IN VIVO AND IN VITRO STUDIES OF *OCIMUM BASILICUM* [LABIATA] LEAF EXTRACTS ON INTESTINAL MOTILITY

BY

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENT OF MASTERS OF SCIENCE IN HUMAN PHYSIOLOGY.

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DECLARATION

I hereby declare that this work reported was carried out by me, under the supervision of Dr A.B Adelaiye and Dr J.A Anuka of the Department of Human physiology, faculty of Human medicine, Ahmadu Bello University Zaria and department of pharmacology and clinical pharmacy, faculty of pharmaceutical sciences Ahmadu Bello University Zaria respectively. The work of other investigators is acknowledged and referred accordingly. I declare that no part of this thesis has been submitted elsewhere for a degree.

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CERTIFICATION

This thesis '' in vivo and in vitro studies of *ocimum basilicum* [labiata] leaf extracts on intestinal motility'' by Shiaki Andokari Fkyabur meets the regulation governing the award of the degree of master of science, Ahmadu Bello University Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my late father Dr Shiaki A.A, who died on the 25th day of November 1999. He had been a great source of inspiration, I thank you very much father. I also dedicate this work to my late younger brother Mr. Teendeh Shiaki who died on the 18th of August 2007. May their souls rest in perfect peace amen.

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ABSTRACT

Ocimum basilicum is a dicotyledonous shrub grown in most parts of Nigeria. Its medicinal properties had gained popularity in traditional medicine. In addition the leaves are eaten in soups in many homes. It consists of a well defined mixture of flavonoids, vitamins, amino acids, sugars, anions, linalool, tannins, rosmarinic acid, salicylic acid, essential oils, fats and terpenes. The acute oral toxicity [determination of median lethal dose (LD_{50})], in vitro and in vivo experiments of aqueous and methanolic extracts were done. Acute toxicity test of aqueous and methanolic extracts of *Ocimum basilicum* was carried out on mice. In this study, fasted mice were given 10, 100, 1000, 1600, 2900 and 5000 mg/Kg of both extracts. Median lethal dose (LD_{50}) was 3807.89 and 2154.07 mg/Kg for aqueous and methanolic extracts respectively. The study to determine in vivo effect of *Ocimum basilicum* leaf extracts of the shrub was on the gastrointestinal transit time in conscious rats. In the in vivo experiment, fasted rats were given 20, 40, 60 and 80 mg/Kg of the extracts orally and fifteen minutes later, 0.5ml of 10% charcoal ($BaSO_4 \cdot H_2O$) was administered into the conscious animals. The control group received the vehicle (normal saline) before the charcoal meal. In the in vitro experiment the effect of 4.00×10^{-6}, 8.00×10^{-6}, 1.60×10^{-5}, 3.20×10^{-5}, 6.40×10^{-5}, 1.28×10^{-4}, 2.56×10^{-4}, 5.12×10^{-4}, 1.024×10^{-3} and 2.048×10^{-3} g/ml of both aqueous and methanolic extracts on the frequency and strength of contraction of an isolated rabbit ileum was determined using a microdynamometer. The result of the study indicates that aqueous and methanolic extracts of *Ocimum basilicum* leaves delays the gastrointestinal transit and inhibits the strength and rate of gastrointestinal smooth muscle contraction in a dose dependent manner. To study the possible mechanisms of action of extracts, phentolamine (α-receptor blocker) at concentration of 4.00×10^{-10}, $8.00 \times 10^{-10,}$ 1.6×10^{-9}, 3.2×10^{-9}, 6.4×10^{-9} and 1.28×10^{-8} g/ml was able to abolish the inhibitory effect of both extracts on smooth muscle contraction. As a result, its inhibitory effect on smooth muscle cells may be mediated via α-receptors located on the cell membrane.

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DEFINITIONS

E_{max}	Maximum effect induced by a drug or chemical
Intrinsic Activity	Proportionality constant used to compare effects of two or more test substances
LD_{50}	Dose that causes 50% mortality to experimental animals
Synergism	A collective effect by two or more substances

CHAPTER ONE - INTRODUCTION

1.1 PLANTS IN MODERN MEDICINE

For a long time, plants have been a valuable source of natural products for maintaining human health especially in the last decade, with more intensive studies on natural therapies. The use of plant sources for pharmaceutical purposes has gradually gained recognition in many parts of the world.

According to WHO medicinal plants would be the best source to obtain a variety of drugs as compared to synthetic drugs used in clinical practice, considering that about 80% of people living in developing countries rely almost completely on traditional medicinal practices for their primary health care (Farnsworth, 1990).

Several researchers have become keenly interested on the medicinal potentials in plants and extensive studies have revealed outstanding results. This outcome has increased the utilization of some of these plants sources as medicinal remedies to cure certain ailments and disorders in the developed world (Ellof, 1998).

For ages, plants have been a good source of food and medicines. Plants such as vegetables have been used to prepare delicacies of different forms for consumption. Fruits are eaten by people and trees have influenced environmental conditions greatly. But unknowingly, these plants have and are still providing essential nutritional and medicinal value to life. Plants have been a good source of vitamins and minerals, which add up to the stores of the human body. Some of these plants provide a protective shield against some toxic agents that could cause diseases.

1.2 HISTORY OF MEDICINAL PLANTS

Nowadays it is interesting to know that plant therapeutics (phytotherapeutics) is gaining grounds and many researchers are exploring various active biochemical components and their mechanisms of action. It will be cheaper for drug development since these plants are grown in our backyards and gardens.

Most plants synthesize certain bioactive substances that have physiological, pharmacological and biochemical activities. Some of these substances could be alkaloids, phenols, flavonoids, essential oils, tannins, vitamins and minerals. However these substances are used for other purposes such as making perfumes and cosmetics as in the case of essential oils found in *Ocimum Basilicum* (Kim et al 1997).

Many plant constituents have been noted for various physiological effects in the human body. Plants with anti-microbial, anti-fungal, anti-diarrheal, hypoglycemic, anti-hypertensive, anti-oxidative, anti-inflammatory and anti-viral potentials have been documented. Some have been said to present cure for conditions such as cancers, asthma and hepatitis. Studies in different countries shown such plant potentials could be efficient (Ikram and Inamul 1984, Almagboul et al 1985, Shapoval et al 1994, Izzo et al 1995 and Artizzu et al 1995) and can be developed upon.

Some of these plant species provide cure for certain ailments or protect the host (providing a protective shield against these diseases). Some of these protective mechanisms may be chemoprotection from free radicals (Aniya et al 2000, Navorro et al 2003 and Giuseppina et al 2003) and radiation protection

(Uma et al 2000). Plant sources have been proven to suppress enzymes that cause chronic diseases such as asthma, diabetes and cancers (Pezzotto 1995, Murakami et al 1996, Kellof et al 2000, Ren and Liece 1997). Diet related medicinal products serve as an apparent benefit and possibly a cure for these conditions (Hakama 1998, Sporn and Suh 2000).

Many studies have elucidated possible mechanisms by which these plant constituents produce their effects. These mechanisms might be via the following:

1. Bioactive constituents in plants might possibly be destructive to disease causative agents (bacteria, viruses or fungi).
2. Bioactive substances might antagonize the effects of toxins secreted by noxious organisms or by suppressing these organisms (e.g. HIV) (Mohmood et al 1997) and carcinogens (Wattenberg 1985, Tanaka 1994, Morse and Stoner 1996 and Dasgupta et al 200).

1.3 HISTORY OF *OCIMUM BASILICUM*

This plant is known as *Ocimum basilicum* (botanical name) in Latin, sweet basil in English and Babui Tulsi or Bantulsi in Ayurvedic. In Nigerian languages, it is known as Efinrin in Yourba, Nchianwu in Ibo, Da'ddoya or Diadoya in Hausa, Eran in Urohobo, Ntong in Efik, Otitoun in Oron, Kungreku in Tiv, Ayenba in Igala and Shimbe in Kuteb.

It belongs to the order-Lamiales, family-Lamiaceae (Mint family), genus-*Ocimum* and species-*Basilicum*. Parts used are the leaves and seeds. There are 150 different tropical species of *Ocimum*. Some of these species are; *Ocimum americanum* (hoary basil), *Ocimum gratissimum* (shrubby basil), *Ocimum*

sanctum (sacral basil), *Ocimum canum* and *Ocimum minimum* as described by Martin and Salguerio (1999) and Mandal and Pattnaik (2000).

It is a hairy plant, growing about 3 feet high (maximum) flowers are white, leaves are grayish-green beneath with dotted dark oil cells, soft and cool to touch and if slightly bruised exhaled a delightful scent. Various species of *Ocimum* may have similarity in shapes of leaves but may differ in sizes. It has a strong aromatic scent just like cloves.

Ocimum basilicum is found in North Africa, France, Cyprus, South America and the United States of America. It is natively to India (Asia), Africa and the pacific islands but was later introduced to Europe as a seasoning for food. In West Africa it is widely distributed and grown in all parts of the sub region including Nigeria.

The origin of the name '*Basilicum*' is uncertain. Some authorities say it comes from the Greek word '*Basileus*' meaning a king because some say 'its aroma is so excellent that it is fit for a king's house or it might have termed royal because it was used in the king's palace for medicinal purposes.

In India this herb is sacred to both Krishna, Vishnu and in every Hindu house. Probably on the accounts of its virtues in disinfecting. It first became inseparable from Hindu homes in India because when dried leaves were burned, the smoke produced served as a repellant to evil spirits. Others say, it is believed to contain a divine spirit and is held sacred to the god of Krishna.

The Egyptians burned a mixture of *Ocimum basilicum* and myrrh to appease their gods. Greeks used it in ceremonies such as anointing of new kings. The Chinese use it as disinfecting agents and in the treatment of gastric and kidney disorders. The Romans used it in massages to relieve aches.

Ocimum basilicum is used for many other purposes such as seasoning of fish and meat in Europe and in Nigeria in particular it is used as a vegetable for making soups. The French use it for making liquors, sophisticated perfumes and cosmetics.



***OCIMUM BASILICUM* PLANT**

1.4 MEDICAL HISTORY OF *OCIMUM BASILICUM*

This medical history was acquired from traditional medicine practitioners and rural inhabitants. They believe that *Ocimum basilicum* plant provides effective remedies for ailments. These beliefs may have physiological explanations and might be incorporated into modern medicine.

Various parts of *Ocimum basilicum* are used for different purposes but the leaves are more regularly used.

Topical administration of cell sap to ruptured capillaries stops bleedings and can be repeated a number of times if bleeding continues.

Physiological explanations might be:

- Enhanced vasoconstriction of injured blood vessels. Enhanced vasoconstriction might probably be due to the increased production and release of vasoconstrictor substances such as serotonin from platelets.
- Stimulation of platelets aggregation at the site of injured blood vessels probably by the increased synthesis and release of thromboxane A_2 (platelet aggregator substance).
- Enhanced platelet adhesion at the site of injured blood vessels probably due to increased production and release of platelet adhesive enhanced.
- Stimulation of clothing factors.

Hypoglycemic activity of this plant has been documented by Chattopadhyay (1993 and 1999). It is used in rural areas in the treatment of diabetes mellitus. A mixture of the cell sap and water is administered orally to a diabetic patient. Active constituents may have physiological effects on the following;

- ✓ Enhanced secretion of insulin by the Islets of Langerhans.
- ✓ Increased mobilization of glucose to the adipose tissue.
- ✓ Decreased breakdown of adipose tissue (lypolysis).
- ✓ Enhanced glucose utilization by tissues.
- ✓ Decreased glucose uptake in the small intestines.
- ✓ Decreased conversion of other endogenous substances to glucose.

