ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

ANTIDIARRHEAL AND ANTISPASMODIC ACTIVITIES OF STEPHANIA ABYSSINICA (MINSPERMASEAE) USED IN ETHIOPIAN TRADITIONAL MEDICINE.

By Tatek Deneke (B.pharm)

June, 2010

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Under the Supervision of

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Department of Pharmacology, School of Pharmacy, Addis Ababa University

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of Master of Science Degree in Pharmacology.

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Acknowledgement

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List of Abbreviations

SALM: Stephania Abyssinica Leaf Methanol Extract
SARM: Stephania Abyssinica Root Methanol Extract
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SARA: Stephania Abyssinica Root Aqueous Extract
SA: Stephania Abyssinica
GI: Gastro intestinal
GIT: Gastro Intestinal tract
ENS: Enteric Nervous systems
CNS: Central Nervous systems
ADI: antidiarrheal index
PI: peristaltic index
DS: diarrhea score
LPRD: loperamide
DRC: dose response curve
PFR: purging frequency reduction
DFT: diarrhea free time
GITR: gastro intestinal transit reduction
Conc: concentration
DW: distilled water
WHO: World health organization
OG: Osmotic Gap
IBS: irritable bowel syndrome
M_3: Muscarinic receptor sub type 3
Ach : Acetylcholine
GPI: Guinea-pig ileum
SEM: Standard error of the mean
ANOVA: analysis of variance
EHNRI: Ethiopia Health and Nutrition Research Institute

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Abstract

Diarrhea is a leading cause of morbidity and mortality in developing countries. Diarrhea may result from disturbance in bowel function in which case there is increased bowel transit, excessive intestinal secretion of water and electrolytes, decreased intestinal reabsorptions as well as more frequent defecations of loose, watery stool.

Many plant preparations have claimed activities and traditional used as antidiarrhea and antispasmodic. *S. abyssinica* is traditionally used for treatment of diarrhea and stomachache in Ethiopia. The aim of this work was to evaluate the antidiarrheal and antispasmodic activities of the aqueous and methanol extract of the root and leaf of *S. abyssinica.*

Antidiarrheal activities were studied in mice using castor oil-induced diarrhea at doses of 25, 50,100, and 200 mg/kg body weight. The extracts significantly prolonged the time of diarrheal induction, increased diarrhea free time, reduced the frequency of diarrhea episodes, decreased the weight of stool, and decreased general diarrheal score in a dose dependent way. With dose of 200 mg/kg the extracts produced higher in-vivo antidiarrheal index (ADI) than the reference loperamide. ADI of loperamide, SALM, SALA, SARM and SARA was 77.33, 88.79, 89.21, 91.08 and 82.23, respectively.

In Entropooling test in mice the extract significantly ($p < 0.01$) inhibited intestinal fluid accumulations of mice in a dose dependent fashion; with dose of 100 mg/kg from1.03±0.093 ml of the control to 0.403±0.019ml, 0.210±0.018 ml, 0.494±0.012ml and 0.288±0.026ml by SALM, SARM, SALA and SARA respectively.

The antispasmodic activity studies were performed as in vitro and in vivo models. The in-vitro antispasmodic activity studies were performed on isolated GPI. The methanol and aqueous extracts of the leaf showed significant and concentration dependent inhibition of acetylcholine induced contraction of isolated GPI. The extracts depressed E_{max} of Ach, and decreased PD_2 value of the Ach. The E_{max} of Ach at conc of 10^{-3}M is decreased (from100 for the control group)

by SALM with concentration of 200 and 100 ug/ml to 45.6±2.13 and 73.2±3.04 respectively, whereas by SALA with 200 and 100 ug/ml to 62.0±2.98 and 74.8±2.46 respectively.

In the in vivo antispasmodic activity test, the extract significantly decreased the peristaltic index (PI). In normal transit test, the PI of SALM, SALA, SARM and SARA with dose of 200 mg/kg was all 0.00 (100% suppression of normal peristalsis). However in castor oil induced transit with dose of 200 mg/kg the peristaltic index (PI) of SALM, SALA, SARM and SARA was 26.67, 36.85, 22.00 and 40.65 respectively.

The result of this study indicated that the plant extract possesses antidiarrheal and antispasmodic activities and proves the fact that this plant is used in traditional medicine for treatment of diarrhea, stomachache and abdominal cramp.

Key words: *S. abyssinica*, antidiarrheal, antispasmodic, antienteropooling, aqueous and methanol extract, animal (mice or guinea pig)

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1. INTRODUCTION

1.1 Overview of diarrhea

Diarrhea is an increased frequency and decreased consistency of fecal discharge as compared with an individual's normal bowel pattern. Frequency and consistency are variable within and between individuals. For example, some individuals defecate as many as 3 times a day, while others defecate only 2 or 3 times per week (Barbara, 2006).

Diarrhea is loosely defined as passage of abnormally liquid or unformed stools at an increased frequency. For adults on a typical Western diet, stool weight >200 g/d can generally be considered diarrheal. Because of the fundamental importance of duration to diagnostic considerations, diarrhea may be further defined as *acute* if <2 weeks, *persistent* if 2 to 4 weeks, and *chronic* if >4 weeks in duration. Conditions, usually associated with the passage of stool totaling <200 gram/day, must be distinguished from diarrhea, as diagnostic and therapeutic algorithms differ (David and Camiller, 2004; Barbara, 2006).

Diarrhea is a leading cause of morbidity and mortality, especially among children in developing countries and it is a major health problem in children under 5 years (Gilani *et al., 2005*). The World Health Organization (WHO) has estimated that 3–5 billion cases occur each year (1 billion in children less than 5 years old) and about 5 million deaths are due to diarrhea (2.5 million in children, less than 5 years old) (Estrada-Soto *et al.*, 2007).

From a mechanistic perspective, diarrhea can be caused by an increased osmotic load within the intestine resulting in retention of water within the lumen; excessive secretion of electrolytes and water into the intestinal lumen; exudation of protein and fluid from the mucosa and altered intestinal motility resulting in rapid transit and decreased fluid absorption. In most instances, multiple processes are affected simultaneously leading to a net increase in stool volume and weight accompanied by increase in fractional water content (Pasricha, 2006).

The major impact of these illnesses is morbidity, because it demands primary medical services, hospital-care time and labor days lost. Furthermore, the most highly used drugs for intestinal disease therapies are very expensive, overused and inadequate use of antibiotics has led to increased prevalence of multi drugs-resistant pathogens. Despite the etiology, chronic diarrhea is linked with dehydration and electrolyte-containing solutions are the first choice for treatment (Estrada-Soto *et al.,* 2007).

Despite the availability of a vast spectrum of approaches for diarrheal management, majority of the population in the developing countries rely on herbal drugs for the management of diarrhea. Medicinal herbs constitute an indispensable component of the traditional medicine practiced worldwide due to the economical viability, accessibility, acceptability and ancestral experience (Afroz *et al.,* 2006).

1.2 Normal physiology

The human small intestine and colon perform important functions including the secretion and absorption of water and electrolytes, the storage and subsequent transport of intraluminal contents. Alterations in fluid and electrolyte handling contribute significantly to diarrhea. Alterations in motor and sensory functions of the human colon result in highly prevalent syndromes such as irritable bowel syndrome, chronic diarrhea, and chronic constipation (David and Camiller, 2004).

The small intestine and colon have intrinsic and extrinsic innervation. The *intrinsic innervation*, also called the enteric nervous system (ENS), comprises myenteric, submucosal, and mucosal neuronal layers. The function of these layers is modulated by interneurons through the actions of neurotransmitter amines or peptides, including acetylcholine, opioids, norepinephrine, serotonin, ATP, and nitric oxide. The myenteric plexus regulates smooth muscle function, and the submucosal plexus affects secretion and absorption.The *extrinsic innervations* of the small intestine and colon are part of the autonomic nervous system and also modulate both motor and secretory functions. The

chief excitatory neurotransmitters controlling motor function are acetylcholine and the tachykinins, such as substance P (Camiller and Murray, 2001; David and Camiller, 2004).

1.2.1 Absorption, secretion and GI motility

Sugars and amino acids are absorbed across the small-intestinal brush border membrane via carriers that couple their movements to that of Na^+. Na^+ coupling permits the organic solute to be transported uphill, i.e., from low luminal to higher cell concentration, a gradient opposite to that for Na^+. The organic solutes then move downhill from enterocyte to blood via basolateral membrane carriers that operate independently of ion movements (Schultz *et al.,* 1966; Ganapath *et al.,* 1985).

The Na^+ gradient, therefore, is the driving force for amino acid, oligopeptide, and sugar absorption. As these organic solutes are absorbed, salt is absorbed with them, and water follows osmotically transport from enterocyte to lateral intercellular space creates a local osmotic gradient that initiates water flow. The coupled transport of Na^+ and organic solute is the theoretical basis for oral rehydration therapy in severe diarrhea (Schultz *et al.,* 1966; Ganapath *et al.,* 1985).

Normally about 8 to 9 liters of fluid enter the small intestine daily from exogenous and endogenous (secretion) sources. Net absorption of the water occurs in the small intestine in response to osmotic gradients that result from the uptake and secretion of ions and the absorption of nutrients (mainly sugars and amino acids), with only about 1 to 1.5 liters crossing the ileocecal valve. The colon then extracts most of the remaining fluid, leaving about 100 ml of fecal water daily (Pasricha, 2006).

Secretions in intestine has role in duodenal alkalinization. The ion exchangers that are localized in small-intestinal and colonic brush border membranes play this role. The individual cell membrane transporters contributing to active Cl^- secretion the three membrane proteins involved are: the apical anion channel, the basolateral membrane K^+ channel and the basolateral membrane NaK2Cl cotransporter (Micheal *et al.,* 2003).

GIT also do two types of motility under autonomic control as fed state (peristalsis) and fast state. This motility ensures the mixing of ingested food and let the bolus in one part of the GI move to the other part. It also works housekeeping activities by moving out the unabsorbed debris out of the GIT (Pasricha, 2006).

1.2.2 Regulation of absorption, secretion and GI motility

The gastrointestinal tract is in a continuous contractile, absorptive, and secretory state. The control of this state is complex, with contributions by the muscle itself, local nerves (*i.e.,* the enteric nervous system, ENS), the central nervous system (CNS), and humoral pathways. Of these, perhaps the most important regulator of physiological gut function is the ENS. Alterations in gastrointestinal motility and in the balance of absorption and secretion in the intestines may underlie irregularities in bowel habits. (Longstereth, 1998; Pasricha and Jefri, 2001).

The ENS is composed of interconnected networks of ganglion cells and nerve fibers mainly located in the submucosa (submucosal plexus) and between the circular and longitudinal muscle layers (myenteric plexus). These networks give rise to nerve fibers that connect with the mucosa and deep muscle. Although extrinsic sympathetic and parasympathetic nerves project onto the submucosal and myenteric plexuses, the ENS can independently regulate gastrointestinal motility and secretion (Mc Quid, 2007).

The neurons within the plexuses secrete different neurotransmitters and a variety of pharmacologically active peptides. The classes of compounds that stimulate active secretion and inhibit active absorption, and those with the opposite effects. The former group includes three kinds of agents: (a) neurotransmitters, including vasoactive intestinal peptide (VIP), acetylcholine, substance P, and the nucleotides ATP; (b) the paracrine agents serotonin and neurotensin, which are released by endocrine (enterochromaffin) cells in the intestinal epithelium; (c) agents released by inflammatory cells, including mainly prostaglandins, histamine, and serotonin (Sellin, 1993; Quigly *et al.,* 1999; Range *et al.,* 2003).

The group of compounds that both inhibit active secretion (HCO_3^- as well as Cl^-) and enhance active absorption includes norepinephrine (via α_2-receptors), neuropeptide Y, enkephalins, somatostatin, and paracrine agents (Sellin, 1993).

The basic motor tool used by the ENS to integrate its GI motility programs is the peristaltic reflex. Physiologically, peristalsis is a series of reflex responses to a bolus in the lumen of a given segment of the intestine; the ascending excitatory reflex results in contraction of the circular muscle on the oral side of the bolus, while the descending inhibitory reflex results in relaxation on the anal side. The net pressure gradient moves the bolus caudal (Furness and Sanger, 2002; Galligan, 2002).

Three neural elements, responsible for sensory, relay, and effector functions, are required to produce these reflexes. Luminal factors stimulate sensory elements in the mucosa, leading to a coordinated pattern of muscle activity that is directly controlled by the motor neurons of the myenteric plexus to provide the effector component of the peristaltic reflex (Furness and Sanger, 2002; Galligan, 2002; Pasricha, 2006).

Motor neurons receive input from ascending and descending interneurons (which constitute the relay and programming systems) that are of two broad types, excitatory and inhibitory. The primary neurotransmitter of the excitatory motor neurons is acetylcholine (ACh). The principal neurotransmitter in the inhibitory motor neurons appears to be nitric oxide (NO), although important contributions may also be made by ATP, and vasoactive intestinal peptide (VIP), all of which are variably co-expressed with NO synthase (Furness and Sanger, 2002; Galligan, 2002; Pasricha, 2006).

1.3 Pathophysiology of diarrhea

1.3.1 General aspects

Osmosis, active secretion, exudation, and altered motility can all drive diarrhea. Specific diarrheal illnesses often involve more than one of these forces.

i) Osmotic diarrhea: When poorly absorbable, low–molecular weight aqueous solutes are ingested, their osmotic force quickly pulls water and, secondarily, ions into the intestinal lumen. Individuals with normal gut function will develop osmotic diarrhea when they ingest large amounts of poorly absorbable solutes, such as lactulose (if they are being treated for hepatic encephalopathy), sorbitol (if they continually chew sugar-free gum), or Mg^{2+} (if they take certain antacids or bowel purgatives) (Micheal *et al.*, 2003)

ii) Secretory diarrhea: Diarrhea resulting from overstimulation of the intestinal tract's secretory capacity can develop in "pure" form (e.g., cholera) or as a component of a more complex disease process (e.g., celiac disease, Crohn disease). "Pure" secretory diarrhea is characterized by (a) large stool volumes (which can exceed 1 liter per hour in well hydrated adults), (b) absence of red or white blood cells in the stool, (c) absence of fever or other systemic symptoms (except those due to dehydration), (d) persistence of diarrhea with fasting (volume may diminish, however), and (e) lack of excess osmotic gap (OG) in stool electrolytes. Osmotic gap is defined as follows: $OG = 290 – 2\{[Na^+] + [K^+]\}$, where 290 is the assumed osmolarity of blood plasma. A gap greater than 50 mM is considered abnormal; the normal gap is made up of Mg^{2+}, Ca^{2+}, NH_4^+, and perhaps organic cations (Farthing *et al.*, 2002; Micheal *et al.*, 2003).

The pattern of stool electrolytes in patients with acute cholera shows Na^+, K^+, and $Cl^–$ concentrations not very different from those in plasma and $HCO_3^–$ concentration somewhat higher than in plasma. In contrast, normal stool shows low $[Na^+]$ and high $[K^+]$ concentrations, due mainly to the colon's reabsorption of Na^+ and secretion (both active and passive) of K^+; and a low $[Cl^–]$ concentration, due to the replacement of $[Cl^–]$ by short-chain organic acid anions generated by colonic bacteria. Normally, $[HCO_3^–]$ concentration is similar to that in plasma (Farthing et al., 2002; Micheal *et al.*, 2003).

iii) Exudative diarrhea: If the intestinal epithelium's barrier function is compromised by loss of epithelial cells or disruption of tight junctions, hydrostatic pressure in blood vessels and lymphatics will cause water and electrolytes, mucus, protein, and sometimes even red and white cells to accumulate luminally (e.g., ulcerative colitis, shigellosis, intestinal lymphangiectasia). If the condition is chronic, the continuing protein loss can

lead to hypoalbuminemia and hypoglobulinemia (Micheal *et al.*, 2003; David and Camiller, 2004).

iv) Diarrhea resulting from motility disturbances: Both increases and decreases in gut motility can lead to diarrhea. Examples of the former are thyrotoxicosis and opiate withdrawal. Decreases in effective motility in the small intestine due to large diverticula, smooth muscle damage, or autonomic neuropathy (diabetic, idiopathic) can result in bacterial overgrowth. And bacterial overgrowth can lead to diarrhea (Chang *et al.*, 1982).

1.3.2 Pathophysiology of chronic diarrhea

i) Hormone-secreting neoplasms: In several uncommon tumors, hormones are produced and released that directly stimulate intestinal secretion, causing profuse diarrhea or, in one instance (gastrinoma), interfering with nutrient absorption (Jensen *et al.*, 1999).

In patients with pancreatic cholera, certain endocrine neoplasms that occur most commonly in pancreatic islets but occasionally in the proximal intestinal mucosa secrete large quantities of VIP, the enteric secretory neurotransmitter. Pheochromocytomas do so also. Profuse diarrhea develops in 30% of patients with medullary carcinoma of the thyroid because of secretion of calcitonin, another secretory stimulus in the intestine (Jensen *et al.*, 1999; Camiller and Murray, 2001).

In Zollinger-Ellison syndrome (gastrinoma), both diarrhea and peptic ulceration can result from the marked increase in gastric acid production that is associated with gastrin-secreting neoplasms. About half of the patients with the rare neoplasm systemic mastocytosis develop diarrhea, likely due to histamine-induced gastric hypersecretion, a cause similar to that of the diarrhea in Zollinger-Ellison syndrome (Jensen *et al.*, 1999; David and Camiller, 2004).

ii) Diabetes mellitus: Diarrhea accompanied by rectal incontinence is an occasional complication of long-standing, insulin-dependent diabetes. It typically occurs in patients with poor diabetic control and peripheral neuropathy. Intestinal biopsies in such patients are usually normal, and nutrient malabsorption or bacterial overgrowth is present only in

a minority of cases. In most instances, the diarrhea is secondary to degeneration of adrenergic nerves in effect less noradrenalin, that, as mentioned above, are antisecretory and/or proabsorptive in intestinal fluid homeostasis (Chang *et al.,* 1985).

1.4 Principles of diarrhea management

Many patients with sudden onset of diarrhea have a benign, self-limited illness requiring no treatment or evaluation. In severe diarrheal cases, dehydration and electrolyte imbalances are the principal risk, particularly in infants, children, and frail elderly patients. Oral rehydration therapy therefore is a cornerstone for patients with acute illnesses resulting in significant diarrhea. This therapy exploits the fact that nutrient-linked co-transport of water and electrolytes remains intact in the small bowel in most cases of acute diarrhea. Sodium and chloride absorption is linked to glucose uptake by the enterocyte; this is followed by movement of water in the same direction. A balanced mixture of glucose and electrolytes in volumes matched to losses therefore can prevent dehydration (Rang *et al.,* 2003; Pasricha, 2006).

