

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

GASTROINTESTINAL EFFECTS OF CRUDE KHAT (*CATHA EDULIS*
F) EXTRACT FOLLOWING ACUTE AND SUB-CHRONIC
ADMINISTRATION IN RODENTS

WINTANA TADESSE

August, 2011

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ADMINISTRATION IN RODENTS

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A Thesis Submitted to Department of Pharmacology and Therapeutics,
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I, the undersigned, declare that this thesis work is my original work and has not been presented for a degree in any other university.

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Acronyms

5-HT:	5- hydroxy tryptamine
ACh:	Acetylcholine
CCK:	Cholecystokinine
COX-1:	Cyclooxygenase-1
COX-2:	Cyclooxygenase-2
DU:	Duodenal ulcer
ECL:	Enterochromafin-like
ENS:	Enteric nervous system
GIT:	Gastrointestinal tract
GMBF:	Gastric mucosal blood flow
H. pylori:	Helicobacter pylori
H ₂ R:	Histamine 2 receptor
IL-12:	Interleukin-12
IPANs:	Intrinsic primary afferent neurons
M ₃ :	Muscarinic receptor sub type 3
NO:	Nitric oxide
NSAIDs:	Non steroidal anti inflammatory drugs
PAF:	Platelet activating factor
PFD:	Pelvic floor dysfunction
PGE ₂ :	Prostaglandin E ₂
PGI ₂ :	Prostacyclin
PGs:	Prostaglandins
PPIs:	Proton pump inhibitors
PPY:	Peptide Y
TG:	Triglyceride
TLC:	Thin layer chromatography
TNF α :	Tumour necrosis factor alpha
VIP:	Vasoactive intestinal polypeptide

Abstract

Khat (*Catha edulis* F) belongs to the Celastraceae family and is a dicotyledonous evergreen shrub/tree used for recreation and alleviation of fatigue in several countries of East Africa and South Arabia. Recently, this habit has spread considerably, raising concern in view of medical and socio-economic consequences of khat consumption. Even though khat has several medicinal, social, and economical values, there are numerous reports regarding its gastrointestinal tract (GIT) adverse effects. As the effects of khat chewing on digestive system mentioned in earlier studies were based on clinical observations, the present study was designed to evaluate the GIT activity of the crude khat extract after acute and sub-chronic administration using different rodent models and parameters used for ulcer and motility measurement were quantified.

For acute study, animals were randomly assigned into different groups. Negative control received Tween 80 2% in distilled water, whereas positive controls were given ranitidine (pylorus ligation and cysteamine HCl models), misoprostol (indomethacin induced gastric ulcer), and loperamide (castor oil induced diarrhea and enteropooling assay). The other group received different doses of crude khat extract: 100 mg/kg, 200 mg/kg and 300 mg/kg. For sub-chronic study, one group was administered vehicle and served as control, whilst the other three were administered three doses of khat extract (100, 200, and 300 mg/kg) for 45 days.

Acute khat administration in pylorus ligation model was shown to aggravate ulcer compared to controls in the highest dose used, as evidenced by increased volume of gastric acid secretion ($p < 0.05$), total acidity ($p < 0.05$) and ulcer index ($p < 0.01$). Similar results were obtained in indomethacin induced gastric ulcer model where 300 mg/kg khat extract increased mucosal damage as demonstrated by a significant reduction in mucin content compared with misoprostol ($p < 0.001$) and vehicle ($p < 0.05$) treated animals. Khat at 300 mg/kg was also shown to accentuate duodenal ulcer induced by cysteamine HCl by significantly increasing ($p < 0.01$) ulcer area as compared to vehicle-treated rats. Whilst ranitidine was shown to offer antiulcer activity in the models tested, khat at 100 mg/kg was devoid of any significant ameliorating or accentuating effect. Surprisingly, sub-

chronic administration of khat at all doses failed to produce neither gastric nor duodenal ulcer.

In castor oil induced diarrhea, 300 mg/kg ($p < 0.001$) and 100 mg/kg ($p < 0.01$) khat extracts significantly decreased the number of watery fecal discharge against vehicle treated rats. In addition, 300 mg/kg dose decreased ($p < 0.05$) weight and volume of intestinal content in castor oil induced enteropooling model. In sub-chronic studies, 300 mg/kg and 200 mg/kg khat extracts significantly inhibited intestinal motility in *in-vivo* gastrointestinal motility model by 53.2% and 48.6%, respectively. On the other hand, weight change on sub-chronic khat extract administration was observed and the change became significant at the 3rd ($p < 0.05$), 4th and 5th week ($p < 0.01$), and 6th week ($p < 0.001$) with 300 mg/kg dose. While in 200 mg/kg decline of weight at 4th week ($p < 0.05$) and for following weeks ($p < 0.01$) was obtained.

In conclusion, acute use of khat at higher dose in the presence of ulcerogenic agents could aggravate gastric and duodenal ulcer, while sub-chronic khat administration alone did not produce ulcer. Furthermore, acute and sub-chronic administration of khat extract at higher dose produced a significant constipating activity. And sub-chronic administration of khat extract at higher dose markedly decreased body weight. These effects collectively indicate that khat consumption in large amount and for a longer period of time is associated with adverse GIT outcomes.

Key words: Catha edulis, Pylorus ligation, Cysteamine HCl, Indomethacin, Castor oil, and Mucin.

1 Introduction

1.1 Peptic ulcer

1.1.1 Regulation of gastric acid secretion

In mammalian gastric mucosa, oxyntic cells project peripherally onto the walls of the gland and thus are commonly called parietal cells (Kobayashi *et al.*, 2000). It is a complex biological structure whose behavior is regulated by a broad variety of extracellular signals that interact with specific receptors present on the cell surface to activate complex signal transduction pathways. Several studies have indicated that the parietal cells express families of protein kinases known as mitogen-activated protein kinases or extracellular signal-regulated protein kinases (Takeuchi *et al.*, 1997) and the Jun NH₂-terminal kinases. These kinases are important elements in signaling cascades that are known to regulate cellular functions such as growth, differentiation, and secretion. Thus, parietal cells have unique structural properties to produce and secrete gastric acid (Kobayashi *et al.*, 2000; Pausawasdi *et al.*, 2000).

The process of gastric acid secretion has been divided into cephalic and peripheral phases. The cephalic phase of gastric acid secretion originates in the central nervous system (CNS) and impacts the hypothalamus. Signals travel via the vagus nerve to the myenteric plexuses of the gastric mucosa. In the succeeding neural network, a variety of secondary neurons signal the gastric fundic and antral epithelia to influence gastric acid secretion by either primary or secondary action, namely, direct effects on parietal cells or gastric epithelial endocrine cells. The peripheral phase of acid secretion regulation involves local signaling within a variety of endocrine cells, transmitting regulatory information to the secretory cells of the gastric mucosa with limited possible mediators (Zeng *et al.*, 1999).

Regulation of gastric acid secretion is a complex physiological process that involves numerous hormones and neurotransmitters. Activation of acid secretion by parietal cells is triggered by paracrine, endocrine via gastrin, histamine and somatostatin and by neural pathways via acetylcholine (ACh) and neuropeptides (Sachs *et al.*, 1997). The effect of

this neural and endocrine cascade is to activate one of the three stimulatory receptors on the parietal cells; H₂ (histamine), M₃ (ACh), and CCK-B (gastrin) receptors (Sachs *et al.*, 1997; Gooz *et al.*, 2000). Stimulation of acid secretion typically involves an initial elevation of intracellular Ca²⁺ and cAMP followed by activation of protein kinase cascades, which trigger the translocation and insertion of H⁺/ K⁺-ATPase (the proton pump) into the apical plasma membrane of the parietal cell (Chi, 2009).

In neural network, the enteric nervous system (ENS) is the only region of the peripheral nervous system that is intrinsically capable of mediating reflex activity. It contains intrinsic neurons and extrinsic efferent and afferent neurons (Falalyeyeva and Beregova, 2007). The efferent fibers are preganglionic and do not directly innervate parietal cells but rather synapse with postganglionic neurons of ENS. The postganglionic neurons contain a variety of transmitters and regulate acid secretion directly and/or indirectly. ACh induces acid secretion either directly by stimulating M₃ receptors on the basolateral membrane of parietal cells or indirectly via stimulation of enterochromafin-like (ECL) cells that release histamine in the fundus and gastrin from the G cells in the gastric antrum (Kobayashi *et al.*, 2000; Schubert and Peura, 2008).

ECL cells, which are histamine-containing endocrine cells in the gastric mucosa, play a central role in the peripheral regulation of acid secretion by release of histamine. Histamine stimulates H₂ receptors on parietal cells (Fig 1) to cause acid secretion by raising intracellular cAMP (Kurbel and Kurbel, 1995; Sachs *et al.*, 1997). The peptide hormone gastrin, produced and released by antral G cells in response to food intake, activate CCK2 on ECL cells leading to release of histamine and on parietal cells directly to stimulate acid secretion (Zhao *et al.*, 2008). Gastrin-stimulated acid secretion is controlled by the release of inhibitory mediators, such as somatostatin (Thompson, 1969; Lindstrom *et al.*, 2001).

Somatostatin, produced by D cells in the antrum as well as in fundus, has no direct contact with the lumen and possesses cytoplasmic processes consistent with a paracrine function (Vuyyuru *et al.*, 1997). The antrum and fundus D cells activated differentially.

In the antrum, antral luminal acidity is thought to stimulate somatostatin release while D cell in the fundus is stimulated by gastrin, ACh and neural peptides (Sachs *et al.*, 1997).

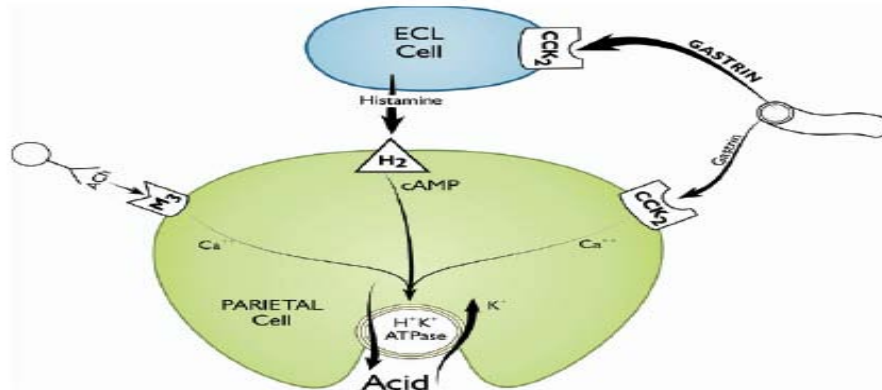


Fig 1. Model illustrating parietal cell receptors and transduction pathways (copied from Schubert and Peura, 2008).

Many studies concerning the central effects of various neurotransmitters and neuropeptides on gastric acid secretion have revealed mechanisms by which the brain regulates gastric acid secretion. Based on these studies, more than 40 peptides have been examined and it is well established that many neuropeptides, such as thyrotropin releasing hormone, corticotropin releasing factor, neuropeptide Y, and bombesin, mediate a CNS induced stimulation or inhibition of gastrointestinal function (Yoneda, 1998).

1.1.2 Gastric mucosal protection

The gastric epithelium is covered by a continuous layer of secreted mucus and bicarbonate which have been widely implicated as an important pre-epithelial protective factor against autodigestion of the gastric mucosa by acid and pepsin. The gastric mucosa is continuously exposed to many noxious factors and substances. Gastric mucosal injury may occur when noxious factors “overwhelm” an intact mucosal defense or when the mucosal defensive mechanisms are impaired (Mojzis *et al.*, 2000).

The mucus-bicarbonate phospholipid “barrier” constitutes the first line of mucosal defense. This barrier is formed by mucus gel, bicarbonate, and surfactant phospholipids,

which cover the mucosal surface. The gastric mucus is secreted by the epithelial surfaces throughout the gastrointestinal tract (GIT) from the stomach to the colon. This gastric mucus occurs in three forms: a soluble mucin presents in gastric juice, insoluble (adherent) mucus covering mucosal cells and mucus present in muciparous cells (Atuma *et al.*, 2001).

The adherent mucus is considered to be the main factor protecting the gastric mucosa. It is a unique secretion in that it forms a gel adherent to the surface that provides a protective barrier between the underlying epithelium and the lumen (Atuma *et al.*, 2001) by forming a stable, unstirred layer at the mucosal surface to prevent immediate mixing of the secreted bicarbonate with the excess of acid in the lumen. The mucus gel, by acting as a mixing barrier, enables the stabilization of a pH gradient from acid in the lumen to near neutral at the mucosal surface to prevent penetration of pepsin and proteolytic digestion of the surface epithelium (Allen and Flemstrom, 2005; Laine *et al.*, 2008).

The mucus gel contains phospholipids, another component of the barrier, and its luminal surface is coated with a film of surfactant phospholipids with strong hydrophobic properties (Laine *et al.*, 2008). This hydrophobic mucosal surface between luminal secretions and the epithelium may repel H⁺ ions and keep the surface of the epithelium dry by preventing the contact of luminal acid (Aakaydin *et al.*, 1991).

