

**ADDIS ABABA UNIVERSITY  
SCHOOL OF GRADUATE STUDIES**

**Effect of Extracts from two *Artemisia* Species: *Artemisia  
afra* and *Artemisia rehan* on Isolated Smooth Muscle  
Tissues *In Vitro***

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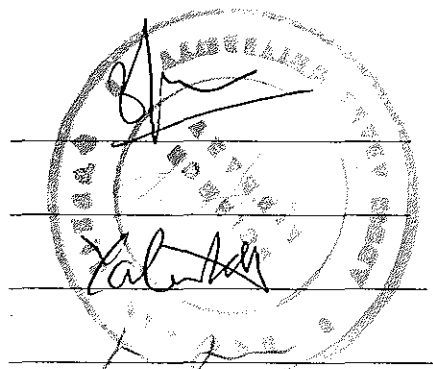
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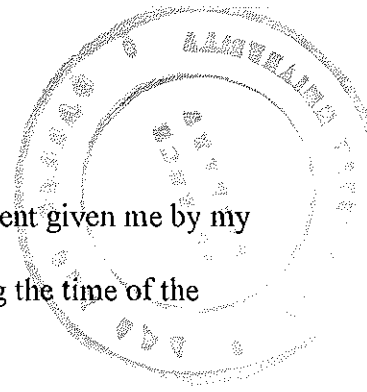
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## **Abbreviations**

**ACh** - Acetylcholine

**ALE** - *Artemisia afra* Leaf Ethanol extract

**ALW** - *Artemisia afra* Leaf Water extract

**ANOVA** - one way analysis of variance

**ARE** - *Artemisia afra* Root Ethanol extract

**ARW** - *Artemisia afra* Root Water extract

**ATP** - Adenosine triphosphate

**a.s.l.** - above sea level

**BMNP** - Bale Mountain National Park

**CaM** - Calmodulin

**cAMP** - Cyclic Adenosine Monophosphate

**CON** - Control

**Conc.** - Concentration

**EHNRI** - Ethiopian Health and Nutrition Research Institute

**ER** - Endoplasmic Reticulum

**GI** - Gastrointestinal

**g/l** - gram per litre

**GPI** - Guinea pig Ileum

**His** - Histamine

**5-HT** - Serotonin

**IBD** - Inflammatory Bowel Disease

**IBS** - Irritable Bowel Syndrome

**ICBN** - International Code of Botanical Nomenclature

**IP<sub>3</sub>** - Inositol triphosphate  
**mAChR** - Muscarinic Acetylcholine Receptor  
**MD** - Mouse Duodenum  
**mg/ml** - milligram per millilitre  
**mm/min** - millimetre per minute  
**M** - Muscarinic  
**MU** - Mouse Uterus  
**MLCK** - Myosin Light Chain Kinase  
**nAChR** - Nicotinic Acetylcholine Receptor  
**NO** - Nitric oxide  
**ng/ml** - nanogram per millilitre  
**OT** - Oxytocin  
**Pav** - Papaverine  
**PGs** - Prostaglandins  
**PI** - Phosphatidylinositol  
**PIP<sub>2</sub>** - Phosphatidylinositol 4,5-biphosphate  
**RLE** - *Artemisia rehan* Leaf Ethanol extract  
**RLW** - *Artemisia rehan* Leaf Water extract  
**ROCs** - Receptor-Operated Calcium Channels  
**SEM** - Standard Error of Mean  
**SR** - Sarcoplasmic Reticulum  
**SPSS** - Statistical Package for the Social Sciences  
**µg/ml** - microgram per millilitre  
**VDCs** - Voltage-Dependent Calcium Channels  
**WHO** - World Health Organization

## Abstract

Ethanollic and aqueous extracts of dried leaf and root of *Artemisia afra* and *Artemisia rehan* were tested on isolated mouse duodenum, guinea pig ileum and mouse uterine strips *in vitro*. The potential spasmolytic (relaxant) and spasmogenic (contractile) effect of the plants were studied in these smooth muscle tissue preparations. Different concentrations of each extract of the plants (ranging from 20-200 µg/ml) were tried out in the presence of agonist controls, ACh (in MD and MU) and histamine (in GPI) as contraction stimulators. A positive antagonist control, papaverine was used as a smooth muscle relaxant in GPI. In most of the ethanolic extracts, there were significant dose-dependent reductions of ACh and histamine-induced contractions in both tissues ( $P < 0.05$ ). Both spontaneous rhythmic and agonist-induced contractions of MD and GPI were greatly reduced by ALE (56% and 35% respectively) and RLE (65% and 56% respectively) at maximal dosages, which suggests spasmolytic property of the crude extracts. All the extracts (200 µg/ml) except ALE and RLE were less potent than papaverine (1.5 µg/ml) in the experiment of GPI. Neither the aqueous nor ethanol extracts of the two plant species caused significant contractile or relaxant effects on MU. All in all, the results in the present study pinpoint that the plants may possess spasmolytic as well as spasmogenic properties based upon the appropriate use of extractive solvent. The results also support the traditional folk use of the aerial and root parts of the plants more often for stomach pains and intestinal cramps than for fertility control purposes.

# 1. INTRODUCTION

Human lore has recognized since prehistory that many substances cause pharmacological and medicinal effects when introduced into the body. A large variety of responses occur: some are beneficial, others are not. The goal of modern pharmacology is to understand how drugs work and discover or design new drugs with optimal therapeutic value but minimal undesirable or toxic side effects. Natural products have been widely used by humans as food or food additives, or as substances in medicinal treatment. However, sometimes the biological effects of these products are not fully known (Dire *et al.*, 2003).

It was not discovered until the late 19<sup>th</sup> century that many drugs function by interacting with macromolecular structures within an organism that are known as receptors. The term receptor means a biological site to which a chemical binds and thus produces a biological action. If a drug inhibits an intracellular enzyme and produces a biological response, that site can be considered a receptor (Carvey, 1998). The macromolecules of organisms have these structurally specific areas to which the micromolecule drug becomes affixed. Paul Ehrlich (cited by Csaky, 1979) expressed that bodies are inactive unless affixed. Later on, this idea was developed further in connection with the effect of acetylcholine on the smooth muscle by considering a "receptive substance" in the muscle. Langley, a pioneer in the field of receptor research, defined a receptor or receptive substance as being the site of competition for agonists and antagonists, and the vehicle for the transmission of the stimulus of agonist interaction to the cell for the production of a physiological response (Kenakin *et al.*, 1992). The agonists such as acetylcholine, histamine and carbachol change the function of receptors as a more or less direct result of binding to it. The pure pharmacologic antagonists such as verapamil, papaverine, and atropine bind to receptors without directly altering the receptor's

function (Bourne, 1995). It is not a very long time since plant extracts were known to exert a wide range of biological effects in multicellular systems. However, by their nature, they necessitate indirect approaches to receptors that binding does not require (Kenakin *et al.*, 1992). The plant extracts as well as drugs can relax or contract isolated vascular, gastrointestinal and reproductive smooth muscle preparations *in vitro*. These isolated tissues are better predictors of drug response in animals and humans.

### **1.1 Autonomic Receptors and Neurotransmitters in the Smooth Muscles of Gastrointestinal and Reproductive tracts**

Unlike that of striated skeletal muscles, which are innervated by the somatic system, unstriated smooth muscles along with striated cardiac muscles are innervated by the autonomic system, which is out of direct conscious control (Gearien and Mede, 1974; Katzung, 1995). The autonomic nervous system can be categorized into two major portions: the sympathetic and parasympathetic (physiologically), thoracolumbar and craniosacral (anatomically), adrenergic and cholinergic (chemically). According to Katzung (1995) the traditional classification of autonomic nerves based on the primary transmitter molecules are cholinergic fibres which act by releasing primary neurotransmitter acetylcholine, and noradrenergic or adrenergic fibres which act by releasing primary neurotransmitter noradrenalin or adrenalin. In recent years most autonomic nerves are known to release several other transmitter substances or co-transmitters such as ATP and NO and thus called purinergic and nitriergic in some peripheral nerves. Previously, drug receptors in isolated tissues from animals have been reviewed by Kenakin (1984) and were found to be adenosine,  $\alpha$ -adrenergic &  $\beta$ -adrenergic, angiotensin, bradykinin, cholinergic (muscarinic and nicotinic), dopamine, histamine, serotonin, opioid and substance P. Cholinoreceptors, adrenoreceptors

and more recently endogenous morphine receptors are the main autonomic receptor subtypes today (Williamson *et al.*, 1996).

The term cholinoreceptors denote receptors that respond to acetylcholine. According to Ganong (1991), Katzung (1995) and Aidley (1998), acetylcholine receptors can be divided into two main types on the basis of their pharmacological properties: muscarinic acetylcholine receptor (mAChR) and nicotinic acetylcholine receptor (nAChR). The mAChRs are members of the large family of G-protein linked receptors which are found in smooth muscle tissues. Classically, they were operationally defined on the basis of activation by selective agonist muscarine and blockage by antagonist atropine. They are responsible for mediating slow synaptic transmission of the physiological effects of parasympathetic nerve activity, which include contraction of wide range of smooth muscles including gastrointestinal and reproductive tracts. Muscarinic receptors are known to be heterogenous in different tissues as five-cloned receptor subtypes: M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub>, M<sub>4</sub>, and M<sub>5</sub>. Among them, M<sub>2</sub>, M<sub>3</sub> and M<sub>4</sub> are found in smooth muscles such as ileum, duodenum and uterus. Response of gut to ACh is mediated by the activation of M<sub>2</sub> and M<sub>3</sub>. Muscarinic M<sub>2</sub> receptors play a role in cAMP-driven relaxation whereas muscarinic M<sub>3</sub> receptors mediate ileal contraction of both longitudinal and circular smooth muscles (Eglen *et al.*, 1996; Broadley and Kelly, 2001). In the myometrium of guinea pig, the contractile event is triggered and modulated by M<sub>3</sub> and M<sub>2</sub> receptor subtypes. In the rat uterus, the two receptors are co-expressed (Munns and Pennefather, 1998). In many smooth muscle tissues, a minor M<sub>3</sub> muscarinic AChR population mediates contraction, despite the presence of a larger M<sub>2</sub> muscarinic AChR except for the guinea pig uterus (Boxall *et al.*, 1997). There is a marked reduction in the sensitivity of the myometrium of humans to acetylcholine during pregnancy due to few myometrial muscarinic

receptors. There is a loss of cholinergic nerves in the uterus of pregnant rats (Munns and Pennefather, 1998).

The role of the following neurotransmitters in the autonomic nervous system must not be overlooked. Acetylcholine (ACh) is considered the most potent agonist and naturally occurring neurotransmitter for the cholinergic nervous system. In order for ACh to produce the chemical and physical changes required for the transmission of nerve impulses across a synapse or from nerve to motor endplate, it must interact with a receptor (Gearien and Mede, 1974). Dale in 1914 showed that ACh was remarkably effective in producing muscarinic-like effects on the frog's heart (Hopkins, 1991). With respect to a possible neurotransmitter function of ACh there are a few bits of information that suggest that ACh may participate in pain perception. The findings that 'nettle (*Urtica urens*) contain ACh and histamine', that 'high concentrations of ACh injected into brachial artery of humans have shown to result in intense pain', and that 'ACh applied to a blister produced a brief but severe pain', all indicate a relationship between ACh and pain (Cooper *et al.*, 1974). The response of smooth muscles to ACh released from cholinergic terminal fibres is expressed as an initiation of contraction and maintenance of other life-maintaining physiological functions (Gearien and Mede, 1974). The effect of ACh in causing contracture of smooth muscles is well documented in all mammals and humans (Prabhakar and Nanda Kumar, 1994). It is well known that ACh stimulates the smooth muscles of the gastrointestinal tract following the stimulation of muscarinic receptors (Ghosh *et al.*, 1993) and thus it is a selective agonist of muscarinic receptors in the guinea pig ileum (Goyal, 1988 cited in Sadraei *et al.*, 2003a). Consequently, it is regarded as the major excitatory neurotransmitter and prime regulator of the gastrointestinal motility in the gastrointestinal tract at the postganglionic parasympathetic neurons (De Man *et al.*, 2003; Sadraei *et al.*, 2003a).

However, it is usual to encounter a guinea pig ileum that will not respond to ACh (Cooper *et al.*, 1974). For instance, histamine is an important mediator of immediate allergic and inflammatory reactions and has an important role in gastric acid secretion and functions as a neurotransmitter and neuromodulator in humans. Sensitivity to histamine varies greatly among species. Humans, guinea pigs, dogs and cats are quite sensitive while mice and rats are much less so. Histamine, like ACh, causes contraction of intestinal smooth muscles. Large doses of histamine may cause diarrhoea, partly as the result of this effect (Burkhalter, 1995).

The effects of histamine are thought to be mediated through 3 (or 4) types of histamine receptors: H<sub>1</sub>, H<sub>2</sub> and H<sub>3</sub> (Hill, 1990; Taylor and Kilpatrick, 1992; Roberts, 1996; Hill *et al.*, 1997) and H<sub>4</sub> (Bytautiene *et al.*, 2003). The histamine receptors are defined by how various antagonists inhibit specific pharmacologic effects of histamine such as secretion, contraction or modulation of other secretagogues. H<sub>1</sub> receptors mediate contraction of gastrointestinal and reproductive tract smooth muscles while H<sub>2</sub> receptors mediate inhibition (i.e. relaxation) of rat uterine contraction (Roberts, 1996; Hill *et al.*, 1997; Bytautiene *et al.*, 2003). Species or tissues variability in response to the same pharmacological agent is also well known. For example, histamine stimulates uterine smooth muscle of guinea pig via H<sub>1</sub> receptors. Conversely, it relaxes the uterine smooth muscles of the rat via H<sub>2</sub> receptors (Black *et al.*, 1972; Adebisi *et al.*, 2002). In guinea pig ileum, histamine produces a well-characterized contractile response via H<sub>1</sub> receptor stimulation (Hew *et al.*, 1990; Hill, 1990). Trzeciakowski (1987) and Hew *et al.* (1990) demonstrated that activation of H<sub>3</sub> receptors in guinea pig ileum inhibited contractions of the smooth muscle mediated by electrically stimulated release of ACh (i.e. inhibition of cholinergically mediated contraction of GPI). Desensitization (tachyphylaxis) to histamine is also a common phenomenon observed in *in vitro* experimental studies of isolated tissue preparations. This has been related to the stimulus of both H<sub>1</sub> and H<sub>2</sub>

receptor subtypes, and recently this phenomenon has also been communicated in relation with histamine H<sub>3</sub> and H<sub>4</sub> receptor subtypes (Perry, 1982; Martinez-Mir, 2002).

Serotonin (5-hydroxytryptamine or 5-HT) is another neurotransmitter found in many tissues. As a local hormone it controls gastrointestinal motility like histamine. Gwee and Read (1994) reported that more serotonin is found in the gut than on any organ system. Smooth muscle serotonin receptors are of 5 types: 5-HT<sub>1</sub>, 5-HT<sub>2</sub>, 5-HT<sub>3</sub>, 5-HT<sub>4</sub> and 5-HT<sub>7</sub>. They may contract or relax the effector cells (De Ponti and Tonini, 2001; Singh *et al.*, 2003). Serotonin causes contraction of gastrointestinal smooth muscle by the direct action of it on smooth muscle receptors plus a stimulating action on ganglion cells located in the enteric nervous system (Burkhalter, 1995).

## **1.2 Factors affecting Smooth Muscle Contraction**

According to Masaki (1990) the factors affecting the contraction and relaxation of smooth muscles are the concentration of free Ca<sup>2+</sup> increase in the cytoplasm, the acto-myosin protein system, neural and hormonal factors, cholinergic agonists and antagonists, etc. Free Ca<sup>2+</sup> ions are the means whereby the contraction and relaxation of all muscle types (skeletal, cardiac and smooth) is switched on and off. A rise in the intracellular Ca<sup>2+</sup> concentration is generally proposed as an activator of contraction of these muscles (Endo *et al.*, 1977; Brading and Sneddon, 1980; Barritt, 1992). However, the mechanism whereby Ca<sup>2+</sup> ions control contraction of smooth muscle differs from that of striated skeletal and cardiac muscles in that the unique enzyme, myosin light chain kinase (MLCK) in the smooth muscle actomyosin can only be activated if Ca<sup>2+</sup> ions combine or interact with Ca<sup>2+</sup> regulating protein called calmodulin (CaM) in order to form the active holoenzyme complex, Ca<sup>2+</sup>. CaM. MLCK (Matthews *et al.*, 1981; Walsh, 1985; Sherwood *et al.*, 1993; Phillippe and Chien, 1998).

