoids and tannins and the difference in anti-enteropooling activity could be due to difference in the level of these active metabolites as described earlier. Terpenoids (Prakash, 2017), flavonoids (Hamalainen *et al.,* 2011) and steroids (Awad *et al.,* 2004) have been shown to inhibit production of prostaglandin E2, which are known to play a crucial role in the stimulation of intestinal secretions through causing secretion of water and electrolytes (Pierce *et al*., 1971). Tannins decrease fluid secretion by inhibiting CFTR and calcium activated chloride channel (CaCC), by generating a protein-precipitating reaction to the gastrointestinal mucosa (Tadesse *et al.,* 2017), which make the intestinal mucosa more resistant to chemical alteration (Balaji *et al.,* 2012; Yaecob *et al.,* 2016).

Ricinoleic acid might also activate the nitric oxide pathway and induce nitric oxide (NO) dependent gastrointestinal secretion (Degu *et al.,* 2016) through the stimulation of cAMP and cGMP concentration (Liang *et al.,* 2005). A growing body of evidence indicates that bioactive compounds such as terpenoids (Jang *et al.,* 2004; Zhao *et al.,* 2016, Prakash, 2017), Alkaloids (Kondo *et al.,* 1993; Rho *et al.,* 2007) and flavonoids (Rupesh *et al.,* 2014) are implicated in the attenuation of NO synthesis. Therefore, the anti-enteropooling effect of both extracts could probably be through NO pathway interruption.

Moreover, small intestine is extrinsically innervated by both sympathetic and parasympathetic divisions of the autonomic nervous system (Tortora and Derrickson, 2009). It stimulates intestinal secretion through neurotransmitters such as acetylcholine and vasoactive intestinal peptides, while, on the other hand, it stimulates intestinal absorption through α_2 adrenergic agents such as enkephalins, and somatostatins (Degu *et al.*, 2016). Degu *et al.*, also suggested that, secondary metabolites such as flavonoids from plant sources could stimulate α_2 adrenergic receptors in the absorptive cells of the gastrointestinal tract and these results suggested that the plant extracts reduced diarrhea by either stimulating reabsorption of electrolytes and water through sympathetic activation or by inhibiting the fluid secretion into the intestine by inhibiting parasympathetic activity.

In order to evaluate the mechanism of antidiarrheal activity, the study was extended to determine the intestinal motility. Gastrointestinal motility is primarily controlled by the parasympathetic and sympathetic nerves, with the parasympathetic innervation acting as the most important factor in regulating motility. In general, increasing activation of the parasympathetic nerves enhances intestinal transit, while increasing activation of the sympathetic nerves inhibits intestinal transit (Tortora and Derrickson, 2009). Atropine, which was used as standard drug, was known to suppress the movement of the charcoal meal due to its anticholinergic effect (Ezenwali *et al.*, 2010; Brown & Laiken, 2011; Kumar *et al.*, 2014). Besides cholinergic receptors, the activation of the sympathetic system via α_2 adrenergic receptors in the gastrointestinal tract was able to inhibit peristaltic activity, reduce muscle tone, alleviate gastric emptying and promote defense of stomach mucosa (Suleyman, 2012; Beserra *et al.*, 2016). The reduction in distance travelled can be used as a tool in order to explain the intestinal smooth muscle relaxation. Contractions of all smooth muscles absolutely depend on the presence of Ca^{2+} which activates the contractile elements and

their relaxation, a mechanism implicated in the antidiarrheal effect of different drugs. According to the work of Baldassano *et al.,* the cactus fruits extract inhibitory effect do not involve potassium channels or voltage dependent Ca^{2+} channels *in vitro* but rather pathways of Ca^{2+} intracellular release (Baldassano *et al.,* 2010). Therefore, the plant could have caused the reduction in distance travelled by the charcoal meal through increasing the intracellular Ca^{2+} release.