Pharmacotherapy of diarrhea should be reserved for patients with significant or persistent symptoms. Nonspecific antidiarrheal agents typically do not address the underlying pathophysiology responsible for the diarrhea; their principal utility is to provide symptomatic relief in mild cases of acute diarrhea. Many of these agents act by decreasing intestinal motility and should be avoided as much as possible in acute diarrheal illnesses caused by invasive organisms. In such cases, these agents may mask the clinical picture, delay clearance of organisms, and increase the risk of systemic invasion by the infectious organisms; they also may induce local complications such as toxic megacolon (Pasricha, 2006; Mc Quid, 2007).

For many chronic conditions, diarrhea can be controlled by suppression of the underlying mechanism. Examples include elimination of dietary lactose for lactase deficiency, use of glucocorticoids or other anti-inflammatory agents for idiopathic inflammatory bowel diseases, adsorptive agents such as cholestyramine for ileal bile acid malabsorption (David and Camiller, 2004).

Proton pump inhibitors such as omeprazole for the gastric hypersecretion of gastrinomas, somatostatin analogues such as octreotide for malignant carcinoid, prostaglandin inhibitors such as indomethacin for medullary carcinoma of the thyroid, and pancreatic enzyme replacement for pancreatic insufficiency. Clonidine, an α_2 adrenergic agonist, may allow control of diabetic diarrhea. For all patients with chronic diarrhea, fluid and electrolyte repletion is an important component (Camiller and Murray, 2001; David and Camiller, 2004).

1.5 Antimotility, antisecretory and antispasmodic agents

i) Opioid agonists: Opioid receptors (μ,κ, and δ) exist in high density in the GIT, located particularly in the myenteric and submucosal plexus, as well as on nociceptive pathways to the brain. In the stomach, motility (rhythmic contraction and relaxation) may decrease. In the small intestine resting tone (persistent contraction) is increased, with periodic spasms, but the amplitude of non propulsive contractions is markedly decreased. In the large intestine, propulsive peristaltic waves are diminished and tone is increased; this delays passage of the fecal mass and allows increased absorption of water, which leads to constipation (Szarka et al., 2007).

They act by several different mechanisms, mediated principally through either mu- or sigma-opioid receptors on enteric nerves, epithelial cells, and muscle. These mechanisms include effects on intestinal motility (μ receptors), intestinal secretion (δ receptors), or absorption (μ and δ receptors) (Szarka *et al.,* 2007).

ii) Chloride channel blockers are effective antisecretory agents *in vitro* but are too toxic for human use and have not proven to be effective antidiarrheal agents *in vivo*.

iii) Calcium channel blockers such as *verapamil* and *nifedipine* reduce motility and may promote intestinal electrolyte and water absorption. Constipation, in fact, is a significant side effect of these drugs. However, because of their systemic effects and the availability

of other agents, they seldom if ever are used for diarrheal illnesses (Pasricha and Jefri, 2001; Pasricha, 2006).

iv) Berberine is a plant alkaloid used most commonly in bacterial diarrhea and cholera, but is also apparently effective against intestinal parasites. The antidiarrheal effects in part may be related to its antimicrobial activity, as well as its ability to inhibit smooth muscle contraction and delay intestinal transit by antagonizing the effects of acetylcholine (by competitive and noncompetitive mechanisms) and blocking the entry of Ca^{2+} into cells. In addition, it inhibits intestinal secretion (Pasricha, 2006).

v) Somatostatin analogues: Octreotide is an octapeptide analog of somatostatin that is effective in inhibiting the severe secretory diarrhea brought about by hormone-secreting tumors of the pancreas and the gastrointestinal tract. In hormone-secreting neoplasms, they block hormones (serotonin, VIP and gastrin) production by the tumor. Since they also appear to have a direct antisecretory effect on the gut epithelium, they have been employed for treating other forms of secretory diarrhea; cancer chemotherapy–induced diarrhea, diarrhea associated with human immunodeficiency virus (HIV), and diabetes-associated diarrhea (Jensen *et al.,* 1999).

vi) Antispasmodics: The antispasmodics are considered useful for relieving or calming colicky pains resulting from spasms of the gut muscles and diarrhea due to hypermotility of the gastrointestinal tract (Gilani *et al*., 1994).

Abdominal pain is a major symptom in IBS, and clinicians have observed that anticholinergic drugs may provide temporary relief for symptoms such as painful cramps related to intestinal spasm. Although controlled clinical trials have produced mixed results, evidence generally supports beneficial effects of anticholinergic drugs (clidinium, prophantiline, dicyclomine and hyoscyamine) for pain (Tally *et al.,* 2003). The most common agents of this class are nonspecific antagonists of the muscarinic receptor and include the tertiary amines dicyclomine and hyoscyamine, and the quaternary ammonium compounds glycopyrrolate and methscopolamine (Rang *et al.,* 2003).

Antispasmodic agents relax smooth muscle in the gut and reduce contractions. They act through anticholinergic or antimuscarinic properties. The anticholinergic effects of antispasmodics limit their use, especially in the long term (Tally *et al.*, 2003).

1.6 Herbal remedies for gastrointestinal motility disorders and use of *Stephania abyssinica*

Plants have been utilized as a medicine for thousands of years. More recently, a WHO study has shown that about 80% of the world's population still relies on traditional medicine *(*Dharmani and Palit *et al., 2006).* A growing numbers of plants have been reported for antidiarrheal and antispasmodic activity.

Various herbal preparations have been used and claimed to have benefits as antidiarrheal and antispasmodic. Among these are *Taverniera abyssinica , Syzygium guineese, Lipdium sitivium, Solaniase gigsa, Moringa stenopetala, Atropa belladonna, Berberis vulgaris, Evodia rutaecarpa, and Linum sitatissimum.* And Studies were done to proof the traditional use the preparations (Abebe and Ayehu, 1993; Abebe *et al.*, 2003).

Aqueous extract of the roots of *Taverniera abyssinica* ("*Dengetegna"*) antagonized Ach and histamine induced contractile responses of the guinea pig ileum and relaxed the smooth muscle of rabbit duodenum, which is suggesstive of its ethnomedical use in stomachache treatment (Noamesi *et al*., 1990). The antihistaminic and anticholinergic activities of aqueous extract of barberry fruits (*Berberis vulgaris*) were investigated on isolated guinea-pig ileum and the extract were found to possess anticholinergic and antihistaminic activities (Shamsa *et al*., 1999).

The leaf ethanol extract of *Moringa stenopetala* was shown to have a potential antispasmodic effect on guinea pig ileum (Mekonnen et al., 1999). The aqueous extract of *Linum sitatissimum* ("*Telba"*) seed was observed to show significant spasmolytic acivitiy and protective effects against experimental ulcerogenesis in *g*uinea pig ileum and mouse stomach (Makonnen *et al.*, 1996).

Muscarinic antagonists like atropine (*Atropa belladonna*) inhibit the contractions of gastrointestinal tract induced by acetylcholine (Ach). This partial inhibition of gastrointestinal motility by atropine has led to their widespread use as antispasmodics in the treatment of disorders associated with intestinal hypermotility (Ghosh *et al*., 1993; Broadley and Kelly, 2001).

The aqueous extract of *Evodia rutaecarpa* fruit was used to examine its effects on castor oil-induced diarrhea and to compare with its anti-transit effect in mice. The results indicated that the extracts had both anti-transit effect and antidiarrheal effects (Li-Li *et al.,* 2000).

Stephania abyssinica is a perennial glabrous creeper with woody or herbaceous stem. It is the most common member of the Menispermaceae indigenous in the mountain rainforest of southern and eastern Africa. The leaves are used for stomachache, abdominal cramp and antidote against snake bites. An aqueous extract of the dried and crushed roots and root powder of this species is used for the treatment of diarrhea, dysentery, vomiting, heart complaints, hypertension, insomnia, and mastitis (Chakraborty *et al.,* 2000; Abebe *et al.*, 2003). The roots are also employed in treatment of round worm, menorrhagia, and boils. Whole of the plant can be used for stomachache cases and syphilis and root decoction is drunk for boils. Root powder or its infusion is used as a remedy against cholera, gonorrhea and syphilis and its root decoction is taken for jaundice. Powder of the leaf is used for treating wound and eczema (Abebe and Ayehu, 1993; Chakraborty *et al.*, 2000; Abebe *et al.,* 2003; Gedif and Hahn *et al.,* 2003).

Previous phytochemical investigations on *S. abyssinica* revealed that isoqiunol alkaloids are the main chemical components. The occurrence of polyphenols (flavonoids and tannins) has been reported. Previous bioactivity test indicate this plant posses antimicrobial, anticancer, antioxidant, anti-inflammatory and antimalaria activities (Chakraborty *et al.,* 2000; Abebe *et al.,* 2003).

It is known that large number of herbal remedies remain unexplored and should be evaluated for their potential therapeutic application (Gedif and Hahn *et al.*, 2003), including antidiarrheal plants. One candidate for such kind of scientific evaluation is Stephania abyssinica and there is lack of scientific proof for claimed antidiarrheal and stomachache treatment use in this country. In this study, therefore, antidiarrheal and spasmolytic activities of the crud extracts of the leaf and root were studied in different experimental models. The present work will help proper utilization of the medicinal plant. On top of this it can be considered a way forward in quest of new antidiarrheal and antispasmodic agent with optimum benefits and less adverse events.

2 OBJECTIVES OF THE STUDY

2.1 General objective

Evaluate the antidiarrheal and antispasmodic activities of the aqueous and methanol extracts of *Stephania abyssinica* which is used in Ethiopian traditional medicine for treatment of diarrhea and stomachache.

2.2 Specific objectives

To evaluate antidiarrheal activities of the aqueous and methanol extracts of the root and leaf in mice,

To evaluate in-vivo spasmolytic activities of the aqueous and methanol extracts of the root and leaf in mice,

To test for in-vitro antispasmodic effect of the aqueous and methanol extracts of the leaf on isolated GPI,

To test for antisecretory effect of the aqueous and methanol extracts of the root and leaf in mice.

3 MATERIALS AND METHODS

3.1 Drugs and Chemicals

Drugs (ACh, Atropine, and Loperamide) used in this study were purchased from Sigma-Aldrich and chemicals (NaCl, KCl, $MgCl_2$, $NaHCo_3$, $NaHPO_4$, Glucose, $CaCl_2$, and gum aciacia) were purchased from Riedel-De Haen, Methanol (TechnoPharmchem, Bahadargarh, India),Charcoal (The British Drug House, Ltd., London) and castor oil was purchased from local market.

3.2 Plant material

The leaves and roots of *S. abyssinica* were collected during July, 2009 G.C from Bela, North Addis Ababa, Ethiopia. The collected plant was identified by taxonomist (Voucher number DT-01) and the specimen was deposited at the National Herbarium, Addis Ababa University. Then the roots and leaves were dried under the shed and crushed to powders.

3.3 Extraction of plant material

Aqueous extract of the root and leaf were prepared by maceration of 100 gram of the powdered plant material in enough distilled water. Flask containing powdered plant material and DW was placed on a shaker for 48 h at room temperature after 48 h each sample was filtered using gauze and filter paper (whatman No1) and lyophilized. The same procedure was followed for methanol extractions except rota – vapor was used to remove methanol from the extracts.

3.4 Animal preparation and dosing

Guinea pigs were purchased from EHNRI and acclimatized to lab condition. The animals were exposed to 12/12 h light-dark cycle and maintained under standard condition for 10 days. Swiss Albino mice were obtained from biology department, Addis Ababa

University animals' house and maintained under standard condition for 10 days (Vogle, 2002).

They were acclimatized under uniform conditions of 12/12 h light and dark cycle and housed at a temperature of 24 ^{0}C. They were fed a standard pellet diet and tap water *ad libitum* at biology department animal house before use for the experiments (Vogle, 2002).

The pilot study was performed with 5, 25, 100, 200 and 300 mg/kg of the extracts in both antidiarrheal and intestinal transit test. The dose of 25 mg/kg showed the minimum effect and there was no much difference in the anti-diarrhea activity of 200 mg/kg compared with 300 mg/kg. All the extracts in castor oil-induced diarrhea at the dose of 5 mg/kg did not inhibit diarrhea significantly when compared with castor oil alone. The same was observed for intestinal transit test. From these preliminary works the doses 25, 50, 100 and 200 mg/kg were selected for subsequent studies.

3.5 Castor-oil induced diarrheal model in mice

The methods described by Vogel, (2002), Aye –than *et al.,* (1989), Ching *et al.,* (2008) and Awouter *et al.,* (1978) were adopted in assaying for the effect of the extracts on caster oil induced diarrhea.

Mice of either sex weiging 25 – 30 g were divided in to control and test groups (n=6). Each animal was then given 0.3 ml of castor oil orally after 30 min of treatment with extracts (25, 50, 100, 200 mg/kg) or vehicle and placed in transparent cages to observe for consistency of stool and to measure on set of diarrhea (Aye –than *et al.,* 1989).

The following parameters were recorded; on set of diarrhea, the total number of both dry and wet stools, number of watery diarrheal stools, and the weight of stools were measured for 4 h (Awouter *et al.,* 1978).

A numerical score based on stool consistency was assigned as follows: normal stool (formed and pelleted stool) =1, mild or semi solid unformed stool=2 and copious or watery stool=3.

The total diarrheal score was calculated as the sum of the recorded diarrheal score based on stool consistence and **%** protection was calculated as reductions in diarrheal score from the control group value. Whereas the in vivo antidiarrheal index (ADI) was expressed as geometric mean of the PFR, GITR and DFT.

ADI= cube root of (PFR x GITR x DFT) where ADI is antidiarrheal index, PFR is purgation frequency reductions (in per cent of control), GITR is gastro intestinal transit reductions (in per cent of control), and DFT is diarrheal free time increase compared with control (in per cent of total time) (Ching *et al.,* 2008).

3.6 In-vivo GI motility

This was carried out according to the method outlined by Aye-than *et al.,* (1989), Ching *et al.,* (2008) and Mujumd *et al.,* (1998) using charcoal meal as a diet marker.

The mice (overnight fasted with free access to water) of either sex were divided in to control and test group of 6 animals per group. The first group (control group) was orally administered the vehicle. The second, third, fourth, and fifth groups received 25, 50, 100, 200 mg/kg body weight of extracts, respectively. The sixth group received the standard drug, atropine (5 mg/kg body weight). Thirty minutes after administration, each animal was given 0.5 ml of charcoal meal orally (10% charcoal in 5% gum acacia), after 30 min each animal was sacrificed and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum was measured and expressed as percentage of total length of the intestine (Peristaltic Index or PI) (Ching *et al.,* 2008).

In the castor oil induced intestinal transit measurements (as part of in vivo antispasmodic model on established gut increased motility unlike the above model which measures

effect on normal peristalsis) the animals (overnight fasted with free access to water) was administered vehicle in control groups, extracts (25, 50, 100, 200 mg/kg), or loperamide (5mg/kg body weight) in test groups 30 min before oral administration of castor oil (0.3 ml), 30 min later orally charcoal meal was administered, after 30 min the animals were sacrifices and the effect on intestinal transit was assessed by calculating peristaltic index (PI) expressed as intestinal transit of the marker (charcoal meal) in cm as % of the total length of the intestine. And % inhibition is calculated as % of control group and considering the control is 100 % (Aye-than *et al.,* 1989; Ching *et al.,* 2008).

3.7 Enteropooling test

This test was done as described by Robert *et al* (1976) and Ching *et al* (2008).
Briefly, mice of either sex were fasted for 24 h but allowed free access to water. The mice was divided in to control and test group. Group 1 was administered distilled water, while group 2 and 3 were pre – treated with 100 and 50 mg/kg of the extracts, and group 4 pre – treated with loperamide (5 mg/kg). After 30 min each animal was administered 10 ml/kg of castor oil. The animals were scarified after 30 min and the small intestine from the pylorus to ceacum was dissected out and its content expelled into a measuring cylinder by milking to measure the volume of the fluid (Robert *et al.,* 1976; Ching *et al.,* 2008).

3.8 In vitro antispasmodic test

This test was carried out according to the method outlined by Gilani *et al* (1994), Galvez *et al* (1996), Mekonnen *et al* (1999) and Vogel (2002).

Fasted (24 hrs) guinea pigs weighing (400-450g) were scarified by a gentle blow at the back and allowed to bleed. The abdominal cavity of the animal was opened by midline incision every time a tissue was required, and the ileum 2-2.5 cm in length was removed immediately and trimmed from surrounding tissues. The contents of the intestine were

washed with Physiological Salt Solution (PSS) called Tyrodes solution (see composition below).

The isolated tissue preparations were used according to the technique described by Gilani *et al.*, (1994) and Mekonnen *et al.*, (1999). Segments of ileum were tied with silk threads at both ends (ileum tied in opposite directions) and suspended in a thermo regulated 25 ml organ bath, maintained at 37 ^{0}C, containing Tyrode solution of the following composition (g/l): NaCl, 8 gram; KCl, 0.2 gram; MgCl2, 0.1 gram; NaHCO3, 1 gram; NaHPO4, 0.05 gram; Glucose, 1 gram; CaCl2, 0.2 gram. One end of the ileum was attached to a tissue holder at the base of the organ bath and the other end to the isometric recording device. The tissues were constantly bubbled with air mixture of 95% O2 and 5% CO2. A suitable weight or resting tension of 1 gram was applied to the individual tissue (Mekonnen *et al*. 1999; Vogel HG, 2002).

The suspended ileum was allowed to equilibrate for 30-45 min before adding acetylcholine, the particular plant extract or the standard drug. After the initial equilibration period, Acetylcholine (10^{-9} to 10^{-3} M) was added to the organ bath and the control cumulative concentration-response curve for acetylcholine was constructed. Each time the added concentration of the acetylcholine was left in contact with the tissues for 30 sec before adding the next concentration. Then the tissue was washed two times with Tyrode solution at the interval of 5 min. It was left to resume its normal contraction. After a stabilized regular contraction, Extracts (SALM and SALA) at conc of 100 and 200µg/ml were added; or atropine was then added to the organ bath 5 min before the corresponding concentration curve was recorded (Gilani *et al*., 1994; Vogel, 2002).

The plant extracts were prepared in physiological Tyrodes salt solutions (PSS) while the stock solutions of all drugs (Ach, and Atropine) were made in distilled water and then serially diluted with PSS. The final dilutions of the drugs were made fresh on the day of the experiment. The calculated concentrations of each plant extracts and standard drugs were final organ bath concentration (Mekonnen *et al*., 1999).

Isometric contractions were recorded with a Grass FT-03 strain gauge transducer coupled to a Grass 79 Polygraph which is equipped with preamplifier, main amplifier, oscillograph and time and event marker (Grass Inc., USA). The chart speed was 5mm/minute. In addition, the anticholinergic effects of the extracts were compared with atropine (6.5 nM) (Mekonnen *et al.*, 1999; Shamsa *et al.*, 1999).