According to Galvez *et al.* (1996), Bytautiene *et al.* (2003) and Sadraei *et al.* (2003b), cytosolic free  $\text{Ca}^{2+}$  levels and  $\text{Ca}^{2+}$ -CaM-MLCK interactions determine two modes of smooth muscle contractions: phasic or tonic. The phasic (spontaneous) smooth muscle contractions result from a transient rise in cytosolic free  $\text{Ca}^{2+}$  concentration and thus they are dependent upon  $\text{Ca}^{2+}$  release from an initial mobilization of intracellular  $\text{Ca}^{2+}$  stores. For instance, myometrial (uterine) contractions are taken as phasic contractions by Phillippe and Chien (1998). Tonic smooth muscle contractions result when the initial peak of  $\text{Ca}^{2+}$  concentration does not return to the baseline but reverts to a sustained lower level which in turn results in a maintained contraction with considerably elevated tension. Tonic contractions are dependent upon  $\text{Ca}^{2+}$  influx from extracellular medium, and they are characteristics of a variety of vascular smooth muscles. Several known physiological agonists such as oxytocin, endothelin, prostaglandin  $\text{F}_{2\alpha}$ , ACh, histamine, KCl and high frequency of electrical stimulation can induce tonic contractions. Thus the calcium need for smooth muscle contraction is provided either from a  $\text{Ca}^{2+}$  influx of extracellular source via voltage-dependent  $\text{Ca}^{2+}$  channels (VDCs) and receptor-operated channels (ROCs), or from a  $\text{Ca}^{2+}$  release of intracellular stores such as sarcoplasmic reticulum (SR), mitochondria and calciosomes (Karakı and Weiss, 1984; da Silva *et al.*, 1993; Buyukokuroglu *et al.*, 2000). Intestinal and reproductive muscles seem to have the two types of ion channels; where VDCs are activated or opened by decreases in membrane potential (i.e. depolarization), and ROCs are regulated or controlled by drug-receptor interactions (Bolton, 1979).

The relative contribution of the external and internal  $\text{Ca}^{2+}$  sources toward contraction is dependent largely upon the nature of the smooth muscle (phasic or tonic) and contractile agents (cholinergic agonists) as well as their concentrations (van Breemen *et al.*, 1982). There is a controversy as to whether a release of SR [ $\text{Ca}^{2+}$ ] plays a significant role in controlling

contractility, or whether the  $\text{Ca}^{2+}$  entry from extracellular space is involved. Previously, intracellular  $\text{Ca}^{2+}$  stores have been proposed to play a role in more  $\text{Ca}^{2+}$  labile responses of the longitudinal gut muscles to muscarinic receptor activation in guinea pig ileum and guinea pig taenia coli (Brading and Sneddon, 1980; Hurwitz and Weissinger, 1980). On the contrary, the phasic spontaneous generated contractions of uterine muscle were reported to be critically dependent upon extracellular  $\text{Ca}^{2+}$  influx via VDCs (Taggart and Wray, 1998).

The intracellular  $\text{Ca}^{2+}$  levels ( $10^{-7}\text{M}$ ) of most cells such as gut epithelial cells and placental barrier are significantly lower than the extracellular bathing fluid concentration ( $10^{-3}\text{M}$ ) (Carafoli, 1991). This intracellular  $\text{Ca}^{2+}$  level may rise from  $10^{-7}$  to  $10^{-6}$  M in these smooth muscles during contraction (Matthews *et al.*, 1981). Cyclic AMP acts as an intracellular messenger in so many different types of cellular functions including muscle contraction. The inhibitory G-protein can slow the conversion of ATP to cAMP by binding to adenylyl cyclase and thus results in reduction of cAMP. In the presence of more ATP, MLCK phosphorylation leads to smooth muscle contractions. It is known by most investigators that drugs which decrease cellular concentration of cAMP can contract smooth muscle (Barritt, 1992; Fehri *et al.*, 1995). It became apparent that for many agonists which bind to receptors on the plasma membrane, cAMP is not responsible for conveying information to intracellular sites. So the PI (Phosphatidylinositol) pathway is considered to be the major mechanism of increased level of calcium in cells. Agonists like ACh induce rapid hydrolysis of  $\text{PIP}_2$  (Phosphatidylinositol 4,5-bisphosphate) into  $\text{IP}_3$  by increasing the activity of phospholipase-C enzymes. Leaving the plasma membrane,  $\text{IP}_3$  binds to a receptor protein and opens  $\text{IP}_3$  gated  $\text{Ca}^{2+}$  channels on smooth ER or ryanodine receptors in the SR of smooth muscle cells. The opening of  $\text{Ca}^{2+}$  channels releases  $\text{Ca}^{2+}$  into the cytosol and this sudden rise of  $\text{Ca}^{2+}$  initiates muscle contraction. The formation of diacyl glycerol activates protein kinase C which can also

contribute to smooth muscle contraction via MLCK phosphorylation (Berridge, 1985; Barritt, 1992; Sadraei *et al.*, 2003ab).

On the other hand, a decrease in the intracellular  $\text{Ca}^{2+}$  concentration is an essential factor for producing smooth muscle relaxation. The relaxant or spasmolytic effect in gastrointestinal tract appear to involve a greater effect on intracellular  $\text{Ca}^{2+}$  release than the blockade of VDCs of the extracellular medium, the inverse occurring in the uterus (da Silva *et al.*, 1993). The relaxant effect may also be due to calcium channel blockers such as papaverine, atropine and verapamil, which markedly reduce the responses to the receptor agonists in the smooth muscles (Mustafa *et al.*, 1995). Three mechanisms of smooth muscle relaxation are reported by Shimizu *et al.* (2000): an intracellular cAMP accumulation, inhibition of cellular respiration and effects on  $\text{Ca}^{2+}$  movement. Papaverine can relax high  $\text{K}^+$  or agonist-induced contractions in various smooth muscles of many animal species. Papaverine inhibits carbachol-induced smooth muscle contraction in guinea pig ileum by inhibiting mitochondrial respiration (Kaneda *et al.*, 1998). In the same manner it can also inhibit oxytocin-induced contraction of uterus of non-pregnant rats in a regular oestrus cycle (Shimizu *et al.*, 2000).

### **1.3 Role of Herbal Remedies in the Society**

In all countries of the world there exists traditional knowledge related to the health of humans and animals. The importance of traditional medicine as a source of primary health care was first officially recognized by the World Health Organization (WHO). The expert group of WHO agreed to define traditional medicine as: "the sumtotal of all the knowledge and practices whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing " (WHO, 1978;

Yilma Desta *et al.*, 1996). The term traditional medicine covers traditional healers and their use of plant materials, animal origins and inorganic mineral substances for health care in the context of social, cultural and religious backgrounds (Attisso, 1983). The use and exploitation of herbal or plant remedies, herbology or phytotherapy, as one component of traditional medicine, in the treatment of various illnesses is also common all over the world. Traditional Chinese, Indian Ayurvedic and Greco-Arab (Pakistani) Unani system of medicine which have been refined and elaborated over several millenia are spreading throughout the world and are used extensively elsewhere in Latin America and African traditional health. The early societies developed this means of healing by herbs guided by thousands of years of taste and experience i.e. by trial and error (Boulos, 1983; Beyerl, 1984; Menassie Gashaw, 1991; De Smet, 1997).

Since half of the world's population still lacked regular access to the most needed essential drugs and pharmaceuticals for disease treatment, a WHO report (1978) proposed that traditional medicine should be integrated into the primary health care. And nowadays, many countries of the world look upon the native medicinal herbs as possible additions to the WHO list of essential drugs once their value is proven in an appropriate scientific way. So, 80% of the world population presently use herbal medicines and over 80% of the population in developing countries relies on them as their major health delivery system (Boulos, 1983; Ernst, 1998). Even in the developed nations the general public is turning to nature i.e. phytotherapy from chemotherapy, stimulated by the growing awareness of the dangers of the overuse of synthetic pharmaceutical drugs as well as their inflated prices (Attisso, 1983; Tyler, 1988; Phillipson, 1999). 80% of the Ethiopian population depends on traditional medicine, too. The use of plants or herbs in religious ceremonies as well as for magic and medicinal purposes is common in Ethiopia as in any other African country. The plants are

prepared for oral intake, fumigant application and local body insertion in order to gain curative, protective and antifertility effects (Menassie Gashaw, 1991; Dawit Abebe and Ahadu Ayehu, 1993; Belachew Desta, 1994).

Nowadays, there is a worldwide 'green' revolution which is reflected in the belief that herbal remedies are generally safer and less damaging to the human body than synthetic drugs. They are wholesome and they provide the body with nutrients such as carbohydrates, proteins, minerals, and vitamins besides curing illnesses (Farnsworth *et al.*, 1975; Tarafder, 1983; Ernst, 1998). An effective curative, preventive and antifertility drug with a minimum of side effects is still derived from higher plants (Cox and Balick, 1994). As Farnsworth (1983) has pointed out, about 25% of the prescribed drugs used in the industrialized nations contain ingredients extracted from higher plants, a situation that has not changed appreciably in the last 40 years. However, only about 10% of the 250,000-750,000 species of higher plants have been used in traditional medicine, and 1% are acknowledged through scientific studies phytochemically to have real therapeutic value when used in extract form by humans (Hansel, 1975; Cox and Balick, 1994).

Hansel (1975) used the term "medicinal plants" as a synonym for "plants and plant constituents with pronounced activity in men and animals". All herbal remedies are result of polyherbal mixtures and thus there is not necessarily a single active ingredient present within the medicinal herb that will help in the development of a new wonder drug (Phillipson, 1999; Izzo and Ernst, 2001). The active plant constituents are scattered throughout the plant kingdom. Some of the classic plant substances in recent developments are rutin, cortisone precursors, reserpine, coumarin, quinine, morphine, atropine, vincristine, digoxin, digitoxin, hyoscine (scopolamine), theophylline, ergometrine, ephedrine, pilocarpine, vinblastine, vinca

alkaloids, khellin, physotigmine, yohimbine,  $\beta$ -carotene, caffeine, capsaicin, colchicine, galanthamine, nicotine, menthol, bishydroxy coumarin, methoxsalen, norhydroguaiaretic acid, etc. (Schultes, 1975; Duke, 1992; De Smet, 1997). Scientific research is in progress to discover these active constituents of potential medicinal interest for a range of useful biological effects such as antifertility, muscle relaxant, spasmolytic or antispasmodic, antimalarial, antihypertensive, antibiotics, cytotoxic, antidiabetic and anticancer. They also provide possible therapies against AIDS and even afflictions of old age (Tyler, 1988; Cox and Balick, 1994; Sanchez de Rojas *et al.*, 1994). These chemical constituents are also used in the classification of receptor systems such as nicotinic, muscarinic and morphine receptors (Williamson *et al.*, 1996).

### **1.3.1 Herbal Remedies for Gastrointestinal Motility Disorders**

According to Gwee and Read (1994) drugs that can modify gut motility fall into three categories:

1. Speeder uppers that induce gastrointestinal propulsion such as cholinergic agonists (acetylcholine, histamine, serotonin), prokinetic agents, laxatives, erythromycin, etc.
2. Slower downers that inhibit excessive gastrointestinal propulsion such as anticholinergics, adrenergic agonists, opiate-like antidiarrhoeal agents.
3. Antispasmodics often called spasmolytics are supposed to diminish painful abdominal spasms and are designed to reduce the force of involuntary and irregular contractions of the body muscle. They are therapeutic agents such as anticholinergics, calcium antagonists and  $\text{Ca}^{2+}$  channel blockers, serotonin antagonists, direct smooth muscle relaxants such as papaverine (Singh *et al.*, 2003).

The plant kingdom is rich in antispasmodics; infact, most remedies used in conventional medicine include at least one antispasmodic of plant origin. They form a very important part of the treatment of gastrointestinal motility disorders such as dyspepsia (indigestion), spasms of intestine, peptic and duodenal ulceration, nausea and vomiting, constipation and diarrhoea, and irritable bowel syndrome (IBS). IBS is a general term characterized by abdominal pain, disturbed defecation, abdominal bloating and altered bowel function affecting primarily the mid and lower gut (Gorard *et al.*, 1994; Williamson *et al.*, 1996; Sadraei *et al.*, 2003b). The antispasmodics are considered useful for relieving or calming colicky pains resulting from spasms of the gut muscles and diarrhoea due to hypermotility of the gastrointestinal tract (Crema and Ponti, 1989 cited in Gilani *et al.*, 1994a), and other characteristic features of IBS.

Among the wide range of plant drugs that have relaxant activities on various smooth muscles, papaverine is the one which is used in the treatment of colic (Mustafa *et al.*, 1995). It is a non-selective smooth muscle relaxant and is used as a control drug for antispasmodic effects. Muscarinic antagonists like atropine (a known competitive antagonist of muscarinic agents) inhibits the contractions of gastrointestinal tract induced by ACh and other muscarinic agonists mediated via  $M_3$  receptors. This partial inhibition of gastrointestinal motility by atropine drugs has led to their widespread use as antispasmodics in the treatment of disorders associated with intestinal hypermotility (Ghosh *et al.*, 1993; Broadley and Kelly, 2001). Drugs also affect the serotonin receptors to exert an antispasmodic or else a prokinetic effect. The development of serotonin 5-HT<sub>3</sub> receptor antagonists offers enormous therapeutic potential as anti-emetic agents, antidiarrhoeal agents, in the control of abdominal pain and discomfort and rectification of gastrointestinal motility (Gwee and Read, 1994). Blocking 5-HT<sub>3</sub> receptors leads to reduced smooth muscle contractility (i.e. an antispasmodic effect) which of clinical significance in chronic diarrhoea (De Ponti and Tonini, 2001; Singh *et al.*,

2003). 5-HT<sub>7</sub> inhibitors are also involved in the inhibitory effect of serotonin in guinea pig ileum and the ligands acting on the receptor may prove to be useful antispasmodic agents to treat gastrointestinal motility disorders such as IBS (Tuladhar *et al.*, 2003).

Ayurvedah, an Indian system of medicine, cited several plants which are useful against various functional gastrointestinal disorders such as abdominal pain, flatulence, colic, dyspepsia, IBS, IBD and the like. There are also a number of plant extracts tested for activity against some of these conditions that mainly involve testing the plant extracts for antispasmodic activity. Plant-derived antispasmodics include some tropane alkaloids (atropine, hyoscine or scopolamine, hyoscycamine), opium alkaloids (papaverine, codeine), flavonoids (luteolin, cirsimartin, quercetin, rutin, apigenin, kaempferol, genkwanin) and essential oils (peppermint, caraway, dill, garlic, chamomile, anise) (Sanchez de Rojas *et al.*, 1994; Williamson *et al.*, 1996). Nowadays, a lot of scientific articles can be cited that report the antispasmodic, muscle relaxant and inhibitory effects of the plant extracts, or their active chemical constituents, or their essential oils.

Some of the chemical constituents of plants for inhibitory or antispasmodic effects are mentioned as follows. Flavonoids are natural products which exhibit various pharmacological effects. Quercetin, one of the flavonoids isolated from aerial parts of *Conyza flaginoides*, caused a concentration dependent inhibition of spontaneous contractions of rat ileum (Mata *et al.*, 1997), and showed antidiarrhoeic activity against castor oil-induced diarrhoea in mice, and also exerted inhibitory effects on guinea pig ileum contractile response (Galvez *et al.*, 1996). Rutin, another flavonoid in *Artemisia scoparia*, was found to cause a concentration dependent inhibition of spontaneous movements of rabbit jejunum with a 30% relaxant effects at a dose of 300 µg/ml. (Gilani *et al.*, 1994b). Flavone cirsimartin, which is isolated from

*Artemisia judaica*, *Artemisia capillaris*, *Artemisia xerophytica* and *Artemisia scoparia* is responsible for the spasmolytic activity of isolated guinea pig ileum and thus support their use in folk medicine for certain gastrointestinal disorders such as ulcer or acute diarrhoea (Abdalla and Abu Zarga, 1987). Four flavonols with spasmolytic activity were isolated from the aerial parts of *Artemisia abrotanum*, which are the active principles for smooth muscle relaxing activity of the plant (Bergendorff and Sterner, 1995; Harborne and Williams, 2000). Flavone luteolin, isolated from *Colchicum richii*, caused a concentration dependent relaxation of the tone of ileum (Abdalla *et al.*, 1994). There are other chemical compounds called alkaloids in plants for spasmolytic effect. Bisnordihydrotoxiferine and villosimine are the indole alkaloids isolated from the roots of *Strychnos divaricans* which antagonized ACh and histamine responses in the guinea pig ileum (da Silva *et al.*, 1993). Another chemical constituent, 7-methoxy coumarin, has been reported to be a smooth muscle relaxant responsible for the spasmolytic activity of *Lavandula stoechas* L. extract (Gilani *et al.*, 2000).