Gastrointestinal disorders (such as diarrhoea) are treated with the leaves. The leaves are blended with salt in water and then filtered. The filtrate is orally administered to patient. Anti-diarrhoeal activity of other species (such as *Ocimum gratissimum*) has been reported by Ilori and Steteolu (1996), Offiah and Chikwendu (1999) and Orafidiya and Elujobi (2000). Diarrhea might be due to:

- ❖ Deranged intestinal motility
- ❖ Toxin produced by bacterial flora present in the intestines

It is used in the treatment of bacterial infections. The leaves are crushed and administered to infected areas for cure. Anti-bacterial activity was reported by Koga et al 1999.

Oral preparations of the cell sap of *Ocimum basilicum* leaves is used in the treatment of gastric and peptic ulcers. Its anti-ulcerogenic activity was reported by Singh (1999). Possibly bioactive components may have the following effects on ulcer patients:

- ⇒ Oral administration of plant extract may enhance decreased production of gastric juices by the gastric glands.
- ⇒ Oral administration of plant extract may enhance the protective coating of the stomach against erosion caused by gastric juice.

Premenstrual pains is relieved by sitting in a bath containing a mixture of cell sap and warm water.

In catarrhs, colds and asthmatic attacks, treatment is achieved by boiling leaves in a pot and patient covers a blanket over the head and inhales effluent from pot.

1.5 AIMS AND OBJECTIVES

The primary aim of this study is to ascertain the effect of *Ocimum basilicum* on intestinal motility. The following parameters will be investigated:

- ◊ The amplitude of phasic spontaneous contraction of rabbit ileum, compared to standard drugs (acetylcholine, histamine, serotonin, epinephrine, norepinephrine and isoprenaline).
- ◊ The frequency of phasic spontaneous contractions of rabbit ileum, compared to standard drugs (acetylcholine, histamine, serotonin, epinephrine, norepinephrine and isoprenaline).
- ◊ The systemic effects of *Ocimum basilicum* extracts in an in-vivo and in-vitro experimental models.
- ◊ The effect of extracts on intestinal transit time in rats.
- ◊ The toxicity studies of extracts in experimental animals. The median lethal dose (LD_{50}) will be determined.
- ◊ Possible mechanisms of action of the extract will be studied using standard receptor antagonists (atropine, propranolol and phentoalmine).
- ◊ The threshold concentration of the extract will be determined.
- ◊ The maximum effective concentration will be determined.

1.7 JUSTIFICATION

The following scientific investigations justify this present study;

- Anti-ulcerogenic activity as documented by Singh (1999). Plant extract in an animal experimental model decreased acid output in the parietal cells and decreased pepsin output in the chief cells of the stomach. It also increased the secretion of hexosamine which provided a protective coating in the gastric mucosa.
- Anti-diarrhoeal activity of *Ocimum gratissimum* (which belongs to the same family with *Ocimum basilicum*) as reported in previous studies showed anti-diarrhoeal effect on castor-oil induced diarrhoea in rats as ascertained by a decrease in the number of wet faeces in the extract treated rats and in addition the plant extract inhibited the propulsive movement of intestinal contents (Ilori and Steteolu 1996, Offiah and Chikwendu 1999 and Orafidiya and Elujobi 2000.).
- The medicinal use of this plant in the treatment of ailments such as gastrointestinal disorder (e.g. diarrhoea), infections and premenstrual tension in rural area prompts the search for its scientific explanations.

These investigations suggest that *Ocimum basilicum* might have modulatory influence on the intestinal motility and smooth muscle contractility.

CHAPTER TWO - LITERATURE REVIEW

2.0 PHYSIOLOGICAL HISTORY OF *OCIMUM BASILICUM*

Ocimum basilicum has many important medicinal properties. It has been used for more than 300 years as a remedy for many common aliments.

Different species of '*Ocimum*' from research reports elicit similarities in physiological effects. A notable example is the anti-oxidative effect of *Ocimum sanctum* (Devi and Ganasoundari 1999), *Ocimum basilicum* (Jayasinghe et al 2003) and *Ocimum micranthum* (Navarro et al 2003). Effects which are common in these plant species might be due to the presence of similar constituents which may differ in quantity. For instance, Roberto et al (2001) documented the presence of flavonoids in *Ocimum gratissimum* and Renée et al (2001) also indicated the presence of flavonoids in nine different species of *Ocimum*.

The literature below highlights, physiological, biochemical and pharmacological properties of various species of '*Ocimum*':

- Hypoglycemic activity (chattopadhyay 1993, chattopadhyay 1999, Aguiyi et al 2000, Nyarkoa et al 2002 and Vats et al 2002).
- Anti-ulcerogenic activity (Mohammed and Mohammed 1989, Singh 1999, Surender and Majumdar 1999, Paul et al 2002 and Poonam et al 2004)
- Anti-diarrheal activity (Ilori and Steteolu 1996, Offiah and Chikwendu 1999 and Orafidiya and Elujobi 2000).
- Anti-depressant activity (Sakina et al 1990 and Maity et al 2000).
- Anti-bacterial activity (Koga and Takumi 1999, Orafidiya et al 2002 and Cimanga et al 2002).
- Anti-fungal activity (Ndounga and Ouamba 1997).
- Antiseptic activity (Orafidiya et al 2002).

- Anti-inflammatory activity (Surender et al 1996 and Klem et al 2000).
- Cutaneous permeation enhancers promoting the absorption of drug via the skin (Fang et al 2004).
- Insecticidal activity (Sekou 2001, Jembere and Ahmed 2001, Josiane et al 2003 and Maedot et al 2004).
- Chemopreventive activity (Prashar et al 1994, Karthikeyan et al 1999 and Prakash and Gupta 2000).
- Radiation protective activity (Uma et al 2000 and sembulingam et al 2005)
- Anti-oxidative activity (Sunanda and Anand 1998, Devi and Ganasoundari 1999 and Yenpallewara et al 2004)
- Anti-HIV activity (Yamasaki et al 1998 and Nana and Caesar 2003)
- Immunostimulant activity (Atal et al 1986, Godhwani et al 1998, Archana and Namasivayam 2000 and Mediratta et al 2002)
- Bone marrow radioprotective activity (Ganasoundri et al 1998)
- Anti-carcinogenic activity (Singh et al 1999 and Gerald et al 2004)
- Analgesic properties (Khanna and Jagriti 2003).
- Anti-hypertensive properties (Surender et al 2001)
- Anti-inflammatory properties (Surender et al 1996 and Otero et al 2000)
- Anti-pyretic properties (Eyasu et al 2003)
- Food preservation potential (Nguefack et al 2004)

2.0.1 HYPOGLYCAEMIC PROPERTIES

The hypoglycaemic effect of the methanolic extract of *Ocimum gratissimum* leaves was evaluated in normal and alloxan-induced diabetic rats. Intraperitoneal injection of the extract significantly reduced plasma levels both in normal and

diabetic rats (chattopadhyay 1993, chattopadhyay 1999, Aguiyi et al 2000 and Vats et al 2002).

2.0.2 ANTI-HIV PROPERTIES

Plant sources have shown anti-HIV properties (Nana and Caesar 2003), such as herbs of the labiatae family (Yamasaki et al 1998). It has been documented that plants rich in flavonoids and tannins have anti-HIV properties (Paul et al 2004 and Gerald et al 2004). An example is the anti-HIV activity of the stem bark of maclura tinctoria (Guiseppina et al 2003). Flavonoids have been implicated to inhibit HIV proteases thereby retarding the development of the virus (Mohmood et al 1997, Gerald et al 2004 and Paul et al 2004). *Ocimum basilicum* is rich in flavonoids (Uma et al 2000) and might contribute to its anti-HIV activity.

2.0.3 ANTI-OXIDATIVE PROPERTIES

Plants could present a source of natural compounds with antioxidant activity (Kim et al 1997, Asai et al 1999 and Aniya et al 2000). Laboratory studies have shown various antioxidant activity of *Ocimum Sanctum* (Devi and Ganasoundari 1999) and *Ocimum micranthum* (Navarro et al 2003). Antioxidant enzymes are the counterparts of oxidative damage that protect the cell. Free radical stress has been implicated in the pathogenesis of a wide range of vascular and metabolic disorders (Lun and Rockbuck 2001). As a consequence, there is increasing evidence of substances with antioxidant properties that may be combined with natural defence mechanisms to inhibit free-radical damage and therefore limit disease progression. Anti-oxidative activity of *Ocimum basilicum* might be due to the presence of flavonoids and proanthocyamides (Hopia and Heinonem 1999).

2.0.4 EFFECTS ON THE SKIN

Ocimum gratissimum Linn (*Ocimum* oil), may be capable of enhancing normal hair growth and promoting follicular proliferation in cyclophosphamide-induced hair loss (Orafidiya et al 2004).

In vitro skin permeation and deposition of indomethacin were significantly enhanced after treatment with *Ocimum basilicum* essential oils (Fang et al 2004).

2.0.5 FLUID RETENTION PROPERTIES

Fluid retention, a common condition in women and is normally unaccompanied by evidence of cardiac, renal or hepatic diseases. Various terms have been used to describe the benign form of fluid retention, idiopathic oedema, orthostatic oedema, cyclical oedema and fluid retention syndrome. Women affected normally complain of heavy, swollen and tight legs and in addition the abdomen, hands, breast and area around the eyes are frequently affected and several hypotheses have been postulated to explain the underlying aetiology of fluid retention (Christie et al 2001).

Physicians in mainland Europe often advice the dietary intake of plants such as *Ocimum basilicum* rich in flavonoids (Christie et al 2004) following the dissemination of findings from German and Italian studies that flavonoids reduce symptoms of fluid retention.

2.0.6 ANTI-CARCINOGENIC PROPERTIES

Studies evaluating the anti-carcinogenic properties of *Ocimum basilicum* were conducted and results have shown excellent prospects.

The modulating influence of *Ocimum basilicum* leaf on benzo (a) pyrene-induced forestomach papillomagenesis at the peri-initiational level and skin carcinogenesis were evaluated. Result showed chemopreventive effects of the leaf extract on carcinomas (Prashar et al 1994, Banerjee and Rao 1995 and Shijun et al 2000). This might stimulate the development of diet derive anti-carcinogenic drugs with reduced resistance, intolerant and unwanted side effects (Kellof et al 2000) which are commonly seen in synthetic anti-carcinogenic drugs.

A study was designed to elucidate the inhibitory efficacy of clocimum (a eugenol rich variety of Ocimum gratissimum; Labiatae) oil on murine skin papillomagensis. Topical application of clocimum oil was observed to have a modulatory potential on skin papillomagenesis (Singh et al 1999).

2.0.7 INSECTICIDAL PROPERTIES

Essential oils from *Ocimum basilicum* and *Ocimum gratissimum* (Labiatae) grown in Guinea were obtained by steam distillation. Following exposure of newly emerged adult beetles (Callosobruchus maculatus) to fumigation, high mortality rates were recorded for *Ocimum basilicum* and *gratissimum* (Sékou et al 2001).

Volatile oils extracted by hydro-distillation from six plant species growing in the Kenyan coast, *Ocimum forskolei Benth, Ocimum fischeri Guerke, Ocimum basilicum and Ocimum gratissimum,* all belonging to the Labiateae, were evaluated for repellency on forearms of human volunteers against Anopheles

mosquito (gambiae sensu stricto). All plants were found to be strong repellents (Josiah et al 2005).

A leaf-extract of *Ocimum basilicum (sweet basil)* was used in formulating an aerosol and mosquito coil, and their efficacy tested against adult mosquitoes (Umerie et al 1998).

2.0.8 ANTI-ULCEROGENIC PROPERTIES OF *OCIMUM BASILICUM*

Anti-ulcerogenic properties of *Ocimum basilicum* was studied against aspirin-induced gastric ulcers in rats. In addition its effect on the output of gastric acid, pepsin and hexosamine concentration in gastric fluid was recorded in ulcerated and non-ulcerated rats. Its aqueous and methanolic extracts decreased the ulcer index significantly (Singh 1999).