Besides, gastric mucosal integrity requires the continuous generation of prostaglandin E2 (PGE2) and prostacyclin (PGI2). Inhibition of their synthesis results in the reduction of gastric mucosal blood flow (GMBF) and gastric mucosal damage (Takeeda *et al.*, 2004). Several studies have focused on novel approaches to enhance the resistance of the gastric mucosa to injury. Prostaglandins (PGs), nitric oxide (NO) and lipoxin A4 have been shown to be important mediators of mucosal defense (Wallace, 2005).

PGs are involved in a variety of physiological processes in the stomach, including acid secretion, production of mucus and mucosal blood flow. The enzyme for PG production exists as two isozymes: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). COX-1 is involved in the maintenance of essential physiological functions, while COX-2,

in response to various proinflammatory stimuli, is responsible for pathological PG production at inflammatory sites (Whittle, 1981; Kargman *et al.*, 1993).

Studies demonstrated that vascular endothelial cells also release a labile humoral vasodilator substance, which is identical with NO acting in concert with PGI₂. Local release of neuropeptides, such as calcitonin gene-related peptide (CGRP) is also likely to be involved in the maintenance of mucosal integrity, since depletion of the neuropeptides from primary afferent nerve terminals results in aggravation of mucosal damage induced by various challenging agents (Gyires, 2005).

1.1.3 Pathogenesis of peptic ulcer

A peptic (or gastric/duodenal) ulcer is damage to the inner surface of the stomach or duodenum, resulting in loss of tissue and inflammation. The defect in the protective lining of the stomach and duodenum may be superficial or become deeply erosive if untreated (Sivri, 2004). The predominant symptom of uncomplicated peptic ulcer is epigastric pain, which can be accompanied by other dyspeptic symptoms such as fullness, bloating, early satiety, and nausea. In duodenal ulcer (DU), epigastric pain occurs typically during the fasting state or even during the night and is usually relieved by food intake or acid-neutralising agents. The most frequent and severe complication of peptic ulcer is bleeding and perforation is less frequent than is bleeding (Malfertheiner *et al.*, 2009).

Gastric ulcer is one of the most common GIT diseases and has affected humans for centuries. It is likely that ulcer develops as a result of imbalance between mucosal defensive (protective) factors such as mucosal blood flow, ischemic preconditioning, NO and PG generation, growth factors, ghrelin, and aggressive factors such as HCl, pepsin, bile acids and others (Tanigawa *et al.*, 2005; Noor *et al.*, 2006). The use of drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs), *Helicobacter pylori* (*H. pylori*) infection and stress are some of the known factors that can cause gastric ulcer (Chi, 2009).

The ability of NSAIDs to cause ulceration is through inhibition of PG synthesis that plays an important role in gastric mucosal defense by forming a cytoprotective layer and increasing the secretion of bicarbonate ions to neutralize the gastric acidity (Dhikav *et al.*, 2003). The component of mucosal defense that appears to be most profoundly altered by NSAIDs is the gastric microcirculatory response to injury. Normally, exposure of the mucosa to an irritant results in a rapid increase in mucosal blood flow. This is mediated via sensory afferent neurons and is probably aimed at removing any toxins or bacterial products that enter the lamina propria, neutralizing back-diffusing acid and contributing to the formation of a microenvironment over sites of superficial mucosal injury that is conducive to repair (Wallace, 2001).

There are another group of mediators such as, tumour necrosis factor α (TNF- α) (Santucci *et al.*, 1994) and leukotrienes that contribute to the increase in neutrophil adherence to damages the mucosa by liberating oxygen-free radicals, releasing proteases, and obstructing capillary blood flow after NSAIDs administration (Chan and Leung, 2002).

H. pylori infection is another major cause of chronic gastritis, peptic ulcer and also affects gastric acid secretion (Gooz *et al.*, 2000; Osawa *et al.*, 2006). Several adaptive and metabolic features (microaerophilic, facultative acidophil and catalase activity) allow the organism to remain unharmed once colonization has ensued and its motility and adherence mechanism to receptors on gastric mucosal cells allow it to colonize the stomach (Behrman, 2005).

Additionally, *H. pylori* infection impairs negative feedback regulation of gastrin release and thus acid secretion. A low antral pH stimulates release of somatostatin from D cells in the antral glands, and this somatostatin exerts paracrine inhibitory control of gastrin release from adjacent G cells. It has very high urease activity by producing ammonia on the surface epithelium and in the glands of the antrum to prevent D cells in the glands from sensing the true level of acidity, leading to inappropriate release of somatostatin and an increase in gastrin, and consequently excess acid secretion (Odum *et al.*, 1994). The neural pathways are also affected through impairing inhibitory neural control and this in

association with hypergastrinaemia leads to further increase of acid output in the presence of DU (Johnson *et al.*, 2007; Malfertheiner *et al.*, 2009).

Despite the development of immune responses against *H. pylori* infection, the bacteria are rarely eliminated and colonization is generally persistent. Chronic gastric inflammation induced by *H. pylori* is considered a Th1-mediated response where the T and B lymphocytes activated by bacterial antigens and proinflammatory cytokines regulate the local and systemic immune response with release of further cytokines (interleukin (IL), TNF α), and antibodies which is implicated in perpetuating the inflammatory changes that lead to disease (Pellicano *et al.*, 2007). As a result of the immunopathogenetic events, additional factors with ulcerogenic potential are released, including platelet activating factor (PAF) and components of the complement pathway (Malfertheiner *et al.*, 2009).

H. pylori cause additional dysregulations in gastric physiologic features through mucosal inflammation that leads to gastric metaplasia within the duodenum. In conjunction with a high duodenal acid load, colonization results in further inflammation and additional gastric metaplasia with a resultant increase in density in the duodenal bulb that make the inflamed duodenal mucosa more susceptible to peptic acid attack and ulceration. The inflammatory reaction and high duodenal acid production impairs bicarbonate secretion and diminishes neutralization of duodenal acid (Behrman, 2005).

The increased maximum acid output may reflect, in part, an increase in the parietal cell mass commonly found in DU patients. The most popular is due to normal or abnormal secretion by an abnormally large number of parietal cells (Marshall, 1995; Wormsley, 1997). The parietal cell hyperplasia has also been hypothesized to result from the trophic stimulus provided by excessive neural (vagal) activity or by excessive amounts of circulating gastrin and releasing mechanisms of gastrins are abnormally resistant to inhibition (Wormsley, 1997). It is proved beyond doubt that the infection with the bacterium plays the dominant role in the pathogenesis of DU (Hurlimann *et al.*, 1998).

Stress is another factor that causes severe structural disruption of the gastric mucosal capillary network and microvascular alterations in genesis of stress ulceration. Studies conducted in cold water immersion restraint rats indicated heterogeneous distribution of corpus mucosal blood flow with alternating regions of high and low blood flow in the gastric corpus that lead to strong gastric contraction (Abdel-Salama *et al.*, 2001). Reduced local mucosal NO generation and increased endothelin-1 (a potent vasoconstrictor) also appear to play an important role in the mechanisms of stress related mucosal damage (Laine *et al.*, 2008).

1.1.4 Treatment of peptic ulcer

Current treatment of gastric ulcer is based on eliminating the *H. pylori* bacterium using antibiotics and to use antacids (sodium bicarbonate, aluminum hydroxide, magnesium hydroxide) and/or acid blockers to relieve pain via neutralization of intraluminal acid and promote healing of inflammatory injuries (Chi, 2009). Historically, anticholinergic drugs were concurrently administered to delay emptying of the agents into the duodenum and to inhibit acid secretion (Aihara *et al.*, 2003; Andersson and Carlsson, 2005).

H₂ receptor antagonists have been first-line therapy for acid-related peptic disease to promote healing ulcers to improve the quality of life (Aihara *et al.*, 2003). These agents gradually replaced with the more potent class of acid-inhibitory drugs, the proton pump inhibitors (PPIs) (Malfertheiner *et al.*, 2009). PPIs, activated at low pH, irreversibly bind to and inactivate the sulphhydryl groups of the H⁺/K⁺-ATPase enzyme of the parietal cell to cause dose-dependent decreases in gastric acid secretion. On the other hand, nonpeptide CCK2 receptor antagonists (Proglumide) have been attempted as antisecretory drugs (Hakanson *et al.*, 1991; Aihara *et al.*, 2003).

The other group of drugs is directed at reinforcement of the mucosal barrier. There are various prophylactic strategies to reduce the gastric toxicity of NSAIDs, which include concurrent treatment with H₂ receptor antagonist, PPIs, and selective COX-2 inhibitors. So these selective COX-2 inhibitors have been developed with no gastric toxicity (Chan and Leung, 2002). Misoprostol, a PGE analogue, is used to prevent NSAIDs-associated

ulcers due to its gastric acid anti-secretory properties and its various mucosal protective properties, mediated by stimulation of gastric mucus, duodenal bicarbonate secretion and enhancement of GMBF (Shield, 1995; Laine *et al.*, 2008).

Sucralfate is also effective in the prevention and treatment of ulcer through the enhancement of gastric mucus secretion (Allen and Flemstrom, 2005). Studies suggest that NO shares most of the muco-protective properties of PG and also platelets make a major contribution to ulcer healing, and the release of several key growth factors from platelets appears to be regulated by proteinase-activated receptors (Laine *et al.*, 2008).

Treatment for *H. pylori*-associated ulcer disease is mainly directed at eradication of infection. Eradication is usually achieved with a combination of acid-inhibiting therapy (PPI) and antibiotics (amoxicillin and clarithromycin) (Malfertheiner *et al.*, 2009). In the case of persistent *H. pylori* infection or early reinfection, the basic approach is to use antibiotics that have not been used before. At present, TNF- α , IL-1 β , COX, PGE and proteases are key players in the control of the healing processes. The dynamic and complicated interactions are under extensive investigation (Chi, 2009).

1.2 Constipation

1.2.1 Normal intestinal physiology

Bowel function is the result of the state of intestinal motility and also of its fluid handling, which is the net result of a dynamic state of absorption and secretion in the gut wall. Most secretion occurs from the mucosal crypt cells and absorption takes place in the villous cells (Cooke *et al.*, 1997). The major goal of small intestine is to absorb nutrients. There are well recognized regional differences between proximal and distal intestine in nutrient absorption. The colon plays an important role in conserving fluid and electrolytes and thus preventing diarrhea (Cook, 1994).

Ions and water can move bidirectionally across the intestinal mucosa, i.e., from luminal (mucosal) to blood (serosal) sides and vice versa. The difference between the two unidirectional fluxes or the “net” ion flux determines the direction of net transport. The active transport of ions (principally Na⁺, Cl⁻, and HCO₃⁻) across the small intestinal

epithelium provides the electrical and chemical forces that drive the coupled absorption of nutrients as well as the net absorption or secretion of water. A variety of absorptive and secretory agents, including local and systemic hormones, neurotransmitters, toxins released by enteric pathogens, and other molecules that gain access to the intestinal lumen stimulate ion and water transport (Camilleri, 2004).

In addition, ENS, neuronal network within digestive wall, consists of two plexi, the myenteric and the submucosal. Both plexi contain so-called secreto-motor nerves that release agonists with either direct or indirect effects on epithelial ion transport. Vasoactive intestinal polypeptide (VIP) and ACh are the predominant secretomotor neurotransmitter with direct epithelial secretory and/or antiabsorptive effects whereas mechanical stimulation of mucosal sensory neurons activates secretion as well as stimulates blood flow and smooth muscle contraction through 5-hydroxy tryptamine (5-HT), substance P, and neurokinins 1 and 2 (Field, 2003; Gershon, 2004).

1.2.2 Absorption, secretion and gastrointestinal motility

Sugars (glucose and galactose) 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 from low luminal to higher cell concentration, a gradient opposite to that for Na^+ . The organic solutes then move from enterocyte to blood via basolateral membrane carriers that operate independently of ion movements. 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 (Ganapathy and Leibach, 1985).

Longitudinally, differences exist in Na^+ entry mechanisms, sites of HCO_3^- secretion, and sites of active K^+ transport. Na^+ crosses small-intestinal and colonic brush borders via Na/H exchange and, in the small bowel, also by Na-organic solute cotransport. In the distal colon, luminal Na^+ is also absorbed via an aldosterone-sensitive Na^+ channel. HCO_3^- is absorbed in the jejunum (via Na/H exchange) and secreted in the duodenum,

ileum, and colon. Its secretion can be explained in part by $\text{Cl}^-/\text{HCO}_3^-$ exchange. Active absorption and secretion of K^+ both occur in the colon, but neither occurs in the small intestine, where K^+ movement is strictly diffusional (Poulsen *et al.*, 1994).

Each day, 7–8 L of fluid are secreted into the GIT or taken by mouth. However, fluxes of fluid during intestinal digestion contribute to the lumen a net 3.0 L of fluid, which flows along osmotic gradients through the highly permeable jejunal mucosa and results in the initial loss of fluids from the intravascular space into the lumen. Subsequently, small bowel reabsorption of water and electrolytes recovers much of the secreted fluid, so that only about 1.2 L of fluid enters the colon each day (Debonnie and Phillips, 1978).