Original computer program Graph pad prism 2.0 (GraphPad-Pism software Inc, San Diago, CA) was used for fitting non – linear curve to do DRC and calculating PD_2 value. Responses in Ach induced GPI contractions were expressed as % of the maximum contractions (E_{max}) induced by Ach (the control) prior to the addition of the plant extracts or atropine.

3.9 Statistical Analysis

The experimental results were expressed as mean ± Standard error of mean (SEM) and the results are analyzed by ANOVA (one way) followed by Turkey-kramer post test for comparing the means differences and were considered statistically significant when $P < 0.05$.

4 RESULTS

4.1 Effects on castor oil induced diarrheal model.

Forty seven min after castor oil administration, the mice in the control group produced copious diarrhea. Pretreatment of mice with the extracts caused dose-dependent and significant ($p<0.05$) delay in the on set of diarrhea, frequency of stooling (reduction in number of wet stools and total stools), decrease in weight of wet stools and the general diarrhea score (Table 1 and 2).

With doses of 200 mg per kg of both root and leaf extracts (aqueous and methanol,) there were no diarrhea episodes. Whereas with dose of 100 mg per kg the on set for SALA and SARA were 171 min ($p<0.001$) and 169 min ($p<0.001$) respectively and with same dose of methanol extracts SARM was 212 min and SALM was 201 min. With the dose of 50 mg per kg the effect of the extracts were significant ($p<0.001$). The on set time was at the third hours and second hour for the 25 mg per kg for all extracts. Long diarrhea free period was observed with dose of 100 mg/kg and no diarrhea was observed with dose of 200 mg/kg.

The extracts produced high antidiarrheal index with dose of 200 mg/kg; SALM, SALA, SARM and SARA produced ADI of 88.79, 89.21, 91.08, and 82.23 respectively and these ADI values of the extracts are all larger than that of the reference loperamide which was 79.33. Least ADI was scored with dose of 25 mg/kg of all extracts (Table 5).

The extracts also reduced diarrheal score significantly ($p< 0.05$) as compared to the control group. With dose of 200 mg/kg least diarrheal score is observed for all extracts; SALM, SARM, SARA and SALA decreased diarrheal score from 29 of the control group to 3.50, 2.3, 3.0 and 3.8 respectively. However, 25 mg/kg produced high diarrhea score for all extracts. See table 1 and 2.

Table1: Effects of methanol extract of *S. abyssinica* on castor oil induced diarrheal model in mice

Treatment	Dose (mg/kg)	on-set time(min)	Total number of Stool	Number of wet stool	Total diarrhea SCORE	Weight of stool (mg)	% Protection
Control	-	47± 2.28	12.67±0.48	10.33±0.67	29±1.92	904±37.22	-
SALM	200	-	3.5±0.4 ^{c}	-	3.5±0.4 ^{c}	132.±1.50 ^{c}	87.93
SALM	100	201±5.78 ^{c}	4.17±0.4 ^{c}	2.00.±0.3 ^{c}	7±1.12 ^{c}	229.±4.12 ^{c}	75.68
SALM	50	176.13±5.17 ^{c}	6.67±0.51 ^{b}	4±0.36 ^{c}	13.15±2.92 ^{c}	399.17±16.32 ^{c}	54.66
SALM	25	93.0±4.41 ^{c}	7.5±0.42 ^{b}	6.83±0.40 ^{a}	20.72±2.62 ^{a}	554.67±13.66 ^{b}	28.55
SARM	200	-	2.33±0.61 ^{c}	-	2.3±0.61 ^{c}	94.±2.40 ^{c}	92.07
SARM	100	212.0±4.13 ^{c}	4±0.58 ^{c}	1.9.±0.12 ^{c}	6±1.54 ^{c}	185.±3.60 ^{c}	79.31
SARM	50	160.83±4.85 ^{c}	5.67±0.21 ^{c}	3.83±0.75 ^{c}	11.02±2.3 ^{c}	259.67±9.75 ^{c}	62
SARM	25	103.33±5.32 ^{c}	7±0.37 ^{b}	5.67±0.53 ^{c}	19.69±3.0 ^{b}	440.17±21.52 ^{c}	32.10
Loperamide	5	195.5±4.50 ^{c}	5.67±0.67 ^{c}	2.16±0.75 ^{c}	8.04±2.10 ^{c}	226.5±11.47 ^{c}	72.28

Values are mean ±SEM, n=6, and P^{a}<0.05, P^{b}<0.01, P^{c}<0.001 are significantly different from the control group

Table 2: Effects of aqueous extract of *S. abyssinica* on castor oil induced diarrheal model in mice

Treatment	Dose (mg/kg)	on-set time(min)	Total number of stool	Number of wet stool	Total Diarrhea SCORE	Weight of stool (mg)	%Protection
Control	-	47.± 4.50	12.67±0.48	10.33±0.67	29±1.92	904±37.22	-
SARA	200	-	3.03±0.40 ^{c}	-	3.03±0.40 ^{c}	102.±3.1 ^{c}	89.55
SARA	100	171.67±8.07 ^{c}	5.67±0.71 ^{c}	3.5±0.22 ^{c}	11.10±2.11 ^{c}	185.83±20.30 ^{c}	61.72
SARA	50	131.67±12.34 ^{c}	6.67±0.49 ^{c}	4.17±0.75 ^{c}	16.19±1.96 ^{c}	237±15.42 ^{c}	44.17
SARA	25	81.83±3.88 ^{b}	8.17±0.70^{b}	7.33±0.82 ^{b}	21.3±2.40 ^{a}	608±17.74 ^{b}	26.55
SALA	200	-	3.83±0.47 ^{c}	-	3.8±1.17 ^{c}	181 ±4.12 ^{c}	86.90
SALA	100	169.5±14.43^{c}	5.5±0.67 ^{c}	3±0.89 ^{c}	14.52±2.62 ^{c}	378±18.45 ^{c}	49.93
SALA	50	97.17±5.23 ^{c}	8.67±0.36^{b}	4.67±0.82 ^{c}	20.78±2.93 ^{a}	445.83±16.09 ^{c}	28.34
SALA	25	65.17±6.07 ^{a}	11.3±0.97^{ns}	8.97.±0.61^{ns}	27.54±1.89^{ns}	885±19.34^{ns}	15.38
Loperamide	5	195.5±4.50 ^{c}	5.67±0.67 ^{c}	2.16±0.75 ^{c}	8.04±2.10 ^{c}	226.5±11.47 ^{c}	72.28

Values are mean ±SEM, n=6, and $P^{a}<0.05$, $P^{b}<0.01$, $P^{c}<0.001$are significantly different from the control group

Ns=not significant

4.2 Effects of the extracts on in-vivo GI motility

The extracts (SALM, SALA, SARM, and SARA) with dose of 100 and 50 mg/kg and the reference drug atropine produced significant ($p<0.001$) inhibition of normal intestinal transit. Whereas with dose of 200 mg/kg the extracts inhibited stomach empting as the marker charcoal was found in stomach.

However with dose of 25 mg/ kg of aqueous extracts showed no significant intestinal transit (Peristaltic Index) reduction even though the methanol extract showed significant inhibition of intestinal transit with same dose. See Table 3.

In castor oil induced intestinal transit model castor oil increased Peristaltic Index from 66.03 ± 2.80 of the normal value to 90.10 ± 1.89. The extracts and the reference drug loperamide produced significant ($p<0.001$) and dose dependent decrease in the castor oil induced intestinal transit when compared to the control group (castor oil) with doses of 200, 100 and 50 mg/kg of all extracts. However with dose of 25 mg/kg no significant reduction of the Peristaltic Index (PI) for all extracts. See table 4.

Table 3: Inhibitory effect of the extract of *S. abyssinica* on normal GI transit of mice

Treatment	Dose (mg/kg)	Peristaltic index (PI) %	Inhibition %
Control	-	66.03±2.8	-
SALM	200	0.0±0.0 ^{c}	100
SALM	100	10.14±1.12 ^{c}	84.64
SALM	50	31.78±1.14 ^{c}	51.87
SALM	25	50.27±2.3 ^{b}	23.87
SARM	200	0±0.00 ^{c}	100
SARM	100	14.46±1.27 ^{c}	69.77
SARM	50	47.79±1.50 ^{c}	27.62
SARM	25	53.31±1.1 ^{b}	19.26
SALA	200	0.00+0.00 ^{c}	100
SALA	100	21.26±1.3 ^{c}	67.8
SALA	50	38.14±1.09 ^{c}	42.23
SALA	25	61.3±1.96 ^{ns}	7.16
SARA	200	0.0±0.0 ^{c}	100
SARA	100	32.83±1.10 ^{c}	50.42
SARA	50	48.04±2.20 ^{c}	27.25
SARA	25	59.9±2.16 ^{ns}	9.28
Atropine	5	25.40±2.00 ^{c}	61.53

Values are mean ± SEM, n= 6 and $P^{a}<0.05$, $P^{b}<0.01$, $P^{c}<0.001$ are significantly different from the control group, Ns=not significant

Table 4: Inhibitory effect of the extract of *S. abyssinica* on castor oil induced intestinal transit in mice.

Treatment	**Dose (mg/kg)**	**Peristaltic Index (%)**	**%Inhibition**
Control	- -	90.10+ 1.89	-
SALM	200	26.67+1.36 ^{c}	70.39
SALM	100	54.00+1.59 ^{c}	40.00
SALM	50	69.10+1.80 ^{c}	23.33
SALM	25	89.50+1.84 ^{ns}	0.66
SALA	200	36.85+2.17 ^{c}	71.11
SALA	100	59.16+2.61 ^{c}	34.44
SALA	50	76.80+2.11 ^{b}	14.67
SALA	25	88.17+1.95 ^{ns}	2.03
SARM	200	22.00+1.60 ^{c}	75.55
SARM	100	46.95+2.94 ^{c}	47.83
SARM	50	63.04+1.61 ^{c}	30.00
SARM	25	79.80+2.84 ^{ns}	11.33
SARA	200	40.67+3.23 ^{c}	55.60
SARA	100	52.34+189 ^{c}	42.22
SARA	50	70.19+2.03 ^{c}	22.22
SARA	25	89.80+1.83 ^{ns}	1.11
Loperamide	5	20.17+2.58 ^{c}	77.78

Values are mean ±SEM, n= 6 and $P^{a}<0.05$, $P^{b}<0.01$, $P^{c}<0.001$ are Significantly different from the control group, Ns=not signify

Table 5: In vivo antidiarrhea index (ADI) of the extracts in mice

Treatment	Dose mg/kg	DFT (%)	GITR (%)	PFR (%)	ADI (%)
control	-		-	-	-
SALM	200	100	70.39	100	88.79
SALM	100	83.75	40	80.64	64.65
SALM	50	73.68	23.33	61.28	47.23
SALM	25	38.75	0.66	33.88	12.70
SALA	200	100	71.11	100	89.21
SALA	100	59.05	34.44	70.96	52.44
SALA	50	40.42	14.67	54.79	31.91
SALA	25	27.08	2.03	20.91	10.35
SARM	200	100	75.55	100	91.08
SARM	100	87.5	47.83	81.6	69.89
SARM	50	66.67	30	62.44	49.98
SARM	25	42.92	11.33	45.11	27.99
SARA	200	100	55.60	100	82.23
SARA	100	71.25	42.22	66.12	58.37
SARA	50	54.58	22.22	59.63	41.66
SARA	25	33.75	1.11	29.04	10.28
Loperamide	5	81.25	77.78	79	79.33

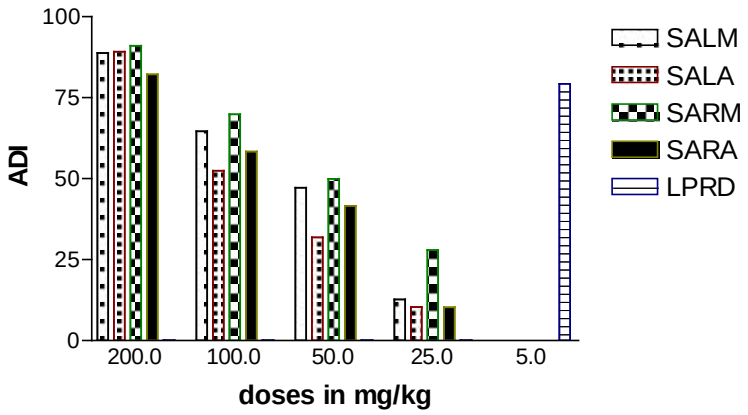

Figure 3: In-vivo antidiarrheal index (ADI) of the extracts.

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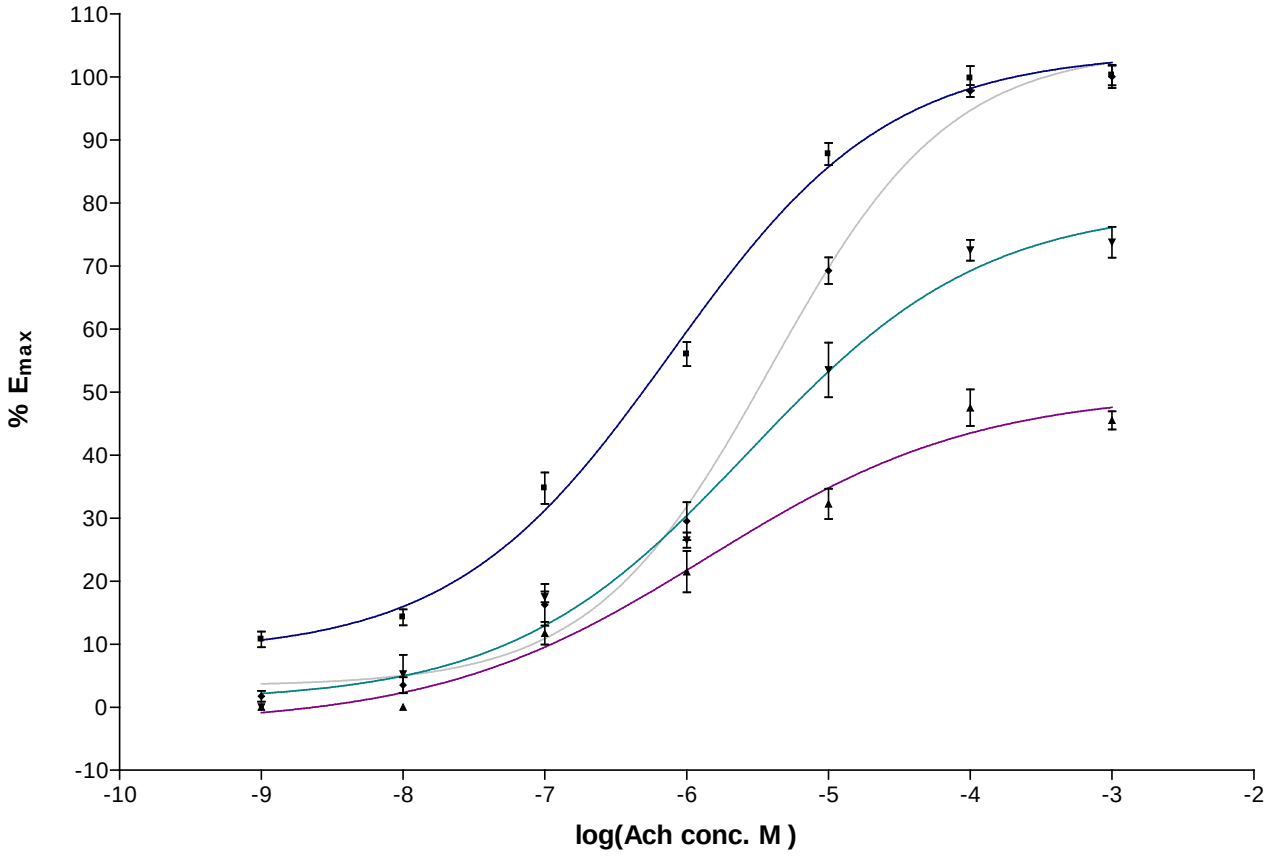
4.3 In vitro antispasmodic activities on isolated GPI

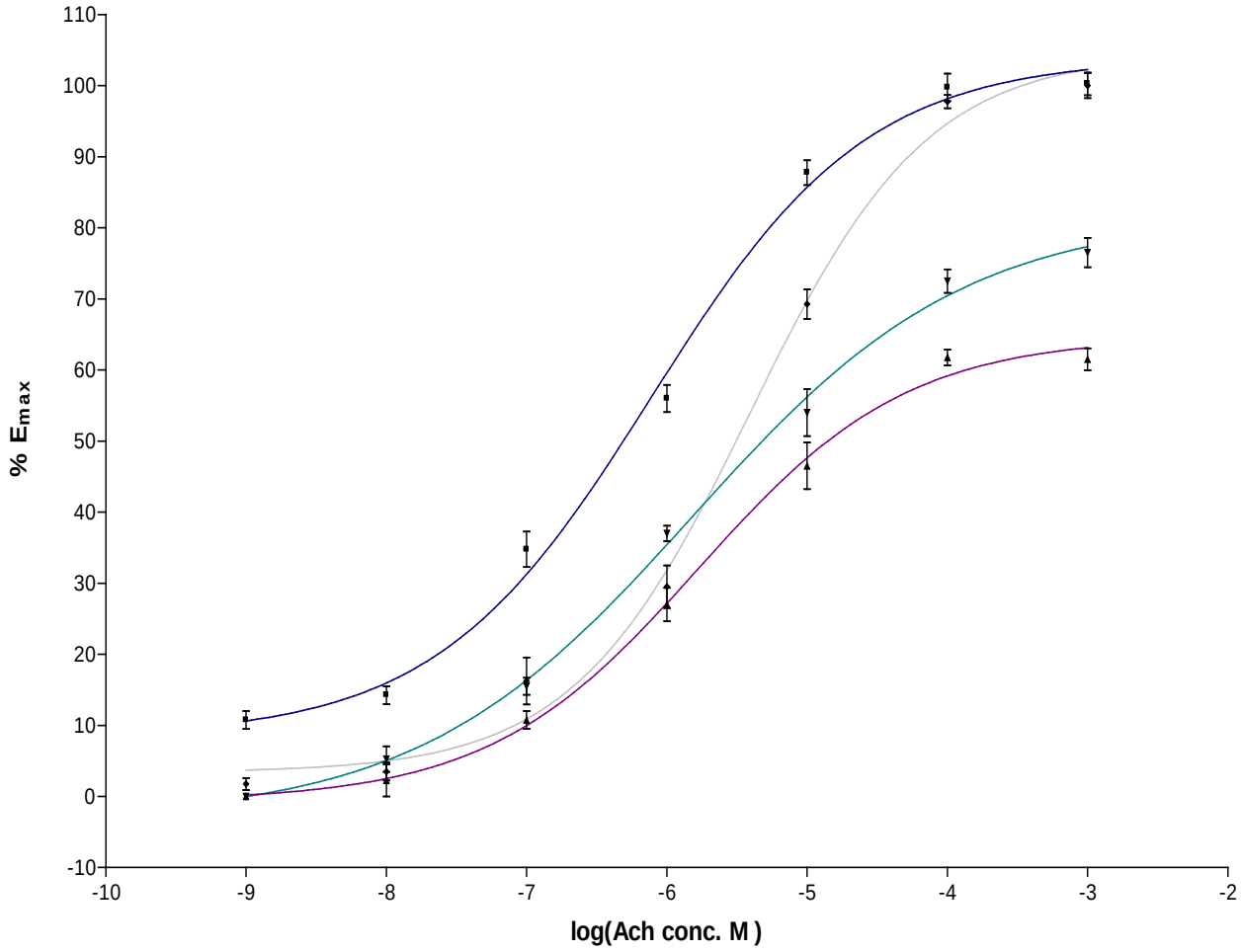
The extracts (SALM and SALA) at conc of 100 ug/ml and 200 ug/ml significantly ($P<0.05$) inhibited the Ach induced contraction when compared with the control. This activity was dose dependent. The inhibition of contraction was significant ($P<0.05$) at all conc of the spasmogen Ach. Methanol extract showed more spasmolytic activities than the aqueous extract at both conc (100 and 200 ug/ml) as E_{max} of Ach at conc of 10^{-3}M was decreased (from 100 for the control group) by SALM with conc of 200 and 100 ug/ml to 45.6±2.13 and 73.2±3.04 respectively, however E_{max} of Ach at conc of 10^{-3} M was decreased (from 100 for the control group) by SALA with conc of 200 and 100 ug/ml to 62.0±2.98 and 74.8±2.46 respectively. However that of atropine (6.5 nM) was 98.1±1.78 (Figure 4 and 5).