Moreover the acid output was decreased by its methanolic extract while hexosamine secretion was enhanced. This suggests that its anti-ulcerogenic effect is due to the decrease in acid and pepsin output which enhances gastric mucosae strength (Singh 1999).

2.0.9 ANALGESIC PROPERTIES

The alcoholic leaf extract of *Ocimum sanctum* (OS, Tulsi) was tested for analgesic activity in mice. In the glacial acetic acid (GAA)-induced writhing test, OS, reduced the number of writhes. *Ocimum sanctum* (OS) also increased the tail withdrawal latency in mice which indicates its analgesic properties (Khanna and Jagriti 2003).

2.1 CONSTITUENTS OF *OCIMUM BASILICUM*

Table 2.1 illustrates a survey of constituents found in *Ocimum basilicum* documented by several authors (Ntezurubanza and Svendsen 1987, Grayer et al 1996, Anger and Morale 1996, Christopher et al 1999, Martin and Salguerio 1999, Uma et al 2000, Javanmardi et al 2002, Opelchenova and Obreshkova 2003, Lee et al 2005 and Fleisher and Fleisher 1992).

This survey illustrates the presences of; vitamins (e.g. beta carotene), amino acids (such as Alanine, Arginine, Aspartic acid, Glutamic acid, Histidine, Tyrosine, Valine), monosaccharides (e.g. D-glucose, D-galactose and D-mannose) and anions (e.g. zinc, boron, calcium, copper, iron, magnesium, manganese and potassium). Further, the presence of linalool, flavonoids (Renée et al 2001 and Roberto et al 2001), tannins, rosmarinic acid, salicylic acid, essential oils, fat, terpenes (Iijima et al 2004), oleanic acid (Njoku and Zeng 1997) and fibre.

2.1.1 IMPORTANT CONSTITUENTS IN *OCIMUM BASILICUM*

A great deal of attention has been drawn to plants containing flavonoids because of their impressive anti-oxidant activity (Kim et al 1997, Asai et al 1999 and Aniya et al 2000). Other physiological activities of flavonoids such as anti-HIV activity, (Mohmood et al 1997) has also been reported.

These compounds appear to be even more effective than the more familiar anti-oxidants like vitamin C and E in their effect of protecting low density

lipoproteins (LDLs) from oxidation (Feng et al 2001). Numerous studies on flavonoids have demonstrated that they may lower cholesterol levels, offering further protection against cardiovascular diseases (Begum and Terao 2002). They also posses anti-carcinogenic, anti-inflammatory and anti-histamine activities and may therefore be useful in preventing and or treating a wide range of disease (Weisburger 2000). Many studies have demonstrated the effectiveness of flavonoids in the prevention and treatment of various cancers, including cancers resistant to chemotherapy (Damianaki et al 2000). In a study of 10,000 Finish men and women, many specific association between flavonoids in the diet and several chronic diseases were studied (Lieberman 2003). It was found that people with higher flavonoid intake have lower neutrality from ischemic heart disease. Men with higher intake of flavonoids was associated with a trend towards a reduction in the risk of type II diabetes mellitus. High levels of estradiol (estrogens) in the plasma has been implicated in breast cancer, prostrate cancer, menopausal symptoms, premenstrual symptoms, endometrosis, fibrocystic breast disease and possibly other hormonally related disease. Flavonoids help to metabolize estradiol to estriol, the safe and protective form of estrogen (Murillo et al 2002).

They are found in fruits, vegetables, nuts, seeds, grains and legumes. They are also found in beverages such as tea coffee and wine.

Quercetin is the major flavonoid in our diet; it is estimated that humans eat approximately 25mg of quercetin per day from onions, apples and other

vegetables and other flavonoids common in foods are curcumin, hesperidin, pycnogenol and nutin (Young et al 1999). From research studies it helps to reduce inflammations, decreases the prevalence of heart disease, acts as an anti-inflammatory agent, anti-oxidative agent and an anti-histaminic agent (Shoskes et al 1999). It enhances cardiovascular parameters, reduces allergic related reactions and can be used in the treatment of asthma, and other inflammatory conditions (Miodini et al 1999). It has been documented to have therapeutic effects on disease such as diabetes, dysentery, gout, cataracts, atomic dermatitis (Begum and Terao 2002), carcinomas (Damianaki et al 2000).

Quercetin has the same biochemical effect as Bestatin® (Ubenimex), which may be a natural remedy against severe acute respiratory syndome (SARS). Bestatin® acts by inhibiting the entry of RNA viruses (SARS Coronavirus) into healthy cells. This effect is achieved by the inhibition of an enzyme known as aminopeptidase N which aids the virus to infest healthy cells of its host. Other antiviral activities are found in herpes simplex, adenoviruses, rotaviruses, paramyxoviruses, polio viruses, parainfluenza, respiratory syncytial viruses and retroviruses (Feng et al 2001). No significant side effect has been documented and it has no proven toxic effects (Rice and Packer 1998).

2.2 SMOOTH MUSCLES

2.2.1 TYPES OF SMOOTH MUSCLES

Smooth muscles are found in most of the internal organs (visceral organs) of the human body, such as the gastrointestinal tract, uterus, ureters, bile ducts, blood vessels and the iris of the eye. Its name is derived from the fact that they do not have microscopic striations which are found in skeletal and cardiac muscles. Muscle fibres in smooth muscles are much smaller than those found in skeletal muscles. They are usually 2 to 5 um (micrometers) in diameter and 50 to 200 um (micrometers) in length, in contrast to the skeletal muscle fibres which are 20 times in diameter and a thousand times longer though contractile mechanisms are similar.

Smooth muscles differ in various organs and distinguishing features are physical dimensions, organization of bundles, characteristic innervations, response to different stimuli and function. They are classified in to two groups, visceral smooth muscles and multi-unit smooth muscles.

Visceral smooth muscle fibres are usually arranged in sheet bundles and have gap junctions between their cell membranes. The gap junctions are multiple points where ions flow to and fro the cell membranes to generate or terminate an action potential of the muscle cells. When an action potential is generated the muscle cells contract together producing an effective shortening of the entire muscle.

Multi-unit smooth muscles are made up of discrete muscle fibres with each fibre contracting separately and usually innervated by a single nerve ending. They rarely exhibit spontaneous contractions and are found in the ciliary muscle of the iris (eye). In lower animal they are found in the nictitating

membrane that covers the eyes and the piloerector muscles that erect cutaneous hair filament when stimulated by sympathetic impulses.

2.2.2 THE CONTRACTILE PROCESS OF SMOOTH MUSCES

Contractile responses are initiated by calcium ions (Ca^{2+}) and energy for this process is derived from the phosphorylation of ATP to ADP. However it has a poorly developed sarcoplasmic reticulum that initiates contraction. Contraction is due to the influx of Ca^{2+} from the extracellular fluid via voltage-gated, ligand-gated and Ca^{2+} channels. Ca^{2+} ions then bind to calmodulin and this complex catalyses the phosphorylation of myosin light chain on serine at position 19. This activates myosin ATPase and the sliding of the actin on myosin, shortening the length of the muscle fibre. Myosin light chain phosphatase catalyses the dephosphorylation of myosin to initiate relaxation. Relaxation is not entirely achieved by this reaction because the myosin remains attached to actin for some time producing sustained contraction after intracellular Ca^{2+} concentration falls. This sustained contraction utilizes little expenditure of energy and has a functional role in maintaining vascular smooth muscle integrity. Relaxation occurs when Ca^{2+}-calmodulin complex dissociates and calmodulin remain unbounded for another contraction.

Smooth muscles contracts 50 to 100 milliseconds (ms) after excitation and reach full contraction about half a second later. Contraction declines 1 to 2 seconds later having a total contraction time of 1-3 seconds, which is slower as compared to skeletal and cardiac muscles. Energy expenditure is reduced (about $^{1}/_{500}$) as compared to skeletal muscles. This might be as a result of fewer number

of myosin filaments in the smooth muscle fibres and this maintains a sustained degree of contraction in visceral organs which regulate their functions.

Spontaneous action potentials are generated when visceral smooth muscles are stretched. This reflex action is necessary for normal function. For example, when the small intestine is filled with ingested food, the walls are stretched and automatic peristaltic contraction begins to propel it contents towards segments that are not stretched.

The autonomic nervous system generally regulates the contractile responses of smooth muscles. Nerve impulses are normally involuntary (are not directly controlled by higher center of the brain). Neuromuscular junctions are absent but these nerve fibres usually diffuse into the muscle mass and at these endings these nerves secrete neurotransmitters that diffuse into the muscle cells via the intracellular fluid. No neuronal endplate is present but the schwann cells have multiple varicosities spread along its terminal axons and vesicles containing neurotransmitters are more towards the cell membrane and release their contents. These vesicles may contain acetylcholine, norepinephrine or any other neurotransmitter depending on the type and function of the neuron.

Two distinctive neurotransmitters have been identified in the autonomic nervous system: acetylcholine and norepinephrine. These neurotransmitters can be excitatory or inhibitory in action; that is when acetylcholine excites, norepinephrine inhibits or vice versa. This is due to the different types of receptors found on different smooth muscles, which defines the type of response elicited.

When excitatory neurotransmitters bind to excitatory receptors, sodium channels open and there is an influx into the intracellular fluid and an action

potential generated. Inhibitory receptors initiate the influx of potassium ions (K^+) and a decline in action potential.

Many physiological conditions potentiate the excitation of the autonomic nervous system and subsequently smooth muscles. These responses are regulatory in function thereby maintaining the normal physiological homeostasis of the body.

These are some examples:

- Decreased oxygen tension in the plasma (hypoxia causing vasodilatation of the smooth muscles of the vascular system thereby increasing blood supply to vital organs.
- Hemorrhages from ruptured blood vessels causing vasoconstriction of capillaries
- Increase in carbon (IV) oxide (CO_2) partial pressure in the blood causing vasodilatation of blood vessels.
- Vasodilatation caused by the increase in lactic acid ion and potassium ion concentration in the plasma.
- Vasoconstriction of cutaneous blood vessels caused by a decrease in body temperature.
- Decrease is gastrointestinal motility by norepinephrine and an increase in motility by acetylcholine.
- Increase in myometrial (uterine smooth muscle) contractions due to increase in intracellular oxytocin concentration.

These neurotransmitters and hormones influence the contractile status of smooth muscles either directly or indirectly. These systems responses are mediated basically via the type of receptors found on the target organs.

Sustained smooth muscle contraction is a state of prolonged partial or full contraction of the muscle fibres and known as tonus contraction or smooth muscle tone. In the GIT this type of contraction is known as basic electrical rhythm (BER) of gastrointestinal smooth muscles. It is characterized by spontaneous contraction and relaxation of the GIT smooth muscle.

This type of contraction can be defined by:

- Summation of contraction in each muscle fibre.
- Prolonged direct smooth muscle excitation without an action potential. This might be caused by local hormones or local factors.

2.2.3 NEUROTRANSMITTERS

Neurotransmitters are chemical substances released at nerve endings that transverse the extracellular fluid, bind to specific receptors and produce a response on the target cell or tissues. They have similar mechanisms of action, compared to hormones but they differ in orientation of their target cells. Neurotransmitters have target cells proximal to their point of secretion whereas hormones have target cells, located distally from their site of secretion. Some drugs and therapeutic agents mimic their mechanisms of action, for example, epinephrine administered locally or directly to ruptured blood vessels cause vasoconstriction and decrease blood loss.

In action potential transmission, arrival of action potential at the neuronal terminal initiates the release of neurotransmitters stored in vesicles into the intracellular fluid. The neurotransmitters then bind to receptors causing a structural change on the cell membrane of the effector organ or tissue. These changes open voltage gated channels thereby initiating ionic influx into the cell.

This movement of ions alters the resting membrane potential thereby stimulating or inhibiting an action potential. After this process is complete, neurotransmitters are deactivated (acetylcholinesterase deactivates acetylcholine) or retaken into the neuron (in the case of norepinephrine).