The presence of smooth muscle relaxant agents isolated from species of Malaysian Medicinal plants (Mustafa *et al.*, 1995), Mexican Medicinal plants (Estrada *et al.*, 1999; Rodriguez-Lopez *et al.*, 2003) and United Arab Emirates Medicinal plants (Tanira *et al.*, 1996) were evidenced by their inhibitory and spasmolytic effect of the cholinergic, histaminergic, nitriergic and ion-induced smooth muscle contractions of guinea pig and rat ileum, and rabbit jejunum. Aqueous extracts of many plants are widely used in therapy in complementary medicines of antispasmodics (Dire *et al.*, 2003). Current therapy for some gastrointestinal disorders is directed towards inhibition of smooth muscle contractions. It is well known that aqueous herbal medicines are traditionally used for their spasmolytic activity in various countries (Hajhashemi *et al.*, 2000; Sadraei *et al.*, 2003b). In an *in vitro* experiment, aqueous extracts of *Prunus spinosa* L. branches were tested to have diminished the response of ACh

and histamine (spasmogens) in mouse duodenum and guinea pig ileum (Rodriguez *et al.*, 1986). A room temperature aqueous extract of the roots of *Taverniera abyssinica* antagonized ACh and histamine induced contractile responses of the guinea pig ileum and relaxed the smooth muscle of rabbit duodenum, which is suggestive of its ethnomedical use in stomachache treatment (Noamesi *et al.*, 1990). The aqueous extract of *Linum usitatissimum* seed was observed to show significant spasmolytic activity and protective effects against experimental ulcerogenesis in guinea pig ileum and mouse stomach (Eyasu Makonnen, 1996). The constipating and spasmolytic effect of khat leaves extract (*Catha edulis Forsk*) were investigated and the plant was found to antagonize the spasmogenic effects of both histamine and carbachol on isolated guinea pig ileum and whole mice in a concentration dependent manner (Eyasu Makonnen, 2000).

The less polar solvents like methanol and ethanol are also known to be very appropriate in extracting the spasmolytic agents of plants. The ethanol extract of *Capparis cartilaginea* inhibited the submaximal contractions of ileum induced by ACh, histamine or serotonin at a dose of 100 µg/ml (Gilani and Aftab, 1994). Gilani *et al.* (1994a) also showed that pure compounds from leaf ethanol extracts of *Moringa oleifera* were found to have inhibitory effect on isolated ileum in a concentration dependent manner. Dichloromethane extracts of *Inula crithmoides L.* (Barrachina *et al.*, 1995a) and, later on, methanol and dichloromethane extracts of *Teucrium* species (Barrachina *et al.*, 1995b) were known to produce a significant inhibition in the maximal contractile effects of ACh, histamine and serotonin in the guinea pig ileum and rat duodenum. The crude methanol extracts of *Erythrina sigmoidea* stem bark were found to have potent anticholinergic effects by decreasing the tone and spontaneous activity of isolated rat ileum induced by carbachol and acetylcholine (Nkeh *et al.*, 1993). They were

also found to inhibit histamine induced contraction of the same tissue showing their potency of antihistaminergic effect (Nkeh *et al.*, 1996).

Results from investigations with animal tissues also suggests that peppermint oil and caraway oil (as categories of essential oils) were found to relax gastrointestinal smooth muscle by reducing  $Ca^{2+}$  influx (Gwee and Read, 1994; Micklefield *et al.*, 2000). The essential oil of *Achillea ageratum L.* was found to be an effective spasmolytic agent capable of inhibiting ACh and  $BaCl_2$  induced contraction of isolated rat duodenum reaching a maximum of 62.8% and 43.6% inhibitions respectively (de la Puerta and Herrera, 1995). *Satureja hortensis L.* essential oil was investigated to have antispasmodic effect on rat isolated ileum *in vitro*. It was found to inhibit the maximum response due to ACh, relax ileum contraction due to depolarization by KCl, and inhibit castor oil-induced diarrhoea (Hajhashemi *et al.*, 2000). Essential oil of *Ocimum gratissimum* was investigated and found to reversibly and concentration dependently relax the basal tone of isolated guinea pig ileum and reverse the tonic contractions induced by KCl and ACh (Madeira *et al.*, 2002). The essential oils of *Artemisia thuscula Cav.* flowers (Perfumi *et al.*, 1995) and *Artemisia alba* (Perfumi *et al.*, 1999) were previously investigated and shown to have dose-dependent and essentially non-competitive spasmolytic effects in guinea pig ileum.

Most medicinal plants which were reported to be anthelmintics were also found to be antispasmodics. Anthelmintics, like smooth muscle relaxants, act by depressing the smooth muscle or by inhibiting the metabolic processes. For instance, the aqueous extract of *Bersama abyssinica* antagonized the spasmogenic effect of histamine on guinea pig ileum in a non-reversible manner (Eyasu Makonnen and Estifanos Hagos, 1993). The mechanisms of taenicidal action of *Hagenia abyssinica* ('Kosso') was suggested to be due to its inhibitory

effect on isolated guinea pig ileum (Messele Arragie *et al.*, 1983). Kosotoxin, a constituent of this plant was tested for spasmolytic properties on guinea pig ileum and rabbit jejunum *in vitro* and was found to inhibit their spontaneous contractions concentration dependently by 40-60% (Mesfin Bogale *et al.*, 1996).

### **1.3.2 Herbal Remedies for Reproductive Tract Disorders**

Fertility control has come to the forefront as a topic of global concern, with important medical, social and political considerations due to population increases. Nowadays, a number of antifertility or contraceptive agents are used so as to combat this unchecked growth of population. These agents usually refer to devices or substances used in the prevention of conception by any mechanism including hormonal agents, which prevent ovulation or interfere with fertilization by altering vaginal or uterine environment. Specific biological effects under the division of fertility regulating category are non-specific contraceptive or antifertility effects, abortifacient, anti-implantation, uterine stimulant and uterine relaxant, labour induction and labour inhibition, oxytocic and anti-oxytocic, oestrogenic and anti-oestrogenic, progesterogenic and anti-progesterogenic, ovulatory and anti-ovulatory, androgenic and anti-androgenic, spermicidal and anti-spermatogenic effects (Soejarto *et al.*, 1978). Antifertility estimation in the female measures the pregnancy rate and includes anti-ovulation, anti-implantation and cytotoxic agents (Williamson *et al.*, 1996). Uterine stimulants are the well-known antifertility agents that excite, facilitate and accelerate myometrial (uterine) contractions. Some of uterine stimulants in *in vitro* and *in vivo* animal and human studies are the abortifacients, ecbolics, oxytocics, uterotonics and emmenagogues. Abortifacients are antifertility agents that act after implantation has taken place. They induce abortion, miscarriage, or premature expulsion of foetus before it is viable or capable of sustaining life. This type of antifertility effect facilitates parturition or childbirth, and is also

effective in expelling placenta (Farnsworth *et al.*, 1975; Tarafder, 1983; Beyerl, 1984). Emmenagogue is synonymous with abortifacient but applies to agents that alter or regulate menstrual cycle by promoting or inhibiting the menstrual flow. Emmenagogues could also be used against dysmenorrhea and amenorrhoea (Boulos, 1983; Beyerl, 1984).

Substances having antifertility effects may exert their actions on areas within the female mammal such as the hypothalamus, anterior pituitary, ovary, oviduct, uterus and vagina, and interrupt reproductive function at some point. The mammalian uterus, which is the main site of antifertility effects, comprises outer myometrial cells which are responsible for the contraction of the uterus, inner endometrial cells which are secretory and non-contractile, and a cervix. The variations in contractility or motility of the uterus are due to complex regulatory mechanisms which include endocrine, neural, mechanical and chemical regulations (Williamson *et al.*, 1996). The rat uterus, particularly the tubal and cervical regions, receives cholinergic innervation though a cholinergic uterine nerve supply are at most sparse in isolated uterine preparations (Bell, 1972). Stimulation of the uterine muscarinic M<sub>3</sub> receptors by agonists such as ACh causes contraction of the uterus and this effect is blocked by a muscarinic competitive antagonist such as atropine (Varol *et al.*, 1989 cited in Veal *et al.*, 1999). However, hormonal control of the uterus is more important than neural control in its regulation. The number of receptors for drugs is also under hormonal control. The influence of hormones (oestradiol and progesterone) on uterine contractile activity was investigated by Downing *et al.* (1981) and Mancinelli *et al.* (1988). There is also a hormonal dependence of uterine responses to catecholamines (adrenalin and noradrenalin) in different species (Bulbring and Tomita, 1987).

The presence of cell surface receptors for classic uterotonic hormones and agonists (including oxytocin, prostaglandins, acetylcholine, histamine, serotonin, noradrenalin and vasopressin) in the myometrium has been well described by Phillippe and Chien (1998) and Veal *et al.* (1999, 2000). The hormone, oxytocin, the most potent of the endogenous uterotonic agents, has a dual action in the uterus. It acts on one subtype of myometrial oxytocin receptors (OT<sub>1a</sub>) to directly cause uterine contraction, and on another subtype of endometrial oxytocin receptors (OT<sub>1b</sub>) to promote synthesis and release of prostaglandins (PGs). The importance of oxytocin in parturition is based on well-documented contractile effects on uterine myometrium at term and perhaps preterm in addition to its role in the maintenance of labour. It can be used close to term to induce labour but is ineffective as an abortifacient agent if used in earlier pregnancy. It is also clear that parturition at term and preterm is highly regulated and likely involves the interplay of many additional factors including oestrogen and progesterone, PGs, and non-classic uterotonic agonists such as growth factors, bacterial endotoxins and cytokines (Pettibone *et al.*, 1990; Phillippe and Chien, 1998)). Prostaglandins are produced in both myometrial and endometrial tissues, but mainly in the latter. As powerful myometrial stimulants, they appear to promote myometrial contractility which in turn generates internal shortening and stretch of myometrial wall as possible trigger mechanisms suggested for labour initiation as well as for abortifacient effects along with oxytocins (Farnsworth *et al.*, 1975). They have also been reported to mediate the activity of most agents that stimulate the uterus (Solloff, 1979; Uguru *et al.*, 1998). Acetylcholine is another myometrial stimulant which is regarded a reliable standard oxytocic agonist in uterine contraction (Veal *et al.*, 2000). In recent years, histamine has also been shown to be potent oxytocic agent with the ability to increase the contractility of isolated myometrium from pregnant and non-pregnant humans, non-pregnant guinea pigs, and pregnant and non-pregnant mice. The release of endogenous histamine may activate uterine

contractility during pregnancy and may lead to preterm labour and delivery (Bytautiene *et al.*, 2003). The accumulation of serotonin in the uterus could also promote uterine contractility and eventually abortion (Pettibone *et al.*, 1990).

On the other hand, uterine relaxants (tocolytic agents) such as  $\beta$ -adrenergic agonists, calcium channel blockers, relaxin and magnesium sulphate inhibit uterine motility and thus they act like antispasmodics (Phillippe and Chien, 1998). Many investigators demonstrated porcine relaxin as a uterine relaxant which reduces the frequency and amplitude of *in vitro* and *in vivo* uterine contractions in non-pregnant rats, pigs and several other species. According to Sherwood *et al.* (1993), relaxin may act directly on the uterus to the extent that they are overridden by contractile agents such as oxytocin and PGs, or other mechanisms that bring about the strong highly coordinated contractions that occur at delivery. It also acts directly on rat uterine myometrial cells where it reduces MLCK activity and availability of  $\text{Ca}^{2+}$  stores. There is good evidence that relaxin suppresses oxytocin's effects on influx and mobilization of  $\text{Ca}^{2+}$  stores by inhibiting oxytocin-induced uterine contractions or oxytocin-induced formation of  $\text{IP}_3$  in uterine cells and myometrial cells (Anwer *et al.*, 1989; Sherwood *et al.*, 1993). Relaxin may be important in restraining uterine contractions during the period of declining progesterone concentrations prior to birth (Porter, 1979 cited in Sherwood *et al.*, 1993).

Higher plants are a rich source of biodynamic agents of all types including fertility regulating agents. There is always a hope that a traditional medicinal plant may provide a reliable contraceptive agent. At the present time, thousands of scientific articles can be cited that report one or more types of anti-reproductive activity exhibited by plant extracts or by substances of known structure derived from plants (Nath *et al.*, 1992). Testing plants that are

known to contain chemical constituents that theoretically could affect the female cycle to produce antifertility effects, or that have the potential to contract the uterus is one of the paths to follow in examining the approximately 750,000 species of higher plants in an animal system for their potential antifertility effects (Farnsworth *et al.*, 1975).

Plant drugs have been used since time immemorial for their effects upon sex hormones, in particular, suppressing fertility, regularizing the menstrual cycle, relieving dysmenorrhoea, treating enlarged prostate, menopausal symptoms, breast pain, and during and after childhood (Williamson *et al.*, 1996). The active chemical constituents of some plants such as oestrogenic sterols, coumesterols and isoflavones that produce antifertility effects have been investigated by Farnsworth *et al.* (1975). For *in vitro* uterine stimulant effects of plants, a large number of compounds of known structures can be cited. The most intriguing example of an unmodified fertility regulating agent of known structure isolated from plants is m-xylohydroquinone which was first isolated from peas (*Pisum sativum*) by Sanyal in 1952 (Soejarto *et al.*, 1978). Other agents such as lithospermic acid (contraceptive first used by Navajo Indians), rutin (impairing fertility of female mice), rottlern (antifertility principle of *Mallotus philippensis*), embelin (abortifacient agent in female), agnus castus and evening primrose oil (for menstrual syndrome and breast pain) ergometrine (standard oxytocic drug for use after childbirth), and saw palmetto berries (taken for prostate enlargement) are documented in Farnsworth *et al.* (1975) and Williamson *et al.* (1996). Apart from plant medicines, there are numerous examples of plants in the diet having accidental biological effects. For instance, gossypol was discovered as a contraceptive when cooking with cotton seed oil led to greatly reduced male fertility in parts of China. More recently, the lower rate of enlarged prostate and prostate cancer in Japanese males was suggested to be due to what is found in soya bean (Leguminacea). Oestrogenic isoflavones in clovers (*Trifolium pratens*) are responsible for

affecting milk levels and reducing fertility in animals ingesting them in forages (Williamson *et al.*, 1996). Plants that are involved in food cooking processes in Ethiopia are also known to induce antifertility. For instance, *Thymus serrulatus Hochst* which is famous condiments (used as spices) in 'wot', butter, oil, tea, 'beso' and bread is also known to possess abortifacient effects (Jansen, 1981; Menassie Gashaw, 1991).

Several other active chemical constituents accountable for uterotonic effects are discovered in various plant species from time to time. For instance, Jansakul *et al.* (1987) isolated and characterized two triterpenic saponins called ardisiacrispin A and B from the crude extract of *Ardisia crispa* root. These compounds were responsible for most utero-contracting properties in treated rats and for washing out dirty blood in women suffering from menstrual pain in Thai traditional medicine. An active indole alkaloid, yuehchukene isolated from the plant *Murraya paniculata Jack.* is used in China to regulate fertility because it has potent anti-implantation activity (Kong *et al.*, 1985; Fabricant and Farnsworth, 2001). Quassinoids discovered as one of the bioactive constituents of *Quassia amara L.* and the most developed antimalarial drug were also found to have antifertility effects (Njar *et al.*, 1995). *Artemisia* species with a santonin constituent was evaluated in mouse to delay in estrus, ovulation and onset of mating and expected to have abortifacient effects in humans (Farnsworth *et al.*, 1975). A mixture of flavonoids from *Artemisia maderaspatana* was found to possess oestrogenicity and anti-implantational activities in the mouse uterus (Jain *et al.*, 1993).