The extracts also produced right ward shift in DRC of Ach and decreased PD_2 value of Ach from 6.13±0.12 to 5.56±0.10 and 5.63±0.09 by SALM with conc of 200 and 100 ug/ml respectively, whereas SALA with conc. of 200 and 100 ug/ml from 6.13±0.12 to 5.77±0.11 and 5.81±0.14 respectively and atropine decreased from 6.13±0.12 to 5.40±0.09. But right ward shift in DRC was in non-parallel way as the slope is different for the DRC of the control group and the test groups.



Control (Ach) ■
SALM 100 ug/ml + ACh ▼
SALM 200 ug/ml + ACh ▲
Atropine + ACh ●

Figure 4: Effect of increasing concentrations of SALM on the DRC of acetylcholine on guinea-pig ileum. Responses were expressed as % of the maximum contractions (Emax) induced by Ach prior to the addition of the extracts (control). Each point is mean ±SEM of six experiments.



Control (Ach) ■
SALA 100 ug/ml + ACh ▼
SALA 200 ug/ml + ACh ▲
Atropine + ACh ●

Figure 5: Effect of increasing concentrations of SALA on the DRC of acetylcholine on guinea-pig ileum. Responses were expressed as % of the maximum contractions (Emax) induced by Ach prior to the addition of the extracts (control). Each point is mean ±SEM of six experiments.

4.4 Effects of the extracts on intestinal fluid accumulations

The results of the effect of the extracts of *S. abyssinica* on intestinal fluid accumulation are presented in Table 6. Oral administration of castor oil produced a significant increase in intestinal fluid (1.03±0.093 ml) as compared to the mice not administered castor oil (0.129±0.04ml). The extracts were found to possess significant anti-enteropooling activities as they significantly ($P<0.001$) reduced intestinal fluid accumulation with dose of 100 mg/kg from1.03 ml of the control to 0.403±0.019 ml, 0.210±0.018 ml, 0.494±0.012 ml and 0.288±0.026 ml by SALM, SARM, SALA and SARA respectively. The standard drug (loperamide) also significantly ($P<0.001$) reduced intestinal fluid accumulation from1.03±0.093 ml of the control to 0.290±0.030ml. The antisecretory activities of the extracts were dose dependent.

Table 6: Effect of the extract on intestinal fluid accumulation in mice.

Treatments	Dose (mg/kg)	Volume of fluid in ml	Inhibition (%)
Control	-	1.030±0.093	-
SALM	100	0.403±0.019^{c}	60.87
SALM	50	0.546±0.038^{b}	46.99
SARM	100	0.210±0.018^{c}	79.61
SARM	50	0.357±0.022^{c}	65.33
SALA	100	0.494±0.012^{c}	52.03
SALA	50	0.653±0.042^{a}	36.60
SARA	100	0.288±0.026^{c}	72.04
SARA	50	0.549±0.090^{b}	46.70
LPRD	5	0.290±0.030^{c}	71.84
Vehicles	-	0.129±0.040^{c}	-

Values are mean ± SEM, n= 6 and $P^{a}<0.05$, $P^{b}<0.01$, $P^{c}<0.001$ are significantly different from the control group.

5 DISCUSSION

In this study, castor oil induced diarrheal model was done in order to test as to whether the extracts of *S. abyssinica* have antidiarrheal activity or not. Then after, other models (antipropulsive and antientropooling) were performed in an attempt to propose some of the possible mechanisms (decrease in GI transit and antisecretory activities) by which they exhibited antidiarrheal activity. And to test antispasmodic activities both in vitro on GPI using Ach as spasmogen and in vivo models in mice using castor oil as spasmogen were performed.

Antidiarrheal activity

Diarrhea may results from disturbance in bowel function in which case there is increased bowel transit, excessive intestinal secretion of water and electrolyte, decreased intestinal reabsorptions as well as more frequent defecations of loose, watery stool (Gurgle *et al*., 2001).

It is known that the active component of castor oil is the ricinoleic acid which is liberated by the action of lipases on castor oil. Castor oil is made up of 90% ricinoleate which when metabolized is responsible for its diarrheal inducing effect. The ricinoleic acid produce irritation and inflammatory actions on intestinal mucosa leading to release of prostaglandins (such as PG-E2). This condition induces an increase in the permeability of the mucosal cells and changes in electrolyte transport, which results in hyper-secretory response (decreasing Na+ and K+ absorption), stimulating peristaltic activity and diarrhea. The castor oil model therefore incorporate both secretory and motility diarrhea (Rouf *et al.,* 2003; Ching *et al.,* 2008).

The result of this study revealed that the extracts possessed antidiarrheal activity in castor oil treated animals. The extract dose-dependently inhibited castor oil-induced transit in mice and this indicates possible antispasmodic activity and possible antidiarrheal mechanism of the extracts. The frequency and severity of castor oil-induced diarrhea in the mice was inhibited in a dose-related manner by the extracts. There was delay in onset

time of diarrhea (increase in diarrhea free time) and the total number of stools, number of wet stools, and weight of wet stools were significantly decreased in dose dependent way, with the highest effect observed with the 200 mg/kg body weight of all extracts (SALM, SALA, SARM and SARA). These effects at 200mg/kg were comparatively more than those produced by loperamide.

Thus, the extracts showed better antidiarrheal activities than the standard loperamide with 200mg/kg and these results suggest efficacy of the extracts as antidiarrheal agents. But the extracts have less potency than the standards loperamide. This less potency is for obvious reason that the extracts are crude whereas loperamide is pure chemical.

The antidiarrhoeal index (ADI) is a measure of the combined effect of the various components of diarrhea such as on-set time of diarrheal (diarrhea free time), purging frequency, and intestinal transit. ADI is used as indicator of in vivo antidiarrheal activities of the extracts. The fact that the extracts produced a significant antidiarrheal index and a significant reduction in diarrheal score reinforces its protective action in diarrhea.

With dose of 200mg/kg of all extracts complete suppression of diarrheal was observed even more than the reference Loperamide and inhibited defecation suggesting constipation could be one of the side effects of the medicinal plant and this speculation is also supported by the marked decrease in GI transit by the extracts especially at larger doses.

The medicinal plant has to be contraindicated in infectious-diarrhea caused by invasive microorganisms as it may cause local GI complication such as toxic mega colon and may decrease elimination of the invasive microorganisms, even though it has been reported *S. abyssinica* has antimicrobial activities (Chacrabotory *et al.* ,2000) unless their antimicrobial activities against specific diarrhea causing microorganisms is established. This proposal is supported by extracts marked decrease of GI motility and extracts marked inhibition of defecation.

The antidiarrheal activities of the extracts of *S. abyssinica* found in the present study is proved to be due to antimotility and antisecretory effect of the extracts as the extracts reduced GI motility in the antipropulsive test and reduced fluid accumulations in antipropulsive test.

The antidiarrheal activities of the extracts of *S. abyssinica* found in the present study could be owing to presence of flavonoids and tannins in this plant as previous studies showed the presence of these metabolites (Chacrabotory *et al.*, 2000; Abebe *et al.*, 2003).

The antidiarrheal activities of flavonoids have been ascribed to their ability to inhibit intestinal motility and water and electrolyte secretions which are known to be altered in diarrheal conditions. Whereas tannins are known to form proteins tannate which make intestinal mucosa more resistant and hence reduce secretion *(*Galvez *et al.,* 1991, 1993; Tripathi, 1994; Borrelli *et al.,* 2000; Venkatesan *et al.,* 2005*).*

Other possible mechanism of action may be due to the extracts binding to mu opiod receptors as previous in vitro study on radioligands binding assay of the methanol extracts of the leaf (SALM) showed affinity of SALM for mu opiod receptor with IC50 of 62 ug/ml (conc. that inhibit 50% nalaxone binding which is opiod antagonist) (Deborah *et al.,* 2002).

The Geiger's criteria for the acceptance of a drug as an antidiarrheal include: (1) inhibition of the production of wet or unformed feaces in animals, (2) inhibition of the production of watery stool or fluid evacuation in animal and (3) inhibition of gastrointestinal propulsive action (Akah *et al.,* 1988).

The extracts (SALM, SALA, SARM and SARA), therefore, meets the Geiger's criteria as antidiarrheal agent as observed from the results of the present study of antidiarrheal and antipropulsive tests.

Antipropulsive activity

Antipropulsive activities were done both for normal transit and castor oil induced transit. This model (antipropulsive test) is useful to test antispasmodic and mechanisms of antidiarrheal activity (Izzo *et al.*, 1992; Vogel, 2002). In the evaluation of effects of the extract on normal Intestinal transit, atropine was used as standard drug. Atropine is known to inhibit intestinal transit (arrest peristalsis) due to its anticholinergic effect (Izzo *et al.*, 1992).

In the evaluation of effects of the extract on normal intestinal transit, the extracts appeared to act on the intestine and the stomach. This is proved by reductions in intestinal propulsive movement of charcoal meal of normal transit at the dose of 25, 50, 100mg/kg. And with dose of 200 mg/kg the extracts inhibited stomach emptying, as the marker charcoal was found in stomach. The results of this model suggested the extracts act not only on the intestine but also on stomach. This effect was comparable even better than standard drug atropine with 200 mg/kg dose though less potent. It is but logical since atropine is pure chemical compared to extracts which are mixture of many compounds, so this is reason for less potency than atropine; however, the extracts showed better efficacy at test dose of 200 mg/kg. ED_{50} of the extracts was not calculated as the extracts are crude and appropriate if determined after isolation of active compound(s).

Studies made on activated charcoal showed that it prevents the absorptions of drugs by adsorbing on the surfaces of charcoal particles (Venkatesan *et al.*, 2005). This may increased the effective dose of the extracts. Activated charcoal was used in GI motility test to serve as marker.

The extracts suppressed the propulsion of charcoal meal which showed the efficacy of the extracts in decreasing the vagal peristaltic movements of GI this also explains muscle relaxant effect of the extracts. This pharmacological action of the extracts suggested one of the possible antidiarrheal activities mechanisms. That is by decreasing hyper motility

in effect increases the time for absorption of water and electrolytes in the intestines also this activity indicates the extracts possible antispasmodic activities (Vogel, 2002).

The antipropulsive activities on normal transit (peristalsis) may be due to anticholinergic activities and/or due to muscle relaxant activities. This speculation is supported by complete arrest of vagal peristalsis by the extract in this in-vivo test and in-vitro antispasmodic tests done on isolated GPI in which the extracts inhibited ACh induced contractions.

In castor oil induced intestinal transits tests the aim is to test the effect of the extracts on established increase in intestinal motility as there is increase in intestinal transit in diarrhea. This experiment was done also as part of in vivo antispasmodic to test effect of the extract on established increase in intestinal motility. The extracts inhibit peristaltic index significantly in this model as compared to the control. However, the extracts produced more reduction in intestinal transit on normal intestinal transit than castor oil induced transit eventhough both were significant. This activity of the extracts was dose dependent.

Antipropulsive effects observed in this model may contribute for antidiarrheal effect of the extracts by increasing intestinal transit time which increase absorption of water and electrolyte.

So the results of this model (antipropulsive tests) indicated the possible antispasmodic activities of the extracts and are consistent with in vitro antispasmodic tests. Also indicated the efficacy of the medicinal plant as antispasmodic agent and support the traditional use of the medicinal plant for stomachache and abdominal spasm.

Lozoya *et al* (2002) reported about the relationship of the spasmolytic, antimotility and antidiarrhoeal activity of *Psidium guajava folia* extract with quercetin flavonoids present in the plant. The significant antidiarrhoeal activity of the methanol fraction of unripe

fruits of *Psidium guajava* extract was also connected to the flavonoids that inhibit Ach release in GI tract (Ghosh *et al*., 1993).

The present study also indicated the relationship of the spasmolytic, antimotility, antisecretory and antidiarrhea activities of the extracts of *S. abyssinica* and which may be due to flavonoids and alkaloids present in the plant.

Antienteropooling activity

Enteropooling model is performed with aim to test possible antisecretory activities of the extracts. Castor oil is known to induce diarrhea in experimental animals and human subjects (Rouf *et al.,* 2003). But it can also be used to induce intestinal fluid accumulation.

Castor oil causes motility and secretory type diarrhea due to its effect on GI motility and electrolyte transport across intestinal mucosa. This is so due to ricinoleic acid which cause irritation to intestinal mucosa resulting in increased release of inflammatory mediators such as PG-E_2 which increase secretions of mucosa fluid and electrolytes by affecting cell permeability (Ammon *et al.,* 1975; Rouf *et al.,* 2003)

In this model, the extracts displayed a significant reduction in volume of fluid as compared to the control group. This antienteropooling activity was dose dependent. The results of this model indicated that the extract has got antisecretory activity as evidenced by the statistically significant reduction in volume of secretion which is important proof of the antisecretory activity of the extracts.

Flavonoids and tannins are some of the constitutes of *S. abyssinica* (Chacrabotory *et al.,* 2000) and Flavonoids have been reported to posses antisecretory activity through inhibition of prostaglandin biosynthesis and also by decreasing histamine and ACh release *(*Gálvez *et al.,*1993, 2003; Ghosh *et al.,* 1993; Borrelli *et al.,* 2000*).* And tannins are known to form proteins tannate which make intestinal mucosa more resistant and hence reduce secretions *(*Gálvez *et al.,* 1991; Tripathi 1994; Borrelli *et al.,* 2000).

Therefore, the antisecretory activity of the extract could have been due to the medicinal plants flavonoids and tannin content.

Apart from the above proposed antisecretory mechanisms (inhibition of prostaglandin biosynthesis) there is possibility that the extracts could act on mu opiod receptors as agonist or prostanoid receptors as antagonist and also anticholinergic mechanism may cause the antisecretory activities. However at this stage and with this work alone difficult to say the exact mechanism of antisecretory action of the extracts.

In vitro antispasmodic activity

ACh induced contraction is mediated via M_3 subtype muscarnic receptor in small intestine. ACh causes depolarization and tonic contraction of intestinal smooth muscles. This is also by increase in concentration of cytoplasm free calcium ions. The activation of muscarnic receptors of longitudinal smooth muscle of small intestine produce an increased frequency of action potential discharge and depolarization, which results in contraction as result of increased efflux of calcium through L-type voltage-operated channel. Calcium also can enter the cell through receptors operated Ca++ channels. In short, calcium ions gain access to the cytoplasm through voltage-activated or receptor-operated calcium channels (Gilani *et al.,* 1994).

The extracts (SALM and SALA) when tested on isolated GPI produced inhibition on ACh induced contractions in a dose dependent manner. The extracts shifted Ach dose response curve (DRC) to the right that indicates the competitive antagonism of Ach receptor as the standard atropine also did the same right ward shift in DRC of Ach and both the extracts and atropine decreased the PD_2 value of Ach meaning increased the EC50 of the ACh. However the extracts (SALM and SALA) also depressed the maximum response to agonist Ach, which suggest non competitive antagonism at concentration of 100ug/ml. The next higher conc. of 200ug/ml also caused further right ward shift in Ach dose response curve in a non-parallel fashion (as the slope of the DRC of the control is different from that of the extracts) and the maximum response was also

further suppressed, which is characteristics of a non competitive antagonism in fact the affinity of the antagonist (the extracts) expressed as PA'_2 was not calculated as the extracts are crude and their molar concentration couldn't be calculated and the formula (to calculate PA'_2) demand molar concentrations of the antagonists.

The spasmolytic activity of the extracts to antagonize Ach on the intestinal smooth muscle indicates their relaxant action on the gut and their efficacy as antispasmodic agent. And they may work through antagonism of cholinergic receptors and/or may act on ion channels such as Ca++ channel or K+ channel and interfere with the process of depolarization (as functional antagonism in effect might cause the observed actions and with this work alone difficult to say exact mechanism). The in-vivo study also support this hypothesis as the extracts abolished in-vivo vagal peristalsis, significantly decreased peristaltic index and inhibited stomach emptying in normal transit test.

The results of in-vitro studies suggest the plant may contain compounds exhibiting antimuscarnic and/or muscle relaxants activities. This hypothesis is supported by right ward shift in dose responses curve of Ach, decrease in PD2 value of the Ach both by extracts and atropine in comparable way and also in vivo study complete arrest of peristalsis was observed. However, the extracts did not act exactly in the same way as atropine. As atropine shifted DRC of Ach rightward but not suppressed Emax of Ach. These facts indicate the extracts may contain more than one compound that act through different mechanism and/or there is possibility that the active compound may have functional antagonism with muscarnic receptors.

Non-competitive antagonism of cholinergic receptors at neuromuscular junction inactivates receptors so that effective complex with agonist (Ach) cannot be formed irrespective of the agonist concentration (Ach). However, in competitive or reversible antagonisms increasing the concentration of agonist at receptor site results in maintenance (eliciting) of pharmacological (physiological) action of the agonist and Emax is maintained as in the absence of the antagonist that is to say Emax is not depressed. But in this study the action of the extracts were non specific as the extracts

suppressed maximum effect elicited by the agonists and also shifted dose response curve of Ach towards right (Ariens *et al.*, 1964).