Cholinergic nerves secrete acetylcholine and their synthesis is catalysed by an enzyme (Choline acetylcoenzyme A). Secreted acetylcholine is degraded in the extracellular fluid after binding to its receptors by an enzyme acetylcholinesterase. For acetylcholine to be effective and available to its receptors during neuromuscular transmission, acetylchlinesterase is deactivated.

Adrenergic transmission includes the secretion of norepinephrine a neurotransmitter secreted by most sympathetic postganglionic nerve fibres. Also neurons that secrete epinephrine and dopamine are considered as adrenergic nerve fibres. Epinephrine is secreted directly into the general circulation by the adrenal gland and it has a sympathetic response on the cardiovascular system, skeletal muscles and the central nervous system (brain). Adrenergic neurotransmitters are secreted in the extrapyramidal system, several mesocortical areas and mesolimbic neuronal path ways. Norepinephrine, epinephrine and dopamine are synthesized by the conversion of tyrosine by monoamine oxidase. Cholinergic neurons are:

- All preganglionic neurons
- The anatomically parasympathetic postganglionic neurons
- The anatomically sympathetic postganglionic neurons that innervate sweat glands
- The anatomically sympathetic neurons which end on blood vessels in skeletal muscles and produce vasodilatation when stimulated.

The remaining postganglionic sympathetic neurons are adrenergic neurons. The adrenal medulla is basically a sympathetic ganglion supplied by cholinergic neurons and has lost its axons thereby secreting epinephrine, norepinephrine and some dopamine directly into the circulation.

TABLE 2.1

DRUGS AND TOXINS THAT AFFECT AUTONOMIC ACTIVITY

Site of Action	Compounds that augment autonomic activity	Compound that depress activity.
Sympathetic and Parasympathetic ganglia	**Stimulate postganglionic neurons** Nicotine Dimethy phenylpiperazinium **Inhibit acetylcholinestrase** DFP(diisopropylfluorophosphate) Physostigmine (Eserine) Neostigmine (prostigmin) Parathion	**Blockers** Hexamethomium Mecamylamine (Inversine) Pentolinium Trimethaphan (Arfonad) High concentration of acetylcholine.
Endings of postganglionic noradrenergic neurons	**Release norepinephrine.** Tyramine Ephedrine Amphetamine	**Block norepinephrine synthesis** Metyrosin (Demser) **Interfere with norepinephrine storage** Reserpine Guanethidine[2] (Ismelin) **Prevent norepinephrine release** Bretylium (Bretylol) Guanethidine[2] (Ismelin) **From false transmitters** Methyldopa (Aldomet)
Muscarinic receptors		Atropine, Scopolamine
α-receptors	**Stimulate α_1 receptors** Methoxamine (Vasoxyl) Phenylephrine (Neo-synephrine) **Stimulate α_2 receptors** Clonidine[3] (Catapres)	**Block α-receptors** Phenoxybenzamine (Dibenzyline) Phentolamine (Regitine) Prazosin (Minipress) Yohimbine
β-receptors	**Stimulate β-receptors** Isoproterenol (Isuprel)	**Blocks β-receptors** Propranolol Atenolol Butoxamine

Acetylcholine binds basically to two types of cholinergic receptors; muscarinic and nicotinic receptors. Muscarine mimics the stimulating action of acetylcholine at muscarinic receptors found in smooth muscles and glands whereas nicotine mimics the action of acetylcholine at nicotinic receptors. Muscarine receptors are found in all target cells stimulated by postganglionic parasympathetic neurons and cholinergic postganglionic neurons of sympathetic neurons system. Nicotine receptors are found at somatic neuromuscular junction and at all synapse between pre and postganglionic neurons.

Adrenergic receptors are classified into 2 subtypes, α and β receptors. These receptors are further classified into $\alpha_{1,}$ α_2, β_1, and β_2 receptors. Muscarine receptors are antagonized by atropine while nicotinic receptors by hexamethonium and trimethaphan. , α_2 by yohimbine, β_1 by atenolol and β_2 by Butoxamine

2.3 THE GASTRINTESTINAL TRACT

The gastrointestinal (GI) system consists of the gastrointestinal tract and certain associated glandular organs that produce secretions that function in the GI tract. The major subdivisions of the GI tract are the mouth, pharynx, esophagus, stomach, duodenum, jejunum and ileum (small intestine), colon, rectum and anus. Associated glandular organs indicate the salivary glands, liver, gallbladder and pancreas.

The major physiological functions of the GI system are to digest food and absorb nutrients into the blood stream. GIT motility refers to the movement of the smooth muscles that mix and circulate its contents propelling it along its length. Usually net propulsion occurs in the orthograde direction that is away

from the mouth and toward the anus. Retrograde propulsion occasionally does occur, vomiting is a notable example. Secretions refer to the processes by which the glands associated with the GI tract secrete water and substances into the tract. Digestion is defined as the processes by which large ingested molecules are chemically degraded to produce smaller molecules that can be absorbed across the wall of the GI tract. Absorption refers to the processes by which nutrient molecules are absorbed by the GI tract which enters the blood stream.

2.3.1 LAYERS OF THE GASTROINTESTINAL TRACT (GIT)

The structure of the GI tract varies greatly from region to region but common features exist in the overall organization of the tissue.

The mucosa consists of an epithelium, the lamina propria and muscularis mucosae. The epithelium is a single layer of specialized cells that lines the lumen of the GI tract. The nature of the epithelium varies greatly from one part of the digestive tract to another. The lamina propria consists largely of lose connective tissue containing collagen and elastin fibrils. The lamina propria is rich with several types of glands and contains lymph nodules and capillaries. The muscularis mucosae is the thin, innermost layer of intestinal smooth muscle. Contraction of the muscularis mucosae throws the mucosa into folds and ridges.

The submucosa consist largely of loose connection tissues with collagen and elastin fibrils. In some regions the submucosal glands are present. The large nerve trunks and blood vessels of the intestinal wall travel in the submucosa.

The muscularis externa characteristically consist of two substantial layers of smooth muscle; an inner circular lager and an outer longitudinal layer.

Contractions of the muscularis external mixes and circulates the contents of the lumen and propels them along the GI tract. The wall of the GI tract contains a network of nerve cells in the submucosa known as the submucosal plexus (Meissner's plexus). The prominent myenteric plexus (Auerbach's plexus) is located between the circular and longitudinal smooth muscle layers. The submucosa and myenteric plexus (intramural plexus), together with the other neurons of the GI tract constitute the enteric nervous system, which helps to integrate the motor and secretory activities of the GI system. If the sympathetic and parasympathetic nerves of the GI tract are cut many motor and secretory activities continue because these processes are controlled by the enteric nervous system.

The serosa, or adventitia, is the outermost layer and consist mainly of connective tissue covered with a layer of squamous mesothelia cell.

2.3.1.1 SYMPATHETIC INNERVATION.

The sympathetic innervations of the GI tract is primarily via postganglionic adrenergic fibres with cell bodies located in the prevertebral and paravertabral plexuses. The coeliac, superior and inferior mesenteric and hypogastric plexuses provide postganglionic sympathetic innervations to various segments of the GI tract. Most of the sympathetic fibres terminate in the submucosa and myenteric plexuses. Activation of the sympathetic nerves usually inhibits the motor and secretory activities of the GI system. Some sympathetic fibres innervate blood vessels of the GI tract, causing vasoconstriction. Other sympathetic fibres innervates glandular structures in the wall of the GI tract. Stimulation of the sympathetic input to the GI tract inhibits motor activity of the muscularis mucosae

and certain sphincters. The inhibitory effect of the sympathetic nerves on the muscularis externa is not a direct action on the smooth muscle cells, since few sympathetic nerve endings are in the muscularis externa. Rather, the sympathetic nerves act to influence neural circuits in the intrinsic plexuses that provide input to the smooth muscle cells. This effect may be reinforced by the action of the sympathetic nerves in reducing blood flow to the muscularis externa.

2.3.1.2 PARASYMPATHETIC INNERVATION

Parasympathetic innervations of the GI tract down to the level of the transverse colon is supplied by branches of the vagus nerves. The colon, rectum and anus receive parasympathetic fibres from the pelvic nerves. These parasympathetic fibres are preganglionic and predominantly cholinergic. Other fibres that travel in the vagus and its branches have other transmitters that in most cases have not been identified. The parasympathetic fibres terminate predominantly on the ganglion cells in the intramural plexuses. The ganglion cells then directly innervate the smooth muscle and secretory cells of the GI tract. Stimulations of parasympathetic nerves usually stimulates the motor and secretory activity of the GI tract

2.3.1.3 ENTERIC NERVOUS SYSTEM

The myenteric and submucosal plexuses are the most well defined plexuses in the wall of the GI tract. The plexuses are networks of nerve fibres and ganglion cell bodies. Some of the incoming axons are preganglionic, whereas others are postganglionic sympathetic fibres. Interneurons in the

plexuses connect afferent sensory fibres with efferent neurons to smooth muscles and secretory cells to form reflex arcs that are wholly within the GI tract wall. Consequently the myenteric and submucosal plexuses can control much coordinated activity in the absence of extrinsic innervations of the GI tract. Axons of these plexuses innervate glands in the mucosa, submucosa, smooth muscle cells (muscularis externa and muscularis mucosae), intramural endocrine and exocrine cells.

2.3.1.4 AFFERENT FIBERS

Afferent fibres in the GI tract provide the afferent limbs of both local and control reflex arcs. Chemoreceptor and mechanoreceptor endings are present in the mucosa and muscularis externa. The axon of some of these receptor cells synapse with other cells in the plexuses to mediate local reflex activity. Other such receptors send their axon back to the central nervous system. The cell bodies of some of the sensory afferent fibres arise from the GI tract. The complex afferent and efferent innervations allow for fine control of secretory and motor activities.

2.3.2 ELECTROPHYSIOLOGY

The resting membrane potential of GI smooth muscle cells ranges from approximately -40 to -80 mV. Na^+ and Cl^- are important in determining the resting membrane potential. Compared to skeletal muscles, GI smooth muscle cells have a higher ratio of Na^+ to K^+ conductance. This contributes to the lower resting membrane potential exhibited in GI smooth muscle cells.

The electrogenic Na^+-K^+ pump contributes significantly to the resting membrane potential in smooth muscle. Because 3 Na^+ ions are extruded from every 2 K^+ taken up, the pump produces a net outward flow of positive charge, which contributes to the membrane potential. If the Na^+-K^+ pump of the teniae coli is inhibited by Ouabain, the resting membrane potential changes from about -60 to -40 mV. When Ouabain is washed out, the resting membrane potential returns to almost -60mV. Based on this observation, about one third of the resting membrane potential in GI smooth muscle results from the electrogenicity of the Na^+-K^+ ATPase. In most other excitable tissues the resting membrane potential is rather constant in time. In GI smooth muscle the resting membrane potential characteristically varies in time. Slow waves are oscillations of the resting membrane potential and are characteristic of GI smooth muscle. The frequency of the oscillations varies from about 3 per minutes in the stomach to 12 per minutes in the duodenum which shows sinusoidal slow waves in rabbit jejunum. Slow waves also are referred to as the basic electrical rhythm.

In each segment of the GI tract the basic electrical rhythm of some cells is faster than that of the slow waves in other regions. Because the smooth muscle cells are well coupled electronically, the waves of depolarization (or repolarization) spreads throughout the segment of the GI tract.

The Amplitude and, to a lesser extent, the frequency of the slow waves can be modulated by the activity of intrinsic and extrinsic nerves. In general, circulating epinephrine (adrenaline) and norepinephrine (noradrenalin) released by sympathetic nerve terminals decrease the amplitude of the slow waves or abolish them, whereas stimulation of parasympathetic nerves increase the size of

the slow waves. Depending on the excitability of the smooth muscle cells, the slow waves may induce action potentials.