1.2.3 Regulation of absorption, secretion and gastrointestinal motility

Intestinal physiology is complex, involving neural, endocrine, and luminal sources of modulation. Neural control has both intrinsic and extrinsic elements; the intrinsic nervous system consists of cell bodies and endings that are positioned between the inner circular and outer longitudinal muscle layers of the gut wall. The cell bodies of the intrinsic nervous system lie in the submucosal ganglia (Meissner's plexus) and the myenteric ganglia (Auerbach's plexus). Thus, the submucosal plexus is concerned with the modulation of fluid and electrolyte transport and the myenteric plexus with control of motility, with additional effects on vascular tone and immune responses (Kunze and Furness, 1999).

Secretion is principally the result of the active transport of Cl^- and HCO_3^- ions through apical chloride channels after the activation of the intracellular mediator's cAMP, cGMP, and Ca^{+2} , although multiple other channels have been identified (Banks and Farthing, 2002). There are classes of compounds that stimulate active secretion and inhibit active absorption (includes neurotransmitters (VIP), paracrine (serotonin), and inflammatory (PG)), and those with the opposite effects (Sellin, 1993; Quigley *et al.*, 1999).

The neuronal regulation of gastrointestinal motility involves intrinsic as well as extrinsic nerves. The extrinsic innervation involves the vagus nerve and splanchnic nerves to the stomach and upper intestine. Extrinsic neurons of the sympathetic and parasympathetic

systems influence smooth muscle indirectly by acting on neurons of the myenteric plexus and the intrinsic innervation involves ENS (Kunze and Furness, 1999).

ENS has semiautonomous specific programmes for motor responses (peristaltic reflexes) and a regional rate of contractions, which contain both the intrinsic primary afferent neurons (IPANs) and the interneurons (Loening-Baucke and Yamada, 1995). Thus, IPANs are analogous neurons that carry information to the interneurons and motor neurons in the ENS. They are present in both plexuses. Peristaltic and secretory reflexes are initiated by submucosal IPANs, which are stimulated by 5-HT acting at 5-HT_{1P} receptors. Myenteric IPANs are involved in other types of gastrointestinal reflexes (Gershon, 2004).

Motor neurons receive input from ascending and descending interneurons that are excitatory and inhibitory type. The neurons within the plexuses secrete different neurotransmitters and a variety of pharmacologically active peptides. Ach is the primary neurotransmitter of excitatory motor neurons, but the same neurons also contain tachykinins. NO is probably the primary inhibitory neurotransmitter, but ATP and VIP are also key contributors to transmission (Shuttleworth *et al.*, 1991; Kunze and Furness, 1999; Gershon, 2004). Studies indicated that effects on the muscle from either excitatory or inhibitory motor neurons are relayed through the interstitial cells of Cajal (ICC), which are electrically coupled to the muscle and determine timing of gastrointestinal smooth muscle contraction (Wingate *et al.*, 2002).

Another important neurotransmitter is 5-HT, which is released by the epithelial enterochromafin cells; this process is mediated by subsets of enteroendocrine cells, which are specialized to act as sensory transducers. These cells trigger either peristaltic or sensory reflexes in response to luminal stimuli by releasing chemical transmitters that activate underlying IPANs (Chen *et al.*, 2001; Atkinson *et al.*, 2006).

In addition, hormones are involved in the regulation of smooth muscle activity and gut motility at several levels, which act and interact directly and indirectly on muscle cells. Thus, the hormones are released locally from endocrine cells in the mucosal lining and

subsequent neural activation of hormones arises within the lumen from the mechanical and chemical properties of food and digestive secretions. Therefore, CCK, ghrelin and somatostatin modulate motility by activating receptors on sensory fibers, extrinsic (vagal) and IPANs, and again back on the endocrine cells in an autoregulatory fashion (Rogers *et al.*, 1995).

1.2.4 Pathogenesis of constipation

Constipation is associated with disordered movement of stool through the colon or anorectum which commonly occurs when the waste (stool) that forms after food is digested moves too slowly (slow transit) as it passes through the digestive tract. In fact, infrequent defecation is an uncommon complaint among those who are constipated and there is little evidence that symptoms predict the presence or absence of colorectal dysfunction (Longstreth *et al.*, 2006).

The underlying etiological factors are still obscure. Dehydration, changes in diet and activity, and certain drugs are frequently to blame to slow transit of stool. When stool moves slowly, too much water is absorbed from the stool, and it becomes hard and dry. Gradual enlargement of the rectum and poor coordination of the pelvic and anal muscles sometimes contribute to or cause constipation (Wald, 2007).

The slow transit constipation is due to motility abnormality, resulting from a decrease in the frequency, duration, and amplitude of the giant migrating contractions, which are also known as high amplitude propagated contractions. The pathogenesis of idiopathic slow transit constipation is unknown in most cases. It is also thought to have as a primary defect slower than normal movement of contents from the proximal to the distal colon and rectum (Lyford *et al.*, 2002). Indeed, it has been suggested that colonic inertia, possibly related to decreased numbers of high amplitude propagated contractions that prolonged residence times of fecal in the right colon and increased, uncoordinated motor activity in the distal colon will offers a functional barrier or resistance to normal transit are the two subtypes in slow transit (AGA, 2000).

Pelvic floor dysfunction (PFD) is the other major pathophysiologic condition. It features normal or slightly slowed colonic transit overall, but a preferential storage of residue for prolonged periods in the rectum (AGA, 2000). In this instance, the primary failure is one of an inability to evacuate adequately contents from the rectum. However, efferent and afferent pathways are involved in mediating the reflexes influencing anal and rectal motility and in mediating sensations. An alteration in the efferent or afferent pathways from the rectum or the pelvic floor could be responsible for disorders of defecation, such as constipation and fecal incontinence (Loening-Baucke and Yamada, 1995).

PFD is characterized by failure of relaxation of the puborectalis and the external anal sphincter muscles, which may even undergo paradoxical contraction during straining. This pathology has been documented by tests of anorectal physiology. The basic mechanism underlying this form of persistent constipation is the failure of the anorectal angle to straighten and the anal canal to shorten during sustained contraction of the puborectalis muscle. In addition, such patients may also endure reduced relaxation of the internal anal sphincter, increased defaecatory sensation thresholds, and higher maximum rectal tolerable volumes (Kuijpers and Bleijenberg, 1985).

In critically ill patients, pathophysiology is incompletely understood, but several conditions play a role. Sepsis and shock impair gastrointestinal motility through elevated levels of circulating endotoxin, inflammatory mediators, and enhanced inducible NO production. Hypotension and hypoperfusion, and also vasoactive and opiates medication, used to treat hypotension, have a negative effect on gastrointestinal motility (Wirthlin *et al.*, 1996; Cullen *et al.*, 1999).

1.2.5 Treatment of constipation

Whether due to slow transit or dyssynergy, in mild cases, the treatment includes general measures like increased intake of water and dietary fibres. A diet with enough fibre helps to form soft, bulky stool. Sufficient dietary fibre is also needed to promote normality in bowel movement frequency over the long term. In severe cases however, treatment is usually directed at the underlying cause as in the case of severe slow transit constipation

a subtotal colectomy that appears to be refractory to medical treatment (simple laxatives) (National Prescribing Centre, 1999).

The various types of laxatives are divided into three general classifications: (i) those causing softening of feces in 1 to 3 days (bulk-forming laxatives, docusates, and lactulose); (ii) those that result in soft or semifluid stool in 6 to 12 hours (bisacodyl and senna); and (iii) those causing water evacuation in 1 to 6 hours (saline cathartics, castor oil, and polyethylene glycol-electrolyte lavage solution) (Wells *et al.*, 2009).

In case of PDF, biofeedback therapy can improve the function and coordination of the abdominal, rectal, and anal sphincter muscles, as well as rectal sensory perception. It includes visual and/or auditory techniques, to provide input during defaecation. The posture and diaphragmatic breathing are also corrected using verbal reinforcement techniques. Progress is monitored by either electromyographic or manometric methods; other biofeedback adjuncts include sensory retraining with an intrarectal balloon. However, there are no controlled trials in adults comparing biofeedback with laxatives, although laxatives are cheaper and more readily available (Chiotakakou-Faliakou *et al.*, 1998; Chiarioni *et al.*, 2006).

1.3 Overview of khat (*Catha edulis* Forsk)

Khat (*Catha edulis* Forsk) belongs to the Celastraceae family and is a dicotyledonous evergreen shrub or tree found growing wild or cultivated in the east region extending from Southern Africa to the Arabian Peninsula (Patel, 2000; Feyissa and Kelly, 2008). The shrub grows to a height of 6 m and the leaves are leathery, glossy, brownish green, with serrated edges, arranged in an alternate fashion on the straight branches (Fig 2). The young shoots and leaves are the parts chewed for their psychoactive properties (Mela and McBride, 2000; Cox and Hagen, 2003).



Fig 2. Picture of Catha edulis.

There are several names for the plant, depending on its origin: chat, qat, qaad/jaad, miraa, mairungi, muhulo, hagigat, cat, catha, gat, tohai, and muraa. The dried leaves of khat are known as Abyssinian tea or Arabian tea (Glenice and Hagen, 2003; Ishraq, 2004). In Ethiopia, it is commonly known as “Chat” and has other local names such as Beleche, Aweday, Abo Mismar, Gelemso, Wondo and others based on place of cultivation. The stimulating power and cost of the product varies depending on the source. It is claimed that the Aweday and Abo Mismar varieties, which are cultivated in Harar highlands of Eastern Ethiopia are the most potent and costly among the local brands and hence are chosen for export (Belew *et al.*, 2000; Gebissa, 2008).

Khat contains cathinone, being a ketoamine base, is extremely unstable and, in particular, it can be transformed into (+)-norpseudoephedrine and (-)-norephedrine by an enzymatic reduction. Pharmacological studies are still being conducted on these compounds, but it is presently believed that the stimulating effect of the plant is principally due to cathinone and cathine (Abdulwaheb *et al.*, 2007). The presence of amphetamine and caffeine in khat has been excluded. As result of structural similarity, cathinone have been termed a ‘natural amphetamine’ (Dhaifalah and Santavy, 2004).

In addition to the two phenylalkamines cathine (norpseudoephedrine) and cathinone (S (-)- α -aminopropiophenone)}, there are more than 40 alkaloids, glycosides, tannins and terpenoids in khat which includes merucathinone, ethereal oils, sterols, triterpenes, flavonoids, ascorbic acid and tannins. The phenylalkylamines and cathedulins are based on a polyhydroxylated sesquiterpene skeleton and are basically polyesters of euonyminol and recently 62 different cathedulins from fresh khat leaves were characterized (Al-Motarreb *et al.*, 2002; AL-Hebshi *et al.*, 2005; Al-Zubairi *et al.*, 2008).

By chewing the khat leaves, cathinone is effectively extracted into the saliva and directly absorbed through the oral mucosa and gastrointestinal lining with higher lipid solubility that provides access to CNS (Mela and McBride, 2000; Odenwald, 2007). The pharmacokinetic parameters for cathinone and other ingredients of khat leaves have been determined over 8 h, with peak plasma levels attained after 1–3.5 h but a pharmacokinetic model has not been developed (Toennes *et al.*, 2000).

The metabolism of cathinone to cathine involves reduction of the ketone group to an alcohol, a fairly common metabolic pathway in humans, catalyzed by liver microsomal enzymes. Only 7% or less of the absorbed α -cathinone is excreted unchanged in the urine, and is mainly excreted in the form of norephedrine and cathine (Cox and Rampes, 2003). Cathinone has a mean terminal elimination half-life of 1.5–4.5 h; for cathine it is approximately 5 h (Hattab and Al-Abdulla, 2001). The amount of norephedrine excreted in urine is much higher than the amount ingested, indicating that cathinone is also metabolized to norephedrine (Feyissa and Kelly, 2008).

The chemical similarity between cathinone and amphetamine suggested that the two substances might have the same mechanism of action. Amphetamine produces its effects by activating neurotransmission mediated by the catecholamines, noradrenaline and dopamine, in particular by releasing these neurotransmitters from their physiological storage sites. Cathinone is capable of releasing dopamine, noradrenaline and serotonin from synaptic terminals (Kalix, 1996; Michael, 2005).

1.3.1 Gastrointestinal effects of khat

Halbach, (1972) asserted that khat-chewing causes certain health disturbances including migraine, cerebral haemorrhage, myocardial insufficiency liver cirrhosis, impotence and pulmonary oedema. In gastrointestine stomatitis followed by secondary infection, oesophagitis, gastritis, constipation, anorexia, malnutrition and general weakness were documented with concomitant khat chewing (Luqman and Danowski, 1976; Kimani *et al.*, 2008). Besides, gastric ulcers and haemorrhoids were reported (Al-Hebshi and Nils, 2005).

The effects of habitual khat chewing on the digestive system mentioned in earlier studies were based on the clinical observation that khat chewers often complained of symptoms suggestive of stomatitis, oesophagitis, gastritis and constipation. In experimental animals, khat extract causes gastritis and duodenitis (Hassan *et al.*, 2002). The dose-related association between khat chewing and gastrointestinal symptoms was confirmed (Kennedy *et al.*, 1983; McKee, 1987). These effects were believed to be caused mainly by the strongly astringent tannins and the alkaloids that affect motility and secretions (WHO, 1980; Dhaifalah and Santavy, 2004).