A lot of higher plants are used traditionally to induce antifertility by interrupting the reproductive processes. The commonly used Indian traditional abortifacient plants were checked for their anti-reproductive potentials in rats orally dosed for 10 days (Nath *et al.*, 1992). Akah (1994) demonstrated that plants and herbs belonging to various families and

species are used by traditional birth attendants and native healers to induce labour, achieve relatively painless delivery, hasten fetal delivery, terminate unwanted pregnancy and evacuate retained placenta in humans, sheep and goats. Previously, the Indian folkloric antifertility studies showed that water extracts of many *Artemisia* species as having abortifacient, ecboic, oxytocic and emmenagogue properties (Farnsworth *et al.*, 1975). The abortifacient effect of a given medicinal plant could be expressed in terms of its ability to increase uterine contraction according to its oxytocic-like property. For instance, the aqueous extracts of *Artemisia arborescens* caused a significant increase in the frequency and amplitude of the phasic and tonic contractions of the isolated uterus and the plant could be taken as a good abortifacient and other antifertility type plant (Abu Zarga *et al.*, 1995). The aqueous extracts of leaves of *Agapanthus africanus* and *Clivia miniata* have been shown to possess similar uterotonic activities in the isolated whole uterus preparation as well as in stripped myometrium (endometrium free preparation). They showed an augmentation of uterine response to oxytocin and ACh due to additive synthesis of uterotonic PGs or to interaction with specific inhibitors such as atropine and indomethacine (Veal *et al.*, 1999, 2000).

The uterine contractile responses may depend on the polarity of the compound found within a given extract. The more polar fraction isolated from *Montana tomentosa* (Zoapatle plant), for instance, was reported to account for most uterine contraction in treated guinea pig (Waller *et al.*, 1987). Extracts obtained with less polar solvents like methanol and ethanol are also known to induce uterine contraction. For instance, the hot methanol extract of *Monechma ciliatum* produced a concentration dependent contraction of the non-pregnant isolated uteri of the rat, mouse and guinea pig, showing oxytocic and oestrogenic effects of the plant *in vitro* (Uguru *et al.*, 1995). Later on, the use of this plant to induce labour in traditional medicine practice was suggested to be due to the contractile effect on the uterus mediated via

muscarinic receptors as well as through prostaglandin synthesis and release (Uguru *et al.*, 1998). The oestrogenic activity of the extract supports its folkloric use to induce labour since oestrogens are known to stimulate prostaglandin synthesis (Solloff, 1979). The increase in the glycogen content of the rat uterus by the oestrogenic nature of *Pueraria tuberosa* DC. is also known to enhance uterine contraction (Shukla, 1996). The leaf ethanol extract of *Moringa stenopetala* was shown to have a potential effect (73.3% antifertility effect) on guinea pig and mouse uteri *in vitro* because of the compounds in the plant that cause uterine contraction or oxytocic-like property (Yalemtsehay Mekonnen, 1999).

On the contrary, other higher plants are used traditionally to maintain fertility and hence to perpetuate reproduction. For instance, a decoction made from a plant *Scaphyglottis livida* is used to maintain pregnancy (Estrada *et al.*, 1999). Ethanol extract of *Moringa oleifera* showed non-specific or general spasmolytic action by completely abolishing the spontaneous activity of the rat uterus (Gilani *et al.*, 1994a). In the same way the ethanol extract of *Capparis cartilaginea* was demonstrated to have such ability by Gilani and Aftab (1994). The tea extract and hydroalcoholic extract of *Leonotis nepetaefolia* relaxed uterine preparations precontracted with KCl in a concentration dependent manner (Calixto *et al.*, 1991). The crude aqueous-ethanol, chloroform and semipurified flavonoid-glycoside extracts of *Sebastiania schottiana* were found to inhibit contractions of rat uterus to agonists like ACh or oxytocin. Tannins which are the common active constituents of medicinal plant crude extracts do this antispasmodic potency by antagonizing calcium-induced contractions of depolarized rat uterus in a non-competitive manner (Calixto *et al.*, 1986). Bisnordihydrotoxiferine, an alkaloid isolated from the root of *Strychnos divaricans*, antagonized oxytocin and ACh-induced contractions in the rat uterus (da Silva *et al.*, 1993). Antispasmodic drug from *Radix paeonia* is used in the treatment of ammenorrhoea and dysmenorrhoea (WHO, 1999). SJ-200

(Himcospaz), a preparation of polyherbal mixture, was found to act like a non-specific antispasmodic by causing significant inhibition of ACh, histamine, BaCl<sub>2</sub> and oxytocin induced contractile responses of rat uterus (Venkataranganna *et al.*, 2002).

Extracts of different parts of a plant may cause different biological effects on isolated uterine preparations. For instance, Prabhakar and Nanda Kumar (1994) demonstrated that *Datura metel* leaf crude extract had antispasmodic (relaxant) effects whereas *Datura metel* root crude extract had spasmogenic (contractile) effects on isolated uterus. Udoh (1999) had also stressed that the leaf and seed extract of *Piper guineense* had cholinergic actions due to their oxytocic-like effect while the root extract had adrenergic actions on the uterus muscle in a concentration dependent manner. However, both excitatory and inhibitory components of seeds of *Abrus precatorius* had oxytocic effects with spasmogenic activities on the uterus eventhough they had spasmolytic activities on other smooth muscle preparations (Nwodo and Botting, 1983).

#### 1.4 Ethnomedical use of *Artemisia* species

The genus *Artemisia* is with nearly 300 species which is found predominantly in the northern temperate region of the world (Esteban *et al.*, 1986). This genus is widely used in many parts of the world either alone or in combination with other plants as herbal remedies for a variety of human illnesses as well as fertility controls. They are members of the great family of *Compositae* (*Asteracea*, as an alternative name used by ICBN, 1972). There are a number of them widely used in the traditional folk medicine for their cicatrizant, chloretic, stomachic, antipyretic, antimalarial, antispasmodic, antihelmintic, carminative, catamenogenic, antihypertensive, antiseptic, antiepileptic, astringent, deobstruent, vermifuge, febrifuge, nervine, narcotic, appetite stimulant, bitter tonic, aromatic, cordial, vulnerary, oxytocic,

cytotoxic, insecticidal and larvicidal properties (Duke, 1985; Yilma Desta *et al.*, 1996; Martinotti *et al.*, 1997). For instance, *Artemisia annua L.* is used in the traditional Chinese medicine for the treatment of haemorrhoids, fever and malaria. In the 1970's it was found that the plant contains an active principle, artemisinin, a novel antimalarial agent which has been shown to be potent against *Plasmodium falciparum in vitro* and *in vivo* (Klayman, 1993 cited in Phillipson, 1999). Crude ethanol extracts of aerial parts of this *Artemisia* species and some sesquiterpene lactones isolated from it were found to have antiulcerogenic property (Dias *et al.*, 2001; Foglio *et al.*, 2002). *Artemisia scoparia Thunb* and *Artemisia scoparia Waldst* were known for their use in the indigenous system of medicine in Pakistan as febrifuge, diuretic and antispasmodics (Chin, 1951 cited in Gilani *et al.*, 1994b). Leaves of *Artemisia maciverae* have medicinal uses of fevers and wound healing and whole parts of *Artemisia ramosa* has antihelmintic, colic and digestive (indigestion) properties (Iwu, 1993). *Artemisia judaica* is reputed for alleviating properties for gastrointestinal colics, diarrhoea and other gastrointestinal disorders (Abdalla and Abu Zarga, 1987). *Artemisia verlotorum Lamotte* is known for its remedy against hypertension (Calderone *et al.*, 1999). *Artemisia vulgaris* (Mugwort), *Artemisia abrotanum L.* (Southernwood), *Artemisia absinthium L.* (European wormwood), *Artemisia afra Jacq.* (African wormwood), and *Artemisia abyssinica Schtz.* (Ethiopian wormwood) are well known in the folk medicine as remedies for stomachaches, diarrhoea, dysentery, constipation, syphilis, jaundice, malaria, relapsing fevers, infectious hepatitis (liver disorders), hypertension, haematuria (urinary retention), cardiac diseases, old cancers, common cold, bronchial asthma, whooping cough, and dropsy (Jansen, 1981; Duke, 1985; Dawit Abebe and Ahadu Ayehu, 1993; Yilma Desta *et al.*, 1996). The folkloric antifertility properties of some *Artemisia* species (*A. abrotanum*, *A. absinthium*, *A. pontica*, *A. siversiana*, *A. vulgaris* and *A. maritima*) has been documented as having anti-implantation,

abortifacient, ecboic, oxytocic and emmenagogue effects regarding the antifertility property (Farnsworth *et al.*, 1975).

#### 1.4.1 *Artemisia afra* - description and its uses

*Artemisia afra* Jacq., commonly known as the African wormwood, is one of the most widely used southern African medicinal plant with essential oil components (Esteban *et al.*, 1986; Graven *et al.*, 1990). It is found throughout Africa as a common species in the southern, central and eastern Africa and extends as far north as Ethiopia. In Ethiopia, this plant is found in Bale, Sidamo, Arba Minch and Keffa (Mesfin Tadesse, in press). Indigenous people of Ethiopia (Bale) refer to *Artemisia afra* as 'Chigugn' (Amharic), Kapani (Oromgna), Kodo (Guragna). It belongs to the family *Compositae* (*Asteraceae*). It is an evergreen perennial herb or deciduous subshrub with grey or green foliage leaves containing yellow florets (Plate1). It is aromatic. It grows up to a height of about 1 meter, at an altitude range of 3070 and 3600metres a.s.l. (Mesfin Tadesse, in press).

*Artemisia afra* has a long history of domestic herbal use in both the Northern and Southern parts of Ethiopia. Juice of chopped leaves of *A. afra* mixed with water is traditionally taken orally for the treatment of roundworm and stomach pains in the Bale area of Ethiopia. The leaves are also chewed or the aroma is inhaled for stomachaches and headaches (Menassie Gashaw, 1991). Charred powder of leaves mixed with honey or edible oil is also used as remedies for eye diseases (crying eyes and cataract) and stomach cramps by the Northern people of Ethiopia. Leaf tea of this plant is used to treat coughs, colds and flu as well as bronchial and intestinal ailments. Milk decoctions of the whole plant or leaves are used in the treatment of haematuria, haemorrhoids, mumps, small pox, malaria, neuralgia, colitis and liver disorders. The plant also has tonic, stimulant, perfumic, antihelminthic (vermifuge) and

antipyretic (febrifuge) properties (Jansen, 1981; Dawit Abebe and Ahadu Ayehu, 1993; Iwu, 1993).

Alpha-thujone, beta-thujone, 1,8-cineole and camphor were reported as the major constituents of *A. afra* from South Africa. On the other hand, yomogi alcohol and artemisia alcohol acetate were reported to be the two major constituents of Ethiopian origin (Graven *et al.*, 1990; Tadele Worku and Rubiolo, 1996). In the previous studies, plant extracts of *A. afra* were studied for antihelmintic property by Berhanu Abegaz and Ermias Dagne (1979). Butanol extracts of *A. afra* were tested to be positive for anti-implantation effect but negative for uterotonic effect. On the other hand, the aqueous and ethanol extracts of the plant were tested to be negative for both uterotonic and anti-implantation effects (Belachew Desta, 1994). Moges Kassa *et al.* (1998) had reported about the *in vitro* anti-malarial activity of the crude extracts from the aerial parts of this plant. Aqueous *A. afra* extract had a hypertensive effect *in vivo* and a dose-dependent biphasic effect on the heart *in vitro* suggesting its potential use for the management of hypertensive conditions (Guantai and Addae-Mensah, 1999). The antimicrobial property of *A. afra* has also been documented by Mangena and Muyima (1999). The plant material has been shown to be devoid of acute toxic effects (Guantai, 1990 cited in Guantai and Addae-Mensah, 1999). The antioxidant (potential free radical scavenging) activities of this plant along with *A. abyssinica* were examined by Burtis *et al.* (2001).

#### **1.4.2 *Artemisia rehan* - description and its uses**

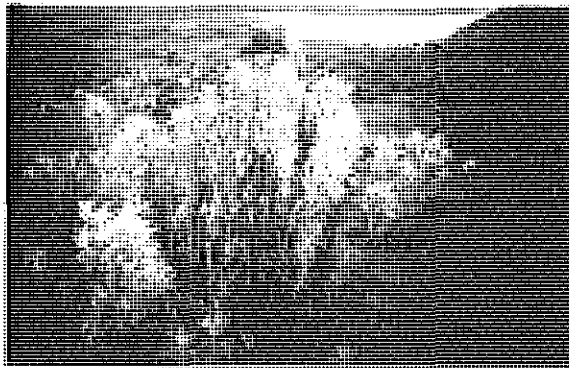
*Artemisia rehan* Chiov. is an endemic plant of *Artemisia* species to Ethiopia with essential oil components (Paulos G. Yohannes, 1980; Esteban *et al.*, 1986). It is found in Tigray, Gondar, Shoa and Bale. It belongs to the same family that *A. afra* belongs. Some investigators group it under *Artemisia absinthium* of the European wormwood and there is a doubt that it may be

introduced in the country by foreigners. It is locally known as 'Ariti' or 'Nech Eyuban'. It is a perennial, very aromatic or odoriferous herb with a light green appearance of foliage leaves and a height of about 30-60cms (Plate 2). It can grow between 1700 and 2350(2440) metres a.s.l. Since the species is only known from cultivation in private gardens and some churches, it may be considered synonymous with the widespread Eurasian and North African species (*Artemisia absinthium*). It may be widely introduced elsewhere as a medicinal herb similar to *Artemisia abrotanum* L. (Mesfin Tadesse, in press).

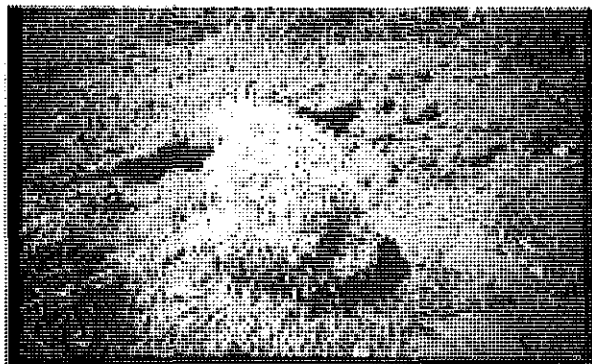
In the rural areas of Bale (southeastern part of Ethiopia) the aerial parts of *Artemisia rehan* is traditionally chewed or taken in as tea decoctions to relieve stomachaches. In Bale and throughout Ethiopia it is also used as a scent preparation having a perfumic property in curing diseases such as measles. The herbs are also used as remedies for malaria, having antihelminthic (vermifuge) and emmenagogue effects (Jansen, 1981).

Previous works on the essential oil of *A. rehan* from Ethiopia revealed camphor and denavonone as major constituents (Berhanu Abegaz and Paulos G.Yohannes, 1982). The green parts of *A. rehan* Chiov. were found to contain vulgarin which is proved to be identical with compounds such as barrellin and judaicin (Berhanu Abegaz *et al.*, 1986). Until now research studies on *A. rehan* were largely concentrated on the antimalarial activity (Moges Kassa *et al.*, 1998). Moges Kassa *et al.* (2001) carried out *in vitro* cytotoxic tests of this antimalarial plant along with *A. afra* against a human leukemia monocyte cell line and the extracts of these plants at 0.23-23 mg/ml concentrations *in vivo* were proved to be not cytotoxic.

The present study on these two closely related *Artemisia* species i.e. *A. afra* and *A. rehan* is to investigate the medicinal effects such as spasmolytic (the most often used term for antispasmodic), spasmogenic, uterotonic and possible antifertility effects of their aqueous and ethanol crude extracts on isolated smooth muscle preparations such as mouse duodenum, guinea pig ileum and mouse uterus. The folk medical use of these plants for gastrointestinal and reproductive tract ailments is a good ground for the study. Moreover, many closely related species of these plants in the Asian and European subcontinents have the above mentioned medicinal properties. The myorelaxant and antispasmodic activity on intestinal and reproductive smooth muscle is an action shared by several components of essential oils of many aromatic plants including *A. afra* and *A. rehan* (Lima *et al.*, 2000). Since these plants were also proved to be best antimalarials (Moges Kassa *et al.*, 1998) their bioactive constituents were expected to have antifertility effects (Njar *et al.*, 1995) as well as antispasmodic effects (Dias *et al.*, 2001; Foglio *et al.*, 2002). However, no pharmacological studies of spasmolytic and spasmogenic properties of these plants have been carried out so far.