Typically the slow waves themselves do not elicit contractions of the smooth muscle layer. Contraction is evoked by the action potentials that are intermittently triggered near the peaks of the slow waves.

Action potentials in GI smooth muscles are more prolonged (10 to 20 ms) as compared to skeletal muscles and have little or no overshoot. The rising phase of the action potential is caused by ionic flow through channels that conduct both Ca^{2+} and Na^{+} and are relatively slow to open. The Ca^{2+} that enters the cell during the action potential plays a significant role in initiating contraction. Repolarization is allowed by a delayed increase in K^{+} conductance.

When the membrane potential of GI smooth muscle reaches the threshold, a train of action potentials (1 to 10 per second), occurs. The extent of depolarization of the cells and the frequency of action potentials are enhanced by excitatory hormones and compounds librated from nerve endings. Inhibitory hormones and neuroeffector substances hyperpolarize the smooth muscle cells and may abolish action potential spikes.

2.3.2.1 ELECTRICAL COUPLING BETWEEN SMOOTH MUSCLE CELLS.

Neighbouring cells are said to be well coupled electrically. Membrane potential of one cell spreads rapidly. The smooth muscle cells of the muscularis externa are well coupled.

Then smooth muscle cells of the circular layer are better coupled than those of the longitudinal layer. The cells of the circular layer are joined by

frequent low-resistance gap junctions that allow the spread of electrical current from one cell to another. Because the electrical resistance of membrane is much higher than the resistance of the cytoplasm, the resistance along the long axis of smooth muscle cells is less than that in the transverse direction. This electrical depolarization in the circular muscle is readily conducted circumferentially around the GI tract wall and quickly depolarizes the ring of circular muscle which includes the original site of depolarization. The ring of depolarization then spreads more slowly along the long axis of the GI tract. The longitudinal smooth muscle cells of the muscularis externa are also well coupled electrically. Therefore an electrical disturbance is rapidly propagated along the axis of the GI in the longitudinal layer.

2.3.2.2 CONTRACTILITY OF INTESTINAL SMOOTH MUSCLE

Gastrointestinal smooth muscle cells fire action potentials and the cells contract in response to these potentials. The action potential usually occurs in burst at the peaks of the slow waves and causes phasic contractions that are superimposed on the baseline of contractions. Because smooth muscle cells contract rather slowly (about one-tenth as fast as skeletal muscle), the individual contractions caused by each action potential in a burst are not visible as district twitches; rather, they sum temporally to produce a smoothly increasing level of tensions. The increase in tension in response to a burst of action potentials is proportional to the number of action potentials in the burst.

Between burst of action potentials the tension developed by GI smooth muscle falls, but not to zero resting, or baseline. Tension developed by the smooth muscle may be altered by neuroeffector, hormones and drugs.

2.3.2.3 INTERGRATION AND CONTROL OF GASTROINTESTINAL MOTOR ACTIVITIES

Control of the contractile activities of GI smooth muscles involves the central nervous system, the intrinsic plexuses of the gut, hormonal factors and electrical coupling among the smooth muscle cells. The GI tract displays much intrinsic control; the intramural plexuses can mediate control and integration of much of the gut's contractile behavior without intervention by central nervous system. The autonomic neurons system typically modulates the patterns of muscular activity that are controlled more directly by the enteric nervous system.

2.3.3 NEUROMUSCULAR INTERACTIONS

Neuromuscular interactions in the GI tract do not involve true neuromuscular junctions with specialization of the post-junctional membrane, as occurs at the motor endplate. The neurons of the intermural plexuses send axon to the smooth muscle layers and each axon branches extensively to innervate many smooth muscle cells.

The circular smooth muscle layer of the muscularis externa is heavily innervated by nerve terminals that form close association with the plasma membranes of smooth muscle cells. The predominant innervation is by fibres that inhibit the electrical and contractile activity of the smooth muscle cells.

The longitudinal smooth muscle cells are much less richly innervated by the neurons of the intrinsic plexuses and the neuromuscular contact are not so intimate. In contrast to the innervations of the circular layer, the longitudinal layer appears to be totally devoid of inhibitory nerve endings. The sparse excitatory nerve fibers are predominantly cholinergic. These neural activities of the plexus

inhibit the circular muscle but stimulate the longitudinal muscle. This is physiologically relevant because the two layers are antagonistic in the sense that contraction of one layer opposes contraction of the other, contraction of the circular layer make the gut longer and thinner whereas contraction of the longitudinal layer makes the gut shorter and wider.

2.3.3.1 NEURONS OF THE ENTERIC NERVOUS SYSTEM

The plexuses of the GI tract wall function as a semi autonomous neuronal system that controls the motor and secretory activities of the digestive system. The enteric nervous system of the small and large intestines alone contains about 10^8 neurons, about as many neurons as in the spinal cord. Both plexuses consist of ganglia that are interconnected by tracts of fine unmyelinated nerve fibers. The neurons in the ganglia of the sensory neurons are sensitive to mechanical deformation, chemical stimuli, temperature. Some of the neurons in the ganglia are effector neurons that send axons to smooth muscle cell of the circular or longitudinal layers, to secretory cells of the GI tract or to GI blood vessels. Many of the neurons in the enteric ganglia are interneurons. They are part of a network of neurons that integrates the sensory input to the ganglia which formulates the output of the effector neurons. Both excitatory and inhibitory enteric motor neurons are closely associated with interstitial cells of Cajal in the deep muscular plexus of the small intestine (ward and Sanders 2001).

2.3.3.2 NEUROMODULATORY SUBSTANCES

Most neuromodulatory substances present in the GI tract wall are also found in the brain. Table (2.3) below list some of the neuroactive substances present in the GI tract.

TABLE 2.2
NEUROTRANSMITTERS AND NEUROMODULATORY SUBSTANCES IN THE ENTERIC NERVOUS SYSTEM

Acetylcholine	Somatostatin	Norepinephrine
Vasoactive intestine peptide (VIP)	Neuropeptide Y	Enkephalin
Purine nucleotides	Dopamine	Neurotensin
R-aminobutyric acid	Cholecystokinin (cck)	Gastrin
5-hydroxyltryptamin (serotonin)	Glycine	Galanin
Substance p	Histamine	Bombesin
Thyrotopin-releasing hormones	Motilin	Angiotensin
Gastrin-releasing peptide (GRP)	Secretin	Histidine
Prostaglandins	Isoleucine (PHI)	

2.3.4 HORMONAL REGULATION OF THE SMALL INTESTINES.

Polypeptides are secreted by the mucosa cells of the gastrointestinal tract which act in a paracrine fashion (secreted proximal to their target tissue) but also enters the circulation to act on other targets. These polypeptides have been identified as gastrointestinal hormones which play an important role in the regulation of gastrointestinal motility. They have been classified into two major families; the gastrin family and the secretin family. The members of the gastrin family are; gastrin and cholecystokinin (CCK) and the secretin family are

secretin, glucagons, glicentin (GLI) and vasoactive intestinal peptide (VIP). Other hormones are also present but they do not readily fall into these two families.

2.3.4.1 GASTRIN

Produced by the G cells located in the lateral walls of the glands in the antral portion of the gastric mucosa. TG cells also secret gastrin in the stomach and the small intestine. They are also secreted in autonomic nerves (vagus nerve) and somatic nerves (Sciatic nerves).

Its major physiological effect is to increase smooth muscle contractility of the gastrointestinal tract. Other effects are trophic action on the mucosa epithelia of the stomach and intestine.

The postganglionic vagal fibres that innervate the G-cells produce gastrin releasing peptide rather than acetylcholine. These fibres cannot be blocked by atropine but blockage of gastrin-releasing peptide receptors on the G cells inhibits gastric emptying and gall bladder contractions but accelerates small intestine transit.

2.3.4.2 CHOLECYSTOKININ-PANCREOZYMIN (CCK)

Secreted by the mucosa epithelia of the upper part of the small intestine. It is found in nerves of the distal ileum and colon. Also in neurons of the brain and other parts of the body. It enhances the motility of the small intestines, arguments the contraction of the pyloric sphincter, then a tropic action on the pancreas and argument the action of secretin.

2.3.4.3 SECRETIN

Secreted by the S cells located in the deep portion of the mucosa of the upper portion of the small intestines. It augments the action of CCK in pancreatic secretion. It decreases gastric acid production and causes contraction of the pyloric sphincter of the body. It enhances the motility of the small intestines, augments the contraction of the sphincter, and has a tropic action on the pancreas.

2.3.4.4 GIP

Secreted by the K cells in the mucosa of the duodenum and jejunum. It inhibits gastric secretions, GI motility and insulin secretion by the islet cells.

2.3.4.5 VASOACTIVE INTESTINAL PEPTIDE (VIP)

It is found in nerves of the gastrointestinal tracts. It stimulates intestinal secretion of electrolyte, relaxes the smooth muscle of the small intestines, causes dilation of the peripheral blood vessels and inhibits gastric acid secretion.

2.3.4.6 MOTILIN

Secreted by enterochromaffin cells and M cells in the stomach, small intestines and colon. It acts on G-protein coupled receptors on enteric neurons in the duodenum and colon, causing smooth muscle contraction in the stomach and intestines.

2.4 THE REFERENCE DRUGS

2.4.1 ACETYLCHOLINE (ACH)

Acetylcholine is a neurotransmitter found at cholinergic synapses and neuroeffector junctions in the peripheral and central nervous system. Its action is mediated via muscarinic and nicotinic receptors. Muscarinic receptors are found on the autonomic effector cell membranes, innervated by postganglionic parasympathetic nerves fibres. They are also found in the brain, autonomic ganglia and blood vessels. Cholinergic agonists mimic the effects of acetylcholine at cholinergic receptor sites. These cholinergic agonists are used clinically for the treatment of gastrointestinal disorders and in ophthalmology. Antagonist inhibits or competes with acetylcholine for muscarinic receptor sites. These antagonists compete with this neurotransmitter at postganglionic cholinergic nerve fibre endings. Pre- and postsynaptic muscarinic antagonists are highly selective to muscarinic cholinergic receptor except for compounds containing quaternary ammonium. Clinical uses of muscarinic antagonist are in the treatment of gastrointestinal disorders and poisoning from cholinesterase inhibitors. They were also used in ophthalmology.

2.4.1.1 MECHANISM OF ACTION OF ACETYLCHOLINE

When an action potential reaches the synaptic junction or the neuromuscular junction, there is quanta release of acetylcholine synthesized and stored in the vesicles of the axon. They are released from the vesicles by exocytosis and acetylcholine binds to its receptors on the cell membrane of effector cells or the effector tissues, which could be a muscle fibre or another neuron. This neurotransmitter-receptor interaction leads to opening of ionic voltage gated channels.

Opening of these channels causes an efflux or influx of ions such as Ca^{2+} or Na^{+} or both or even K^{+} and Cl^{-} depending on the specific receptor activated. This movement of ions causes the following;

- Influx of Ca^{2+} in muscles fibres causes contraction.
- Influx of Na^{+} into a cell or another neuron causes depolarization, a decrease in negativity of the cells and an action potential.
- Efflux of Cl^{-} and K^{+} causes hyperpolarization in the effector cells, increasing negativity and decreasing the excitability of the effector cell.

Acetylcholine is deactivated at neuromuscular junction and synapses by cholinesterase and in the plasma by butyrylcholinesterase terminating its effect. Acetylcholine is found at the following sites.

- ✓ Autonomic effector sites innervated by postganglionic parasympathetic fibres
- ✓ Sympathetic and parasympathetic ganglion cells
- ✓ The preganglionic nerves that innervate the adrenal medulla
- ✓ Motor end plates on skeletal muscles innervated by somatic nerves.
- ✓ Certain synapses in the central nervous system

Muscarine acting selectively as a potent agonist at muscarinic receptors, acetylcholine and all drugs producing similar effects are term muscarinic in action. All actions of muscarinic substances are antagonized by atropine (Pesic et al 1999). All drugs that bind to nicotinic receptors are termed nicotinic because they produce the same effect as nicotine and are antagonized by drugs referred in table 2.2.