Gastric symptoms were also attributed to a hypotonic stomach resulting from the sympathomimetic action of cathine and its precursor. Haymann *et al.*, (1995) reported a significant decrease in gastric emptying time after khat chewing. Cathinone assumed to be responsible for the slowed gastric emptying, a similar effect with the sympathomimetic agents (isoprenaline and salbutamol), and it was substantiated by *in vitro* experiments where cathinone caused relaxation of the rat stomach. Delayed gastric emptying may contribute to an increased rate of gastrooesophageal reflux manifested as heartburn and acid regurgitation, and to an increased risk of Barrett oesophagus, a precancerous condition (Haymann *et al.*, 1995).

Constipation, the other common complaint of khat chewers, is associated to significant delay of both the oro-caecal transit time and the whole gut transit time by the khat leaves (Hassan *et al.*, 2007). Meanwhile, anorexia may be attributed to combined central and

gastric effects of cathinone in fresh leave of khat (Murray *et al.*, 2008). Undesirably, khat might also lead to malnutrition and increased susceptibility to infectious diseases such as tuberculosis as result of its contribution to prolonged anorexia (Kalix, 1992).

An earlier study by Al meshal *et al.* (1985) on the flavonoid fraction showed khat to possess significant anti-inflammatory and gastric anti-ulcer activities. Another study suggested that pure cathinone at 10 and 30 mg/kg showed significant dose dependent decrease in the intensity of gastric mucosal damage induced by aspirin and pylorus-ligation model at 1 and 6 h after treatment indicating antigastric ulcer activity (Al-Shabanah *et al.*, 1994). On the other hand, a study performed by Rajaa *et al.* (2000) indicated that khat chewing is associated with DU.

The effect of khat on human GIT remains an area of considerable interest. However, controversy remains regarding the potential effects and mechanisms by which khat induces duodenal and gastric ulcer. Performing the present study on crude khat extract would at least, in part, shed light on whether temporal dependent administration of crude khat extract have protective or aggravating effect on gastroduodenal ulcer and the possible mechanisms associated with the effect. Furthermore, this study enables us to observe the association between weight change pattern and sub-chronic administration of crude khat extract. Thus, given the extensive use of khat use in East Africa and Arabian Peninsula as well as the fact that khat use has become a growing concern in the developed world, initiating such a comprehensive study would add to the existing body of knowledge on khat and GIT. Moreover, the knowledge of khat pharmacology is increasing, but its interpretation remained fairly controversial in some aspects. Some of the peripheral effects may be over looked or simply escaped interpretation for various reasons. Therefore, elucidating the effects of this plant is still valuable (Mahmood *et al.*, 2009).

2 Objectives

2.1 General objective

- To evaluate the gastrointestinal tract effects of crude khat extract after acute and sub-chronic administration in rodents.

2.2 Specific objectives

- To examine gastric ulcer aggravating/protective effects of crude khat extract on pylorus ligation induced gastric ulcer model.
- To test gastric ulcer aggravation/protection of crude khat extract using indomethacin induced gastric ulcer model.
- To assess duodenal ulcer aggravation/protection on cysteamine induced ulcer model after crude khat extract administration.
- To propose the possible mechanism how crude khat extract aggravates / protects ulcer upon acute administration.
- To test whether crude khat extract induces/impedes gastric and duodenal ulcer after sub-chronic administration.
- To evaluate constipating effect of crude khat extract after acute administration.
- To study antimotility activity of crude khat extract in *in-vivo* gastrointestinal motility model.
- To assess the dose-dependent weight change patterns after sub-chronic administration of crude khat extract.

3 Materials and Method

3.1 Drugs and chemicals

Cathinone was obtained from Laboratory and Scientific Section of the United Office on Drugs and Crime (UNODC), silica gel 60 (Kieselgel F₂₅₄) and chloroform (Research-Lab Fine Chem. Industries, Mumbai), diethyl ether (Fisher Scientific Limited, UK), ethyl acetate (Nice Chemicals Limited, India), methanol (Reagent Chemical Services Limited, USA), aqueous ammonia (Research Lab, fine chemicals, India), Tween 80 (Sigma-Aldrich, UK), Ninhydrin (Belami fine chemicals Limited, India), ranitidine injection (Cadila Pharmaceuticals, India), loperamide (Sigma-Aldrich, UK), activated charcoal (Lab. Reagent, India), indomethacin (Merck Shsrp and Dohm, Haarlem-Netherlands), sucrose, misoprostol (Cipla Ltd, India), cysteamine HCl and Alcian Blue 8GX (Himedia Laboratories PVT. Ltd, Mumbai, India), HCl, carboxymethylcellulose (CMC), Na acetate, phenolphthalin, NaOH, MgCl₂ and gum acacia (BDH Chemicals Limited Poole, England), and castor oil (Shiba Pharmaceutical and Chemical Limited, Yemen) were purchased from the respective sources.

3.2 Collection of plant material

Bundles of fresh khat shoots and small branches were purchased at a local market from Aweday, its natural habitat, 525 km South of Addis Ababa, Ethiopia. The fresh bundles were packed in plastic bags and transported in an icebox to the School of Pharmacy, Addis Ababa University. The plant was identified by a taxonomist and a voucher specimen (WT001) was deposited at the National Herbarium, Addis Ababa University. The fresh leaves were immediately kept at -20 °C till the time of extraction.

3.3 Animal preparation

Either sex of Sprague Dawley rats (150-280 g) and Swiss albino mice (25–35 g) were bred in the animal house of School of Pharmacy, Addis Ababa University and housed in a standard plastic cage at a controlled room temperature of (21 ± 1⁰C), maintained at 12/12 hrs light/ dark cycle and relative humidity of 50%. They were fed on pellets and given drinking water *ad libitum*. The care and handling of animals were in accordance with the

internationally accepted standard guidelines for use of animals (Hiruma-Lima *et al.*, 2006).

3.4 Extraction

The extract was prepared as described elsewhere with slight modification (Connor *et al.*, 2002; Banjaw and Schmidt, 2004; Bedada and Engidawork, 2010). Briefly, the freeze-dried plant was finely minced with knife on glass plate, weighed by electronic digital balance and placed in Erlenmeyer flasks (200 g per flask) wrapped with aluminum foil to avoid light induced decomposition. Chloroform (112.5 mL) and diethyl ether (337.5 mL) (1:3 v/v) were added to cover the minced leaves. The resulting mixture was stirred using a rotary shaker at 120 rpm (New Brunswick Scientific Co, USA) and 20 °C under dark condition for 24 h. The content was later filtered through folded Whatman No 1. filter paper with the help of a mini filter pump. Fractions of the organic filtrate collected in this way were kept in wide mouth bottles and placed in a hood for 24 h to remove the organic solvents. The fraction was left overnight in a deep freezer and then lyophilized using freeze dryer (Christ 100400 Bioblock Scientific, France). Afterward the yield was calculated and found to be 1.0% which was similar with previous work (Abdelwahib *et al.*, 2007).

3.5 Thin layer chromatography

The process was based on thin layer chromatography (TLC) separation as described by Lehmann *et al.*, (1990) and a specific test was used for detection of the main khat amines, cathinone, and norpseudoephedrine. Analytical TLC of about 0.25 mm thick was prepared using silica gel and dried at 110^oc for about 4 h. Small amount of the crude extract was dissolved in methanol (1mg/5ml) and few droplets (5 µl) of the crude extract were then applied band-wise to the TLC plate and developed using a mixture of solvents, ethyl acetate, methanol and ammonia (EtOAc-MeOH-NH₃, 85:10:5).

A methanolic solution of about 1mg/ml of cathinone hydrochloride was used as standard. Prior to visualization of the plate, it was dried by hot air blower. The plate was then observed under UV visible light (254 nm) and then sprayed with fresh ninhydrin reagent (0.3 g in 100 ml n-butanol + 3 ml concentrated acetic acid) and heated at 110⁰c for 2 min. The plate was visualized under UV at a wave length of 254 nm and retardation factor (Rf) values were calculated.

3.6 Animal grouping and dosing

In all models, animals were divided into four groups each comprising of six. On every day of the experiment, all groups were taken from the cage and weighed using electronic digital balance in order to determine the dose. Accordingly, these different groups were treated and administered as follows: first group (control) was treated with vehicle (Tween 80, 2% v/v in distilled water, negative control). The second group (positive control) was treated with ranitidine (50 mg/kg) and misoprostol (200 µg/kg) as antisecretory and cytoprotective respectively in ulcer models (Pathaka *et al.*, 2007) whereas in the constipation model loperamide (5 mg/kg) was considered as the positive control. The other groups were treated with different doses of crude khat extract; 100 mg/kg (K100), 200 mg/kg (K200) and 300 mg/kg (K300). However, only the 200 mg/kg dose of khat extract was used in the sub-chronic study for both ulcer and constipation evaluation protocols.

The standard drugs and crude khat extract were administered orally (by gavage) and by intraduodenal route. The khat extract was weighed, mixed with Tween 80 in water (2%, v/v) to a predetermined concentration, stirred continuously while filling the syringe. The doses for the khat extracts were selected from previous reports as estimated based on human daily consumption (Abdulwaheb *et al.*, 2007; Bedada and Engidawork, 2010; Mohammed and Engidawork, 2011) and yield value was then converted into rats based on the body surface area (Reagon-Shaw *et al.*, 2008).

3.7 Acute study

3.7.1 Pylorus-ligation induced gastric ulcer

The assay was performed using the method of Shay *et al* (1945) with a slight modification. The animals were divided into four groups (each containing 6 rats). After 36 h of fasting with water *ad libitum*, the animals were anesthetized with ether, the abdomen was opened with midline incision and the pylorus was ligated carefully so as to avoid damage to the blood supply. Immediately after pylorus ligation, animals received either vehicle, ranitidine or khat extract intraduodenally. The stomach was then placed back and the abdominal wall was closed with sutures. The animals were scarified 6 h after ligation using ether overdose and the stomach was removed. A small incision was made at the junction of the pylorus ligation and greater curvature then the stomach content was collected and centrifuged (Gallenbamp Junior, England) at 2000 rpm for 15 min and examined for lesions in the glandular portion.

The supernatant volume was measured and pH was recorded with a digital pH meter (Schott Glas Mainz, Germany). The mean ulcer lesion area (mm²) and ulcer score was estimated as described elsewhere (Hemmati *et al.*, 1974) i.e., 0.5= minute, sporadic, punctuate lesions, 1= several small lesions, 2= one large extensive lesion or multiple moderate sized lesions, 3 = several large lesions. The ulcer index was calculated by the following equation according to Ganguly, (1969).

$$Ulcer\ index = 10/x$$

Where x=total mucosal area/ ulcerated area.

Measurement of total acidity

After collecting and centrifugation of the stomach contents, the total acidity was also determined by taking the gastric juice (1 ml) into a conical flask containing 2-3 drops of phenolphthalein and this was titrated against 0.01N NaOH. The volume of NaOH consumed was assumed to correspond to the total acidity. Acidity was determined using the following equation as described previously (Hawk *et al.*, 1947; Melese *et al.*, 2011).

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1} \text{ ml/lit}$$

3.7.2 Indomethacin induced gastric ulcer

Indomethacin was suspended in 1% CMC in distilled water (6 mg/mL) and administered to fasted rats in a dose of 30 mg/kg (0.5 mL/100 g). Rats were treated with crude khat extract 30 min before indomethacin. Negative and positive control rats were treated similarly with an equivalent volume of vehicle and misoprostol (Bhargava *et al.*, 1973) before induction of gastric lesion. The animals were sacrificed 4 h after administration of the ulcerogenic agent. The stomach of animals was removed, rinsed with normal saline and gastric mucous content was determined according to standard procedures (Szabo *et al.*, 1985; Al-Howiriny *et al.*, 2005).

Determination of gastric mucous content

The modified procedure of Corne *et al* (1974) was used to determine gastric-wall mucus. The glandular segments from the stomach were removed and weighed. Each segment was transferred immediately to 10 ml 0.1% Alcian 8GX blue solution (in 0.16 M sucrose solution, buffered with 0.05 M sodium acetate, pH 5.8) for 2 h and the excess dye was removed by two successive rinsing for 15 and 45 min with 0.25 M sucrose solution. The dye complexed with the gastric wall mucus was extracted with 0.05 M magnesium chloride for 1 h. A 10 ml sample of blue extract was then shaken with an equal volume of diethyl ether for 2 h with an interval of 30 min. The resulting emulsion was centrifuged at 3000 rpm for 15 min and the absorbance of the aqueous layer was recorded at 580 nm using UV-Visible spectrophotometer (CECIL, model UV 1601, England). The quantity of Alcian blue extracted/g (net) of glandular tissue was then calculated (Al-Harbi *et al.*, 1997).

Standard calibration curve (Fig 3) for Alcian blue was generated with 95% confidence interval by taking different concentrations of Alcian blue (7.8, 15.6, 31.25, 62.5, 125, 250, and 500 µg) so as to determine the content of Alcian blue attached to mucin of glandular tissues of the stomach.