In this in-vitro study spasmolytic activities of the leaf extracts were proved. And the results of this in-vitro model is in agreement with in vivo spasmolytic model done on mice in which the extracts showed muscle relaxant effect by inhibiting (abolished) vagal spontaneous and rhythmic contraction of the small intestine and stomach of mice with dose of 200 mg/kg in antipropulsive effect test on normal GI transit and also in castor oil induced GI transit there were reduction in propulsive movement of the GI.

The results of this in-vitro model and in vivo spasmolytic model are in agreement with each other and prove the traditional use of this medicinal plant for stomachaches and abdominal cramp.

Other studies revealed flavonoid constituents of different plants showed spasmolytic activity in different tissues preparations *in vitro* (Gilani *et al.,* 1994b; Abdalla *et al.,*1994; Galvez *et al*., 1996;).

In the previous phytochemical study flavonoids and isoquinol alkaloid were found in SA (Chacrabotory et al., 2000; Abebe et al 2003). These metabolites might be responsible for the observed spasmolytic activity of the extracts.

6 CONCLUSION AND RECOMMENDATION

Results of this study indicated that the methanol and aqueous extracts of Stephania abyssinica leaves and roots possess significant and dose dependent antidiarrheal activity due to their inhibitory effect both on GI propulsion and fluid secretion. And also the extracts possess significant antispasmodic activity due to anticholinergic effect. So the findings of this study provide scientific bases and prove for the claimed antidiarrheal and antispasmodic activities and consistent with the utility of the medicinal plant in traditional medicine for treatment of diarrheal diseases, stomach ache and abdominal cramp.

The following are recommended for further work

● PG-E2 and MgSo4 diarrheal models need to be further investigated,

●Effect of the extracts on intestinal ion conc such as, measurement of K^+, Na^+ and Cl^- concentration of intestinal fluid to assess effect on electrolytes transports,

● Other models of antispasmodics activities test such as effect on KCl (potassium chloride), serotonin and histamine induced contractions,

● Detailed phytochemical screening and activity guided fractionation to find out the active principle(s) responsible for the pharmacological activities, and

● Precise mechanism(s) of action should be elucidated.

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1. INTRODUCTION

1.1 Overview of diarrhea

Diarrhea is an increased frequency and decreased consistency of fecal discharge as compared with an individual's normal bowel pattern. Frequency and consistency are variable within and between individuals. For example, some individuals defecate as many as 3 times a day, while others defecate only 2 or 3 times per week (Barbara, 2006).

Diarrhea is loosely defined as passage of abnormally liquid or unformed stools at an increased frequency. For adults on a typical Western diet, stool weight >200 g/d can generally be considered diarrheal. Because of the fundamental importance of duration to diagnostic considerations, diarrhea may be further defined as *acute* if <2 weeks, *persistent* if 2 to 4 weeks, and *chronic* if >4 weeks in duration. Conditions, usually associated with the passage of stool totaling <200 gram/day, must be distinguished from diarrhea, as diagnostic and therapeutic algorithms differ (David and Camiller, 2004; Barbara, 2006).

Diarrhea is a leading cause of morbidity and mortality, especially among children in developing countries and it is a major health problem in children under 5 years (Gilani *et al., 2005*). The World Health Organization (WHO) has estimated that 3–5 billion cases occur each year (1 billion in children less than 5 years old) and about 5 million deaths are due to diarrhea (2.5 million in children, less than 5 years old) (Estrada-Soto *et al.*, 2007).

From a mechanistic perspective, diarrhea can be caused by an increased osmotic load within the intestine resulting in retention of water within the lumen; excessive secretion of electrolytes and water into the intestinal lumen; exudation of protein and fluid from the mucosa and altered intestinal motility resulting in rapid transit and decreased fluid absorption. In most instances, multiple processes are affected simultaneously leading to a net increase in stool volume and weight accompanied by increase in fractional water content (Pasricha, 2006).

The major impact of these illnesses is morbidity, because it demands primary medical services, hospital-care time and labor days lost. Furthermore, the most highly used drugs for intestinal disease therapies are very expensive, overused and inadequate use of antibiotics has led to increased prevalence of multi drugs-resistant pathogens. Despite the etiology, chronic diarrhea is linked with dehydration and electrolyte-containing solutions are the first choice for treatment (Estrada-Soto *et al.,* 2007).

Despite the availability of a vast spectrum of approaches for diarrheal management, majority of the population in the developing countries rely on herbal drugs for the management of diarrhea. Medicinal herbs constitute an indispensable component of the traditional medicine practiced worldwide due to the economical viability, accessibility, acceptability and ancestral experience (Afroz *et al.,* 2006).

1.2 Normal physiology

The human small intestine and colon perform important functions including the secretion and absorption of water and electrolytes, the storage and subsequent transport of intraluminal contents. Alterations in fluid and electrolyte handling contribute significantly to diarrhea. Alterations in motor and sensory functions of the human colon result in highly prevalent syndromes such as irritable bowel syndrome, chronic diarrhea, and chronic constipation (David and Camiller, 2004).

The small intestine and colon have intrinsic and extrinsic innervation. The *intrinsic innervation*, also called the enteric nervous system (ENS), comprises myenteric, submucosal, and mucosal neuronal layers. The function of these layers is modulated by interneurons through the actions of neurotransmitter amines or peptides, including acetylcholine, opioids, norepinephrine, serotonin, ATP, and nitric oxide. The myenteric plexus regulates smooth muscle function, and the submucosal plexus affects secretion and absorption.The *extrinsic innervations* of the small intestine and colon are part of the autonomic nervous system and also modulate both motor and secretory functions. The

chief excitatory neurotransmitters controlling motor function are acetylcholine and the tachykinins, such as substance P (Camiller and Murray, 2001; David and Camiller, 2004).

1.2.1 Absorption, secretion and GI motility

Sugars and amino acids are absorbed across the small-intestinal brush border membrane via carriers that couple their movements to that of Na^+. Na^+ coupling permits the organic solute to be transported uphill, i.e., from low luminal to higher cell concentration, a gradient opposite to that for Na^+. The organic solutes then move downhill from enterocyte to blood via basolateral membrane carriers that operate independently of ion movements (Schultz *et al.,* 1966; Ganapath *et al.,* 1985).

The Na^+ gradient, therefore, is the driving force for amino acid, oligopeptide, and sugar absorption. As these organic solutes are absorbed, salt is absorbed with them, and water follows osmotically transport from enterocyte to lateral intercellular space creates a local osmotic gradient that initiates water flow. The coupled transport of Na^+ and organic solute is the theoretical basis for oral rehydration therapy in severe diarrhea (Schultz *et al.,* 1966; Ganapath *et al.,* 1985).

Normally about 8 to 9 liters of fluid enter the small intestine daily from exogenous and endogenous (secretion) sources. Net absorption of the water occurs in the small intestine in response to osmotic gradients that result from the uptake and secretion of ions and the absorption of nutrients (mainly sugars and amino acids), with only about 1 to 1.5 liters crossing the ileocecal valve. The colon then extracts most of the remaining fluid, leaving about 100 ml of fecal water daily (Pasricha, 2006).

Secretions in intestine has role in duodenal alkalinization. The ion exchangers that are localized in small-intestinal and colonic brush border membranes play this role. The individual cell membrane transporters contributing to active Cl^- secretion the three membrane proteins involved are: the apical anion channel, the basolateral membrane K^+ channel and the basolateral membrane NaK2Cl cotransporter (Micheal *et al.,* 2003).

GIT also do two types of motility under autonomic control as fed state (peristalsis) and fast state. This motility ensures the mixing of ingested food and let the bolus in one part of the GI move to the other part. It also works housekeeping activities by moving out the unabsorbed debris out of the GIT (Pasricha, 2006).

1.2.2 Regulation of absorption, secretion and GI motility

The gastrointestinal tract is in a continuous contractile, absorptive, and secretory state. The control of this state is complex, with contributions by the muscle itself, local nerves (*i.e.,* the enteric nervous system, ENS), the central nervous system (CNS), and humoral pathways. Of these, perhaps the most important regulator of physiological gut function is the ENS. Alterations in gastrointestinal motility and in the balance of absorption and secretion in the intestines may underlie irregularities in bowel habits. (Longstereth, 1998; Pasricha and Jefri, 2001).

The ENS is composed of interconnected networks of ganglion cells and nerve fibers mainly located in the submucosa (submucosal plexus) and between the circular and longitudinal muscle layers (myenteric plexus). These networks give rise to nerve fibers that connect with the mucosa and deep muscle. Although extrinsic sympathetic and parasympathetic nerves project onto the submucosal and myenteric plexuses, the ENS can independently regulate gastrointestinal motility and secretion (Mc Quid, 2007).

The neurons within the plexuses secrete different neurotransmitters and a variety of pharmacologically active peptides. The classes of compounds that stimulate active secretion and inhibit active absorption, and those with the opposite effects. The former group includes three kinds of agents: (a) neurotransmitters, including vasoactive intestinal peptide (VIP), acetylcholine, substance P, and the nucleotides ATP; (b) the paracrine agents serotonin and neurotensin, which are released by endocrine (enterochromaffin) cells in the intestinal epithelium; (c) agents released by inflammatory cells, including mainly prostaglandins, histamine, and serotonin (Sellin, 1993; Quigly *et al.,* 1999; Range *et al.,* 2003).

The group of compounds that both inhibit active secretion (HCO_3^- as well as Cl^-) and enhance active absorption includes norepinephrine (via α_2-receptors), neuropeptide Y, enkephalins, somatostatin, and paracrine agents (Sellin, 1993).

The basic motor tool used by the ENS to integrate its GI motility programs is the peristaltic reflex. Physiologically, peristalsis is a series of reflex responses to a bolus in the lumen of a given segment of the intestine; the ascending excitatory reflex results in contraction of the circular muscle on the oral side of the bolus, while the descending inhibitory reflex results in relaxation on the anal side. The net pressure gradient moves the bolus caudal (Furness and Sanger, 2002; Galligan, 2002).

Three neural elements, responsible for sensory, relay, and effector functions, are required to produce these reflexes. Luminal factors stimulate sensory elements in the mucosa, leading to a coordinated pattern of muscle activity that is directly controlled by the motor neurons of the myenteric plexus to provide the effector component of the peristaltic reflex (Furness and Sanger, 2002; Galligan, 2002; Pasricha, 2006).

Motor neurons receive input from ascending and descending interneurons (which constitute the relay and programming systems) that are of two broad types, excitatory and inhibitory. The primary neurotransmitter of the excitatory motor neurons is acetylcholine (ACh). The principal neurotransmitter in the inhibitory motor neurons appears to be nitric oxide (NO), although important contributions may also be made by ATP, and vasoactive intestinal peptide (VIP), all of which are variably co-expressed with NO synthase (Furness and Sanger, 2002; Galligan, 2002; Pasricha, 2006).

1.3 Pathophysiology of diarrhea

1.3.1 General aspects

Osmosis, active secretion, exudation, and altered motility can all drive diarrhea. Specific diarrheal illnesses often involve more than one of these forces.

i) Osmotic diarrhea: When poorly absorbable, low–molecular weight aqueous solutes are ingested, their osmotic force quickly pulls water and, secondarily, ions into the intestinal lumen. Individuals with normal gut function will develop osmotic diarrhea when they ingest large amounts of poorly absorbable solutes, such as lactulose (if they are being treated for hepatic encephalopathy), sorbitol (if they continually chew sugar-free gum), or Mg^{2+} (if they take certain antacids or bowel purgatives) (Micheal *et al.*, 2003)

ii) Secretory diarrhea: Diarrhea resulting from overstimulation of the intestinal tract's secretory capacity can develop in "pure" form (e.g., cholera) or as a component of a more complex disease process (e.g., celiac disease, Crohn disease). "Pure" secretory diarrhea is characterized by (a) large stool volumes (which can exceed 1 liter per hour in well hydrated adults), (b) absence of red or white blood cells in the stool, (c) absence of fever or other systemic symptoms (except those due to dehydration), (d) persistence of diarrhea with fasting (volume may diminish, however), and (e) lack of excess osmotic gap (OG) in stool electrolytes. Osmotic gap is defined as follows: $OG = 290 – 2\{[Na^+] + [K^+]\}$, where 290 is the assumed osmolarity of blood plasma. A gap greater than 50 mM is considered abnormal; the normal gap is made up of Mg^{2+}, Ca^{2+}, NH_4^+, and perhaps organic cations (Farthing *et al.*, 2002; Micheal *et al.*, 2003).

The pattern of stool electrolytes in patients with acute cholera shows Na^+, K^+, and $Cl^–$ concentrations not very different from those in plasma and $HCO_3^–$ concentration somewhat higher than in plasma. In contrast, normal stool shows low $[Na^+]$ and high $[K^+]$ concentrations, due mainly to the colon's reabsorption of Na^+ and secretion (both active and passive) of K^+; and a low $[Cl^–]$ concentration, due to the replacement of $[Cl^–]$ by short-chain organic acid anions generated by colonic bacteria. Normally, $[HCO_3^–]$ concentration is similar to that in plasma (Farthing et al., 2002; Micheal *et al.*, 2003).

iii) Exudative diarrhea: If the intestinal epithelium's barrier function is compromised by loss of epithelial cells or disruption of tight junctions, hydrostatic pressure in blood vessels and lymphatics will cause water and electrolytes, mucus, protein, and sometimes even red and white cells to accumulate luminally (e.g., ulcerative colitis, shigellosis, intestinal lymphangiectasia). If the condition is chronic, the continuing protein loss can

lead to hypoalbuminemia and hypoglobulinemia (Micheal *et al.*, 2003; David and Camiller, 2004).

iv) Diarrhea resulting from motility disturbances: Both increases and decreases in gut motility can lead to diarrhea. Examples of the former are thyrotoxicosis and opiate withdrawal. Decreases in effective motility in the small intestine due to large diverticula, smooth muscle damage, or autonomic neuropathy (diabetic, idiopathic) can result in bacterial overgrowth. And bacterial overgrowth can lead to diarrhea (Chang *et al.*, 1982).

1.3.2 Pathophysiology of chronic diarrhea

i) Hormone-secreting neoplasms: In several uncommon tumors, hormones are produced and released that directly stimulate intestinal secretion, causing profuse diarrhea or, in one instance (gastrinoma), interfering with nutrient absorption (Jensen *et al.*, 1999).

In patients with pancreatic cholera, certain endocrine neoplasms that occur most commonly in pancreatic islets but occasionally in the proximal intestinal mucosa secrete large quantities of VIP, the enteric secretory neurotransmitter. Pheochromocytomas do so also. Profuse diarrhea develops in 30% of patients with medullary carcinoma of the thyroid because of secretion of calcitonin, another secretory stimulus in the intestine (Jensen *et al.*, 1999; Camiller and Murray, 2001).

In Zollinger-Ellison syndrome (gastrinoma), both diarrhea and peptic ulceration can result from the marked increase in gastric acid production that is associated with gastrin-secreting neoplasms. About half of the patients with the rare neoplasm systemic mastocytosis develop diarrhea, likely due to histamine-induced gastric hypersecretion, a cause similar to that of the diarrhea in Zollinger-Ellison syndrome (Jensen *et al.*, 1999; David and Camiller, 2004).

ii) Diabetes mellitus: Diarrhea accompanied by rectal incontinence is an occasional complication of long-standing, insulin-dependent diabetes. It typically occurs in patients with poor diabetic control and peripheral neuropathy. Intestinal biopsies in such patients are usually normal, and nutrient malabsorption or bacterial overgrowth is present only in

a minority of cases. In most instances, the diarrhea is secondary to degeneration of adrenergic nerves in effect less noradrenalin, that, as mentioned above, are antisecretory and/or proabsorptive in intestinal fluid homeostasis (Chang *et al.,* 1985).

1.4 Principles of diarrhea management

Many patients with sudden onset of diarrhea have a benign, self-limited illness requiring no treatment or evaluation. In severe diarrheal cases, dehydration and electrolyte imbalances are the principal risk, particularly in infants, children, and frail elderly patients. Oral rehydration therapy therefore is a cornerstone for patients with acute illnesses resulting in significant diarrhea. This therapy exploits the fact that nutrient-linked co-transport of water and electrolytes remains intact in the small bowel in most cases of acute diarrhea. Sodium and chloride absorption is linked to glucose uptake by the enterocyte; this is followed by movement of water in the same direction. A balanced mixture of glucose and electrolytes in volumes matched to losses therefore can prevent dehydration (Rang *et al.,* 2003; Pasricha, 2006).

Pharmacotherapy of diarrhea should be reserved for patients with significant or persistent symptoms. Nonspecific antidiarrheal agents typically do not address the underlying pathophysiology responsible for the diarrhea; their principal utility is to provide symptomatic relief in mild cases of acute diarrhea. Many of these agents act by decreasing intestinal motility and should be avoided as much as possible in acute diarrheal illnesses caused by invasive organisms. In such cases, these agents may mask the clinical picture, delay clearance of organisms, and increase the risk of systemic invasion by the infectious organisms; they also may induce local complications such as toxic megacolon (Pasricha, 2006; Mc Quid, 2007).

For many chronic conditions, diarrhea can be controlled by suppression of the underlying mechanism. Examples include elimination of dietary lactose for lactase deficiency, use of glucocorticoids or other anti-inflammatory agents for idiopathic inflammatory bowel diseases, adsorptive agents such as cholestyramine for ileal bile acid malabsorption (David and Camiller, 2004).

Proton pump inhibitors such as omeprazole for the gastric hypersecretion of gastrinomas, somatostatin analogues such as octreotide for malignant carcinoid, prostaglandin inhibitors such as indomethacin for medullary carcinoma of the thyroid, and pancreatic enzyme replacement for pancreatic insufficiency. Clonidine, an α_2 adrenergic agonist, may allow control of diabetic diarrhea. For all patients with chronic diarrhea, fluid and electrolyte repletion is an important component (Camiller and Murray, 2001; David and Camiller, 2004).

1.5 Antimotility, antisecretory and antispasmodic agents

i) Opioid agonists: Opioid receptors (μ,κ, and δ) exist in high density in the GIT, located particularly in the myenteric and submucosal plexus, as well as on nociceptive pathways to the brain. In the stomach, motility (rhythmic contraction and relaxation) may decrease. In the small intestine resting tone (persistent contraction) is increased, with periodic spasms, but the amplitude of non propulsive contractions is markedly decreased. In the large intestine, propulsive peristaltic waves are diminished and tone is increased; this delays passage of the fecal mass and allows increased absorption of water, which leads to constipation (Szarka et al., 2007).