2.4.1.2 EFFECTS OF ACETYLCHOLINE ON THE GI TRACT

Acetylcholine causes an increase in tone, amplitude of contraction and peristaltic activity of the GT smooth muscles. It also increases the secretions of glands in the gastrointestinal tract. The increased motility may result to nausea, belching, vomiting, intestinal cramps and defecation. Nicotinic receptors are ligand-gated ionic channels are activated rapidly causing an increase in intracellular Ca^{2+} and Na^{+}. This increases the polarity (depolarization) of the effector cells eventually potentiating an action potential. On the other hand muscarinic receptors are slower as compared to nicotinic receptors in their unset of action and they are G-protein coupled receptors. These muscarinic receptors could be either excitatory or inhibitory.

2.4.1.3 PROPERTIES OF CHOLINERGIC RECEPTORS

Muscarinic receptors have been cloned and five subtypes have been identified, expressed as M_1, M_2, M_3, M_4 and M_5 and they are said to respond similarly to pharmacological expressed subtype receptors M_1, M_2, M_3, M_4 and M_5 (Dorge et al 1991 and Caulfield 1993) and all these subtypes are coupled to guanine nucleotide binding regulatory proteins (G-proteins) that have regulatory effects in the inner portion of the cell membrane (Yu et al 1992 and Obi et al 1994). Stimulation of M_1, or M_3 receptors stimulators hydrolysis of polyphosphoinositides and the mobilization of intracellular Ca^{2+} by binding to G-proteins and activating phospholipase C which catalysis the reaction. (Zang et al 2001). M_2 and M_4 receptors inhibit adenylyl cyclase and regulate specific ion channels.

Nicotinic receptors have been classified into 2 types. α and β receptors. There are eight and subtypes; α_2-α_9 nicotinic receptors and four β subtypes (β_2 - β_5) in the nervous system. (Walker et al 2001).

2.4.1.4 SYNERGISMS AND ANTAGONISMS OF ACETYLCHOLINE

Acetylcholine is hydrolyzed by cholinesterase in the neuromuscular junctions and synapses, butyrylcholinesterase in the plasma. Administration of anti-cholinesterase antagonist such as neostigmine causes prolonged binding of acetylcholine receptors and sustained contraction. Atropine is a competitive blocker for muscarinic receptors and is highly selective, whereas nicotinic receptors are blocked by hexamethonium and tubocurarine.

2.4.2 EPINEPHRINE AND NOREPINEPHRINE

These are catecholamines secreted by the sympathetic nerves and the adrenal medulla. They are involved in regulatory mechanisms. Norepinephrine is a neurotransmitter secreted by adrenergic nerves of the sympathetic autonomic nervous system (ANS), while epinephrine is secreted by the adrenal medulla directly into the blood circulation. The physiological and metabolic responses preceding stimulation of the sympathetic nerves are mediated via the neurotransmitter Norepinephrine.

The effects of both substances when secreted are similar in some organs and differ in others. Both substances stimulate the myocardium (cardiac muscle) when secreted but epinephrine causes vasodilatation of blood vessels in the skeletal muscles (norepinephrine has no effect) and norepinephrine causes

vasoconstriction in capillaries of the skin, mucosa and kidney whereas epinephrine has no effect in these organs.

These catecholamines are synthesized from tyrosine by monoamine oxidase (MAO) and stored in vesicles in the cells when secreted. They bind to receptors on the neuroeffector organ to produce a response. However after acting, they are retaken into the cells and stored. These catecholamines are potent stimulants of α and β adrenergic receptors, leading to various effects in the body;

- Stimulates the cardiac muscle increasing the rate and force of contraction.
- Causes vasodilatation of blood vessels supplying skeletal muscles.
- Relaxes gastrointestinal smooth muscles and contraction of sphincters. Inhibits gastrointestinal secretion.
- Causes vasoconstriction of blood vessels in the skin and kidney.
- Increases the rate and depth of respiration.
- Increases the metabolic rate; glycolysis and glyconeogenesis

2.4.2.1 ADRENERGIC RECEPTOR ANTAGONIST

Adrenergic receptor antagonists compete for binding sites with catecholamines. Examples are phentolamine, prazosin, Yohimbine and phenoxybenzamine. They bind with α -adrenergic receptor but do not produce any effect as in the case of catecholamines. β-adrenergic receptors antagonist are propranolol (blocks β_1 and β_2 receptors), atenolol (blocks β_1) and Butoxamine (β_2 receptors). These receptors are highly selective for receptor binding sites.

2.4.3 5- HYDROXYTRYPTAMINE (SEROTONIN)

This compound has been noted for its effects on various types smooth muscles, platelet aggregation and as a neurotransmitter in the central nervous system (Adam 2001). 5-HT is found in high concentration in enterochromattin cells in the gastrointestinal tract, platelets and in the central nervous system. Molecular cloning elucidates the subtypes of 5-HT, which fall into 5 distinct functional groups, 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4 and 5-HT_5. The subtype present in the gastrointestinal tract is 5-HT_{2A}. When secreted it increases K^+ efflux from the intracellular fluid, depolarizing the cells and triggering an action potential (Trim et al 2001) (stimulating smooth muscle contractility).

2.4.5 HISTAMINE

Histamine is stored in mass cells and has been implicated as a mediator of allergic responses. It has major physiological regulating function on the gastric acid secretion and recently it has been documented to function as a neurotransmitter in the central nervous system (Adams 2001).

Histamine potentiates the following responses;

- It initiates contraction of smooth muscles of the bronchi and gastrointestinal tract via, H_1-receptors.
- It stimulates gastric acid secretion via H_2-receptors
- Histamine causes vasodilatation of vascular smooth muscles. Hypotension caused by this response is via H_1 and H_2 receptors on the vascular smooth muscles.
- Histamine potentiates the contraction of uterine smooth muscles

H_1 and H_2 receptor have been cloned and they belong to the super family of G-proteins-coupled receptors. H_1 receptors are coupled to phospholipase C and when activated initiates the formation of inositol-1,4,5-triphosphate (IP_3) and diacylglycerol from the phospholipids on the cell membrane. IP_3 stimulates rapid release of Ca^{2+} from the endoplasmic reticulum. Diacylglycerols activate protein Kinase C and Ca^{2+} activates Ca^{2+} calmodulin-dependent protein kinase and phspholipase A_2. H_2 receptors stimulate adenylyl cyclase which leads to the activation of cyclic AMP-dependent protein Kinase. Antihistamines such as diphenhydramine (Benadryl) are potent on H_1 and H_2 receptors.

2.4.5 CALCIUM ION (Ca^{2+}) CHANNEL ANTAGONIST

These drugs block Ca^{2+} channels, inhibiting the influx of Ca^{2+} into the intracellular fluid, thereby inhibiting contraction of muscles. Examples of antagonist are nifedipine and verapamol. From research studies, these drugs are effective in the treatment of hypertension. They decreased the inotropic effect of the cardiac muscles. Calcium channel blockers have diverse chemical structure which belongs to five classes. They include; verapamol (a phenylalkylamine), a diltiazem (a benzothiazepine), nicardine, nifedepine, amlodipine, felodipine, nimodipine (dihydropyridines) and bepridil (a diarylaminopropylamine ether).

Some plants have been identified to have Ca^{2+} channel blocking effects such as Bacopa monniera (Ahsana and Shaban 1999) and Andrographis paniculata (Burgos et al 2001).

2.4.6 MECHANISM OF DRUG ACTION

The effects of most drugs result from their interactions with macromolecular compounds present in the cell. These interaction acts on the function of the pertinent compound, thereby initiating biochemical and physiological changes. The term receptor was established to denote the component of the cell with which chemical substances interact and produce responses.

2.4.7 DRUG RECEPTOR

Receptors are predominantly made up of protein molecules. Many drugs act on receptors and are often selective because receptors are specialized to recognize and respond to particular signaling molecules with great sensitivity. Drugs bind to these receptors and mimic the effect of endogenous regulatory substances such as neurotransmitters and hormones. These drugs are also called agonist. Some other drugs interact with receptors but do not mimic the effect of these endogenous substances. These drugs are known as antagonist. The binding of drugs to receptors can be by ionic bonding, hydrogen bonding, hydrophobic, van der waal bonding or covalent bonding.

The interaction of a drug and its receptor depends on their chemical structures. Relatively minor modification of a drug or receptor modifies their interaction and pharmacological properties.

2.4.8 RELATIONSHIP BETWEEN DRUG CONCENTRATION AND EFFECT

Extending the analysis of drug-receptor interactions beyond the initial binding of drug to receptor raises important questions as to the relationship between the concentration of drug-receptor complex and the magnitude of the

observed effect. In the classical receptor theory developed, it was assumed that the effect of a drug is proportional to the fraction of receptors occupied by drug and that maximal effect results when all receptors are occupied. While these assumptions may be true in limited cases, exceptions are common. Consequently, Ariens (1954) introduced the term intrinsic activity (or r a "proportionality constant") to describe the relationship between the effect, E, elicited by a drug, D, and the concentration of drug-receptor complexes: $E = \alpha [DR]$. This relationship also addressed the anomalous observation that some drugs did not elicit a maximal response even at apparently maximal receptor occupancy. It was Stephenson (1956), however who advanced the concept of concentration-response relationships even further by offering an explanation for nonlinear relationships between occupancy and response, and for the ability of a group of agonists to produce equal responses while occupying different proportions of the receptor population. This explanation involves a property of an agonist called efficacy. The response, R, of a tissue is some function of the stimulus, S, given to that tissue: $R = f(S)$ and $S = ey$, where e = efficacy and y = fractional receptor occupancy. Differences in drug effects in different tissues are thus a reflection of the contributions of properties of the drug, properties of its receptor and properties of a tissue in terms of receptor density and the coupling of receptor occupancy to the ultimate response. Today, the terms intrinsic activity and efficacy are commonly used together and are operationally synonymous. Although the mathematical description of dose-response relationships cannot reveal molecular mechanisms, the basic descriptive concepts are instructive and provide an appreciation of how the concentration of a drug at its target organs determines the therapeutic response.

CHAPTER THREE - MATERIALS AND METHODOLOGY

3.2 PLANT MATERIALS

3.2.1 COLLECTION OF PLANT LEAVES

Ocimum basilicum plant was obtained from Ahmadu Bello University (A.B.U) farms located in Zaria. The leaves were collected in mid August and identified by Mallam Musa Mohammed in the herbarium of the department of biological sciences; A.B.U Zaria with voucher no 1875. The leaves were dried for two weeks in a well-ventilated room and ground to powder form and stored at room temperature until required.

3.2.2 PREPARATION OF EXTRACTS

Extraction of active constituents in dried powder was by the method of maceration. Solvents (water and methanol) were used to dissolve the powder.

The crude extracts were prepared by weighing 250g of dry powdered ground leaves into conical flasks. 200 ml of deionized water was mixed with the powder and placed on a mechanical shaker for 12 hours at 37^0C. Mixture was then allowed to stand for another 24 hour before filtered using a cotton plug. The filtrate was then placed in an evaporating dish and concentrated in a water bath at 40^oC until solid residue was obtained. 54g (aqueous extract) and 72g (methanolic extract) of brownish residue was obtained. Extracts were stored in a refrigerator at 4^oC until required for use.