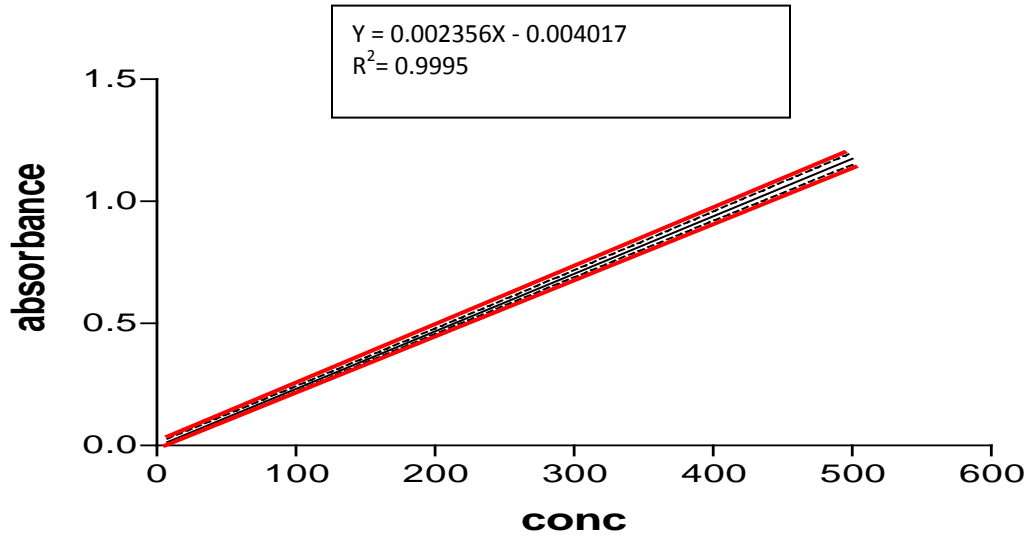


Fig 3. Calibration curve for Alcian blue 8Gx in aqueous solution.

The equation for the linear calibration graph was $Y = 0.002356X - 0.004017$, where “Y” represents the dependent variable (Absorbance) and “X” stands for the independent variable (concentration of Alcian blue in microgram). Thus, the value of the absorbance measured in the different treatment groups was used to get the corresponding concentration of Alcian blue which was complexed with the mucin on the wall of the glandular portion of the stomach. Finally, the amount of mucin per gram of net glandular tissue was calculated using the formula shown below (Corne *et al.*, 1974; Shine *et al.*, 2009).

$$\text{Mucin Content} = \frac{\mu\text{g Alcian blue}}{\text{gm wt of grandular tissue}}$$

3.7.3 Cysteamine HCL induced duodenal ulcer

Duodenal ulcer was induced by administering cysteamine hydrochloride (400 mg/kg, p.o.) twice at an interval of 4 h. Vehicle, ranitidine or crude khat extract at was administered 30 min prior to each dose of cysteamine hydrochloride for different groups. After 24 h, all the animals were sacrificed and the duodena were excised carefully and cut open along the antimesentric side. The duodenal ulcer area, ulcer score and ulcer index were determined. Based on their intensity, the ulcers were given scores as follows: 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis, 3 = perforated or penetrated ulcer. The ulcer index was calculated using the following equation: (Asad *et al.*, 2001; Khare *et al.*, 2008).

$$\text{Ulcer index} = \frac{\text{Arithmetic mean of intensity in a group} + \text{Number of ulcer positive animals}}{\text{Total number of animals}} \times 2$$

3.7.4 Castor oil induced diarrhea in mice

Mice were fasted for 12 h prior to the commencement of the experiment and were randomly divided into four groups of six mice each. Mice either received vehicle, loperamide or crude khat extract (100 and 300 mg/kg) orally. After 30 min of administration of the extract or loperamide, castor oil 0.2 ml/ mouse was administered orally. The mice were then placed on individual special transparent cages covered with absorbent white clean paper to collect faeces (Mukherjee *et al.*, 1998). The cages were inspected for the presence of the characteristic diarrhea droppings. The absence was recorded as a protection from diarrhea (Diurno, *et al.*, 1996) and the percentage inhibition was calculated (Mohammed *et al.*, 2009)

$$\% \text{Inhibition} = \frac{(\text{control} - \text{test})}{\text{control}} \times 100\%$$

In addition, other parameters were observed: onset of diarrhea, number of watery diarrhea, number of wet faeces, total number of faecal output, and total weight of wet faeces (Adeyemi *et al.*, 2009).

3.7.5 Castor oil induced enteropooling in mice

This was determined as described and modified by Dicarlo *et al* (1994). Intestinal secretion was indirectly analyzed by enteropooling assay. Groups of overnight fasted mice were treated with 100 mg/kg and 300 mg/kg khat extract, vehicle or loperamide orally 30 min before the oral administration of castor oil, 0.2 ml per mouse. The mice were sacrificed 30 min later, and the entire small intestine from each animal was weighed and their group average was calculated. In the mean time, the volume of intestinal fluid was measured through expulsion of its content into a measuring cylinder (Dahiru *et al.*, 2006). The difference in the weight and volume of small intestine in all castor oil treated groups was considered as the castor oil induced accumulation of intestinal fluid (Mujumdar *et al.*, 2000)

3.8 Sub-chronic studies

3.8.1 Crude khat extract on ulcer induction

This experiment was performed to find out whether sub-chronic administration of crude khat extract would induce ulcer by its own virtue without concomitant administration of beverages. The animals were divided into four groups each containing six rats: the first group was given vehicle while group two, three and four were administered crude khat extract at 100 mg/kg, 200 mg/kg and 300 mg/kg dose respectively for forty-five consecutive days. At the 45th day, the animals were sacrificed using ether overdose then the stomach were taken out and opened along the greater curvature and similarly duodena were excised and cut opened along antimesentric side if there were any induction of gastric and duodenal ulcer through observation of ulcer production.

3.8.2 Crude khat extract and gastrointestinal motility

The effect of sub-chronic administration of crude khat extract on intestinal propulsion in rats was tested using the charcoal meal method (Olatokunboh *et al.*, 2010). Rats were randomized and placed in four plastic cages of six animals per cage corresponding to four groups of animals. Group 1 was administered vehicle orally by gavage. Group 2, 3 and 4 were pretreated with 100 mg/kg, 200 mg/kg and 300 mg/kg dose of khat extract, respectively for forty-five consecutive days. At the forty fifth day the rats were given 1 ml of 10% activated charcoal suspended in 5% gum acacia orally after 30 min of administration of vehicle and extracts. The rats were sacrificed 30 min later by inhalation of ether and the abdomen was exposed after dissection; The peristaltic index (PI) which is the distance traveled by the charcoal meal relative to the total length of small intestine expressed in percentage was determined for each rats (Mukherjee *et al.*, 1998; Adeyemi *et al.*, 2009; Jebunnessa *et al.*, 2009).

3.8.3 Crude khat extract on body weight change pattern

This procedure was conducted if sub-chronic administration of khat extract produces progressive change in weight from week to week by comparing their weight in each week. The animals were randomly allocated to four groups each comprising of six rats: first group was treated with vehicle whereas group two, three and four were given crude khat extract at doses of 100 mg/kg, 200 mg/kg and 300 mg/kg respectively for forty-five consecutive days and every week the animals were placed in electronic digital balance to record the weight change pattern.

3.9 Statistical analysis

The results were expressed as mean±S.E.M. The total variation and difference among means were analyzed through one-way analysis of variance (ANOVA) followed by Tukey's comparison test analysis and the $p < 0.05$ was considered significant. For ulcer score, the analysis was performed using one way ANOVA followed by Dune's post test. The GraphPad InStat3 Demo (Graphpad Software Inc, San Diego) was employed for all statistical analysis.

4 Results

4.1 Thin layer chromatography

Spraying the TLC plate with ninhydrin, a reagent used to detect ammonia, primary and secondary amines; and visualizing under UV light at a wavelength of 254 nm produced a violet zone with an Rf value of about 0.42 corresponding to the standard cathinone.

4.2 Effect of crude khat extract on acute administration

4.2.1 Crude khat extract on pylorus ligation induced gastric ulcer

The plant extract at dose of 300 mg/kg had significantly ($p < 0.05$) increased volume of gastric acid secretion compared with negative control. Whilst 100 mg/kg dose of khat extract exhibited a tendency to decreased volume of gastric acid secretion that failed to reach statistical significance. By contrast ranitidine at 50 mg/kg dose had significantly reduced ($p < 0.001$) volume of gastric acid secretion compared to control as well as khat treated rats (Fig 4).

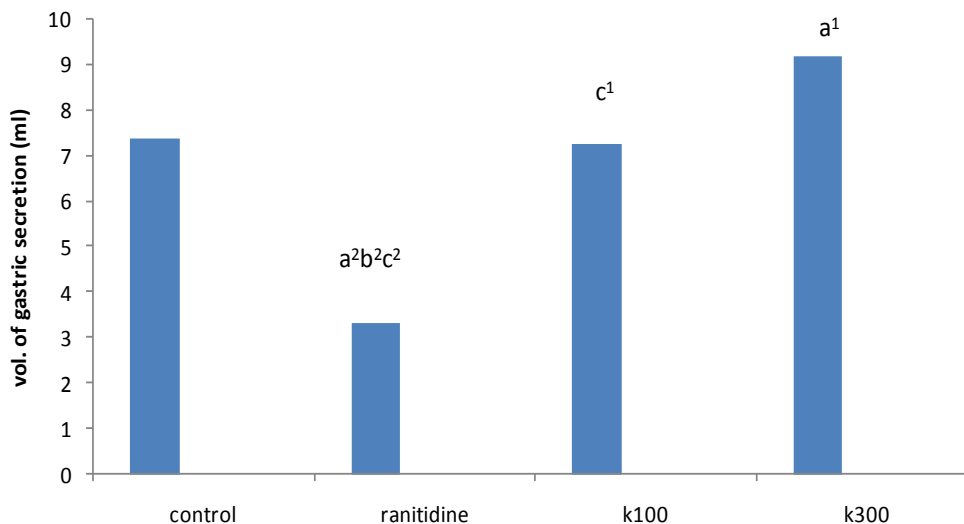


Fig 4. Effect of crude khat extract on volume of gastric acid secretion following pylorus ligation in rats. ^a: compared with control, ^b: compared with k100, ^c: compared with k300, ¹: $p < 0.05$, ²: $p < 0.001$.

As it can be seen from Fig 5, the standard drug, ranitidine did show a significant rise in pH of gastric contents compared to control and 300 mg/kg (($p < 0.01$) dose as well as 100 mg/kg ($p < 0.05$) dose of khat extract. When comparing control with both doses of the khat extracts, it did not show statistically significant difference among the groups. However, the plant at 100 mg/kg produced a slight percentage (15.6%) increase in pH and in 300 mg/kg dose slight percentage (7.14%) reduction was observed compared to negative control.

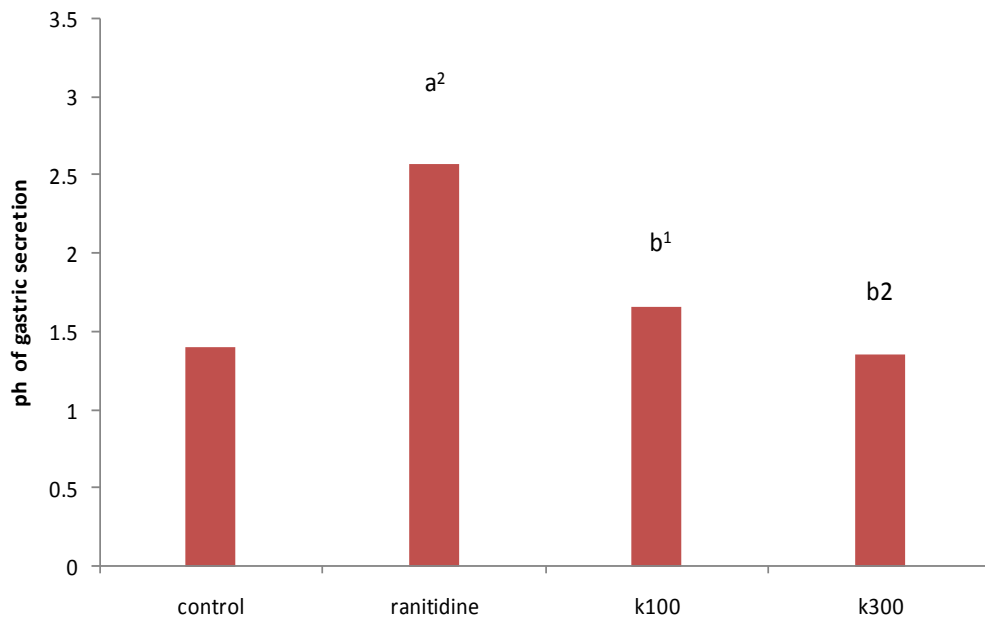


Fig 5. Effect of crude khat extract on pH of gastric acid secretion in rats. ^a: compared with control, ^b: compared with ranitidine (50 mg/kg), ^c: compared with k300, ¹: $p < 0.05$, ²: $p < 0.01$.

Total acidity was significantly ($p < 0.001$) reduced by ranitidine at 50 mg/kg compared with control and both doses of khat extract (Fig 6). The 100 mg/kg dose of khat extract didn't produce statistically significant reduction in total acidity but still there was a slightly reduction with percentage of 2.46% compared with the negative control. While in 300 mg/kg total acidity raised significantly ($p < 0.01$) and ($p < 0.05$) compared with 100 mg/kg and negative control, respectively.