They act by several different mechanisms, mediated principally through either mu- or sigma-opioid receptors on enteric nerves, epithelial cells, and muscle. These mechanisms include effects on intestinal motility (μ receptors), intestinal secretion (δ receptors), or absorption (μ and δ receptors) (Szarka *et al.,* 2007).

ii) Chloride channel blockers are effective antisecretory agents *in vitro* but are too toxic for human use and have not proven to be effective antidiarrheal agents *in vivo*.

iii) Calcium channel blockers such as *verapamil* and *nifedipine* reduce motility and may promote intestinal electrolyte and water absorption. Constipation, in fact, is a significant side effect of these drugs. However, because of their systemic effects and the availability

of other agents, they seldom if ever are used for diarrheal illnesses (Pasricha and Jefri, 2001; Pasricha, 2006).

iv) Berberine is a plant alkaloid used most commonly in bacterial diarrhea and cholera, but is also apparently effective against intestinal parasites. The antidiarrheal effects in part may be related to its antimicrobial activity, as well as its ability to inhibit smooth muscle contraction and delay intestinal transit by antagonizing the effects of acetylcholine (by competitive and noncompetitive mechanisms) and blocking the entry of Ca^{2+} into cells. In addition, it inhibits intestinal secretion (Pasricha, 2006).

v) Somatostatin analogues: Octreotide is an octapeptide analog of somatostatin that is effective in inhibiting the severe secretory diarrhea brought about by hormone-secreting tumors of the pancreas and the gastrointestinal tract. In hormone-secreting neoplasms, they block hormones (serotonin, VIP and gastrin) production by the tumor. Since they also appear to have a direct antisecretory effect on the gut epithelium, they have been employed for treating other forms of secretory diarrhea; cancer chemotherapy–induced diarrhea, diarrhea associated with human immunodeficiency virus (HIV), and diabetes-associated diarrhea (Jensen *et al.,* 1999).

vi) Antispasmodics: The antispasmodics are considered useful for relieving or calming colicky pains resulting from spasms of the gut muscles and diarrhea due to hypermotility of the gastrointestinal tract (Gilani *et al*., 1994).

Abdominal pain is a major symptom in IBS, and clinicians have observed that anticholinergic drugs may provide temporary relief for symptoms such as painful cramps related to intestinal spasm. Although controlled clinical trials have produced mixed results, evidence generally supports beneficial effects of anticholinergic drugs (clidinium, prophantiline, dicyclomine and hyoscyamine) for pain (Tally *et al.,* 2003). The most common agents of this class are nonspecific antagonists of the muscarinic receptor and include the tertiary amines dicyclomine and hyoscyamine, and the quaternary ammonium compounds glycopyrrolate and methscopolamine (Rang *et al.,* 2003).

Antispasmodic agents relax smooth muscle in the gut and reduce contractions. They act through anticholinergic or antimuscarinic properties. The anticholinergic effects of antispasmodics limit their use, especially in the long term (Tally *et al.*, 2003).

1.6 Herbal remedies for gastrointestinal motility disorders and use of *Stephania abyssinica*

Plants have been utilized as a medicine for thousands of years. More recently, a WHO study has shown that about 80% of the world's population still relies on traditional medicine *(*Dharmani and Palit *et al., 2006).* A growing numbers of plants have been reported for antidiarrheal and antispasmodic activity.

Various herbal preparations have been used and claimed to have benefits as antidiarrheal and antispasmodic. Among these are *Taverniera abyssinica , Syzygium guineese, Lipdium sitivium, Solaniase gigsa, Moringa stenopetala, Atropa belladonna, Berberis vulgaris, Evodia rutaecarpa, and Linum sitatissimum.* And Studies were done to proof the traditional use the preparations (Abebe and Ayehu, 1993; Abebe *et al.*, 2003).

Aqueous extract of the roots of *Taverniera abyssinica* ("*Dengetegna"*) antagonized Ach and histamine induced contractile responses of the guinea pig ileum and relaxed the smooth muscle of rabbit duodenum, which is suggesstive of its ethnomedical use in stomachache treatment (Noamesi *et al*., 1990). The antihistaminic and anticholinergic activities of aqueous extract of barberry fruits (*Berberis vulgaris*) were investigated on isolated guinea-pig ileum and the extract were found to possess anticholinergic and antihistaminic activities (Shamsa *et al*., 1999).

The leaf ethanol extract of *Moringa stenopetala* was shown to have a potential antispasmodic effect on guinea pig ileum (Mekonnen et al., 1999). The aqueous extract of *Linum sitatissimum* ("*Telba"*) seed was observed to show significant spasmolytic acivitiy and protective effects against experimental ulcerogenesis in *g*uinea pig ileum and mouse stomach (Makonnen *et al.*, 1996).

Muscarinic antagonists like atropine (*Atropa belladonna*) inhibit the contractions of gastrointestinal tract induced by acetylcholine (Ach). This partial inhibition of gastrointestinal motility by atropine has led to their widespread use as antispasmodics in the treatment of disorders associated with intestinal hypermotility (Ghosh *et al*., 1993; Broadley and Kelly, 2001).

The aqueous extract of *Evodia rutaecarpa* fruit was used to examine its effects on castor oil-induced diarrhea and to compare with its anti-transit effect in mice. The results indicated that the extracts had both anti-transit effect and antidiarrheal effects (Li-Li *et al.,* 2000).

Stephania abyssinica is a perennial glabrous creeper with woody or herbaceous stem. It is the most common member of the Menispermaceae indigenous in the mountain rainforest of southern and eastern Africa. The leaves are used for stomachache, abdominal cramp and antidote against snake bites. An aqueous extract of the dried and crushed roots and root powder of this species is used for the treatment of diarrhea, dysentery, vomiting, heart complaints, hypertension, insomnia, and mastitis (Chakraborty *et al.,* 2000; Abebe *et al.*, 2003). The roots are also employed in treatment of round worm, menorrhagia, and boils. Whole of the plant can be used for stomachache cases and syphilis and root decoction is drunk for boils. Root powder or its infusion is used as a remedy against cholera, gonorrhea and syphilis and its root decoction is taken for jaundice. Powder of the leaf is used for treating wound and eczema (Abebe and Ayehu, 1993; Chakraborty *et al.*, 2000; Abebe *et al.,* 2003; Gedif and Hahn *et al.,* 2003).

Previous phytochemical investigations on *S. abyssinica* revealed that isoqiunol alkaloids are the main chemical components. The occurrence of polyphenols (flavonoids and tannins) has been reported. Previous bioactivity test indicate this plant posses antimicrobial, anticancer, antioxidant, anti-inflammatory and antimalaria activities (Chakraborty *et al.,* 2000; Abebe *et al.,* 2003).

It is known that large number of herbal remedies remain unexplored and should be evaluated for their potential therapeutic application (Gedif and Hahn *et al.*, 2003), including antidiarrheal plants. One candidate for such kind of scientific evaluation is Stephania abyssinica and there is lack of scientific proof for claimed antidiarrheal and stomachache treatment use in this country. In this study, therefore, antidiarrheal and spasmolytic activities of the crud extracts of the leaf and root were studied in different experimental models. The present work will help proper utilization of the medicinal plant. On top of this it can be considered a way forward in quest of new antidiarrheal and antispasmodic agent with optimum benefits and less adverse events.

2 OBJECTIVES OF THE STUDY

2.1 General objective

Evaluate the antidiarrheal and antispasmodic activities of the aqueous and methanol extracts of *Stephania abyssinica* which is used in Ethiopian traditional medicine for treatment of diarrhea and stomachache.

2.2 Specific objectives

To evaluate antidiarrheal activities of the aqueous and methanol extracts of the root and leaf in mice,

To evaluate in-vivo spasmolytic activities of the aqueous and methanol extracts of the root and leaf in mice,

To test for in-vitro antispasmodic effect of the aqueous and methanol extracts of the leaf on isolated GPI,

To test for antisecretory effect of the aqueous and methanol extracts of the root and leaf in mice.

3 MATERIALS AND METHODS

3.1 Drugs and Chemicals

Drugs (ACh, Atropine, and Loperamide) used in this study were purchased from Sigma-Aldrich and chemicals (NaCl, KCl, $MgCl_2$, $NaHCo_3$, $NaHPO_4$, Glucose, $CaCl_2$, and gum aciacia) were purchased from Riedel-De Haen, Methanol (TechnoPharmchem, Bahadargarh, India),Charcoal (The British Drug House, Ltd., London) and castor oil was purchased from local market.

3.2 Plant material

The leaves and roots of *S. abyssinica* were collected during July, 2009 G.C from Bela, North Addis Ababa, Ethiopia. The collected plant was identified by taxonomist (Voucher number DT-01) and the specimen was deposited at the National Herbarium, Addis Ababa University. Then the roots and leaves were dried under the shed and crushed to powders.

3.3 Extraction of plant material

Aqueous extract of the root and leaf were prepared by maceration of 100 gram of the powdered plant material in enough distilled water. Flask containing powdered plant material and DW was placed on a shaker for 48 h at room temperature after 48 h each sample was filtered using gauze and filter paper (whatman No1) and lyophilized. The same procedure was followed for methanol extractions except rota – vapor was used to remove methanol from the extracts.

3.4 Animal preparation and dosing

Guinea pigs were purchased from EHNRI and acclimatized to lab condition. The animals were exposed to 12/12 h light-dark cycle and maintained under standard condition for 10 days. Swiss Albino mice were obtained from biology department, Addis Ababa

University animals' house and maintained under standard condition for 10 days (Vogle, 2002).

They were acclimatized under uniform conditions of 12/12 h light and dark cycle and housed at a temperature of 24 ^{0}C. They were fed a standard pellet diet and tap water *ad libitum* at biology department animal house before use for the experiments (Vogle, 2002).

The pilot study was performed with 5, 25, 100, 200 and 300 mg/kg of the extracts in both antidiarrheal and intestinal transit test. The dose of 25 mg/kg showed the minimum effect and there was no much difference in the anti-diarrhea activity of 200 mg/kg compared with 300 mg/kg. All the extracts in castor oil-induced diarrhea at the dose of 5 mg/kg did not inhibit diarrhea significantly when compared with castor oil alone. The same was observed for intestinal transit test. From these preliminary works the doses 25, 50, 100 and 200 mg/kg were selected for subsequent studies.

3.5 Castor-oil induced diarrheal model in mice

The methods described by Vogel, (2002), Aye –than *et al.,* (1989), Ching *et al.,* (2008) and Awouter *et al.,* (1978) were adopted in assaying for the effect of the extracts on caster oil induced diarrhea.

Mice of either sex weiging 25 – 30 g were divided in to control and test groups (n=6). Each animal was then given 0.3 ml of castor oil orally after 30 min of treatment with extracts (25, 50, 100, 200 mg/kg) or vehicle and placed in transparent cages to observe for consistency of stool and to measure on set of diarrhea (Aye –than *et al.,* 1989).

The following parameters were recorded; on set of diarrhea, the total number of both dry and wet stools, number of watery diarrheal stools, and the weight of stools were measured for 4 h (Awouter *et al.,* 1978).

A numerical score based on stool consistency was assigned as follows: normal stool (formed and pelleted stool) =1, mild or semi solid unformed stool=2 and copious or watery stool=3.

The total diarrheal score was calculated as the sum of the recorded diarrheal score based on stool consistence and **%** protection was calculated as reductions in diarrheal score from the control group value. Whereas the in vivo antidiarrheal index (ADI) was expressed as geometric mean of the PFR, GITR and DFT.

ADI= cube root of (PFR x GITR x DFT) where ADI is antidiarrheal index, PFR is purgation frequency reductions (in per cent of control), GITR is gastro intestinal transit reductions (in per cent of control), and DFT is diarrheal free time increase compared with control (in per cent of total time) (Ching *et al.,* 2008).

3.6 In-vivo GI motility

This was carried out according to the method outlined by Aye-than *et al.,* (1989), Ching *et al.,* (2008) and Mujumd *et al.,* (1998) using charcoal meal as a diet marker.

The mice (overnight fasted with free access to water) of either sex were divided in to control and test group of 6 animals per group. The first group (control group) was orally administered the vehicle. The second, third, fourth, and fifth groups received 25, 50, 100, 200 mg/kg body weight of extracts, respectively. The sixth group received the standard drug, atropine (5 mg/kg body weight). Thirty minutes after administration, each animal was given 0.5 ml of charcoal meal orally (10% charcoal in 5% gum acacia), after 30 min each animal was sacrificed and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum was measured and expressed as percentage of total length of the intestine (Peristaltic Index or PI) (Ching *et al.,* 2008).

In the castor oil induced intestinal transit measurements (as part of in vivo antispasmodic model on established gut increased motility unlike the above model which measures

effect on normal peristalsis) the animals (overnight fasted with free access to water) was administered vehicle in control groups, extracts (25, 50, 100, 200 mg/kg), or loperamide (5mg/kg body weight) in test groups 30 min before oral administration of castor oil (0.3 ml), 30 min later orally charcoal meal was administered, after 30 min the animals were sacrifices and the effect on intestinal transit was assessed by calculating peristaltic index (PI) expressed as intestinal transit of the marker (charcoal meal) in cm as % of the total length of the intestine. And % inhibition is calculated as % of control group and considering the control is 100 % (Aye-than *et al.,* 1989; Ching *et al.,* 2008).

3.7 Enteropooling test

This test was done as described by Robert *et al* (1976) and Ching *et al* (2008).
Briefly, mice of either sex were fasted for 24 h but allowed free access to water. The mice was divided in to control and test group. Group 1 was administered distilled water, while group 2 and 3 were pre – treated with 100 and 50 mg/kg of the extracts, and group 4 pre – treated with loperamide (5 mg/kg). After 30 min each animal was administered 10 ml/kg of castor oil. The animals were scarified after 30 min and the small intestine from the pylorus to ceacum was dissected out and its content expelled into a measuring cylinder by milking to measure the volume of the fluid (Robert *et al.,* 1976; Ching *et al.,* 2008).

3.8 In vitro antispasmodic test

This test was carried out according to the method outlined by Gilani *et al* (1994), Galvez *et al* (1996), Mekonnen *et al* (1999) and Vogel (2002).

Fasted (24 hrs) guinea pigs weighing (400-450g) were scarified by a gentle blow at the back and allowed to bleed. The abdominal cavity of the animal was opened by midline incision every time a tissue was required, and the ileum 2-2.5 cm in length was removed immediately and trimmed from surrounding tissues. The contents of the intestine were

washed with Physiological Salt Solution (PSS) called Tyrodes solution (see composition below).

The isolated tissue preparations were used according to the technique described by Gilani *et al.*, (1994) and Mekonnen *et al.*, (1999). Segments of ileum were tied with silk threads at both ends (ileum tied in opposite directions) and suspended in a thermo regulated 25 ml organ bath, maintained at 37 ^{0}C, containing Tyrode solution of the following composition (g/l): NaCl, 8 gram; KCl, 0.2 gram; MgCl2, 0.1 gram; NaHCO3, 1 gram; NaHPO4, 0.05 gram; Glucose, 1 gram; CaCl2, 0.2 gram. One end of the ileum was attached to a tissue holder at the base of the organ bath and the other end to the isometric recording device. The tissues were constantly bubbled with air mixture of 95% O2 and 5% CO2. A suitable weight or resting tension of 1 gram was applied to the individual tissue (Mekonnen *et al*. 1999; Vogel HG, 2002).

The suspended ileum was allowed to equilibrate for 30-45 min before adding acetylcholine, the particular plant extract or the standard drug. After the initial equilibration period, Acetylcholine (10^{-9} to 10^{-3} M) was added to the organ bath and the control cumulative concentration-response curve for acetylcholine was constructed. Each time the added concentration of the acetylcholine was left in contact with the tissues for 30 sec before adding the next concentration. Then the tissue was washed two times with Tyrode solution at the interval of 5 min. It was left to resume its normal contraction. After a stabilized regular contraction, Extracts (SALM and SALA) at conc of 100 and 200µg/ml were added; or atropine was then added to the organ bath 5 min before the corresponding concentration curve was recorded (Gilani *et al*., 1994; Vogel, 2002).

The plant extracts were prepared in physiological Tyrodes salt solutions (PSS) while the stock solutions of all drugs (Ach, and Atropine) were made in distilled water and then serially diluted with PSS. The final dilutions of the drugs were made fresh on the day of the experiment. The calculated concentrations of each plant extracts and standard drugs were final organ bath concentration (Mekonnen *et al*., 1999).

Isometric contractions were recorded with a Grass FT-03 strain gauge transducer coupled to a Grass 79 Polygraph which is equipped with preamplifier, main amplifier, oscillograph and time and event marker (Grass Inc., USA). The chart speed was 5mm/minute. In addition, the anticholinergic effects of the extracts were compared with atropine (6.5 nM) (Mekonnen *et al.*, 1999; Shamsa *et al.*, 1999).

Original computer program Graph pad prism 2.0 (GraphPad-Pism software Inc, San Diago, CA) was used for fitting non – linear curve to do DRC and calculating PD_2 value. Responses in Ach induced GPI contractions were expressed as % of the maximum contractions (E_{max}) induced by Ach (the control) prior to the addition of the plant extracts or atropine.

3.9 Statistical Analysis

The experimental results were expressed as mean ± Standard error of mean (SEM) and the results are analyzed by ANOVA (one way) followed by Turkey-kramer post test for comparing the means differences and were considered statistically significant when $P < 0.05$.

4 RESULTS

4.1 Effects on castor oil induced diarrheal model.

Forty seven min after castor oil administration, the mice in the control group produced copious diarrhea. Pretreatment of mice with the extracts caused dose-dependent and significant ($p<0.05$) delay in the on set of diarrhea, frequency of stooling (reduction in number of wet stools and total stools), decrease in weight of wet stools and the general diarrhea score (Table 1 and 2).

With doses of 200 mg per kg of both root and leaf extracts (aqueous and methanol,) there were no diarrhea episodes. Whereas with dose of 100 mg per kg the on set for SALA and SARA were 171 min ($p<0.001$) and 169 min ($p<0.001$) respectively and with same dose of methanol extracts SARM was 212 min and SALM was 201 min. With the dose of 50 mg per kg the effect of the extracts were significant ($p<0.001$). The on set time was at the third hours and second hour for the 25 mg per kg for all extracts. Long diarrhea free period was observed with dose of 100 mg/kg and no diarrhea was observed with dose of 200 mg/kg.

The extracts produced high antidiarrheal index with dose of 200 mg/kg; SALM, SALA, SARM and SARA produced ADI of 88.79, 89.21, 91.08, and 82.23 respectively and these ADI values of the extracts are all larger than that of the reference loperamide which was 79.33. Least ADI was scored with dose of 25 mg/kg of all extracts (Table 5).

The extracts also reduced diarrheal score significantly ($p< 0.05$) as compared to the control group. With dose of 200 mg/kg least diarrheal score is observed for all extracts; SALM, SARM, SARA and SALA decreased diarrheal score from 29 of the control group to 3.50, 2.3, 3.0 and 3.8 respectively. However, 25 mg/kg produced high diarrhea score for all extracts. See table 1 and 2.