In this study, the charcoal meal test demonstrates that the graded doses of both pulp and seed extracts of the *Opuntia ficus-indica* significantly reduced intestinal propulsive movement in both normal and castor oil induced intestinal transit as compared to the negative controls. Although significant effect is observed in both cases, the inhibitory effect of the extracts in castor oil-induced intestinal transit was greater as compared to that of the normal intestinal transit. According to literatures, drugs with antidiarrheal effects are well known for reducing gastrointestinal contractions and thereby slows the intestinal transit (Kumar *et al.*, 2014), allowing more time for better absorption of water and electrolytes (Ihekwereme *et al.,* 2016; Yaecob *et al.,* 2016). The observed effect is therefore possible due to the extracts' ability to inhibit the intestinal movement, which in turn accounts for the antidiarrheal effect of the extracts of the plant. In this study, the inhibitory effect on the intestinal transit seems higher with the seed extract than that of the pulp extract which could be attributed to the difference in the level of secondary metabolites (Mostafa *et al.,* 2014; Rtibi *et al.,* 2018). Moreover, Rtibi *et al.,* also showed that with increased dose of the juice of the pulp the plant was observed to have laxative activity and in line with this, Osuna-Martínez *et al.,* citing the work of others, has reported that orally ingested *opuntia ficus-indica* may cause mild diarrhea (Osuna-Martínez *et al.,* 2014). As described by Rtibi *et al.,* laxative effect could be attributed to the presence of high amounts of sugars essentially the sucrose which as a

major laxative produces the enhancement of the gastrointestinal tract mechanism in healthy and constipated rats (Rtibi *et al.,* 2018).

The inhibition of intestinal movement could be due to the presence of phenolics, alkaloids, terpenoids, flavonoids and tannins that were reported for their antidiarrheal activities through inhibition of intestinal motility as described earlier. Tannins were shown to inhibit peristaltic movements and intestinal secretions by reducing the intracellular Ca^{2+}inward current or by activation of the calcium pumping system, which induces the muscle relaxation, attributed by spasmolytic and calcium channel blocking (CCB) activities (Yaecob *et al.,* 2016; Wansi *et al.,* 2017). Flavonoids are also known to inhibit intestinal motility (AL-Maamori, 2011) through relaxing intestinal smooth muscles (Damabi *et al.,* 2010; Yaecob *et al.,* 2016), while terpenoids, on the other hand, were reported to inhibit intestinal motility and secretion induced by castor oil by inhibiting the release of autacoids and prostaglandins (Yaecob *et al.,* 2016). Furthermore, anticholinergic agents are known to inhibit gastrointestinal hypermotility as indicated earlier. Therefore, although not directly supported by our experimental data, showing a fair effect as compared to the standard drug atropine it is likely that the fruit extracts of the plant inhibits gastrointestinal hypermotility through anticholinergic effect. Whatever mechanisms, however, are involved, the results of this study showed reduction in gastric contents and watery texture of diarrheal stools as well as gastrointestinal motility thus leading to the much desired reduction in frequency of stooling in diarrheal disease.

The antidiarrheal index (ADI) is a measure of the combined effects of different components of diarrhea such as purging frequency, onset of diarrheal stools, and intestinal fluid accumulation (Yaecob *et al.,* 2016). As indicated in the literature, the higher the ADI value, the better the effectiveness of the extract in curing diarrhea (Tadesse *et al*., 2017). The aqueous extract of both

pulp and seed of the *Opuntia ficus-inidca* produced a dose-dependent antidiarrheal index which depicts that the plant extracts produce antidiarrheal activity in a dose-related manner. Higher antidiarrheal activity was observed with the pulp extract as compared to the seed, which could probably be due to the difference in the level of bioactive compounds such as sterols, flavonoids and phenolic compounds indicated in the literature (Mostafa *et al.*, 2014).

Furthermore, during acute toxicity test on both extracts in rats no side effects were observed, suggesting that both extracts of the plant have high margins of safety. The high degree of safety is also consistent with its popular use as food.

6. Conclusion

The results obtained in the present study suggest that both pulp and seed extracts of the fruits of *Opuntia ficus-inidca* have beneficial effect in controlling diarrhea. Pretreatment of rats at the doses of 100, 200 and 400 mg/kg of the extracts caused a significant reduction in the frequency of total number of stools and wet stooling as well as in delaying the onset of diarrhea as compared to the controls. Both extracts also showed a significant inhibition on the castor oil induced intestinal fluid accumulation as well as on normal and castor oil induced intestinal transit. The result from *in vivo* ADI revealed that there was a dose-dependent increment in the antidiarrheal index value for both extracts, the maximum effect being observed with the pulp extract at 400mg/kg. The present data, therefore, provided a support for the traditional use of the plant as an antidiarrheal remedy.

7. Recommendation

Based on the findings of the present study, the following recommendations are forwarded for further studies:

- Further studies should be conducted to isolate, purify and identify bioactive principle(s) responsible for the antidiarrheal activities of both extracts.
- Further studies are required to ascertain the precise mechanism of action of the antidiarrheal activity of the plant
- Performing antidiarrheal activity tests with various solvent fractions.

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