Table 1: Effect of crude khat extracts on ulcer score in pylorus ligation induced gastric ulcer.

Treatment	Dose (mg/kg)	Ulcer score	% of reduction in ulcer score	% of increment in ulcer score
Control		2.33 \pm 0.21	-	-
Ranitidine	50 mg/kg	0.66 \pm 0.11 ^{a1c2}	72	-
Khat extract	100 mg/kg	1.16 \pm 0.16 ^{c1}	50.1	-
Khat extract	300 mg/kg	2.83 \pm 0.16	-	17.67

Results are mean \pm S.E.M. for six rats. Statistical comparison was performed using one way ANOVA followed by Dune's post test. ^a: compared with control, ^b: compared with k100, ^c: compared with k300; ¹: $p < 0.05$. ²: $p < 0.001$.



Control



R50



K100



K300

Fig 7. Picture of pylorus ligation induced ulcer in rats.

The effect of khat extract at dose of 100 mg/kg and 300 mg/kg on the ulcerative area and in ulcer index was depicted in Table 2. The extract at 300 mg/kg dose indicated significant ($p < 0.05$) increment in ulcer area compared with negative control. Although 100 mg/kg extract did not show significant reduction but still indicated slight reduction (6.66%) as compared with control. A significant ($p < 0.001$) reduction of lesion area was shown by reference compound, ranitidine against control and both doses of the extract. However, 100 mg/kg dose produced a significant ($p < 0.01$) reduction in mean ulcer area compared with the 300 mg/kg dose of extract.

Table 2: Effect of extracts of crude khat on ulcer area and ulcer index in pylorus ligation model.

Treatment	Mean \pm SEM ulcer area (mm ²)	% change in ulcer area (mm ²)	Ulcer index	% change in ulcer index
Control	30 \pm 3.376	-	0.12 \pm 0.01	-
Ranitidine (50 mg/kg)	9.5 \pm 0.846 ^{a3b3c3}	-68.33	0.018 \pm 0.003 ^{a3b3c3}	-85
Extract (100 mg/kg)	28 \pm 2.47 ^{c2}	-6.66	0.1 \pm 0.001 ^{c2}	-16.6
Extract (300 mg/kg)	39.6 \pm 1.783 ^{a1}	+24.2	0.176 \pm 0.014 ^{a2}	+31.8

Values are in mean \pm S.E.M for six rats. The comparison was performed using one way ANOVA followed by Tukey's post test. ^a: compared with control, ^b: compared with k100, ^c: compared with k300: ¹: $p < 0.05$, ²: $p < 0.01$, ³: $p < 0.001$.

Table 2 also revealed ulcer index, where the 300 mg/kg dose significantly ($p < 0.01$) increased ulcer index against negative control and significant ($p < 0.001$) reduction in ulcer index was produced by ranitidine compared with vehicle and both doses of the extract. Although, the 100 mg/kg did not show significant difference, there was a slight reduction (16.6%) in ulcer index compared to control. Meanwhile, the 100 mg/kg dose of extract showed a significant ($p < 0.01$) inhibition in ulcer index against 300 mg/kg dose of extract.

4.2.2 Crude khat extract on indomethacin induced gastric ulcer

Effect of khat extract on gastric wall mucus is shown in Table 3. Pretreatment with positive control, misoprostol was found to preserve the functional cytoarchitecture of the entire gastric mucus significantly ($p < 0.001$) compared to vehicle and the two doses of khat extract. While comparing 300 mg/kg dose of khat extract with negative control, significant ($p < 0.05$) reduction in gastric wall mucus was produced by khat extract. Similarly, 300 mg/kg produced significant ($p < 0.05$) reduction in mucus content against 100 mg/kg khat extract. Whereas 100 mg/kg dose of khat extract didn't show significant difference compared with negative control.

Table 3: Effect of crude khat extract on the induction of changes in gastric wall mucus

Treatment	Gastric wall mucus (Alcian blue $\mu\text{g/g}$ wet glandular tissue)
Control	54.9 \pm 3.182
Misoprostol (200 $\mu\text{g/kg}$)	125.863 \pm 15.264 ^{a2b2c2}
Khat extract (100 mg/kg)	55.50 \pm 2.866
Khat extract (300 mg/kg)	13.71 \pm 1.515 ^{a1b1}

Tabular values represent mean \pm S.E.M of six rats/group. The comparison was performed using one way ANOVA followed by Tukey's post test. ^a:compared with control, ^b:compared with k100, ^c:compared with k300: ¹: $p < 0.05$, ²: $p < 0.001$.

4.2.3 Crude khat extract on cysteamine HCl induced duodenal ulcer

As indicated below in Table 4, the antisecretory standard drug, ranitidine at dose of 50 mg/kg produced a significant ($p < 0.001$) reduction in ulcer area compared with negative control and khat extract at 300 mg/kg in cysteamine induced duodenal ulcer. Furthermore, ranitidine showed a significant ($p < 0.01$) reduction in ulcer area compared with 100 mg/kg dose of extract. In addition, 300 mg/kg dose of khat extract significantly

($p < 0.01$) increased the ulcer area compared with vehicle. In the mean time, 100 mg/kg dose of extract produced a significant ($p < 0.001$) reduction in ulcer area against 300 mg/kg dose of khat extract.

Table 4: Effect of crude khat extract on ulcer score and ulcer area on cysteamine HCL induced duodenal ulcers in rats.

Treatment	Dose	Ulcer score	Ulcer positive animals	Ulcer area	% ulcer protection
Control		2.33 ±0.211	6/6	23.16±1.54	-
Ranitidine	50 mg/kg	0.16±0.166 ^{a1b1c3}	1/6	0.83±0.83 ^{a3b2c3}	93.13
Khat extract	100 mg/kg	1.166±0.307 ^{c1}	6/6	15.83±3.34 ^{c3}	49.95
Khat extract	300 mg/kg	3.00 ±0.00	6/6	36.5±3.22 ^{a2}	

Values are in mean±S.E.M for six rats. The comparison was performed using one way ANOVA followed by Tukey's test. While ulcer score followed by Dune's post test. ^a:compared with control, ^b:compared with k100, ^c:compared with k300. ¹: $p < 0.05$, ²: $p < 0.01$, ³: $p < 0.001$.

In Table 4 ranitidine also indicated statistically significant ($p < 0.05$) and ($p < 0.001$) reduction in ulcer score against vehicle and 300 mg/kg dose of khat extract, respectively. Whereas 100 mg/kg dose of khat extract revealed significant ($p < 0.05$) reduction in ulcer score compared with 300 mg/kg khat extract. While comparing ranitidine with control and both doses of extract there was significant difference in all parameters and hence better protection from the ulcerogenic effect of cysteamine.

The ulcer index of cysteamine hydrochloride induced duodenal ulcer was indicated in Fig 8; the 300 mg/kg dose was increasing ulcer index from 2.77 to 3.00 and 100 mg/kg dose of khat extract showed reduction of ulcer index from 2.77 to 2.05 compared with negative control. Mean while, ranitidine (50 mg/kg) dose was effective in reducing the ulcer index from 2.77 to 0.39 compared with control.

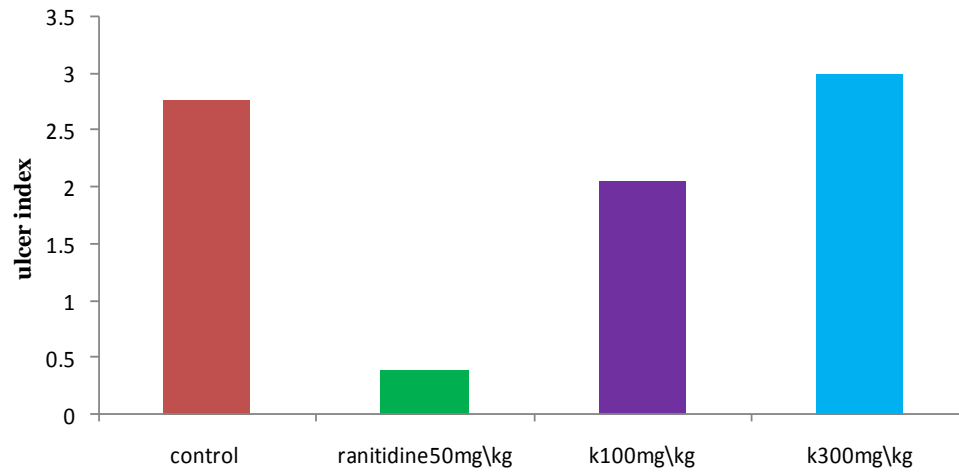
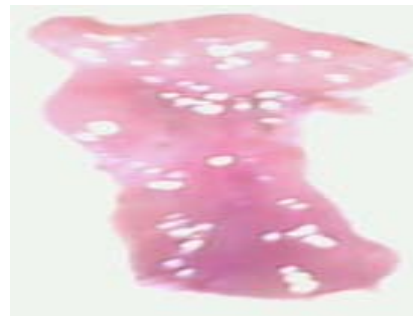


Fig 8. Effect of crude khat extract on ulcer index in cysteamine HCL induced duodenal ulcer. Ranitidine produced higher reduction on the ulcer index. k100:100 mg/kg, k300:300 mg/kg of khat extract.



a. Control



b. ranitidine 50 mg/kg



c. khat extract 100 mg/kg



d. khat extract 300 mg/kg

Fig 9. Pictures of cysteamine induced duodenal ulcer of rats.

4.2.4 Crude khat extract on castor-oil induced diarrhea

Based on different parameters, the effect of khat on castor oil induced diarrhea was evaluated. As indicated in Table 5: the oral administration of 300 mg/kg of khat extract significantly ($p < 0.01$) increased the onset time of faeces compared with control group but no significant difference while comparing 100 mg/kg dose of extract and negative control group. Loperamide (positive control) at 5 mg/kg dose significantly ($p < 0.001$) and ($p < 0.05$) increased the onset time for defecation against control and 100 mg/kg, respectively.

Table 5: Effect of crude khat extract on the onset time, number of watery diarrhea and weight of wet faeces in castor-oil induced diarrhea.

Treatment	Dose	Onset time of faeces (min)	Number of watery diarrhea	Weight of wet faeces
Control	-	41.66±5.044	18.167±1.40	756.6±19.01
Loperamide	5 mg/kg	148.33±10.54 ^{a3b1}	0.833±0.40 ^{a3}	96±4.00 ^{a3}
Khat extract	100 mg/kg	55±11.76 ^{c1}	8.8±2.88 ^{a2}	386.4±9.69 ^{a3c1}
Khat extract	300 mg/kg	121.17±22.44 ^{a2}	4.5±1.56 ^{a3}	218±69.35 ^{a3}

Values are in mean±S.E.M for six mice. The comparison was performed using one way ANOVA followed by Tukey's test. ^a:compared with control, ^b:compared with k100, ^c:compared with k300 mg/kg.¹: $p < 0.05$, ²: $p < 0.01$, ³: $p < 0.001$. k100 mg/kg: 100 mg/kg khat extract, k300: 300 mg/kg of khat extract.

In addition to this the 100 mg/kg dose did show a significant ($p < 0.01$) reduction in number of watery faecal pellet. Similarly, this dose produced significant ($p < 0.001$) diminution in the weight of wet faeces compared with control group. Furthermore, comparing both extracts (100 mg/kg, 300 mg/kg), the result revealed a significant ($p < 0.05$) difference in weight of wet faeces and onset time of defecation.

The number of watery fecal discharge for at least 4 h post-treatment with 300 mg/kg dose of extract decreased significantly ($p < 0.001$) compared to negative control. The 100 mg/kg dose showed a significant ($p < 0.01$) reduction in number of watery faeces

compared with control group. The standard drug, loperamide (5 mg/kg) revealed a significant ($p<0.001$) decline in watery fecal discharge and there was no significant difference when comparing 100 mg/kg and 300 mg/kg dose in the number of watery faeces. Similarly, administration of 300 mg/kg, 100 mg/kg and loperamide produced significant ($p<0.001$) reduction in the weight of wet fecal discharge against vehicle. Administration of the 100 mg/kg dose significantly ($p<0.05$) increased the weight of wet faeces against 300 mg/kg dose of extract.

As specified in Table 6, there was a marked ($p<0.001$) and ($p<0.05$) reduction in the total number of faeces after administration of loperamide at 5 mg/kg dose compared with control group and 100 mg/kg dose, respectively. The 300 mg/kg khat extract produced a significant ($p<0.05$) and ($p<0.001$) reduction in the number of wet faeces and total number of faeces compared to negative control group. The effect of highest dose of the extract was almost similar to that of loperamide (5 mg/kg) with 26% difference in inhibition of diarrhea. Besides, 100 mg/kg dose indicated a significant ($p<0.01$) reduction in total number of faeces and number of wet faeces against control group.

Table 6: Effect of the crude khat extract on the total number of stools and wet faeces against castor- oil induced diarrhea in mice.