Table1: Effects of methanol extract of *S. abyssinica* on castor oil induced diarrheal model in mice

Treatment	Dose (mg/kg)	on-set time(min)	Total number of Stool	Number of wet stool	Total diarrhea SCORE	Weight of stool (mg)	% Protection
Control	-	47± 2.28	12.67±0.48	10.33±0.67	29±1.92	904±37.22	-
SALM	200	-	3.5±0.4 ^{c}	-	3.5±0.4 ^{c}	132.±1.50 ^{c}	87.93
SALM	100	201±5.78 ^{c}	4.17±0.4 ^{c}	2.00.±0.3 ^{c}	7±1.12 ^{c}	229.±4.12 ^{c}	75.68
SALM	50	176.13±5.17 ^{c}	6.67±0.51 ^{b}	4±0.36 ^{c}	13.15±2.92 ^{c}	399.17±16.32 ^{c}	54.66
SALM	25	93.0±4.41 ^{c}	7.5±0.42 ^{b}	6.83±0.40 ^{a}	20.72±2.62 ^{a}	554.67±13.66 ^{b}	28.55
SARM	200	-	2.33±0.61 ^{c}	-	2.3±0.61 ^{c}	94.±2.40 ^{c}	92.07
SARM	100	212.0±4.13 ^{c}	4±0.58 ^{c}	1.9.±0.12 ^{c}	6±1.54 ^{c}	185.±3.60 ^{c}	79.31
SARM	50	160.83±4.85 ^{c}	5.67±0.21 ^{c}	3.83±0.75 ^{c}	11.02±2.3 ^{c}	259.67±9.75 ^{c}	62
SARM	25	103.33±5.32 ^{c}	7±0.37 ^{b}	5.67±0.53 ^{c}	19.69±3.0 ^{b}	440.17±21.52 ^{c}	32.10
Loperamide	5	195.5±4.50 ^{c}	5.67±0.67 ^{c}	2.16±0.75 ^{c}	8.04±2.10 ^{c}	226.5±11.47 ^{c}	72.28

Values are mean ±SEM, n=6, and P^{a}<0.05, P^{b}<0.01, P^{c}<0.001 are significantly different from the control group

Table 2: Effects of aqueous extract of *S. abyssinica* on castor oil induced diarrheal model in mice

Treatment	Dose (mg/kg)	on-set time(min)	Total number of stool	Number of wet stool	Total Diarrhea SCORE	Weight of stool (mg)	%Protection
Control	-	47.± 4.50	12.67±0.48	10.33±0.67	29±1.92	904±37.22	-
SARA	200	-	3.03±0.40 ^{c}	-	3.03±0.40 ^{c}	102.±3.1 ^{c}	89.55
SARA	100	171.67±8.07 ^{c}	5.67±0.71 ^{c}	3.5±0.22 ^{c}	11.10±2.11 ^{c}	185.83±20.30 ^{c}	61.72
SARA	50	131.67±12.34 ^{c}	6.67±0.49 ^{c}	4.17±0.75 ^{c}	16.19±1.96 ^{c}	237±15.42 ^{c}	44.17
SARA	25	81.83±3.88 ^{b}	8.17±0.70^{b}	7.33±0.82 ^{b}	21.3±2.40 ^{a}	608±17.74 ^{b}	26.55
SALA	200	-	3.83±0.47 ^{c}	-	3.8±1.17 ^{c}	181 ±4.12 ^{c}	86.90
SALA	100	169.5±14.43^{c}	5.5±0.67 ^{c}	3±0.89 ^{c}	14.52±2.62 ^{c}	378±18.45 ^{c}	49.93
SALA	50	97.17±5.23 ^{c}	8.67±0.36^{b}	4.67±0.82 ^{c}	20.78±2.93 ^{a}	445.83±16.09 ^{c}	28.34
SALA	25	65.17±6.07 ^{a}	11.3±0.97^{ns}	8.97.±0.61^{ns}	27.54±1.89^{ns}	885±19.34^{ns}	15.38
Loperamide	5	195.5±4.50 ^{c}	5.67±0.67 ^{c}	2.16±0.75 ^{c}	8.04±2.10 ^{c}	226.5±11.47 ^{c}	72.28

Values are mean ±SEM, n=6, and $P^{a}<0.05$, $P^{b}<0.01$, $P^{c}<0.001$are significantly different from the control group

Ns=not significant

4.2 Effects of the extracts on in-vivo GI motility

The extracts (SALM, SALA, SARM, and SARA) with dose of 100 and 50 mg/kg and the reference drug atropine produced significant ($p<0.001$) inhibition of normal intestinal transit. Whereas with dose of 200 mg/kg the extracts inhibited stomach empting as the marker charcoal was found in stomach.

However with dose of 25 mg/ kg of aqueous extracts showed no significant intestinal transit (Peristaltic Index) reduction even though the methanol extract showed significant inhibition of intestinal transit with same dose. See Table 3.

In castor oil induced intestinal transit model castor oil increased Peristaltic Index from 66.03 ± 2.80 of the normal value to 90.10 ± 1.89. The extracts and the reference drug loperamide produced significant ($p<0.001$) and dose dependent decrease in the castor oil induced intestinal transit when compared to the control group (castor oil) with doses of 200, 100 and 50 mg/kg of all extracts. However with dose of 25 mg/kg no significant reduction of the Peristaltic Index (PI) for all extracts. See table 4.

Table 3: Inhibitory effect of the extract of *S. abyssinica* on normal GI transit of mice

Treatment	Dose (mg/kg)	Peristaltic index (PI) %	Inhibition %
Control	-	66.03±2.8	-
SALM	200	0.0±0.0 ^{c}	100
SALM	100	10.14±1.12 ^{c}	84.64
SALM	50	31.78±1.14 ^{c}	51.87
SALM	25	50.27±2.3 ^{b}	23.87
SARM	200	0±0.00 ^{c}	100
SARM	100	14.46±1.27 ^{c}	69.77
SARM	50	47.79±1.50 ^{c}	27.62
SARM	25	53.31±1.1 ^{b}	19.26
SALA	200	0.00+0.00 ^{c}	100
SALA	100	21.26±1.3 ^{c}	67.8
SALA	50	38.14±1.09 ^{c}	42.23
SALA	25	61.3±1.96 ^{ns}	7.16
SARA	200	0.0±0.0 ^{c}	100
SARA	100	32.83±1.10 ^{c}	50.42
SARA	50	48.04±2.20 ^{c}	27.25
SARA	25	59.9±2.16 ^{ns}	9.28
Atropine	5	25.40±2.00 ^{c}	61.53

Values are mean ± SEM, n= 6 and $P^{a}<0.05$, $P^{b}<0.01$, $P^{c}<0.001$ are significantly different from the control group, Ns=not significant

Table 4: Inhibitory effect of the extract of *S. abyssinica* on castor oil induced intestinal transit in mice.

Treatment	**Dose (mg/kg)**	**Peristaltic Index (%)**	**%Inhibition**
Control	- -	90.10+ 1.89	-
SALM	200	26.67+1.36 ^{c}	70.39
SALM	100	54.00+1.59 ^{c}	40.00
SALM	50	69.10+1.80 ^{c}	23.33
SALM	25	89.50+1.84 ^{ns}	0.66
SALA	200	36.85+2.17 ^{c}	71.11
SALA	100	59.16+2.61 ^{c}	34.44
SALA	50	76.80+2.11 ^{b}	14.67
SALA	25	88.17+1.95 ^{ns}	2.03
SARM	200	22.00+1.60 ^{c}	75.55
SARM	100	46.95+2.94 ^{c}	47.83
SARM	50	63.04+1.61 ^{c}	30.00
SARM	25	79.80+2.84 ^{ns}	11.33
SARA	200	40.67+3.23 ^{c}	55.60
SARA	100	52.34+189 ^{c}	42.22
SARA	50	70.19+2.03 ^{c}	22.22
SARA	25	89.80+1.83 ^{ns}	1.11
Loperamide	5	20.17+2.58 ^{c}	77.78

Values are mean ±SEM, n= 6 and $P^{a}<0.05$, $P^{b}<0.01$, $P^{c}<0.001$ are Significantly different from the control group, Ns=not signify

Table 5: In vivo antidiarrhea index (ADI) of the extracts in mice

Treatment	Dose mg/kg	DFT (%)	GITR (%)	PFR (%)	ADI (%)
control	-		-	-	-
SALM	200	100	70.39	100	88.79
SALM	100	83.75	40	80.64	64.65
SALM	50	73.68	23.33	61.28	47.23
SALM	25	38.75	0.66	33.88	12.70
SALA	200	100	71.11	100	89.21
SALA	100	59.05	34.44	70.96	52.44
SALA	50	40.42	14.67	54.79	31.91
SALA	25	27.08	2.03	20.91	10.35
SARM	200	100	75.55	100	91.08
SARM	100	87.5	47.83	81.6	69.89
SARM	50	66.67	30	62.44	49.98
SARM	25	42.92	11.33	45.11	27.99
SARA	200	100	55.60	100	82.23
SARA	100	71.25	42.22	66.12	58.37
SARA	50	54.58	22.22	59.63	41.66
SARA	25	33.75	1.11	29.04	10.28
Loperamide	5	81.25	77.78	79	79.33

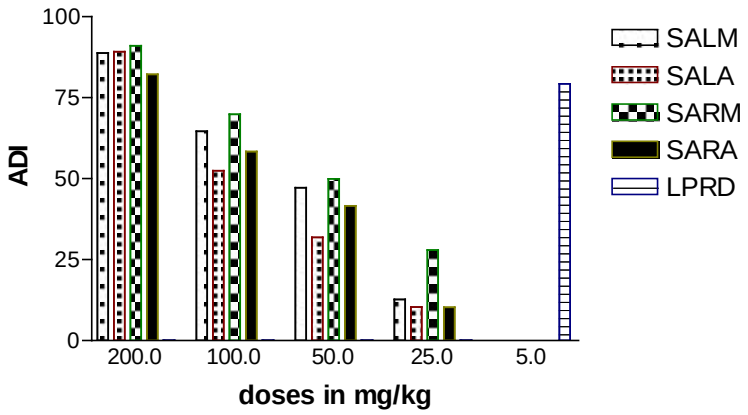

Figure 3: In-vivo antidiarrheal index (ADI) of the extracts.

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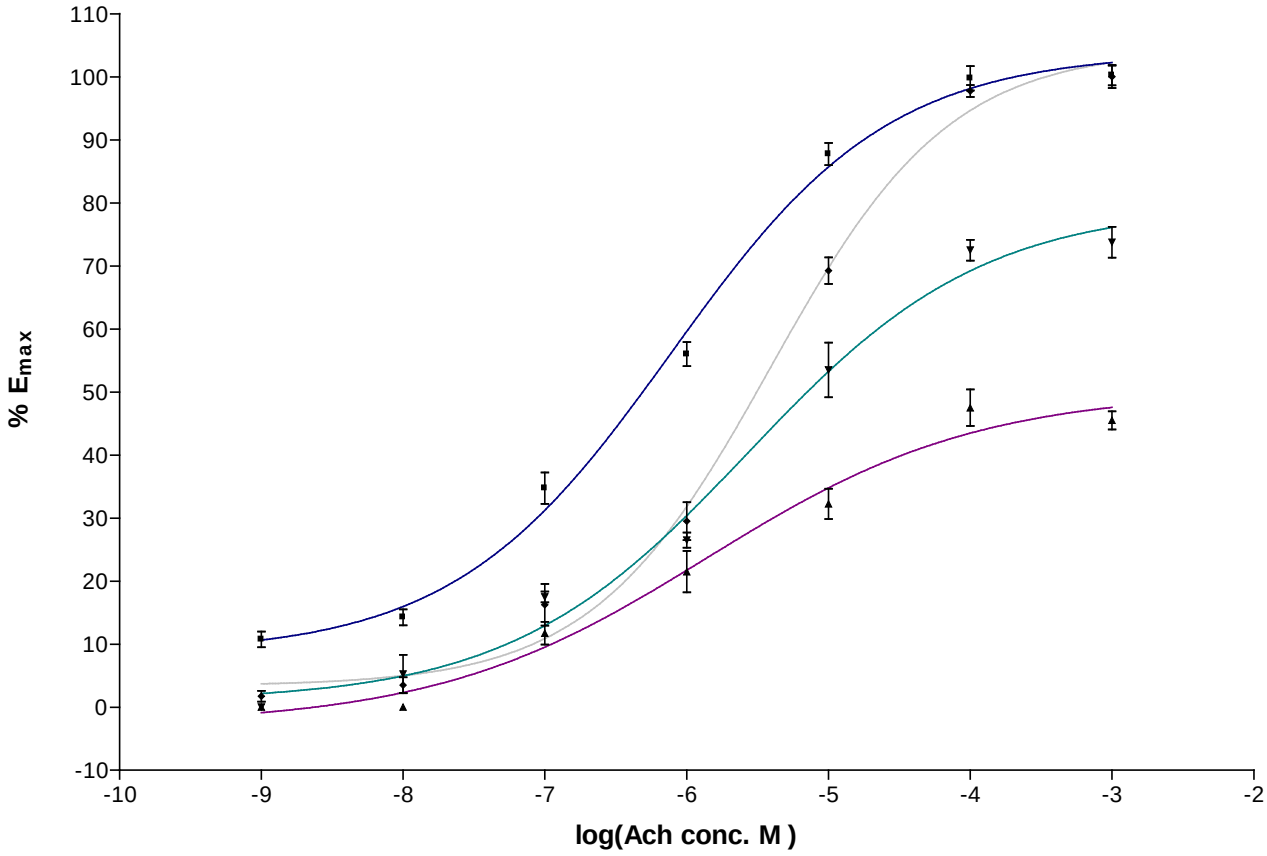
4.3 In vitro antispasmodic activities on isolated GPI

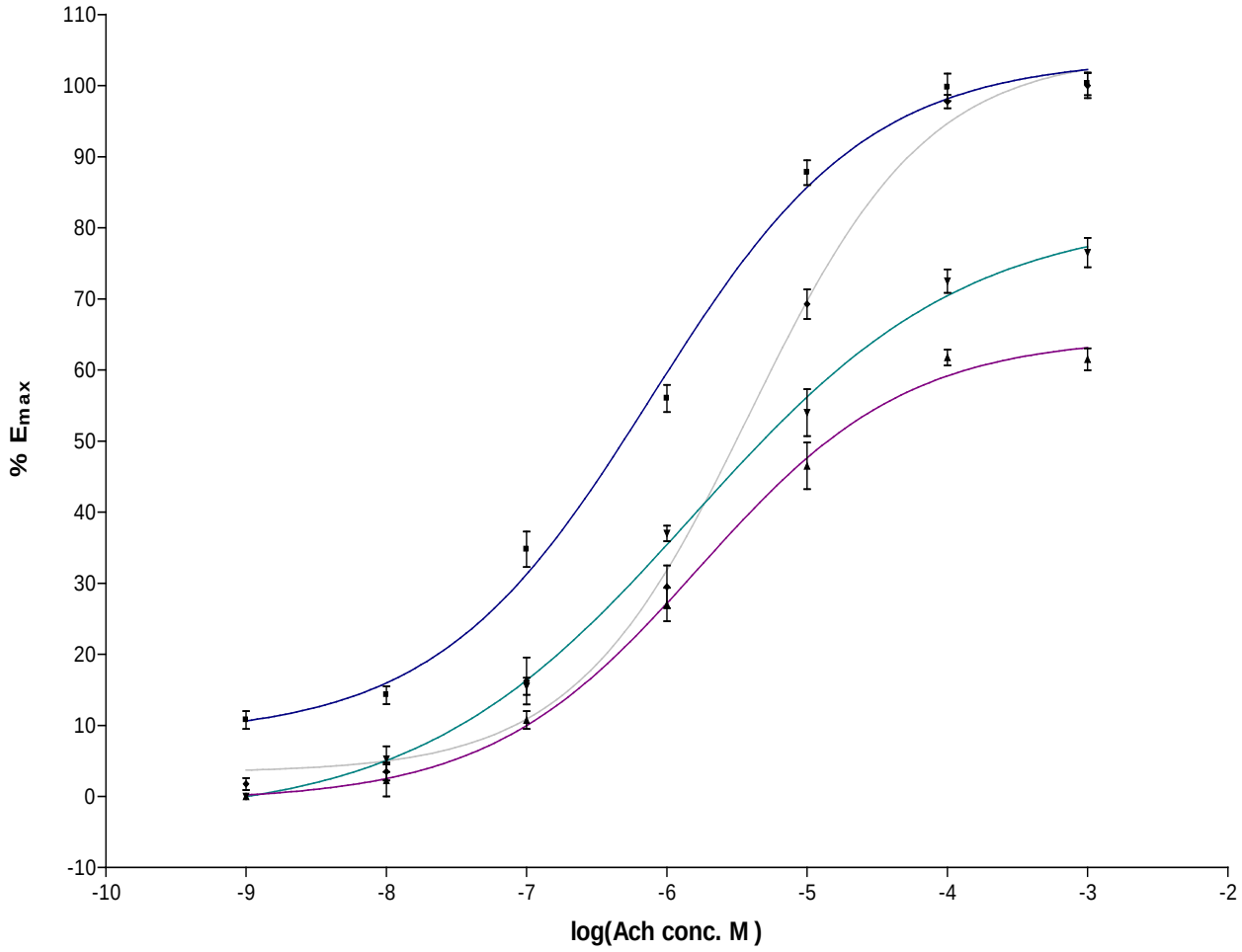
The extracts (SALM and SALA) at conc of 100 ug/ml and 200 ug/ml significantly ($P<0.05$) inhibited the Ach induced contraction when compared with the control. This activity was dose dependent. The inhibition of contraction was significant ($P<0.05$) at all conc of the spasmogen Ach. Methanol extract showed more spasmolytic activities than the aqueous extract at both conc (100 and 200 ug/ml) as E_{max} of Ach at conc of 10^{-3}M was decreased (from 100 for the control group) by SALM with conc of 200 and 100 ug/ml to 45.6±2.13 and 73.2±3.04 respectively, however E_{max} of Ach at conc of 10^{-3} M was decreased (from 100 for the control group) by SALA with conc of 200 and 100 ug/ml to 62.0±2.98 and 74.8±2.46 respectively. However that of atropine (6.5 nM) was 98.1±1.78 (Figure 4 and 5).

The extracts also produced right ward shift in DRC of Ach and decreased PD_2 value of Ach from 6.13±0.12 to 5.56±0.10 and 5.63±0.09 by SALM with conc of 200 and 100 ug/ml respectively, whereas SALA with conc. of 200 and 100 ug/ml from 6.13±0.12 to 5.77±0.11 and 5.81±0.14 respectively and atropine decreased from 6.13±0.12 to 5.40±0.09. But right ward shift in DRC was in non-parallel way as the slope is different for the DRC of the control group and the test groups.