**Plate 1.** The plant *Artemisia afra* with its grey or green foliage leaves containing inconspicuous yellow florets (The picture was taken nearby Dinshu area of BMNP)



**Plate 2.** The plant *Artemisia rehan* with light green appearance of foliage leaves (The picture was taken in the private garden of a villager near the Dinshu area).

## **1.5. Objectives of the Present Study**

### **1.5.1. General Objective**

- ◆ Examine the effects of ethanol and aqueous extracts of *Artemisia afra* and *Artemisia rehan* on isolated duodenal, ileal and uterine smooth muscle *in vitro* preparations from mice and guinea pigs.

### **1.5.2. Specific Objectives**

- ◆ Determine whether the leaves and roots of these plants have spasmolytic and spasmogenic activities in mammals.
- ◆ Determine whether leaves and roots have uterotonic and possible antifertility effects.
- ◆ Compare the contracting and relaxing trends of the respective smooth muscles by these plant extracts.

## **2. MATERIALS AND METHODS**

### **2.1. Plant Material Collection**

The leaves and roots of *Artemisia afra* and *Artemisia rehan* plant samples were collected during the flowering period in November 2002. *Artemisia afra* was collected from Bale Mountains National Park (BMNP) at the gate of the head quarter (Dinshu) 400km south east of Addis Ababa. *Artemisia rehan* was bought from the compound of villagers around the same place. After authentication by botanists, the representative plant specimens were kept in the National Herbarium of Addis Ababa University and given voucher numbers.

### **2.2. Preparation of Crude Extracts**

The leaves and roots of each plant were air-dried at room temperature separately in a shade. Their dried parts were ground to moderately coarse to fine powder. The crude extracts were prepared using leaf and root part of the plant of each species. Each of the powdered plant materials was macerated (soaked) in two types of solvents separately: water and 98.8% ethanol.

Aqueous and ethanol extracts of leaves and roots of each plant were prepared by suspending a known amount of the powdered pieces in gram in a known amount of distilled water in ml. The macerated samples of both aqueous and ethanol extracts were rotated on a shaker for 24 hours at room temperature. After 24 hrs, each sample was filtered out using a Whatman No. 1 filter paper. The methods followed were adopted from previous workers (Jansakul *et al.*, 1987; Belachew Desta, 1994; Calderone *et al.*, 1999; Yalemtehay Mekonnen, 1999; Asfaw Debella, 2002). The filtered aqueous extracts were concentrated by lyophilization or freeze-drying, and powdery solid extracts were obtained after 24 hours. The filtered ethanol extracts

were allowed to evaporate their alcohol using a rotavapour at 40<sup>0</sup>C and semi-solid extracts with dark greenish brown colour were obtained. Finally after lyophilization and rotavapouring, sumtotals of 6 extracts with different yields were obtained. They were named (with respective percentage yields put in brackets) as ALW (2.8%), ARW (6.3%) ALE (7.7%), ARE (2%), RLW (6%) and RLE (7.5%). The extracts were stored at -20<sup>0</sup>C until used for experiments.

### **2.3. Experimental Animals**

Male and female animals of swiss albino mice (25-35 grams) and Guinea pigs (300-400 grams) of specific age were obtained from the animal house of the Department of Biology of Addis Ababa University. They were maintained under uniform conditions of light illumination and housed at a temperature of 24 ± 2.0<sup>0</sup>C. They were given a standard pellet diet and tap water *ad libitum*, based on previous works by other authors (Nath *et al.*, 1992; Shukla, 1996; Yalemtehay Mekonnen, 1999).

### **2.4. *In vitro* testing on Mouse Duodenum and Guinea pig Ileum**

The animals of either sex were killed by a chosen way: by dislocating the vertebrae (Mice) and by a stunning blow to the head and then bleeding by cutting the neck blood vessels (Guinea pig). The abdominal cavity of each animals were opened by midline incision everytime a tissue was required, and the particular tissue (duodenum or ileum) was removed immediately and cleaned of excess and attached tissues. The contents of the intestine were washed off with a physiological salt solution called Tyrode solution. The isolated tissue preparations were carried out according to the technique of Perry (1982) and Williamson *et al.* (1996).

Segments of duodenum or ileum of about 2-3cm in lengths were immediately removed from a freshly killed mouse and guinea pig fasted overnight respectively. They were tied with silk threads at both ends (ileum tied in opposite directions) and suspended in a thermoregulated 25ml organ bath, maintained at 37<sup>0</sup>C, containing a Tyrode solution of the following composition (g/l): NaCl, 8 gram; KCl, 0.2 gram; MgCl<sub>2</sub>, 0.1 gram; NaHCO<sub>3</sub>, 1 gram; NaH<sub>2</sub>PO<sub>4</sub>, 0.05 gram; Glucose, 1 gram; CaCl<sub>2</sub>, 0.2 gram. One end of the duodenum or ileum was attached to a tissue holder at the base of the organ bath and the other end to the isometric recording device. The tissues were constantly bubbled with air mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>. A suitable weight or resting tension of 1 gram was applied to the individual tissue, the method used by de la Puerta and Herrera (1995) and Galvez *et al.* (1996).

The suspended duodenum or ileum was allowed to equilibrate for 20-30 minutes before adding acetylcholine or histamine or the particular plant extract. After the initial equilibration period of 30 minutes, acetylcholine (in the case of duodenum) or histamine (in the case of ileum) was added to the organ bath in geometric series at final organ bath concentrations of 40, 80, 160 and 320 ng/ml (in the case of duodenum), 4, 8, 16, and 32 ng/ml (in the case of ileum) with a time interval of 3 minutes. Dose response relationships of ACh and histamine upon the tissues were done so as to choose the submaximal concentration. Each time the added acetylcholine or histamine was left in contact with the tissues for 30 seconds and then rinsed with Tyrode solution. The tissue was then left to resume its normal contraction. After a stabilized regular contraction, ACh (of 80 ng/ml) or histamine (of 8 ng/ml) was added and once again left in contact with the tissue (duodenum or ileum) for 30 seconds and then washed with the physiological solution. This was done each time before the addition of the specific extract (in the case of duodenum) and the addition of papaverine and specific extract (in the case of ileum) in order to observe their similar contractile or inhibitory effects. The

final organ bath concentrations that effected the submaximal stimulation of the tissue i.e. 80 ng/ml of ACh and 8 ng/ml of histamine were taken as controls in each experiment. After continual regular contraction, different doses of the particular plant extract (aqueous or ethanolic) of each *Artemisia* species (ALE, ARE, RLE, ALW, ARW, RLW) in a measured concentration were injected to the organ bath so that their effects would stay with the respective tissues for 5 minutes. Then the control ACh or histamine was added at the end of 5 min in the presence of the plant extract and then washed after 30 seconds. After rhythmic contraction of the tissue resumed, the control ACh or histamine was again added in order to establish the reversible contraction capacity of the tissue and also to test the subsequent concentration of the plant extract. The same procedure was repeated whenever different plant extracts at different final organ bath concentrations were tested. Each plant extract of the two *Artemisia* species was tested at final organ bath concentrations ranging from 20-200 µg/ml. At least in one of the experiments of each plant extract, 87.5 µg/ml of papaverine at final organ bath concentration of 0.375, 0.75 and 1.5 µg/ml was added as a positive control (antagonist) to an organ bath containing ileum before the addition of specific plant extracts. Solutions of plant extracts were made in physiological Tyrode salt solutions. On the other hand, stock solutions of all drugs (ACh, histamine, and papaverine) were made in distilled water and then serially diluted with physiological salt solution. The final dilutions of the drugs were made fresh on the day of the experiment.

Isometric contractions to ACh or histamine and papaverine or plant extracts were recorded with a Grass FT-03 strain gauge transducer coupled to a Grass 79 Polygraph which is equipped with preamplifier, main amplifier, oscillograph and time and event marker (Jansakul *et al.*, 1987; Belachew Desta, 1994; Yalemtehay Mekonnen, 1999).

### **2.5. *In vitro* testing on Mouse Uterine tissue**

The utero-contracting (uterotonic) activities of the plant extracts were tested on uterine strips of the experimental animal. Non-pregnant or virgin female albino mice were used for *in vitro* uterine preparation test. Each mouse was killed by cervical dislocation. The abdomen was opened by midline incision and the uterine horns or strips were severed at their junctions with the fallopian tubes, and placed in a dish containing a physiological salt solution called De Jalon's solution of the following composition (g/l): NaCl, 9 gram; KCl, 0.42 gram, NaHCO<sub>3</sub>, 0.5 gram; D-glucose, 0.5 gram; CaCl<sub>2</sub>, 0.06 gram. Approximately 1.5-2cm long uterine strip was cut and each strip was suspended in a thermostatically regulated 25ml organ bath containing a De Jalon solution that was maintained at 37<sup>0</sup>C and constantly gassed with air of 95% O<sub>2</sub> and 5% CO<sub>2</sub> (Uguru *et al.*, 1995; Williamson *et al.*, 1996). The two ends of the uterus were tied with silk threads and one end of the uterus strip was tied to the same transducer used before. A resting basal tension of 1 gram was applied to the uterine tissue and was allowed to equilibrate or stabilize for at least 30 minutes before adding the drug ACh or plant extract. Acetylcholine as a control drug and then various doses of plant extracts of the two *Artemisia* species were added to the organ bath as it was exactly done in the previous *in vitro* experiment. Solutions of the plant extracts were made in physiological salt solution of De Jalon.

### **2.6. Measurement of Tissue Responses by Polygraph machine**

Contraction peaks of duodenum, ileum and uterus recorded by the Polygraph were measured in centimetres. Contractions were measured as maximum changes in tension from pre-drug baseline within the contact time. The contraction peak of the control ACh (80 ng/ml) or histamine (8 ng/ml) was taken as a reference (100%). The peak made due to ACh or histamine alone was compared with that formed due to ACh or histamine in the presence of

the plant extract. The effect of the plant extract at a given bath concentration was then recorded by measuring the length of the peak due to the plant extract + ACh (or plant extract + histamine). Each value was then converted to a percentage contraction by considering a 100% ACh or histamine contraction. The responses of the tissues to the plant extracts (or to papaverine in cases of ileum) were calculated as per cent of the control values of either ACh or histamine obtained immediately before the addition of the plant extracts or papaverine. This method was adopted from previous workers (Clark and Sharma, 1969; Nkeh *et al.*, 1993,1996; Galvez *et al.*, 1996; Dar and Channa, 1999; Yalentsehay Mekonnen, 1999; Tubaro *et al.*, 2003).

## **2.7. Data Analysis**

Mean and standard error of mean (SEM) were calculated for each group of *in vitro* results on mouse duodenum, guinea pig ileum and mouse uterus. Significance of differences between the means was calculated by one way analysis of variance (ANOVA). This was followed by Scheffe post-hoc test (using SPSS, version 10.0) for dose response comparisons of the various plant extracts on the respective tissues. Values were expressed as the mean  $\pm$  SEM from six experiments of each dose of the extract. A 100% tissue contraction due to ACh or histamine were compared with the mean  $\pm$  SEM percentage tissue contraction due to ACh (histamine) + plant extract at each final organ bath concentrations. The differences between various doses of plant extracts on each tissue were considered significant if the p-value is 0.05.

### 3. RESULTS

To study the nature of spasmolytic or relaxant component of the plant extracts, isolated mouse duodenum and guinea pig ileum preparations were used which yielded similar results.

#### 3.1. *In vitro* effects on Mouse Duodenum ( MD )

Some of the tested extracts showed a considerable inhibition of ACh-induced contractions of duodenum and some showed only slight inhibitions. All the extracts (except ARW) significantly inhibited the ACh-induced duodenal contraction as compared with that evoked by ACh alone. ALE and RLE extracts showed great inhibitions very significantly ( $P < 0.05$ ,  $F = 90.5$  and  $146$  respectively). ARE and RLW extracts exhibited moderate inhibitions significantly ( $P < 0.05$ ,  $F = 33.7$  and  $32.6$  respectively). While ALW showed slight inhibition ( $P < 0.05$ ,  $F = 12.3$ ), ARW exceptionally did show a slight spasmogenicity ( $P < 0.05$ ,  $F = 13.4$ ). However, the initial mild stimulatory effect by ARW was neither dose-dependent nor reproducible, and disappeared after repeated administration of the subsequent doses of the plant extract. The dose-dependent effects of both ethanolic and aqueous extracts of *Artemisia afra* and *Artemisia rehan* expressed as the mean % contraction  $\pm$  SEM are given in Table 1. As shown in the table, all the extracts significantly reduce the ACh induced duodenal contractions in a concentration-related manner. Ethanol extracts, particularly ALE and RLE were found to show the most inhibitory contractions of the isolated mouse duodenum reaching a maximum of  $44.3 \pm 0.9$  (at a dose of  $160 \mu\text{g/ml}$ ) and  $35 \pm 1.8$  (at a dose of  $120 \mu\text{g/ml}$ ) inhibitory contractions respectively. These extracts reduced the maximal responses to ACh in this tissue by 55.7% and 65% respectively at those higher organ bath concentrations. The dose dependent changes of these extracts are also much more pronounced. For instance, ALE, at dose ranges of 40-160  $\mu\text{g/ml}$ , reduced 22-56% of the ACh-induced contractions, and

RLE, at dose ranges of 20-120  $\mu\text{g/ml}$ , reduced 25-65% of ACh-induced contraction of the mouse duodenum.

Table 1. The effect of extracts of *Artemisia afra* and *Artemisia rehan* on ACh-induced contraction of Mouse Duodenum (MD).

Type of Extracts	Group	Extract conc.( $\mu\text{g/ml}$ )	Contractile response (%)	F- value	
ALE	a	1	40	77.6 $\pm$ 0.9* <sup>2a,3a,4a</sup>	90.5
		2	80	68.2 $\pm$ 2.0* <sup>4a</sup>	
		3	120	67.9 $\pm$ 1.8* <sup>4a</sup>	
		4	160	44.3 $\pm$ 0.9	
ALW	b	1	40	98.8 $\pm$ 3.3* <sup>3b,4b</sup>	12.3
		2	80	97.3 $\pm$ 4.2* <sup>3b,4b</sup>	
		3	120	79.8 $\pm$ 3.4	
		4	160	77.1 $\pm$ 1.4	
ARE	c	1	40	90.3 $\pm$ 1.2* <sup>3c,4c</sup>	33.7
		2	80	85.8 $\pm$ 1.3* <sup>3c,4c</sup>	
		3	120	71.8 $\pm$ 2.8	
		4	160	68.5 $\pm$ 1.6	
ARW	d	1	40	87.2 $\pm$ 0.7* <sup>4d</sup>	13.4
		2	80	80.8 $\pm$ 2.2* <sup>4d</sup>	
		3	120	84.5 $\pm$ 0.7* <sup>4d</sup>	
		4	160	94.2 $\pm$ 1.8	
RLE	e	1	20	75.1 $\pm$ 0.7* <sup>2e,3e,4e</sup>	146
		2	40	60.5 $\pm$ 1.9* <sup>3e,4e</sup>	
		3	80	39.3 $\pm$ 1.5	
		4	120	35.0 $\pm$ 1.8	
RLW	f	1	20	104.5 $\pm$ 3.1* <sup>3f,4f</sup>	32.6
		2	40	92.5 $\pm$ 0.8* <sup>3f,4f</sup>	
		3	80	77.0 $\pm$ 0.8	
		4	120	66.6 $\pm$ 3.9	

Control ACh = 80 ng/ml, n = 6 for Mouse Duodenum

Responses were expressed as % of the initial contractions induced by spasmogen ACh prior to the addition of the plant extracts. Data are Mean  $\pm$  SEM of six duodenum preparations.

Significant relative to different extract concentrations \* P < 0.05.

Sample Polygraph tracings showing the effect of ALE and RLE on ACh-induced contractions of mouse duodenum for a 5-minute contact time are given in Figure 1 and Figure 2. For lower doses (20 and 40  $\mu\text{g/ml}$ ), the inhibitory effects were partially reversible and the spontaneous rhythmic contractions of the duodenum returned to normal after washing the tissue a couple of times. In most of the experiments, higher doses (eg. 120-160  $\mu\text{g/ml}$ ) completely abolished the rhythmic spontaneous and ACh-induced contractions irreversibly i.e. the tissues were not able to recover to their normal tones even after repeated washings.