3.2.3 PRECENTAGE YIELD OF EXTRACTS OF *OCIMUM BASILICUM* AFTER EXTRACTION

The initial weight of *Ocimum basilicum* leaves obtained from the field before drying was 1.056 Kg. Final weight of powdered leaves (ground) was 0.543 Kg (543g), representing 48.58% moisture content and 51.42% dry matter $^{w}/_{w}$. Quantity of dry leaves used for extraction was 250g to yield 54g of aqueous extract and 72g of methanolic extract constituting the original sample taken.

$$\% \text{ yield} = \frac{\text{weight of extract (concentrated)}}{\text{weight of powdered leaves taken}} \times 100\%$$

$$\% \text{ yield for aqueous extract} = \frac{54}{250} \times 100\%$$

$$= 0.216 \times 100\%$$

$$= 21.6\%$$

$$\% \text{ yield for methanolic extract} = \frac{72}{250} \times 100\%$$

$$= 0.288 \times 100\%$$

$$= 28.8\%$$

3.3 PREPERATION OF SOLUTIONS

Each drug was freshly prepared each day before use. Stock solutions of drugs were prepared by weighing 0.01g of drugs (acetylcholine, histamine, serotonin, epinephrine, norepinephrine and isoprenaline) and dissolved in 10ml of deionized water to give a concentration of 100µg/ml. Further dilutions were made to 1µg/ml, 10µg/ml and 100µg/ml by diluting appropriately (see appendix 47).

A stock solution of the plant extracts was also prepared. 1g of extracts was dissolved in 10ml of deionized water to give a concentration of 100mg/ml. Further dilutions were made to 1mg/ml and 10mg/ml (see appendix 47).

3.4 ANIMALS

Nine adult rabbits weighing 2.0-2.5 Kg, 50 Wister rats weighing 150-200g and twenty-six mice weighing 20-30g of both sexes were obtained from the department of pharmacology animal house A.B.U Zaria. The animals were maintained on standard laboratory animal feeds and tap water, which was given, ad libitum. They were kept in cages at room temperature. The animals were fasted 24 hours before all the experiments.

3.5 DETERMINATION OF MEDIAN LETHAL DOSE (LD_{50}) OF *OCIMUM BASILICUM* EXTRACTS IN MICE

The median lethal doses (LD_{50}) of plant extracts were determined by the method of Lorke (1983) using thirteen mice (150-200g) for each extract. Nine mice were divided into three groups (three each). Testing was done in two procedures: firstly, oral doses of plant extracts dissolved in normal saline were

given to mice in groups I, II and III at 10, 100 and 1000 mg per body weight (Kg) respectively. The animals were observed for 24 hours for toxic signs and death. Based on the results in the initial procedure, further specific doses of 1000, 1600, 2900 and 5000 mg/Kg were selected in the final procedure according to Lorke's (1983) method. Extracts were orally administered to animals (one animal per group) and observed for another 24 hours for toxic signs and death. Results were recorded as a ratio of number of animals that died over the total number of animals in the group. The median lethal doses (LD_{50}) of the extracts were analyzed by obtaining the geometrical mean of the highest non-lethal ($^0/_1$) dose [highest dose that produced 0% mortality] and the lowest lethal ($^1/_1$) dose [lowest dose that produced 100% mortality].

3.6 EXPERIMENTAL PROTOCOLS

3.6.1 IN-VIVO STUDIES (INVESTIGATING THE EFFECT OF *OCIMUM BASILICUM* EXTRACTS ON INTESTINAL TRANSIT TIME IN RATS)

Twenty-five rats (150-200g) were divided into five groups with five animals in each group. Oral doses of aqueous extract of *Ocimum basilicum* were given through a cannular to animals in groups II-V at 20, 40, 60 and 80mg/kg respectively and group I animals served as the control group (animals received normal saline). Fifteen minutes later 0.5 ml of 10% charcoal ($BaSO_4{\cdot}H_2O$) suspended in normal saline was orally administered to conscious animals. An additional 5ml of air was added to flush the residual charcoal suspension remaining in the cannular into the rats. Animals were sacrificed by decapitation 30 minutes later. The stomach and small intestines were exposed immediately by lapratomy. The oesophagogastric, gastroduodenal and ileocaecal junctions were

ligated and the whole stomach and intestines were carefully removed. They were laid out on a wooden board to observe the charcoal front. This was indicated by the leading edge, which the charcoal had moved in the intestines. The gastro-intestinal transit time was analyzed by dividing the length at which the charcoal had travelled to the total length of the small intestines. Intestinal transit time was also assessed in twenty-five rats using methanolic extract of *Ocimum basilicum* with the experimental procedure described above. This experimental procedure was described by Chen et al 1995.

3.6.2 IN-VITRO STUDIES (INVESTIGATING THE EFFECT OF *OCIMUM BASILICUM* EXTRACTS ON SMOOTH MUSCLE CONTRACTILITY IN RABBIT ILEUM)

In this experiment, rabbits were dissected and 2-3cm strips of the ileum was removed and suspended vertically in a 25ml thermostatically controlled organ bath under tension of 5g containing tyrode solution at 37^0C and a pH of 7.4. After an equilibrating period of 90 minutes, spontaneous contractions was recorded by a microdynamometer on a tracing paper by adding non-cumulative concentrations of standard drugs (acetylcholine, histamine, serotonin, epinephrine, norepinephrine and isoprenaline) in concentrations of 4.00×10^{-10}, 8.00×10^{-10}, 1.60×10^{-9}, 3.20×10^{-9}, 6.40×10^{-9}, 1.28×10^{-8}, 2.56×10^{-8}, 5.12×10^{-8}, 1.024×10^{-7}, 2.048×10^{-7} and 4.06×10^{-7} g/ml into the organ bath. Non-cumulative concentrations of plant extracts was also added to organ bath at concentrations of 4.00×10^{-6}, 8.00×10^{-6}, 1.60×10^{-5}, 3.20×10^{-5}, 6.40×10^{-5}, 1.28×10^{-4}, 2.56×10^{-4}, 5.12×10^{-4}, 1.024×10^{-3} and 2.048×10^{-3} g/ml. and

spontaneous contractions recorded as described above. Experiment as described by Rebollar et al 2002.

3.6.3 TO INVESTIGATE THE POSSIBLE MECHANISMS OF ACTION OF *OCIMUM BASILICUM* EXTRACTS

Ocimum basilicum extracts probably mediate their effects via α and β receptors located on the cell membrane. Receptor antagonists were administered into the organ bath containing rabbit ileum before administering extracts. Phentolamine (α-antagonist) and propranolol (β-antagonist) at concentrations of 4.00×10^{-10}, 8.00×10^{-10}, 1.6×10^{-9}, 3.2×10^{-9}, 6.4×10^{-9} and 1.28×10^{-8} g/ml were administered into organ bath. 10 seconds after aqueous extract that produced maximum response (E_{max}) in rabbit ileum was added into the same organ bath. The same procedure was performed for the methanolic extract. Spontaneous contractions in rabbit ileum were recorded. This protocol was described by Rebollar et al 2002.

3.7 ANALYSIS OF DATA

All data were expressed as mean ± S.E.M. Statistical difference between the groups were evaluated using the student's t-test (Snedecor and Cochran 1980), probability value less than 0.05 ($P<0.05$) was considered as statistically significant.

CHAPTER FOUR – RESULTS

4.1 ACUTE ORAL TOXICITY STUDIES (LD_{50}) OF *OCIMUM BASILICUM* EXTRACTS IN MICE

The extracts were readily soluble in normal saline. The number of animals that died after oral administration of both aqueous and methanolic extracts was recorded in Tables 4.1, 4.2, 4.3 and 4.4. Median lethal doses (LD_{50}) were then analyzed using the formula in section 4.1.3.

4.1.1 ACUTE ORAL TOXICITY STUDIES (LD_{50}) OF AQUEOUS EXTRACT OF *OCIMUM BASILICUM* IN MICE

In Table 4.1, animals (n=3) that received 10, 100 and 1000 mg/kg of aqueous extract recorded 0% mortality ($^0/_3$). In Table 4.2, 0% mortality was recorded in animals that received 1000, 1600 and 2900 mg/kg ($^0/_1$). 100% mortality was recorded in the group (n=1) that received 5000 mg/kg ($^1/_1$). Median lethal dose was estimated as 3807.89 mg/Kg (see section 4.1.3).

4.1.2 ACUTE ORAL TOXICITY STUDIES (LD_{50}) OF METHANOLIC EXTRACT OF OCIMUM BASILICUM IN MICE

0% mortality ($^0/_3$) was recorded in animals (n=3) that received 10, 100 and 1000 mg/Kg of methanolic extract (Table 4.3). 0% mortality ($^0/_1$) was recorded in animals (n=1) that received 1000 and 1600 mg/Kg. 100% mortality ($^1/_1$) was recorded in animals (n=1) that received 2900 and 5000 mg/Kg (Table 4.4). LD_{50} for methanolic extract was calculated to be 2154.07 mg/Kg (see section 4.1.3).

DETERMINATION OF MEDIAN LETHAL DOSE (LD_{50}) OF *OCIMUM BASILICUM* EXTRACTS IN MICE

$$LD_{50} = \sqrt{\text{Highest non-lethal dose x Lowest lethal dose}}$$

From the formula above the LD_{50} for extracts were calculated as;

MEDIAN LETHAL DOSE (LD_{50}) AQUEOUS EXTRACT OF *OCIMUM BASILICUM* IN MICE

LD_{50} for aqueous extract $= \sqrt{2900 \text{ X } 5000}$ mg/Kg

$= \sqrt{14\ 500\ 000}$ mg/Kg

$= 3807.89$ mg/Kg

MEDIAN LETHAL DOSE (LD_{50}) OF METHANOLIC EXTRACT OF *OCIMUM BASILICUM* IN MICE

LD_{50} for methanolic extract $= \sqrt{1600 \text{ X } 2900}$ mg/Kg

$= \sqrt{4\ 640\ 000}$ mg/Kg

$= 2154.07$ mg/Kg

Table 4.1: Initial studies (acute oral toxicity for aqueous extract). Animals in this study received oral doses of aqueous extracts and were observed. Doses of extracts were calculated and administered according to individual weights of animals. Clinical signs of toxicity were considered. At this stage, doses selected had a wide range [n = 3 (where n - number of animals per group)].

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GROUP	DOSES (mg/Kg)	MORTALITY RATE	CLINICAL SIGNS
1	10	$^{0}/_{3}$	NIL
2	100	$^{0}/_{3}$	NIL
3	1000	$^{0}/_{3}$	NIL

Table 4.2: Final studies (acute oral toxicity for aqueous extract). After an initial oral administration to animals in Table 4.1, new doses of aqueous extract were selected and administered. Animals were observed for signs of toxicity [n = 1 (where n - number of animals per group)].

GROUP	DOSES (mg/Kg)	MORTALITY RATE	CLINICAL SIGNS
1	1000	$^{0}/_{1}$	NIL
2	1600	$^{0}/_{1}$	NIL
3	2900	$^{0}/_{1}$	NIL
4	5000	$^{1}/_{1}$	Restlessness, excitation, jerking and death

Table 4.3: Initial studies (acute oral toxicity for methanolic extract). Methanolic extract was administered orally to animals. Weights of animals were considered. Signs of toxicity were observed in animals after administration of extracts and recorded below [n = 3 (where n - number of animals per group)].

GROUP	DOSES (mg/Kg)	MORTALITY RATE	CLINICAL SIGNS
1	10	$^{0}/_{3}$	NIL
2	100	$^{0}/_{3}$	NIL
3	1000	$^{0}/_{3}$	NIL

Table 4.4: Final studies (acute oral toxicity for methanolic extract). After an initial oral administration of methanolic extract to animals, a second dose range was selected and administered to animals and signs of toxicity were observed and recorded [n = 1 (where n - number of animals per group)].