Control (Ach) ■
SALM 100 ug/ml + ACh ▼
SALM 200 ug/ml + ACh ▲
Atropine + ACh ●

Figure 4: Effect of increasing concentrations of SALM on the DRC of acetylcholine on guinea-pig ileum. Responses were expressed as % of the maximum contractions (Emax) induced by Ach prior to the addition of the extracts (control). Each point is mean ±SEM of six experiments.



Control (Ach) ■
SALA 100 ug/ml + ACh ▼
SALA 200 ug/ml + ACh ▲
Atropine + ACh ●

Figure 5: Effect of increasing concentrations of SALA on the DRC of acetylcholine on guinea-pig ileum. Responses were expressed as % of the maximum contractions (Emax) induced by Ach prior to the addition of the extracts (control). Each point is mean ±SEM of six experiments.

4.4 Effects of the extracts on intestinal fluid accumulations

The results of the effect of the extracts of *S. abyssinica* on intestinal fluid accumulation are presented in Table 6. Oral administration of castor oil produced a significant increase in intestinal fluid (1.03±0.093 ml) as compared to the mice not administered castor oil (0.129±0.04ml). The extracts were found to possess significant anti-enteropooling activities as they significantly ($P<0.001$) reduced intestinal fluid accumulation with dose of 100 mg/kg from1.03 ml of the control to 0.403±0.019 ml, 0.210±0.018 ml, 0.494±0.012 ml and 0.288±0.026 ml by SALM, SARM, SALA and SARA respectively. The standard drug (loperamide) also significantly ($P<0.001$) reduced intestinal fluid accumulation from1.03±0.093 ml of the control to 0.290±0.030ml. The antisecretory activities of the extracts were dose dependent.

Table 6: Effect of the extract on intestinal fluid accumulation in mice.

Treatments	Dose (mg/kg)	Volume of fluid in ml	Inhibition (%)
Control	-	1.030±0.093	-
SALM	100	0.403±0.019^{c}	60.87
SALM	50	0.546±0.038^{b}	46.99
SARM	100	0.210±0.018^{c}	79.61
SARM	50	0.357±0.022^{c}	65.33
SALA	100	0.494±0.012^{c}	52.03
SALA	50	0.653±0.042^{a}	36.60
SARA	100	0.288±0.026^{c}	72.04
SARA	50	0.549±0.090^{b}	46.70
LPRD	5	0.290±0.030^{c}	71.84
Vehicles	-	0.129±0.040^{c}	-

Values are mean ± SEM, n= 6 and $P^{a}<0.05$, $P^{b}<0.01$, $P^{c}<0.001$ are significantly different from the control group.

5 DISCUSSION

In this study, castor oil induced diarrheal model was done in order to test as to whether the extracts of *S. abyssinica* have antidiarrheal activity or not. Then after, other models (antipropulsive and antientropooling) were performed in an attempt to propose some of the possible mechanisms (decrease in GI transit and antisecretory activities) by which they exhibited antidiarrheal activity. And to test antispasmodic activities both in vitro on GPI using Ach as spasmogen and in vivo models in mice using castor oil as spasmogen were performed.

Antidiarrheal activity

Diarrhea may results from disturbance in bowel function in which case there is increased bowel transit, excessive intestinal secretion of water and electrolyte, decreased intestinal reabsorptions as well as more frequent defecations of loose, watery stool (Gurgle *et al*., 2001).

It is known that the active component of castor oil is the ricinoleic acid which is liberated by the action of lipases on castor oil. Castor oil is made up of 90% ricinoleate which when metabolized is responsible for its diarrheal inducing effect. The ricinoleic acid produce irritation and inflammatory actions on intestinal mucosa leading to release of prostaglandins (such as PG-E2). This condition induces an increase in the permeability of the mucosal cells and changes in electrolyte transport, which results in hyper-secretory response (decreasing Na+ and K+ absorption), stimulating peristaltic activity and diarrhea. The castor oil model therefore incorporate both secretory and motility diarrhea (Rouf *et al.,* 2003; Ching *et al.,* 2008).

The result of this study revealed that the extracts possessed antidiarrheal activity in castor oil treated animals. The extract dose-dependently inhibited castor oil-induced transit in mice and this indicates possible antispasmodic activity and possible antidiarrheal mechanism of the extracts. The frequency and severity of castor oil-induced diarrhea in the mice was inhibited in a dose-related manner by the extracts. There was delay in onset

time of diarrhea (increase in diarrhea free time) and the total number of stools, number of wet stools, and weight of wet stools were significantly decreased in dose dependent way, with the highest effect observed with the 200 mg/kg body weight of all extracts (SALM, SALA, SARM and SARA). These effects at 200mg/kg were comparatively more than those produced by loperamide.

Thus, the extracts showed better antidiarrheal activities than the standard loperamide with 200mg/kg and these results suggest efficacy of the extracts as antidiarrheal agents. But the extracts have less potency than the standards loperamide. This less potency is for obvious reason that the extracts are crude whereas loperamide is pure chemical.

The antidiarrhoeal index (ADI) is a measure of the combined effect of the various components of diarrhea such as on-set time of diarrheal (diarrhea free time), purging frequency, and intestinal transit. ADI is used as indicator of in vivo antidiarrheal activities of the extracts. The fact that the extracts produced a significant antidiarrheal index and a significant reduction in diarrheal score reinforces its protective action in diarrhea.

With dose of 200mg/kg of all extracts complete suppression of diarrheal was observed even more than the reference Loperamide and inhibited defecation suggesting constipation could be one of the side effects of the medicinal plant and this speculation is also supported by the marked decrease in GI transit by the extracts especially at larger doses.

The medicinal plant has to be contraindicated in infectious-diarrhea caused by invasive microorganisms as it may cause local GI complication such as toxic mega colon and may decrease elimination of the invasive microorganisms, even though it has been reported *S. abyssinica* has antimicrobial activities (Chacrabotory *et al.* ,2000) unless their antimicrobial activities against specific diarrhea causing microorganisms is established. This proposal is supported by extracts marked decrease of GI motility and extracts marked inhibition of defecation.

The antidiarrheal activities of the extracts of *S. abyssinica* found in the present study is proved to be due to antimotility and antisecretory effect of the extracts as the extracts reduced GI motility in the antipropulsive test and reduced fluid accumulations in antipropulsive test.

The antidiarrheal activities of the extracts of *S. abyssinica* found in the present study could be owing to presence of flavonoids and tannins in this plant as previous studies showed the presence of these metabolites (Chacrabotory *et al.*, 2000; Abebe *et al.*, 2003).

The antidiarrheal activities of flavonoids have been ascribed to their ability to inhibit intestinal motility and water and electrolyte secretions which are known to be altered in diarrheal conditions. Whereas tannins are known to form proteins tannate which make intestinal mucosa more resistant and hence reduce secretion *(*Galvez *et al.,* 1991, 1993; Tripathi, 1994; Borrelli *et al.,* 2000; Venkatesan *et al.,* 2005*).*

Other possible mechanism of action may be due to the extracts binding to mu opiod receptors as previous in vitro study on radioligands binding assay of the methanol extracts of the leaf (SALM) showed affinity of SALM for mu opiod receptor with IC50 of 62 ug/ml (conc. that inhibit 50% nalaxone binding which is opiod antagonist) (Deborah *et al.,* 2002).

The Geiger's criteria for the acceptance of a drug as an antidiarrheal include: (1) inhibition of the production of wet or unformed feaces in animals, (2) inhibition of the production of watery stool or fluid evacuation in animal and (3) inhibition of gastrointestinal propulsive action (Akah *et al.,* 1988).

The extracts (SALM, SALA, SARM and SARA), therefore, meets the Geiger's criteria as antidiarrheal agent as observed from the results of the present study of antidiarrheal and antipropulsive tests.

Antipropulsive activity

Antipropulsive activities were done both for normal transit and castor oil induced transit. This model (antipropulsive test) is useful to test antispasmodic and mechanisms of antidiarrheal activity (Izzo *et al.*, 1992; Vogel, 2002). In the evaluation of effects of the extract on normal Intestinal transit, atropine was used as standard drug. Atropine is known to inhibit intestinal transit (arrest peristalsis) due to its anticholinergic effect (Izzo *et al.*, 1992).

In the evaluation of effects of the extract on normal intestinal transit, the extracts appeared to act on the intestine and the stomach. This is proved by reductions in intestinal propulsive movement of charcoal meal of normal transit at the dose of 25, 50, 100mg/kg. And with dose of 200 mg/kg the extracts inhibited stomach emptying, as the marker charcoal was found in stomach. The results of this model suggested the extracts act not only on the intestine but also on stomach. This effect was comparable even better than standard drug atropine with 200 mg/kg dose though less potent. It is but logical since atropine is pure chemical compared to extracts which are mixture of many compounds, so this is reason for less potency than atropine; however, the extracts showed better efficacy at test dose of 200 mg/kg. ED_{50} of the extracts was not calculated as the extracts are crude and appropriate if determined after isolation of active compound(s).

Studies made on activated charcoal showed that it prevents the absorptions of drugs by adsorbing on the surfaces of charcoal particles (Venkatesan *et al.*, 2005). This may increased the effective dose of the extracts. Activated charcoal was used in GI motility test to serve as marker.

The extracts suppressed the propulsion of charcoal meal which showed the efficacy of the extracts in decreasing the vagal peristaltic movements of GI this also explains muscle relaxant effect of the extracts. This pharmacological action of the extracts suggested one of the possible antidiarrheal activities mechanisms. That is by decreasing hyper motility

in effect increases the time for absorption of water and electrolytes in the intestines also this activity indicates the extracts possible antispasmodic activities (Vogel, 2002).

The antipropulsive activities on normal transit (peristalsis) may be due to anticholinergic activities and/or due to muscle relaxant activities. This speculation is supported by complete arrest of vagal peristalsis by the extract in this in-vivo test and in-vitro antispasmodic tests done on isolated GPI in which the extracts inhibited ACh induced contractions.

In castor oil induced intestinal transits tests the aim is to test the effect of the extracts on established increase in intestinal motility as there is increase in intestinal transit in diarrhea. This experiment was done also as part of in vivo antispasmodic to test effect of the extract on established increase in intestinal motility. The extracts inhibit peristaltic index significantly in this model as compared to the control. However, the extracts produced more reduction in intestinal transit on normal intestinal transit than castor oil induced transit eventhough both were significant. This activity of the extracts was dose dependent.

Antipropulsive effects observed in this model may contribute for antidiarrheal effect of the extracts by increasing intestinal transit time which increase absorption of water and electrolyte.

So the results of this model (antipropulsive tests) indicated the possible antispasmodic activities of the extracts and are consistent with in vitro antispasmodic tests. Also indicated the efficacy of the medicinal plant as antispasmodic agent and support the traditional use of the medicinal plant for stomachache and abdominal spasm.

Lozoya *et al* (2002) reported about the relationship of the spasmolytic, antimotility and antidiarrhoeal activity of *Psidium guajava folia* extract with quercetin flavonoids present in the plant. The significant antidiarrhoeal activity of the methanol fraction of unripe

fruits of *Psidium guajava* extract was also connected to the flavonoids that inhibit Ach release in GI tract (Ghosh *et al*., 1993).

The present study also indicated the relationship of the spasmolytic, antimotility, antisecretory and antidiarrhea activities of the extracts of *S. abyssinica* and which may be due to flavonoids and alkaloids present in the plant.

Antienteropooling activity

Enteropooling model is performed with aim to test possible antisecretory activities of the extracts. Castor oil is known to induce diarrhea in experimental animals and human subjects (Rouf *et al.,* 2003). But it can also be used to induce intestinal fluid accumulation.

Castor oil causes motility and secretory type diarrhea due to its effect on GI motility and electrolyte transport across intestinal mucosa. This is so due to ricinoleic acid which cause irritation to intestinal mucosa resulting in increased release of inflammatory mediators such as PG-E_2 which increase secretions of mucosa fluid and electrolytes by affecting cell permeability (Ammon *et al.,* 1975; Rouf *et al.,* 2003)

In this model, the extracts displayed a significant reduction in volume of fluid as compared to the control group. This antienteropooling activity was dose dependent. The results of this model indicated that the extract has got antisecretory activity as evidenced by the statistically significant reduction in volume of secretion which is important proof of the antisecretory activity of the extracts.

Flavonoids and tannins are some of the constitutes of *S. abyssinica* (Chacrabotory *et al.,* 2000) and Flavonoids have been reported to posses antisecretory activity through inhibition of prostaglandin biosynthesis and also by decreasing histamine and ACh release *(*Gálvez *et al.,*1993, 2003; Ghosh *et al.,* 1993; Borrelli *et al.,* 2000*).* And tannins are known to form proteins tannate which make intestinal mucosa more resistant and hence reduce secretions *(*Gálvez *et al.,* 1991; Tripathi 1994; Borrelli *et al.,* 2000).

Therefore, the antisecretory activity of the extract could have been due to the medicinal plants flavonoids and tannin content.

Apart from the above proposed antisecretory mechanisms (inhibition of prostaglandin biosynthesis) there is possibility that the extracts could act on mu opiod receptors as agonist or prostanoid receptors as antagonist and also anticholinergic mechanism may cause the antisecretory activities. However at this stage and with this work alone difficult to say the exact mechanism of antisecretory action of the extracts.

In vitro antispasmodic activity

ACh induced contraction is mediated via M_3 subtype muscarnic receptor in small intestine. ACh causes depolarization and tonic contraction of intestinal smooth muscles. This is also by increase in concentration of cytoplasm free calcium ions. The activation of muscarnic receptors of longitudinal smooth muscle of small intestine produce an increased frequency of action potential discharge and depolarization, which results in contraction as result of increased efflux of calcium through L-type voltage-operated channel. Calcium also can enter the cell through receptors operated Ca++ channels. In short, calcium ions gain access to the cytoplasm through voltage-activated or receptor-operated calcium channels (Gilani *et al.,* 1994).

The extracts (SALM and SALA) when tested on isolated GPI produced inhibition on ACh induced contractions in a dose dependent manner. The extracts shifted Ach dose response curve (DRC) to the right that indicates the competitive antagonism of Ach receptor as the standard atropine also did the same right ward shift in DRC of Ach and both the extracts and atropine decreased the PD_2 value of Ach meaning increased the EC50 of the ACh. However the extracts (SALM and SALA) also depressed the maximum response to agonist Ach, which suggest non competitive antagonism at concentration of 100ug/ml. The next higher conc. of 200ug/ml also caused further right ward shift in Ach dose response curve in a non-parallel fashion (as the slope of the DRC of the control is different from that of the extracts) and the maximum response was also

further suppressed, which is characteristics of a non competitive antagonism in fact the affinity of the antagonist (the extracts) expressed as PA'_2 was not calculated as the extracts are crude and their molar concentration couldn't be calculated and the formula (to calculate PA'_2) demand molar concentrations of the antagonists.

The spasmolytic activity of the extracts to antagonize Ach on the intestinal smooth muscle indicates their relaxant action on the gut and their efficacy as antispasmodic agent. And they may work through antagonism of cholinergic receptors and/or may act on ion channels such as Ca++ channel or K+ channel and interfere with the process of depolarization (as functional antagonism in effect might cause the observed actions and with this work alone difficult to say exact mechanism). The in-vivo study also support this hypothesis as the extracts abolished in-vivo vagal peristalsis, significantly decreased peristaltic index and inhibited stomach emptying in normal transit test.

The results of in-vitro studies suggest the plant may contain compounds exhibiting antimuscarnic and/or muscle relaxants activities. This hypothesis is supported by right ward shift in dose responses curve of Ach, decrease in PD2 value of the Ach both by extracts and atropine in comparable way and also in vivo study complete arrest of peristalsis was observed. However, the extracts did not act exactly in the same way as atropine. As atropine shifted DRC of Ach rightward but not suppressed Emax of Ach. These facts indicate the extracts may contain more than one compound that act through different mechanism and/or there is possibility that the active compound may have functional antagonism with muscarnic receptors.

Non-competitive antagonism of cholinergic receptors at neuromuscular junction inactivates receptors so that effective complex with agonist (Ach) cannot be formed irrespective of the agonist concentration (Ach). However, in competitive or reversible antagonisms increasing the concentration of agonist at receptor site results in maintenance (eliciting) of pharmacological (physiological) action of the agonist and Emax is maintained as in the absence of the antagonist that is to say Emax is not depressed. But in this study the action of the extracts were non specific as the extracts

suppressed maximum effect elicited by the agonists and also shifted dose response curve of Ach towards right (Ariens *et al.*, 1964).

In this in-vitro study spasmolytic activities of the leaf extracts were proved. And the results of this in-vitro model is in agreement with in vivo spasmolytic model done on mice in which the extracts showed muscle relaxant effect by inhibiting (abolished) vagal spontaneous and rhythmic contraction of the small intestine and stomach of mice with dose of 200 mg/kg in antipropulsive effect test on normal GI transit and also in castor oil induced GI transit there were reduction in propulsive movement of the GI.

The results of this in-vitro model and in vivo spasmolytic model are in agreement with each other and prove the traditional use of this medicinal plant for stomachaches and abdominal cramp.

Other studies revealed flavonoid constituents of different plants showed spasmolytic activity in different tissues preparations *in vitro* (Gilani *et al.,* 1994b; Abdalla *et al.,*1994; Galvez *et al*., 1996;).

In the previous phytochemical study flavonoids and isoquinol alkaloid were found in SA (Chacrabotory et al., 2000; Abebe et al 2003). These metabolites might be responsible for the observed spasmolytic activity of the extracts.

6 CONCLUSION AND RECOMMENDATION

Results of this study indicated that the methanol and aqueous extracts of Stephania abyssinica leaves and roots possess significant and dose dependent antidiarrheal activity due to their inhibitory effect both on GI propulsion and fluid secretion. And also the extracts possess significant antispasmodic activity due to anticholinergic effect. So the findings of this study provide scientific bases and prove for the claimed antidiarrheal and antispasmodic activities and consistent with the utility of the medicinal plant in traditional medicine for treatment of diarrheal diseases, stomach ache and abdominal cramp.

The following are recommended for further work

● PG-E2 and MgSo4 diarrheal models need to be further investigated,

●Effect of the extracts on intestinal ion conc such as, measurement of K^+, Na^+ and Cl^- concentration of intestinal fluid to assess effect on electrolytes transports,

● Other models of antispasmodics activities test such as effect on KCl (potassium chloride), serotonin and histamine induced contractions,

● Detailed phytochemical screening and activity guided fractionation to find out the active principle(s) responsible for the pharmacological activities, and

● Precise mechanism(s) of action should be elucidated.

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