The dose-response curve (line graph) of the extracts ALE, ARE, RLE, RLW along with the control ACh on mouse duodenum was shown in Figure 3. RLE followed by ALE showed the most significant inhibition of duodenal contraction as compared with that of the control ACh alone. They showed greater inhibitory contractions as the dose increases from 40-120  $\mu\text{g/ml}$ . ARE and RLW showed moderate inhibitions as the dose increases.

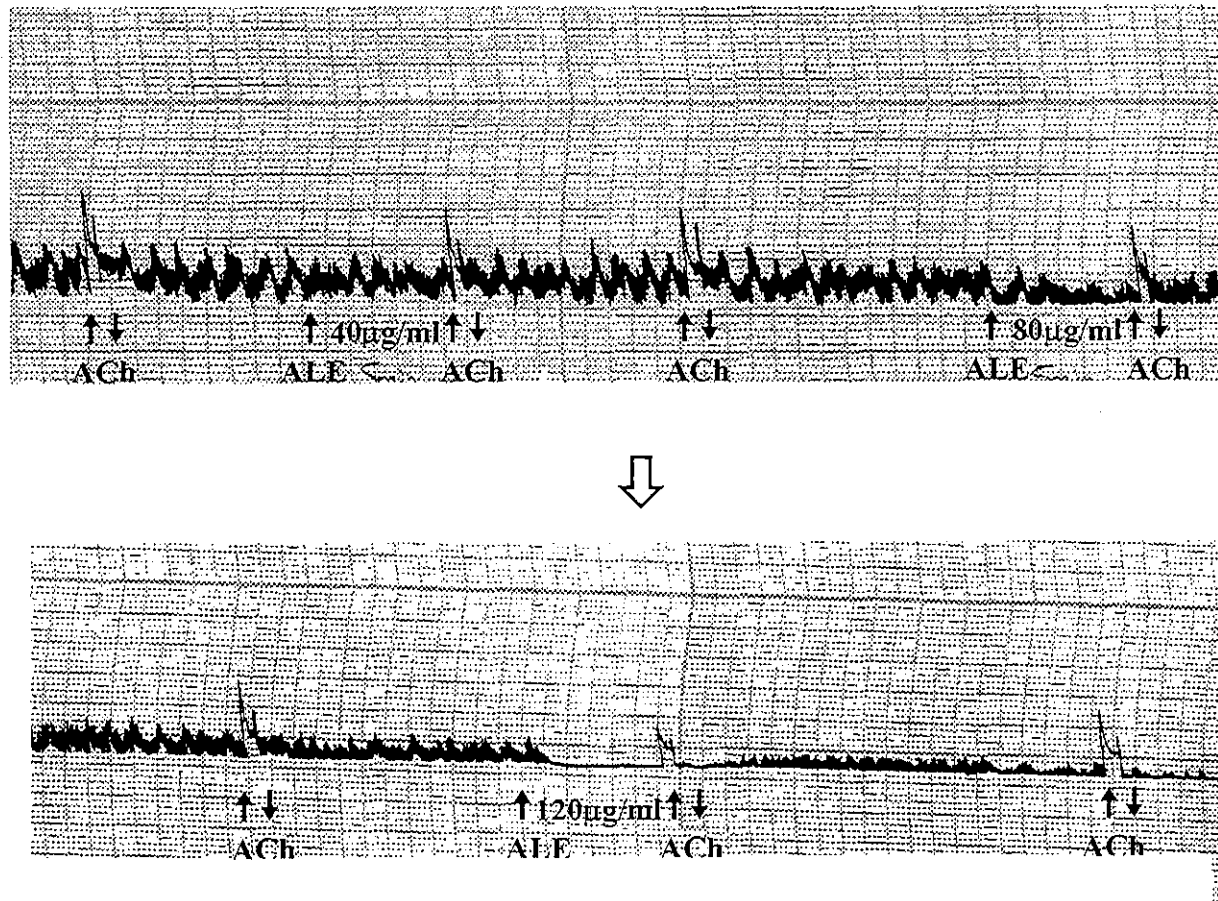


Figure 1. Polygraph tracing showing the effect of ALE on ACh-induced contraction of isolated mouse duodenum at different organ bath concentrations. ↑ shows the addition & ↓ shows the washing out of ACh and the extract. The concentrations expressed in  $\mu\text{g/ml}$  are final organ bath concentration values of the extract. The big arrow in the middle shows continuation. Each square represents 5mm and the chart speed of Polygraph runs at 5mm/min.

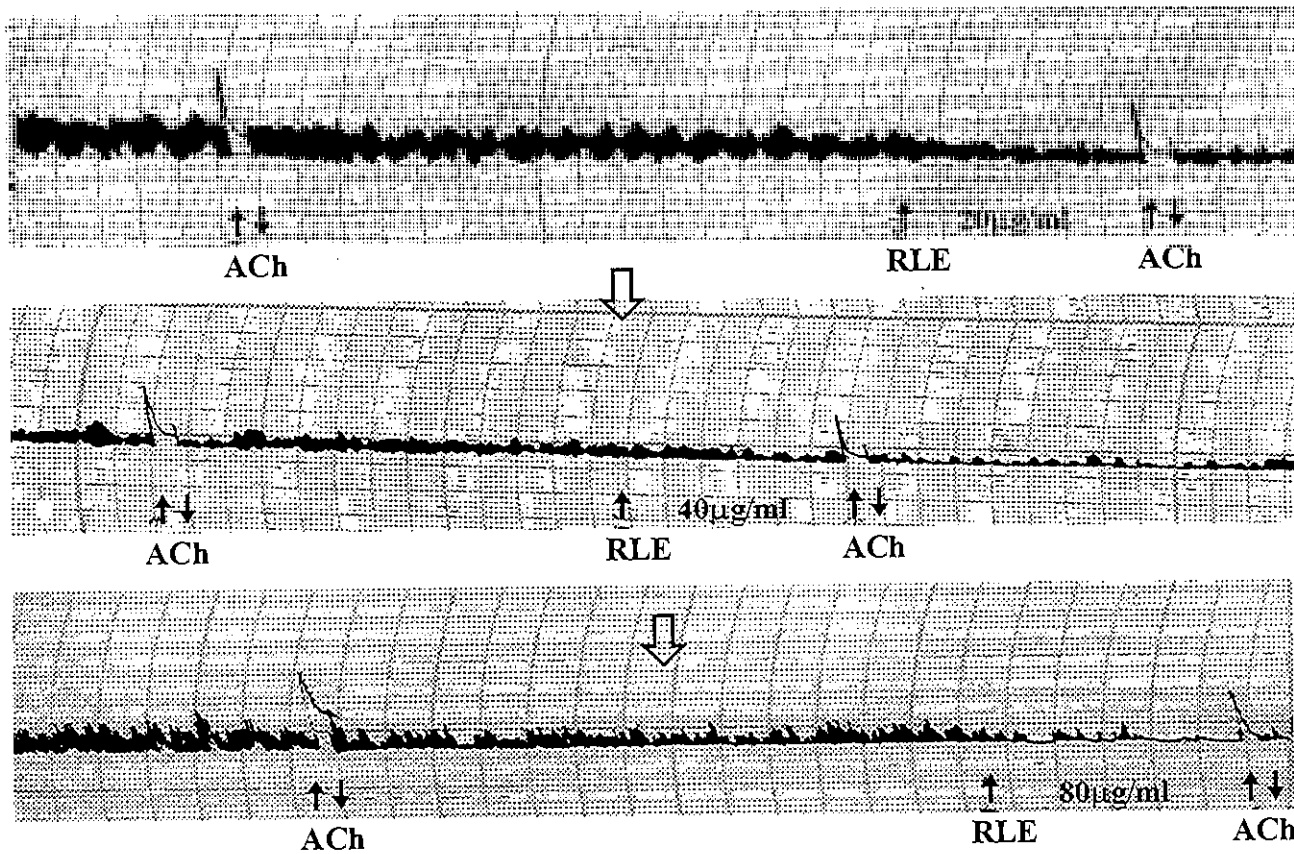


Figure 2. Polygraph tracing showing the effect of RLE on ACh-induced contraction of isolated mouse duodenum at different organ bath concentrations. ↑ shows the addition & ↓ shows the washing out of ACh and the extract. The concentration expressed in µg/ml are the final organ bath concentration values of the extract. The big arrow in the middle shows continuation. Each square represents 5mm and the chart speed of Polygraph runs at 5mm/min

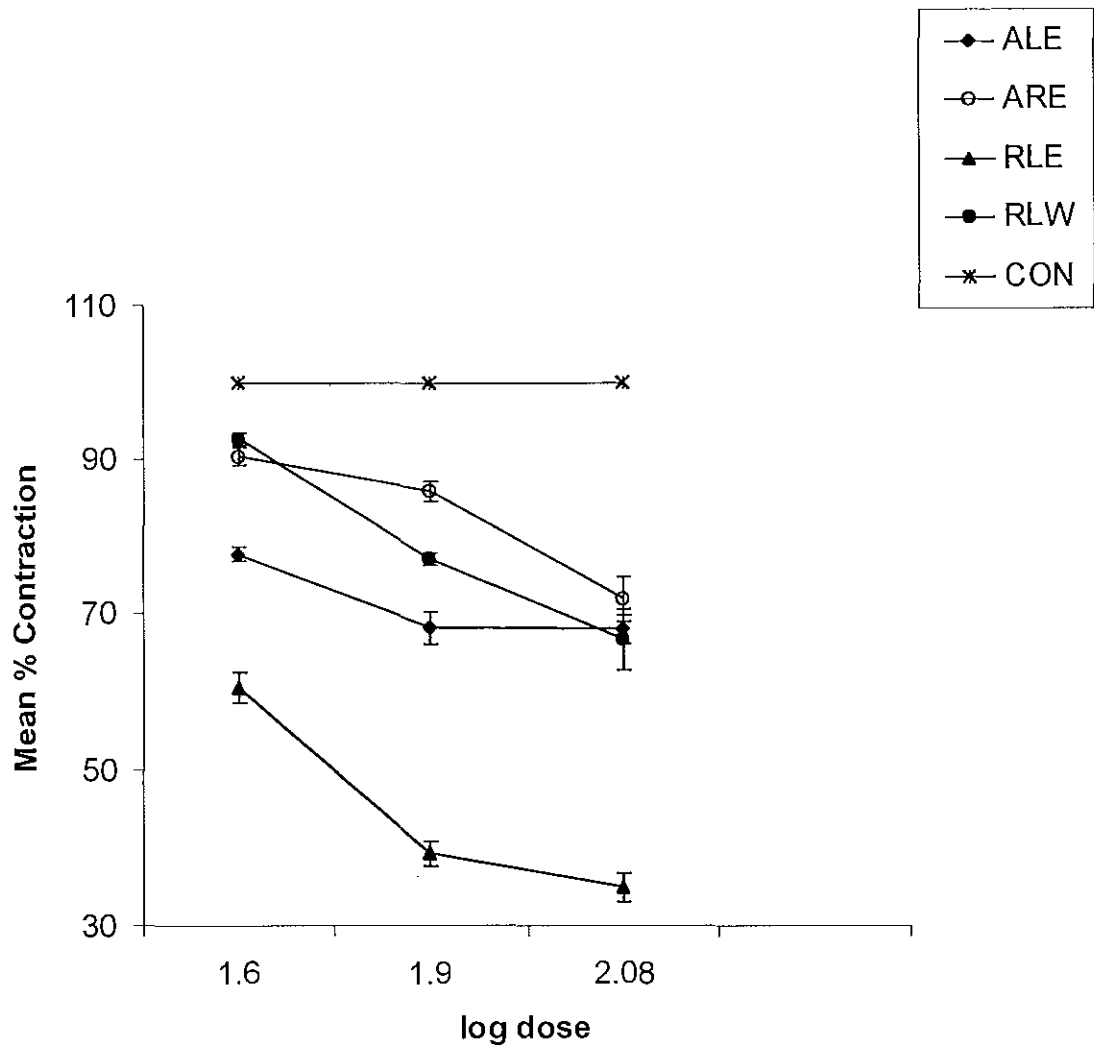


Figure 3. Dose-response curve (line graph) showing the mean percentage contraction recorded by ACh in the presence of the extracts (ALE, ARE, RLE, and RLW) at different organ bath concentrations as compared with the control ACh alone on isolated mouse duodenum. Vertical bars represent standard errors of the mean. RLE and ALE showed greater decreases in ACh-induced contraction as the dose increases.

### 3.2. *In vitro* effects on Guinea pig Ileum (GPI)

As in the case of mouse duodenum, some of the tested extracts showed considerable inhibitions and some only slight inhibitions of the histamine-induced contractions of GPI. All extracts except ARW showed a dose-dependent inhibition of histamine-induced contractions as compared with that evoked by histamine alone. The dose-dependent effects of ethanolic and aqueous extracts of *A. afra* and *A. rehan* extracts on GPI expressed as the mean % contraction  $\pm$  SEM values are given in Table 2. As shown in the table, inhibition of the control histamine (8 ng/ml) induced contractions was carried out by different concentrations of the extracts ranging from 80-200  $\mu$ g/ml. The extracts ALE, ALW, ARE, RLE and RLW caused concentration dependent inhibitions significantly ( $P < 0.05$ ,  $F = 53, 35.2, 36.1, 88.4$  and  $44.4$  respectively). ARW did not show any significant effect though it showed a bit tendency of contractility or spasmogenicity ( $P > 0.05$ ,  $F = 7.1$ ). ALE and RLE resulted in a greater inhibition of histamine-induced ileal contractions at the afore-mentioned dose regimens. They were capable of reducing contractions reaching a maximum of  $60.9 \pm 2.7$  and  $43.5 \pm 2.7$  inhibitory contractions respectively at higher bath concentrations (200  $\mu$ g/ml). That is, they reduced the maximal responses to histamine in GPI by 39% and 56% respectively. Between dose regimens of 80-200  $\mu$ g/ml, ALE reduced 11-39% and RLE reduced 10-56% of the histamine-induced contractions.

Here, papaverine (a smooth muscle relaxant) was used as a positive control to investigate the comparable relaxant effectiveness of the extracts on GPI at the respective dosages. The dose-dependent effects of papaverine (at doses of 0.375, 0.75 and 1.5  $\mu$ g/ml of final organ bath concentrations) were applied for a contact time of 5 minutes before the addition of histamine. Their effects expressed as the mean % contraction  $\pm$  SEM values were expressed in Table 3. It

was found that all the extracts except RLE and ALE were less potent than the papaverine. RLE (at dose 200  $\mu\text{g/ml}$ ) is approximately 1.5 fold more potent than the most antagonist papaverine (1.5  $\mu\text{g/ml}$ ) used in the experiment. ALE is equivalent in its relaxant potency to papaverine at the respective doses.

Table 2. The effect of extracts of *A. afra* and *A. rehan* on His-induced contraction of GPI  
(Guinea pig Ileum)

Type of Extracts	Group	Extract conc. ( $\mu\text{g/ml}$ )	Contractile response (%)	F- value	
ALE	a	1	80	88.8 $\pm$ 0.6 <sup>*3a,4a</sup>	53
		2	120	82.7 $\pm$ 0.7 <sup>*4a</sup>	
		3	160	77.2 $\pm$ 1.9 <sup>*4a</sup>	
		4	200	60.9 $\pm$ 2.7	
ALW	b	1	80	103.8 $\pm$ 4.6 <sup>*3b,4b</sup>	35.2
		2	120	91 $\pm$ 1.9 <sup>*4b</sup>	
		3	160	77.6 $\pm$ 1.2	
		4	200	68.4 $\pm$ 1.2	
ARE	c	1	80	96.9 $\pm$ 4.6 <sup>*3c,4c</sup>	36
		2	120	89.7 $\pm$ 1.7 <sup>*3c,4c</sup>	
		3	160	80.4 $\pm$ 1.3	
		4	200	73.3 $\pm$ 1.7	
ARW	d	1	80	93.2 $\pm$ 1.3 <sup>*3d,4d</sup>	7.1
		2	120	100.8 $\pm$ 2.8	
		3	160	106.4 $\pm$ 3.9	
		4	200	111.7 $\pm$ 3.3	
RLE	e	1	80	90.4 $\pm$ 1.9 <sup>*2e,3e,4e</sup>	88.4
		2	120	70.9 $\pm$ 2.5 <sup>*3e,4e</sup>	
		3	160	59.3 $\pm$ 0.3 <sup>*4e</sup>	
		4	200	43.5 $\pm$ 2.7	
RLW	f	1	80	97.1 $\pm$ 2.0 <sup>*2f,3f,4f</sup>	44.4
		2	120	88.9 $\pm$ 1.6 <sup>*3f,4f</sup>	
		3	160	80.2 $\pm$ 0.5 <sup>*4f</sup>	
		4	200	69 $\pm$ 2.5	

Control His = 8 ng/ml of final organ bath concentration, n = 6 for GPI

Responses were expressed as % of the initial contractions induced by agonist histamine prior to the addition of the plant extracts. Data of contractile response were expressed as mean  $\pm$  SEM of 6 guinea pig ileum preparations. Significant relative to different extract concentrations: \* P< 0.05

Table 3. The effect of papaverine on Histamine-induced contraction of GPI

Pav conc. ( $\mu\text{g/ml}$ )		Contractile response (%)	F- value
1	0.375	$88.0 \pm 1.8$ * <sup>2,3</sup>	28.3
2	0.75	$75.7 \pm 2.9$ * <sup>3</sup>	
3	1.5	$63.3 \pm 2.2$	

Control His = 8 ng/ml of final organ bath concentration.