Treatment	Dose	Total number of faeces	Number of wet faeces	Inhibition of diarrhea (%)
Control	-	19.833±1.352	19.83±1.352	0.00
Loperamide	5mg/kg	3.00±0.7303 ^{a3b1}	1.33±0.33 ^{a3b1}	93.3
Khat extract	100mg/kg	14.6±4.41 ^{a1}	9.8±2.69 ^{a2}	50.5
Khat extract	300mg/kg	8.5±2.141 ^{a1}	6.5±1.82 ^{a3}	67.22

Values are in mean±S.E.M for six mice. The comparison was performed using one way ANOVA followed by Tukey's test. ^a:compared with control, ^b:compared with k100, ^c:compared with k300mg/kg:¹: $p < 0.05$, ²: $p<0.01$, ³: $p<0.001$.

4.2.5 Crude khat extract on castor oil induced enteropooling in mice

As indicated in Table 7, 300 mg/kg dose significantly ($p < 0.05$) decreased weight of intestine contents compared with control and loperamide (5 mg/kg). Loperamide also produced a significant ($p < 0.001$) and ($p < 0.01$) reduction in weight of intestinal contents compared with control and 100 mg/kg dose, respectively. Similar result was obtained in the volume of intestinal content.

In addition, the weight of intestinal content inhibition was 29.7% at 100 mg/kg and 54.5% at 300 mg/kg khat extract. Similarly, 100 mg/kg and 300 mg/kg doses produced a significant inhibition 32.69% and 51.9% in the volume of intestinal fluid accumulation, respectively. Moreover, loperamide showed a significant percentage inhibition to both volume and weight of the intestinal content against two doses of extract.

Table 7: Effect of crude khat extract on castor oil induced enteropooling in mice.

Group	Dose	Weight of intestinal content (g)	% inhibition of Wt. intestinal content	Volume of intestinal content (ml)	% inhibition of vol. intestinal content
Control	-	1.01±0.16	-	1.04±0.21	-
Loperamide	5 mg/kg	0.16±0.03 ^{a3b2c1}	84.15	0.105±0.02 ^{a3b2c1}	89.9
Khat extract	100 mg/kg	0.71±0.04	29.7	0.7±0.07	32.69
Khat extract	300 mg/kg	0.46±0.04 ^{a1}	54.45	0.5±0.06 ^{a1}	51.9

Effect of crude khat extract on castor oil induced enteropooling in mice. Values are expressed as mean±S.E.M. The comparison was performed using one way ANOVA followed by Tukey's test. ^a:compared with control, ^b:compared with k100, ^c:compared with k300:¹: $p < 0.05$, ²: $p < 0.01$, ³: $p < 0.001$.

4.3 Effect of crude khat extract on sub-chronic administration

4.3.1 Crude khat extract on ulcer induction

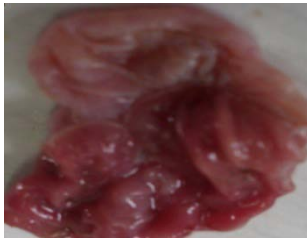
This study was performed in order to reveal the induction of gastric and duodenal ulcer after administration of crude khat extract at doses of 100 mg/kg, 200 mg/kg and 300 mg/kg for forty- five consecutive days and the result indicated that there was no statically significant difference with the control and hence administration of khat per se at doses of 100 mg/kg, 200 mg/kg and 300 mg/kg didn't induce ulcer (Fig 10).



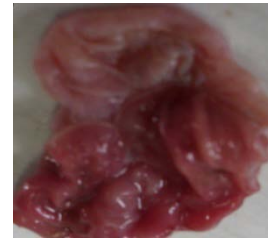
a. control



b. khat 100 mg/kg



c. 200 mg/kg



d. 300 mg/kg

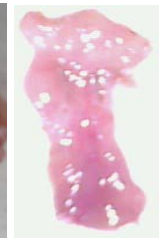


Fig 10. Pictures for sub-chronic administration of crude khat extract. The left side in all groups indicated stomach while the pictures in right side showed duodenum.

4.3.2 Crude khat extract in *in-vivo* gastrointestinal motility

As illustrated in Table 8, the result of present study revealed that the extract at dose of 300 mg/kg significantly ($p < 0.001$) decreased length passed by charcoal meal and propulsion of the marker through the GIT when compared with control group. Likewise, 300 mg/kg dose showed a significant ($p < 0.05$) reduction in length passed by marker and in intestinal motility compared to 100 mg/kg khat extract. However, there was no statistical significance difference comparing 200 mg/kg and 300 mg/kg doses of extract in both length passed and motility. Besides, 300 mg/kg dose of extract showed 53.18% inhibition in gastrointestinal motility.

Table 8: Effect of crude khat extract on gastrointestinal motility with activated charcoal after sub-chronic administration in rats.

Treatment	Gastrointestinal motility			% inhibition
	Length of GIT (cm)	Length passed by marker	% peristaltic index (motility)	
Control	102.6±4.28	59.5±2.78	57.99±2.15	-
Khat extract 100 mg/kg	97.4±3.36	37±3.74 ^{a2d1}	38.16±3.64 ^{a2d1}	34.19
Khat extract 200 mg/kg	103.4±0.75	30.8±0.86 ^{a2}	29.82±1.01 ^{a2}	48.57
Khat extract 300 mg/kg	98±1.31	26.4±1.03 ^{a2}	27.15±1.05 ^{a2}	53.18

Values are expressed as mean±S.E.M. The comparison was performed using one way ANOVA followed by Tukey's test. ^a:compared with control, ^b:compared with 100 mg/kg, ^c:compared with 200 mg/kg, ^d:compared with 300 mg/kg: ¹: $p < 0.05$, ²: $p < 0.001$.

4.3.3 Crude khat extract on weight change pattern

As shown in Fig 11, the crude khat extract at dose of 300 mg/kg produced a significant ($p < 0.05$) weight loss starting at the 3rd week and the reduction kept on the following weeks (4, 5) with marked ($p < 0.01$) decline compared to control. In 6th week, 300 mg/kg dose produced significant ($p < 0.001$) weight loss compared with 100 mg/kg dose of extract. The khat extract at 200 mg/kg started to lose weight at the 4th week ($p < 0.05$) and

lose maintained with ($p < 0.01$) in the following weeks compared to control. In contrast, 100 mg/kg dose of khat extract indicated a weight gain and no statically significance difference in the weight except for last week (6th) which indicated significant ($p < 0.05$) difference compared with control. While comparing 200 mg/kg and 300 mg/kg dose of extract there was no significant difference in weight loss.

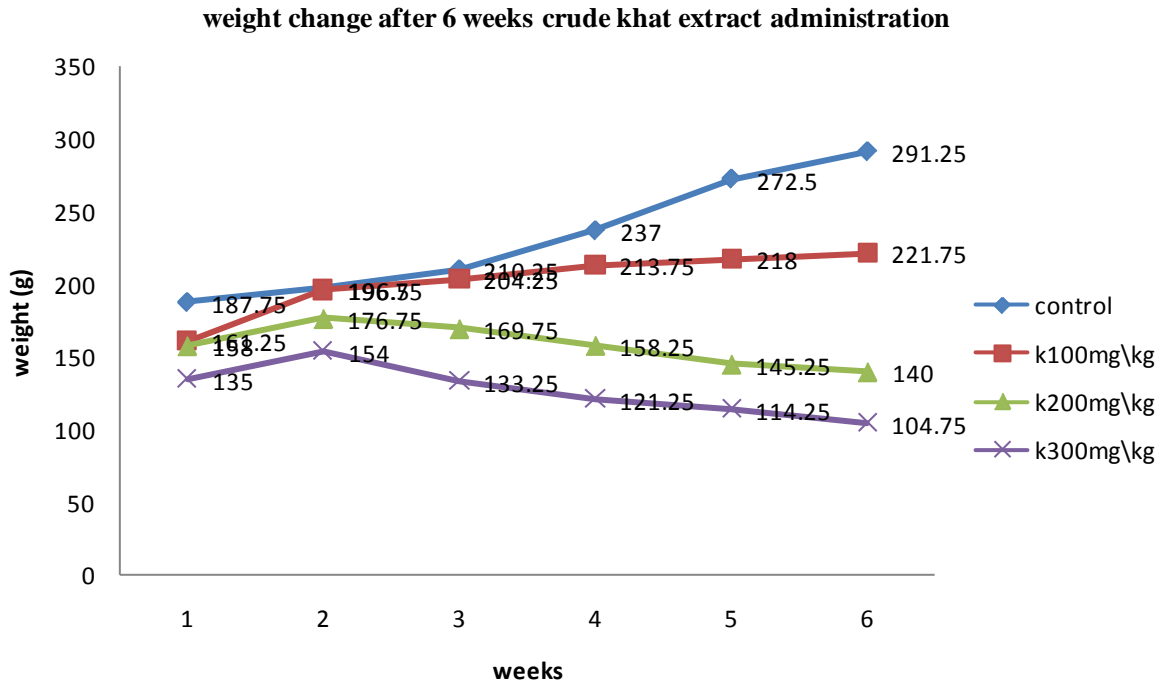


Fig 11. Weekly weight change pattern after administration of crude khat extract and control for 6 weeks. The comparison was performed using one way ANOVA followed by Tukey's test. ^a:compared with control, ^b:compared with k100, ^c:compared with k200mg/kg, ^d:compared with 300mg/kg..

5 Discussion

5.1 Effect of khat on gastric and duodenal ulcer

5.1.1 Pylorus ligation induced gastric ulcer

Pylorus ligation induced ulcer is one of the most widely used methods for studying the effect of drugs on gastric secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric content on the stomach with resultant hypersecretion of gastrin and therefore gastric acid secretion, leading to the auto digestion of gastric mucosa for the development of ulcers in the stomach. Thus, agents that decrease gastric acid secretion and/or increase mucus secretion are effective in preventing the ulcers induced by this method (Khare *et al.*, 2008).

The present work attempted to comprehensively assess effect of crude khat extract at two different doses on rat's volume of gastric acid secretion, pH of gastric content, total acidity, ulcer score and ulcer index after the pylorus ligation. In the present study, this model can provide whether khat reduce or increase the secretion in the already induced ulcer. Previous studies revealed the effect of pure cathinone with antisecretory and cytoprotection action in comparison with amphetamine (Al-Shabanah *et al.*, 1994). To our best knowledge there is no previous experimental studies performed on the effect of crude khat extract on gastric and duodenal ulcer.

The results of this study indicated aggravation of gastric ulcer formation after administration of crude khat extract at a dose of 300 mg/kg in rats induced by pylorus ligation. This finding can be evidenced by the significant increment in the volume of gastric acid secretion and total acidity. However, the pH of gastric content in 300 mg/kg administered rat did not show a significant reduction, which might be attributed to the small sample size. On the other hand, 300 mg/kg dose might have an effect on the volume and total acidity rather than reducing the pH.

Al-Shabanah *et al* (1994) reported that pure cathinone at dose of 10 mg/kg and 30 mg/kg protect gastric ulcer induced by pylorus ligation through antisecretory action. In contrast, amphetamine dose-dependently increased secretion of endogenous CCK and Ca²⁺ levels,

which may lead to hypersecretion of acid through stimulation of H⁺/K⁺ ATPase (Doong *et al.*, 1998; Giambalvo *et al.*, 2004).

Our finding is different from Al-Shabanah *et al* (1994) that could arise from the substance used (pure vs. crude) in the two experimental protocols and probably the antisecretory effect of cathinone present in the crude extract could have been overwhelmed by other constituents of the extract. Khat contains several chemical constituents such as tannin/tannic acid which are responsible for the negative effect of GIT (Halbach, 1972; Ali *et al.*, 2006). Similarly, Kemper (1999) and Morris (2010) reported about high amount of tannin being present in plants were suspected of its ulcerogenic effect including mouth, esophagus and GIT in the test animals. Subsequently, the amount of these tannins increases with the amount of khat extract administered though the cathinone level can also increase proportionally but its amount is smaller comparing with tannins and hence cathinone may not have the ability to overwhelm this high concentration of tannins or other constituents that might have role in aggravating ulcer.

The other factors that wire this result could be the effect of pesticides on khat leaves. These days particularly in Yemen, khat is subjected to wide range of pesticides and therefore people chewing leaves of khat shrub were exposed to severe health problems as consequence of pesticides (Kalix 1992; Awadh *et al.*, 2006). Date *et al*, (2004) reported that chewing khat grown with chemical pesticides causes considerable acute and chronic adverse effects on the digestive system. Another proposition that entail additional investigation might be effect of khat on increasing prevalence and colonization of *H. pylori* that has major role in pathogenesis of ulcer.

5.1.2 Indomethacin induced gastric ulcer

NSAIDs such as indomethacin, have the ability to cause gastroduodenal ulceration via suppression of PG synthesis. In the stomach, PG play a vital protective role by stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow, and regulating mucosal cell turnover and repair. Thus, the suppression of PG synthesis by

NSAIDS results in increased susceptibility to mucosal injury and gastroduodenal ulceration (Hiruma-Lima *et al.*, 2006).

In this model, crude khat extract at 300 mg/kg dose displayed an increased mucosal damage, which could be evidenced by significant $p < 0.001$ and $p < 0.05$ reduction in the mucus content compared with misoprostol and control, respectively. Meanwhile, 100 mg/kg dose of extract didn't show significant difference when comparing with control. These results suggest the possible involvement of PG and/or mucus in the aggravation of ulcer at 300 mg/kg dose.