Responses were expressed as % of the initial contractions induced by agonist histamine prior to the addition of the antagonist papaverine. Data of contractile responses are expressed as mean  $\pm$  SEM of 6 guinea pig ileum preparations. Relative significant to different Pav concentrations: \* P< 0.05

The sample Polygraph tracings of the effect of ethanolic extract RLE on isolated GPI for a 5 minute contact time are given in Figure 4. Higher doses (160-200  $\mu\text{g/ml}$ ) of this extract decreased and finally completely abolished the spontaneous and histamine-induced contractions of the ileum. The inhibitory effect of the extract was fully reversible since the contractions resumed after repeated and intermittent washings of the ileal preparations with the physiological Tyrode solution. The Polygraph tracings of the effect of aqueous extract ARW on GPI for a 5 minute contact time are given in Figure 5. The extract ARW initially caused mild to high stimulatory effect with a sustained contraction, and this behavior did not change during the 5-min contact time with the tissue, which is suggestive of the presence of some spasmogenic component. The histamine-induced contractions were also potentiated by the extract when it was observed from the baseline.

The dose response curve (line graph) of the GPI to histamine alone (control) and to histamine in the presence of the extracts like ALE, ARE, RLE and RLW was shown in Figure 6. Here again, RLE and ALE showed greater inhibitions while ARE and RLW exhibited moderate inhibitions of the ileum as the dose increases from 80-200  $\mu\text{g/ml}$ .

### **3.3. *In vitro* effects on Mouse Uterus (MU)**

The *in vitro* experiment on MU tissue did not give a good result. Not any one of the extracts used gave significant uterine contractions or relaxations.

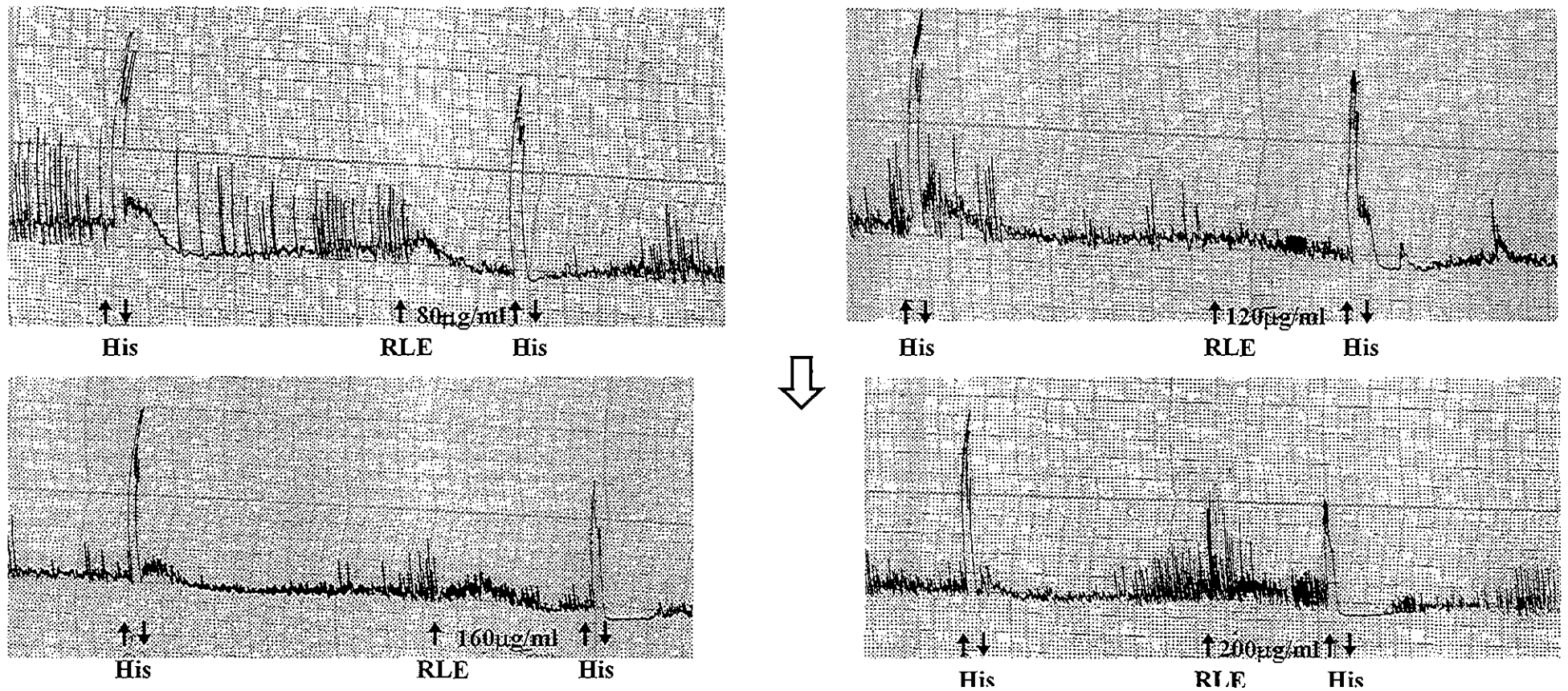


Figure 4. Polygraph tracing showing the effect of RLE on histamine-induced contraction of isolated guinea pig ileum in the absence and presence of RLE extract at different organ bath concentrations.  $\uparrow$  shows addition and  $\downarrow$  shows washing out of histamine and extract. The big arrow in the middle shows continuation. Each square represents 5mm and the chart speed of Polygraph runs at 5mm/min.

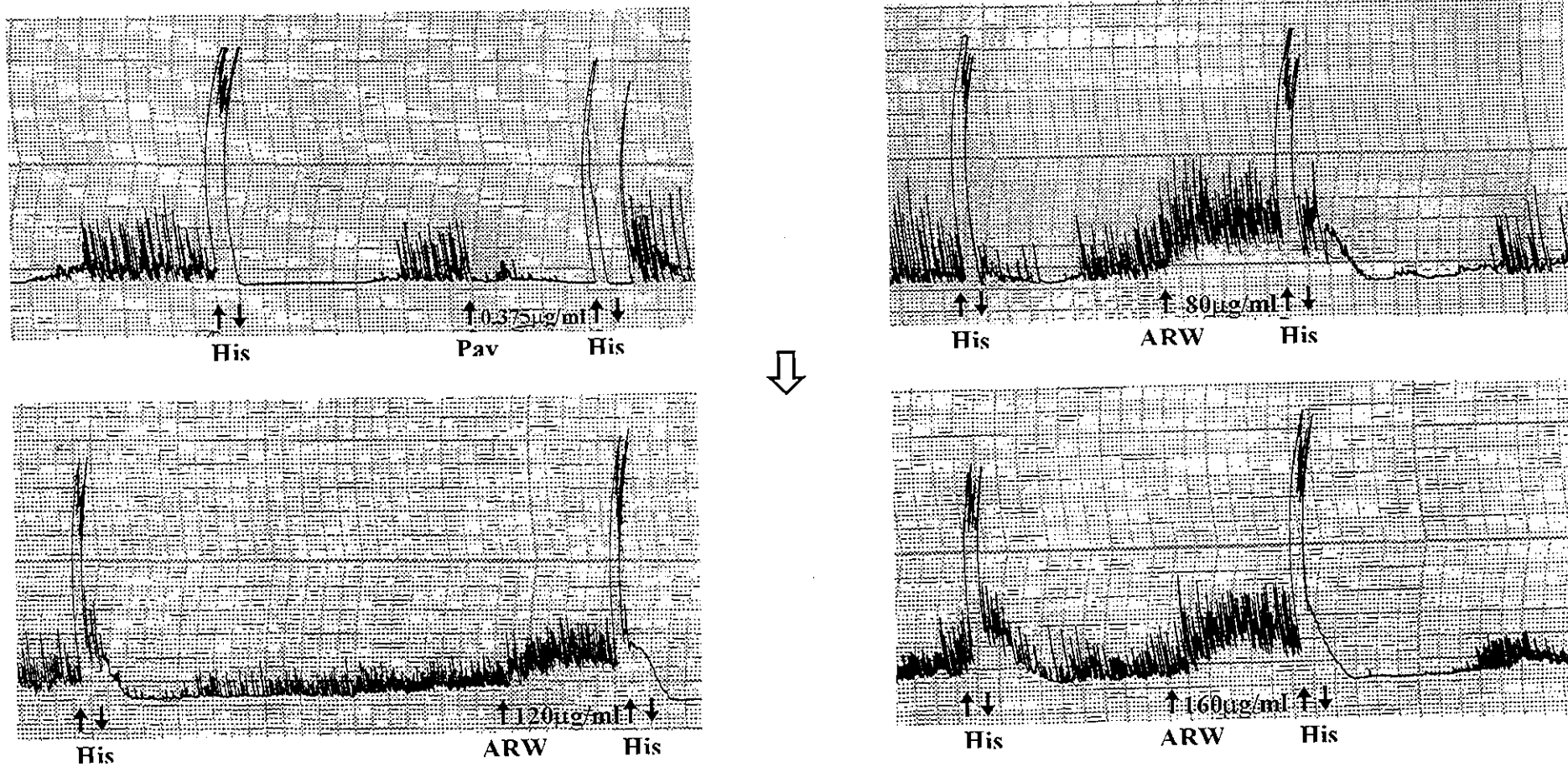


Figure 5. Polygraph tracing showing the effect of ARW on histamine-induced contraction of isolated Guinea pig ileum in the absence and presence of ARW extract at different organ bath concentrations.  $\uparrow$  shows addition and  $\downarrow$  shows washing out of histamine & extract. The big arrow in the middle shows continuation. Each square represents 5mm and the chart speed of polygraph runs at 5mm/min.

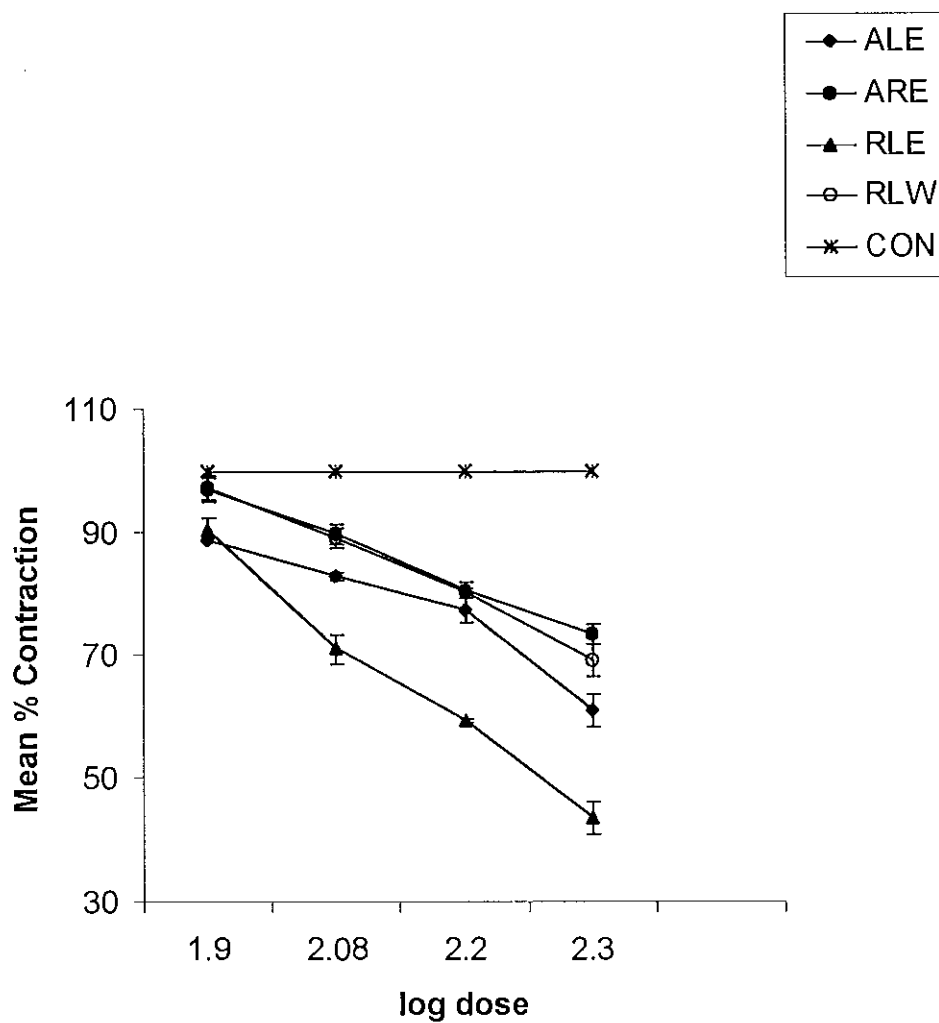


Figure 6. Dose-response curve (line graph) showing the mean percentage contraction recorded by histamine in the presence of the extracts (ALE, ARE, RLE, and RLW) at different organ bath concentrations as compared with the control histamine alone on isolated GPI. Vertical bars represent standard errors of the mean. RLE and ALE have shown greater decreases in His-induced contraction as the dose increases.

#### 4. DISCUSSION

On Mouse duodenum and Guinea pig ileum experiments, leaf ethanol extracts of *A. afra* and *A. rehan* (ALE and RLE) showed more inhibition of ACh and histamine induced contractions of the respective tissues than any of the other extracts. The inhibitory activities of all extracts except ARW were also significant in a dose dependent manner from 20 - 200 µg/ml (Table 1 and Table 2). These results were depicted as parts of spasmolytic properties. Similar results were reported for closely related plants of *Artemisia* by different investigators. As reported by Abu Zarga *et al.* (1995) the aqueous extract of aerial parts of *Artemisia arborescens* caused a concentration dependent reduction in the amplitude of phasic contractions and tone of the isolated rat ileum. Hydromethanolic extract of the whole parts of *Artemisia scoparia* were found to result in a concentration dependent inhibition of the spontaneous movement of the rabbit jejunum reaching a 30% relaxant at a dose of 300 µg/ml (Gilani *et al.*, 1994b). The aqueous crude extracts of *Artemisia verlotorum* Lamotte herb was investigated by Martinotti *et al.* (1997) to have inhibitory effects on guinea pig and rat ileum preparations *in vitro*. The flavonoids in *Artemisia judaica* also inhibited the amplitude of phasic and tonic contractions of the GPI in the same manner (Abdalla and Abu Zarga, 1987).

Less polar solvents like ethanol and methanol were found to be the most effective in showing spasmolytic effects more readily on tissues. For instance, ethanol extract of *Capparis cartilaginea* (Gilani and Aftab, 1994) and pure compounds from leaf ethanol extract of *Moringa oleifera* (Gilani *et al.*, 1994a), were found to have inhibitory effects on ACh, histamine and serotonin induced contractions of isolated ileum in a concentration related manner. This is more or less coinciding with the findings for ALE, ARE and RLE of *A. afra* and *A. rehan* species in the present study. On the other hand, the more polar solvents (like water) employed for extraction were turned out to be the most effective in exhibiting

spasmogenic effects. For example, Gilani *et al.* (1999) proved that the spasmogenic component of crude extract of *Sida pakistanica* being separated in aqueous fraction whereas its spasmolytic component being concentrated in the ethylacetate fraction. In the present study the aqueous extract of *A. afra* root (ARW) showed a spasmogenic activity in a dose dependent manner (with reproducible result on GPI but not on MD) while the ethanol extracts of the plant leaves and roots showed the opposite effect. The different parts of the plant used may also be attributable to the spasmolytic or spasmogenic effects of the extract. Caceres *et al.* (1992) found out that only the seed part of *Moringa oleifera* had shown significant antispasmodic activity on the intestinal spasm of rat duodenum while the other parts (leaves, root, stalk and flowers) hadn't. The leaf extract of *A. afra* (ALW) and *A. rehan* (RLW) had spasmolytic effect on MD and GPI but the root extract of *A. afra* had spasmogenic effect.

The aqueous extracts ALW (on GPI) and RLW (on MD) seemed to have spasmogenic (contractile) effects at lower doses (80 µg/ml and 20 µg/ml respectively) but they became obviously more spasmolytic (relaxant) at higher doses. Similarly, there is a profile of such effects of crude extracts of certain other medicinal plants. Fehri *et al.* (1995) showed that dried leaf extracts of *Olea europea L.* exerted two opposite effects on basal tone of rat isolated ileum and mouse duodenum characterized by a contraction at lower doses (0.1-100 µg/ml) and a relaxation at higher doses (300-3000 µg/ml). Gilani *et al.* (1999) also recently reported that the crude ethanolic extract of *Sida pakistanica* exerted two antagonistic effects on isolated guinea pig ileum and rabbit jejunum, which is characterized by a mild spasmogenic effect at low concentration ranges of 100-300 µg/ml, and spasmolytic effect at high concentration ranges of 5000-10,000 µg/ml. The methanol extract of roots of *Lasiosiphon kraussianus* had no effect on the contractions of the ileum at lower doses but

resulted in slight inhibition up to complete abolishment of rhythmic contractions of ileum at higher doses (Ebong and Nwude, 1981).

The partial to complete abolishment of spontaneous and rhythmic contractions of both tissues (GPI and MD) even after many washings gave a solid basis for the spasmolytic property of most of the extracts at higher doses. According to Yalemtehay Mekonnen (1999) spontaneous and rhythmic contractions of both MD and GPI were abolished by leaf ethanol extract of *Moringa stenopetala* with increasing extract concentration. In most cases of the present MD experiments (e.g. Figure 1 and Figure 2), the tissue failed to recover for a longer period of time after the addition of maximal doses of the extracts. This effect of the extracts could be attributed to the nature of antagonism, which may be irreversible binding of the extracts, or may be an action interfering with various metabolic processes such as cAMP. As for GPI experiments of the present study (e.g. Figure 4 and Figure 5), the recovery of the tissue took a short period of time, which may be suggestive of reversible binding of the extracts.

The results in the *in vitro* MD and GPI experiments (Table 1 and Table 2) revealed that crude extracts of *A. afra* and *A. rehan* must have active chemical constituents with possible spasmolytic effects. Previous investigators supported this possibility for closely related species of *Artemisia*. Bergendorff and Sterner (1995) reported that flavonoids are the most active chemical constituents of most *Artemisia* species to bring about the general spasmolytic actions in isolated smooth muscle preparations. According to these scientists, four flavonols with spasmolytic activity were isolated from the aerial parts of *Artemisia abrotanum*. Previously, Abdalla and Abu Zarga (1987) had reported about the flavone cirsimartin, isolated from *Artemisia judaica*, *Artemisia capillaris*, *Artemisia xerophytica* and *Artemisia scoparia*

that exerted similar spasmolytic effects on GPI. Rutin as one of the flavonoids in *Artemisia scoparia* was also found to cause a concentration dependent inhibition of spontaneous movements of rabbit jejunum (Gilani *et al.*, 1994b). Flavonoids, which are responsible for smooth muscle relaxation, are also common in other plants that are not closely related to *Artemisia*. Flavone luteolin, isolated from *Colchicum richii* caused a concentration dependent relaxation of the tone of the ileum (Abdalla *et al.*, 1994). Quercetin, one of the flavonoids isolated from the aerial parts of *Conyza flaginoides* exerted inhibitory effects on GPI contractile response, and also caused a concentration dependent inhibition of the spontaneous contractions of rat ileum (Galvez *et al.*, 1996; Mata *et al.*, 1997). The distinct relaxant effect of *Satureja obovata* varieties on the isolated rat duodenum was suggested to be the polar compounds, flavonoids (Sanchez de Rojas *et al.*, 1994). According to Harborne and Williams (2000) and Karamenderes and Apaydin (2003) the antispasmodic effect of total extract of *Achillea nobilis L.* on rat duodenum, was investigated to be due to the inhibitory effects of some flavonoids in it. These flavonoids are responsible for other useful medicinal properties in the folk medicine. Lozoya *et al.* (2002) reported about the relationship of the spasmolytic, antimotility and antidiarrhoeic activity of *Psidium guajava folia* extract with its content in quercetin flavonoids. The significant antidiarrhoeal activity of the methanolic fraction of unripe fruits of *Psidium guajava* extract was also connected to the flavonoids that inhibit ACh release in GI tract (Lutterodt, 1989 cited in Ghosh *et al.*, 1993).

The crude extracts may also get their inhibitory effect from the composition of essential oils in the plant. For instance, the spasmolytic activity of essential oil of related species, *Artemisia thuscula Cav.* flowers (Perfumi *et al.*, 1995) and *Artemisia alba* (Perfumi *et al.*, 1999) were previously investigated and shown to have dose dependent and essentially non-competitive spasmolytic effects in GPI. *Artemisia afra* and *Artemisia rehan* of the present study have also

got their own essential oil composition which may be responsible for biological activities like antispasmodics (Paulos G.Yohannes, 1980; Berhanu Abegaz and Paulos G.Yohannes, 1982; Graven *et al.*, 1990; Tadele Worku and Rubiolo, 1996). Since total crude extracts were used, it is also possible that the relaxant action of the plants may be linked to the combined effect of several chemical constituents in a synergistic way. For instance, the *in vitro* antispasmodic effect of the hydroalcoholic extract of *Pycnocyla spinosa* on ileum contractions were suggested to be due to components in the alkaloid fraction to a greater extent, flavonoid-rich fraction to a lesser extent, and saponin-rich fraction to the least extent (Sadraei *et al.*, 2003a). The antispasmodic effect of polyherbal preparation, SJ-200 was also ascribed to such combination of active chemical constituents (Venkantaranganna *et al.*, 2002). This could be similar to the presently studied *Artemisia* species. All in all, the antispasmodic effects exerted by them on the GPI and MD *in vitro* may be due to one or more of the previously mentioned reasons.

In the GPI experiment (comparing Table 2 and Table 3), extract RLE (200 µg/ml) was tested to have 1.5 more times antispasmodic potency than papaverine (1.5 µg/ml), and the extract ALE (200 µg/ml) with an equivalent spasmolytic potency to papaverine (1.5 µg/ml). This must be the consequence of the most active chemical constituents of the extracts that may be responsible for a greater or similar antispasmodic potency with that of papaverine. This finding is supported by other investigators. For instance, the compounds rutamarin and arborinine, isolated from *Ruta graveolens* were found to have antispasmodic effect on isolated ileum with a comparable antispasmodic potency to that of papaverine (Minker *et al.*, 1979). The smooth muscle relaxant, onitin which is isolated from *Onychium siliculosum* inhibited the contractions of GPI provoked by agonists like ACh, serotonin, K<sup>+</sup> and Ba<sup>2+</sup> by showing a non-specific antispasmodic action like papaverine (Ho *et al.*, 1985; Yang, 1986). There is also a

report that pterosins such as onotisin, otinoside along with onitin as having smooth muscle relaxant properties like papaverine (Sheridan *et al.*, 1999). Methyleugenol, an essential oil component of many aromatic medicinal plants, exerted a concentration dependent reversible relaxant and antispasmodic effects on GPI similar to that of well-characterized smooth muscle relaxant papaverine (Lima *et al.*, 2000). Considering the fact that the extracts used in the present study are in total crude forms and possibly contain many active chemical constituents, ALE and RLE extracts were expected to show equivalent or even more antihistaminergic effects than is papaverine.

The contractions of smooth muscle preparations including GPI, MD and MU are dependent upon an increase in the cytoplasmic free  $[Ca^{2+}]_i$  which activates the contractile elements (Endo, 1977; Karaki and Weiss, 1984). In general, tonic and phasic contractions of these tissues induced by ACh and histamine are dependent upon  $Ca^{2+}$  influx from extracellular  $Ca^{2+}$  medium, or  $Ca^{2+}$  release from intracellular  $Ca^{2+}$  stores, or due to membrane depolarization (Bolton, 1979; Brading and Sneddon, 1980). It is also known that the contraction of the gut muscle GPI is mediated through  $H_1$  histamine receptors (Black *et al.*, 1972; Hill, 1990) and that of gut muscle MD is mediated through  $M_2$  and  $M_3$  muscarinic receptors (Eglen *et al.*, 1996; Broadley and Kelly, 2001). Bolton and Kitamura (1983) reported a striking relationship between smooth muscle contractions (whether phasic or tonic) and  $Ca^{2+}$  influx during activation of muscarinic and histaminergic receptors. Therefore, binding of histamine to  $H_1$  receptors (GPI) and ACh to  $M_2$  and  $M_3$  receptors (MD) results in the opening of ROCs (for  $Na^+$  influx) and then VDCs (for  $Ca^{2+}$  influx). The present findings suggest that relaxant effects of the plant extracts on MD and GPI may be attributed mainly to the inhibition of  $Ca^{2+}$  entry from extracellular medium via these channels in the membrane of the smooth muscles since the tonic contractile effects induced by ACh and histamine were reduced by the plant

extracts. The inhibition of spontaneous movements as well as agonist-induced contractions of the respective tissues by the extracts (except ARW) may also be due to the interference with  $\text{Ca}^{2+}$  release from intracellular stores, or with depolarization process, or with one of the multiple biochemical processes associated with the influx of  $\text{Ca}^{2+}$  into the smooth muscles (Sanchez de Rojas *et al.*, 1994). One or more of these mechanisms may decrease the  $\text{Ca}^{2+}$  concentration available for contractile machinery so that the tissues show those spasmolytic effects. It is also possible that the extracts act directly on the smooth muscle of the gut by partially or reversibly blocking the contractile effects on ACh and histamine (Nkeh *et al.*, 1993,1996). Since the inhibitory effects of the plant extracts on MD are long-lasting and sometimes irreversible upon washout of the extracts and ACh, it is likely that the plant extracts impair energy production in the smooth muscle. However, the inhibitory effects of the plant extracts on GPI are rapid and readily reversible upon washout of the extract and histamine and it is unlikely that the plant extracts impair energy production in GPI. The spasmolytic agents in the extracts may act on muscarinic  $\text{M}_2$  and  $\text{M}_3$  receptors (in MD) and histamine  $\text{H}_1$  receptors (in GPI). Consequently, the plant extracts may irreversibly bind to the muscarinic receptors of MD but may reversibly bind to the histamine receptors of GPI.

As the histamine-induced responses (in GPI) were reduced in the presence of the plant extracts, the possibility of interaction of plant extracts with intracellular  $\text{Ca}^{2+}$  ions cannot be ruled out. This raises a question, whether the antispasmodic activity of extracts is mediated through the mechanisms of  $\text{Ca}^{2+}$  channel blockade since calcium channel blockers such as verapamil are well known to be antispasmodics (Gilani *et al.*, 1994b; Brunton, 1996 cited in Gilani *et al.*, 2000).

The *in vitro* experiment on mouse uterine tissue gave us non-significant results. The lack of either significant spasmolytic or spasmogenic effects of all the extracts on MU might be due to various factors. Appropriate extractive solvents might not be used to get the above effects. Moreover, the contractile and relaxant effects of the crude extracts may be best characterized in a quiescent smooth muscle preparation such as GPI but not in the MU with spontaneous contractions at 37°C (Tsai and Ochillo, 1991 cited in Gilani *et al.*, 1999). That may be why the aqueous extract ARW clearly showed a pattern of spasmogenic or contractile effect during the 5-min contact time with the ileal tissue (Figure 5) while not showing any significant effect with uterine tissues. The slight or no effect of the extracts on mouse uterine contractions may also be due to the crudeness of the extracts which in turn may contain many different components, or due to the antagonistic activity of one or more of the active principles in the crude extracts on uterine muscarinic receptors which may result in the inactivation of ROCs in smooth muscles. Therefore, it is difficult to say that there is antifertility effect in the real case.

On the other hand, the observation that the extracts showed little stimulation of mouse uterine contraction *in vitro* although they are used to induce labour in humans may be due to species variability. Pettibone *et al.* (1990) reported that significant species differences exist between rodent and human uterus in the oxytocin receptor binding affinity of certain compounds related to oxytocin. Alternatively, it is also possible that the extracts may contract pregnant mouse uterus *in vivo*, though not non-pregnant or virgin mouse uterus *in vitro*. A cholinergic uterine nerve supply are at most sparse in isolated uterine preparations (Bell, 1972). It can also be said that the slight uterotonic or oxytotic activity of the extracts is more or less in consistent with the pharmacological findings of *A. afra* by Belachew Desta (1994). However, the butanol fraction of *A. afra* was investigated to have a positive anti-implantation effect

which implies that other less polar solvents have to be used in order to check whether or not much significant contractile effects could be achieved. So other extraction procedures such as with butanol should be tried out before the folkloric antifertility use of *A. afro* is rejected once and for all.

## 5. CONCLUSION AND RECOMMENDATIONS

In this study the possible spasmolytic and spasmogenic effects of *A. afra* and *A. rehan* extracts were demonstrated. Some extracts have shown higher spasmolytic activities while others low or even non-significant results. The spasmolytic activities shown by some extracts might suggest the presence of active chemical components such as flavonoids or the combined effect of several chemical constituents. The spasmogenic or contractile effects might also suggest the presence of other chemical substances such as alkaloids or the type of solvent used to extract the specific chemical. Possible mechanisms of the spasmolytic and spasmogenic effects of the extracts were also suggested. However, further investigations have to be carried out in order to get at the exact mechanisms.

The spasmolytic and spasmogenic effects of plants can be affected by different factors like solvent employed, concentration of extracts used, and even parts of the plant tested. Spasmogenic effects seem to be active in more polar solvents like water, and spasmolytic effects appear to be active in less polar solvents like ethanol. So, in order to get more efficacious result the most suitable solvent must be used. Moreover, further studies must be conducted in order to clarify which constituent of the extracts is responsible for the respective activities i.e. the exact chemical constituents to result in those effects have to be isolated through pharmacological fractionation and other related activities. This is very useful to identify the real spasmolytic or spasmogenic agent in the traditional folk use of the plant that is responsible for the remedy of many gastrointestinal and reproductive ailments.

In this experiment it was shown that ALE, ARE and RLE are good relaxants of GPI and MD whereas only ARW is good contractant of MD and GPI. These double-faced effects justify their use in the folk medicine. It is concluded that the spasmolytic activity of *A. afra* and *A.*

*rehan* on intestinal smooth muscles (MD and GPI) may be responsible for their usefulness in the traditional herbal treatment of gastrointestinal disorders (spasms) such as stomach pains, diarrhoea and intestinal cramps. The spasmogenic ARW may, rather, be used in the treatment of constipation and as anthelmintics. At present, nothing can be said about the uterine stimulant and uterine relaxant property of the extracts supporting the folk medical use of these plants. However, further studies need to look for their possible action on spontaneous contraction-free uterine tissues (maintained at 32<sup>0</sup>C) and any biological effect on other smooth muscles since there are many traditional folk uses of these medicinal plants in the country. Nowadays, these natural remedies become more reliable and with less side effects than the reliefs and remedies obtained from expensive pharmaceutical drugs of adverse side effects on organs of the body. Furthermore, the folk use of these plants must be strengthened with scientific evaluation of their efficacy and harmlessness in clinical use. However, from our sample collection and survey in the Dinshu area of Bale, *Artemisia rehan* and *Artemisia abyssinica* (a close relative of *Artemisia afra*) are in danger of extinction due to either excessive use of them for some cultural practices or due to unnecessary clearing-off. These tendencies should be stopped in order to investigate their other would-be nature-endowed useful biological effects such as anti-cancer, anti-aging and even anti-AIDS potentials.

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