The concomitant administration of indomethacin with 300 mg/kg dose of crude khat extract could have a synergistic effect on the gastric injury. This finding is consistent with the pylorus ligation model where the result showed an increased secretion of acid in the stomach. It is well known that indomethacin induces gastric ulcer by inhibition of PG, which are cytoprotective to gastric mucosa. Gastric mucus is an important protective factor for the gastric mucosa and consists of a viscous, elastic, adherent and transparent gel formed by water and glycoproteins. Pre-treatment with 300 mg/kg dose of khat induced a significant reduction in mucus protective effect in the stomach of animal subjected to indomethacin treatment. The ability of khat extract to decrease gastric mucus content might be through inhibition of formation of PGE₂. Although the exact nature and mechanism of action of ulcer aggravating phytoconstituents of khat extract is not known, results of present study add support to the pylorus ligation results.

5.1.3 Cysteamine HCL induced duodenal ulcer

Cysteamine hydrochloride inhibits the alkaline mucus secretion from the Brunner's glands which is an important epidermal growth factor in the protection of the duodenal mucosa in the proximal duodenum. This growth factor present in large amount in Brunner's gland. Thus, gastric hypersecretion, decreased alkaline mucosal secretions and decreased PG synthesis may act together in inducing DU (Khare *et al.*, 2008). In addition, cysteamine has been demonstrated to inhibit gastric emptying that accelerates duodenal ulcer formation (Poulsen *et al.*, 1982; Lee *et al.*, 1987). In the present investigation,

cysteamine induced duodenal ulcer model was used to show the effect of crude khat extract on DU.

The result indicated aggravation of DU after administration of crude khat extract at dose of 300 mg/kg which can be evidenced by significant increment in ulcer area and score compared with control and ranitidine. Besides, 300 mg/kg dose of khat extract increased the ulcer index from 2.77 to 3.00 compared with control. Result of this work could be supported by observational study that documented a significant association of DU with khat chewing aside from concomitant use of beverages, smoking and chemical used to grow plant (Rajaa *et al.*, 2000).

Eventhough the mechanism by which 300 mg/kg crude khat extract aggravates DU cannot be explained by the present data, high dose of khat extract might have increased level of aggressive phytoconstituents which gives the extract higher capacity in aggravating the ulcer. Synergistic inhibition of PG synthesis by cysteamine and extract was speculated as probable mechanism. This could be corroborated by the results of indomethacin induced gastric ulcer and castor oil induced diarrhea via stimulation of PG production as one mechanism among several others. The delayed gastric emptying effect of khat, reported by Haymann *et al.*, (1995), might enhance inhibition of gastric emptying by cysteamine that leads to pool of undiluted gastric secretion aggravating formation of DU (Poulsen *et al.*, 1982). Hypersecretory, hypergastrinemia, and increased Ca/cAMP level may also contribute to aggravation of DU.

5.1.4 Sub-chronic effect of crude khat extract on ulcer induction

The effects of habitual khat chewing on the digestive system mentioned in earlier studies were based on the clinical observation that khat chewers often complained of gastrointestinal symptoms and thus studies were performed in health volunteers to show the relation of khat and DU (Rajaa *et al.*, 2000). So this work could shed some light on the likelihood of khat chewing per se for a prolonged time as a causative factor for ulcer. Accordingly, it was found out that sub-chronic administration of crude khat extract, regardless of the doses, produced neither gastric nor duodenal ulcer in rats. These

findings are not entirely surprising, as similar negative result has been reported in previous study where sub-chronic administration of khat did not have any adverse effect on liver function. This could be explained in part by the presence of flavonoids and/or polyphenols that have been suggested to act as antioxidant through free radical scavenging activity (Al- Zubairi *et al.*, 2008).

5.2 Khat induced constipation

5.2.1 Castor oil induced diarrhea in mice

Castor oil is made up of 90% ricinoleate which when metabolized is responsible for the observed effects of the oil. The active metabolite ricinoleic acid is responsible for its diarrhea inducing property, which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa (Mohammed *et al.*, 2009; Bakare *et al.*, 2011); it is also associated with endogenous stimulation of PG release (Dahiru *et al.*, 2006). Thus, the liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to liberation PGE₂ content in the gut lumen, that stimulate motility and net secretion of the water and electrolytes (Pierce *et al.*, 1971; Mathad *et al.*, 2005). In addition, according to Mascolo *et al* (1994), castor oil induces the release of NO, which in turn provokes the generation of PG by colonic cells, thereby further exacerbating the diarrhoeal condition (Adeyemi *et al.*, 2009).

In this model, khat extract at 300 mg/kg produced more effect than 100 mg/kg, probably indicating the dose-dependent like effect of the response. This effect of crude khat extract could emanate from one of the several mechanisms that had been previously proposed to explain the diarrheal effect of castor oil. These include, among others, reduction of active Na⁺ and K⁺ absorption and inhibition of Na⁺/K⁺ ATPase activity in small intestine and colon (Gaginella *et al.*, 1978), thus reducing normal fluid absorption, stimulation of PG formation, and PAF (Galvez *et al.*, 1993; Pinto *et al.*, 1999; Meite *et al.*, 2009). Likewise, castor oil increases peristaltic activity and produces permeability changes in the intestinal mucosal membrane to electrolytes and water in addition to elevation of PG production (Palombo *et al.*, 2006; Meite *et al.*, 2009). From these observations, it can be deduced

that the extract may act through inhibition of PG which could be evidenced by data derived from ulcer studies or reduction in propulsive movement of small intestinal tract that was verified in antipropulsive/gastrointestinal motility test.

In addition to those suggested mechanisms, the constipating activity of crude khat extract in the present study could also be owing to the presence of different chemical constituents that include flavonoids, alkaloids, tannins, and terpenes as it was reported in other antidiarrheal plants (Di Carlo *et al.*, 1993; Borrelli *et al.*, 2004; Palombo, 2006; Adeyemi *et al.*, 2009).

The antidiarrheal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretions induced by PGE₂, whereas tannins make intestinal mucosa more resistant (Meite *et al.*, 2009). Therefore, these activities of khat extract could be due to the presence of flavonoids and tannins that account for inhibition of motility and secretions on top of khat's inhibitory effect on PG synthesis that was alluded to the ulcer and enteropooling studies particularly with 300 mg/kg dose of the extract.

5.2.2 Castor oil induced enteropooling in mice

The crude khat extract in castor oil induced diarrhea model in albino mice indicated remarkable decreased in different parameters at 300 mg/kg dose of extract. This observation was also substantiated by castor oil induced intaluminal accumulation of fluid by indirect enteropooling assay in mice as castor oil is reported to induce diarrhea by increasing the volume of intestinal content through prevention of the reabsorption of water via ricinoleic acid, causing an increase in the release of inflammatory mediators such as PGE₂. Hence, their mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport. In addition to this, PGE₂ also inhibit the absorption of glucose, a major stimulus to intestinal absorption of water and electrolytes (Mathad *et al.*, 2005).

In this model, both doses of khat extract significantly decreased weight and volume of intestinal content implying the crude khat extract at tested doses reduced diarrhea

probably by inhibiting PGE₂ production. The chemical constituents of khat also have the ability to provide this effect. Inhibition of PG synthesis, decreasing histamine and ACh release were proposed for the antidiarrheal activity of flavonoids. Tannins are known to form proteins tannate which makes intestinal mucosa more resistant (Meite *et al.*, 2009). Therefore, khat extract at 300 mg/kg dose provided antidiarrheal activity through inhibition of PG synthesis in harmony with other studies (Perianayagam *et al.*, 2005, Mehmood *et al.*, 2011). Apart from the above proposed antisecretory mechanisms, anticholinergic mechanism could be another probable mechanism.

5.2.3 Sub-chronic effect of crude khat extract in *in vivo* gastrointestinal motility

The activated charcoal avidly absorbs drugs and chemicals on the surface of charcoal particles thereby preventing absorption (Mathad *et al.*, 2005). This experiment carried out in gastrointestinal motility with activated charcoal meal that showed a reduction in the propulsive movement of small intestine after pre-treatment with crude khat extract at different doses for forty-five consecutive days.

The suppression of the intestinal fluid accumulation by the extract might also suggest inhibition of gastrointestinal function. The above speculation was further supported by the inhibitory action of the extract on intestinal charcoal meal motility. The result indicated that the crude khat extract at dose of 300 mg/kg decreased length passed by charcoal meal and propulsion of the marker through the GIT. In the meantime, 300 mg/kg dose of extract showed 53.18% inhibition in gastrointestinal motility. Similarly, 200 mg/kg khat extract did also show significant reduction in both parameters measured during this particular model. Thus, this result showed the effect of crude khat extract on the gastrointestinal transit time through marked reduction in the intestinal motility. Hence, suppressing the propulsion of charcoal meal leads to an increase in absorption water and electrolytes.

Agents with such property have been reported as possessing constipating activity. The extract produced a decrease in propulsive movement at the standard charcoal meal in the small intestine, suggesting an antispasmodic activity. This activity was dose dependent

with the greatest effect shown at 300 mg/kg of the extract. This finding is consistent with the previous work on the constipating and spasmolytic effects of khat leaves extract and it was found that the khat extract antagonizes the spasmogenic effects of both histamine and carbachol on isolated guinea pig ileum and whole mice in a concentration dependent manner (Makonnen *et al.*, 2000).

Furthermore, some compounds of khat have been shown to inhibit intestinal motility in a dose related manner. Tannins are best known to decrease the irritability of the bowel thereby reducing peristaltic index and flavonoids are known for inhibiting the motility and secretion (Galvez *et al.*, 1993; Chitme, 2004). Therefore, the plant demonstrated the constipating activity through inhibition of castor oil induced diarrhea, intraluminal fluid accumulation and peristaltic activity in the small intestine.

5.3 Sub-chronic effect of crude khat extract on weight change pattern

In this study, dose-dependent decline in body weight was observed after sub-chronic administration of crude khat extract. This finding could be associated with the anorexic effects of khat or it may also be linked with interference of food absorption from GIT (Favrod-Coune and Broers, 2010). Zelger and Carlini, (1980) also documented reduced food intake when administered acutely and body weight when given chronically. Moreover, amphetamine-like compounds affect appetite centrally, by acting in the hypothalamus and peripherally, by delaying gastric emptying (Heymann *et al.*, 1995; Doong *et al.*, 1998; Tucci, 2010). In other study, khat consumption has also been shown to interfere with gastric emptying and food absorption which may lead to malnutrition (Al-Motarreb *et al.*, 2002).

A significant increase in plasma leptin, associated with khat chewing, leads to loss of appetite accompanied by decreased body weight (Al-Dubai *et al.*, 2006) that may substantiate our findings. The role of ghrelin and PYY in khat induced weight loss has been excluded (Heymann *et al.*, 1995; Murray *et al.*, 2008). Mean while, recent study indicated khat extract play an important role in enhancing the anti-obesity effect in rats (Aziz *et al.*, in press).

6 Conclusion and recommendations

6.1 Conclusion

The results of the present study suggest that the 300 mg/kg dose of crude khat extract aggravate gastric and duodenal ulcer after acute administration in different models. This activity might be partly attributed to its dose, phytochemical constituents and amphetamine like action. In sub-chronic study, there was no ulcer induction in all tested groups which inferred sub-chronic chewing may not produced ulcer although studies done on animals cannot fully represent effect of agents in humans but still it is an indicative of the situation in humans. Besides, sub-chronic administration of crude khat extract showed dose-dependent constipating activity and reduction on the body weight.

Hence, it could be concluded that, acute use of khat at higher dose may aggravate gastric and duodenal ulcer in those chewers with existing ulcer problem. Meanwhile, sub-chronic usage of khat at higher dose produced significant weight reduction, constipating and anorexic activity.

6.2 Recommendations

- In present study, sub-chronic administration of khat extract and ulcer induction was examined thus it will be appropriate to perform experimental and epidemiological studies on the association of chronic khat extract administration and ulcer induction.
- The GIT adverse effect of khat and the contribution of concomitantly taken beverages should be thoroughly investigated. Since khat is commonly chewed together with other agents like coffee, tea, cigarette, and alcohol (taken during and/or after session of khat chewing).
- Comparative studies on the different varieties of khat leaves available in the market should be performed in order to rule out the effect of geographical variation on composition of khat constituents.
- Experimental study should be conducted on the collective effect of pesticides with crude khat extract.

- Other antispasmodics activities test such as effect on serotonin and KCl induced contractions should be performed to substantiate the constipation effect of khat.
- Another proposal that requires an investigation might be effect of khat in enhancing Na⁺/K⁺ ATPase activity.
- Effect of crude khat extract on those different small intestine ions (Na⁺, K⁺ and Cl⁻) should be conducted so that it can provide a clue about mechanism of khat extract on intestinal fluid accumulation model.
- Fractionation should be carried out in order to find out the active principle(s) responsible for the ulcer aggravating effect of khat.
- Detailed molecular mechanism (s) of action should be elucidated so as to clarify the activity of crude khat extract on those aforementioned models in the present work.

7 References

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