GROUP	DOSES (mg/Kg)	MORTALITY RATE	CLINICAL SIGNS
1	1000	$^{0}/_{1}$	NIL
2	1600	$^{0}/_{1}$	NIL
3	2900	$^{1}/_{1}$	Restlessness, excitation, jerking and death
4	5000	$^{1}/_{1}$	Restlessness, excitation, jerking and death

4.2.0 EFFECT OF AQUEOUS EXTRACT ON INTESTINAL TRANSIT TIME

Oral administration of aqueous extract of *Ocimum basilicum* to animals, produced a significant ($P<0.001$) decrease in the distance traveled by the charcoal fronts in groups III, IV and V as compared with the control group (Table 4.5) and ($P<0.05$) for group II. Table 4.5 (refer to appendix 6 -10) also illustrates a significant decrease ($P<0.05$) in intestinal transit time in groups II and III as compared with the control and ($P<0.001$) for groups IV and V.

In Figures 4.1 and 4.2, aqueous extract of *Ocimum basilicum* was able to decrease the length traveled by charcoal front and the intestinal transit time in a dose dependent manner.

4.2.1 EFFECT OF METHANOLIC EXTRACT ON INTESTINAL TRANSIT TIME

Oral administration of methanolic extract of *Ocimum basilicum* in Table 4.6 (appendix 1-6) produced a significant ($P<0.001$) decrease in distance traveled by charcoal fronts in groups II, III, IV and V as compared with the control group. Groups II and III showed a non-significant ($P<0.05$) decrease in intestinal transit time and groups IV and V produced a significant ($P<0.001$) decrease as compared with the control group.

Methanolic extract of *Ocimum basilicum* decreased the length traveled by charcoal front (Figure 4.3) and intestinal transit time (Figure 4.4) in a dose dependent manner which indicates an inhibition in peristaltic movement of the intestines.

Table 4.5: Effects of aqueous extract of Ocimum basilicum on the length of charcoal front (cm) of total length (cm) and intestinal transit time (mm/min) expressed as Mean ± SEM.

Parameters	Groups/Doses				
	I (Control)	II (20mg/Kg)	III (40mg/Kg)	IV (60mg/Kg)	V (80mg/Kg)
Total Length of small Intestine (cm)	119.42 ± 8.19	109.76 ± 8.60	97.16 ± 4.91	111.60 ± 9.75	107.60 ± 9.33
Charcoal Front (cm)	52.46 ± 2.39	30.38 ± 5.77 *	29.74 ± 3.89 **	21.14 ± 3.18 **	7.64 ± 2.21 **
Gastrointestinal Transit (mm/min)	0.44 ± 0.03	0.28 ± 0.05 *	0.31 ± 0.06 *	0.19 ± 0.01 **	0.07 ± 0.02 **

*P < 0.05 (significant difference), **P < 0.001 (highly significant difference), ^{NS} P>0.05 (non significant difference) as compared to control: student t 'test' (n = 5).

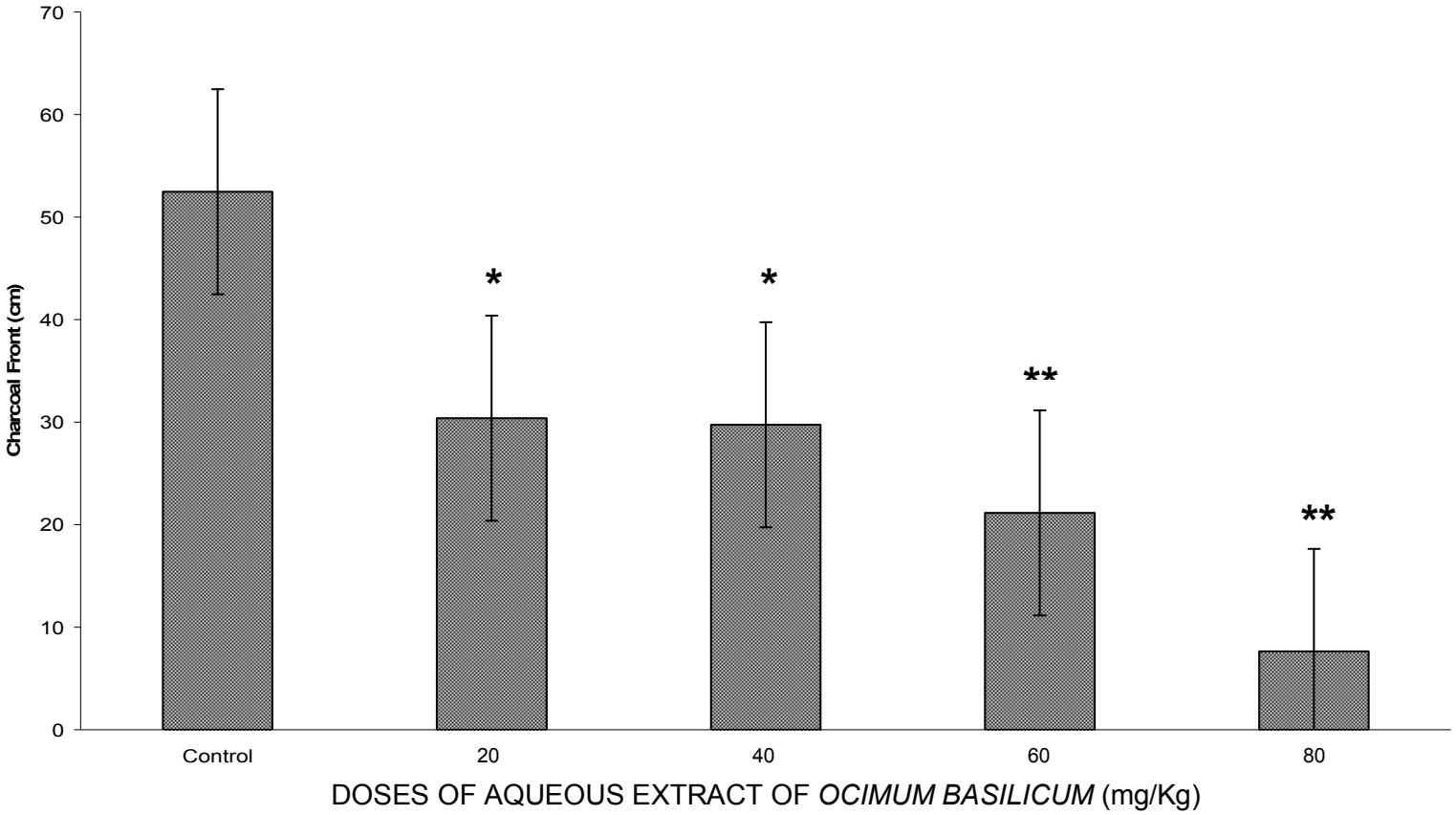


Figure 4.1: Effect of oral administration of aqueous extract of *Ocimum basilicum* on the distance traveled by charcoal front (cm). Data shown indicate Mean ± SEM * P<0.05 (significant difference), **P<0.001 (highly significant difference) and ^{NS} P>0.05 (non significant difference) as compared with control group (n = 5).

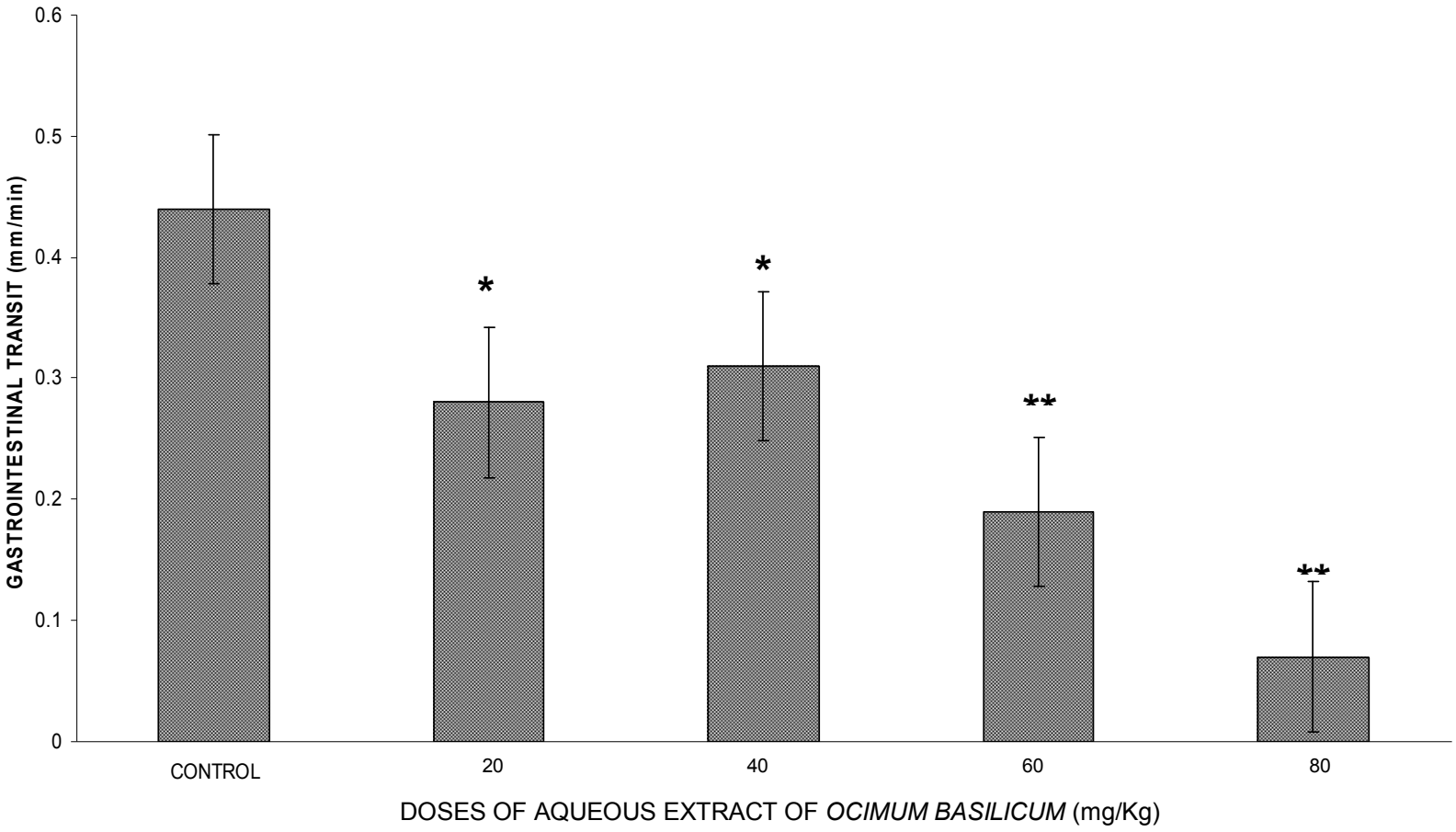


Figure 4.2: Effects of oral administration of aqueous extract of *Ocimum basilicum* on gastrointestinal transit time (mm/min). Data indicate Mean ± SEM *P<0.05 (significant difference), **P<0.001 (highly significant difference) and ^{NS} P>0.05 (non significant difference) as compared with control group (n = 5).

Table 4.6 Effects of methanolic extract of *Ocimum basilicum* on the length of charcoal front (cm) of total length (cm) and intestinal transit time (mm/min) expressed as Mean ± SEM.

Parameters	Groups/Doses				
	I (Control)	II (20mg/Kg)	III (40mg/Kg)	IV (60mg/Kg)	V (80mg/Kg)
Total Length of small Intestine (cm)	178.26 ± 4.75	78.46 ± 3.00	86.34 ± 0.38	92.76 ± 2.26	90.20 ± 2.38
Charcoal Front (cm)	54.74 ± 3.72	16.60 ± 0.69**	24.88 ± 2.47**	16.08 ± 0.91**	5.56 ± 2.24**
Gastrointestinal Transit (mm/min)	0.31 ± 0.04	0.21 ± 0.05 ^{NS}	0.29 ± 0.01^{NS}	0.17 ± 0.02*	0.06 ± 0.01**

*P < 0.05 (significant difference), **P < 0.001(highly significant difference), ^{NS}P>0.05 (non significant difference) as compared to control (n = 5).

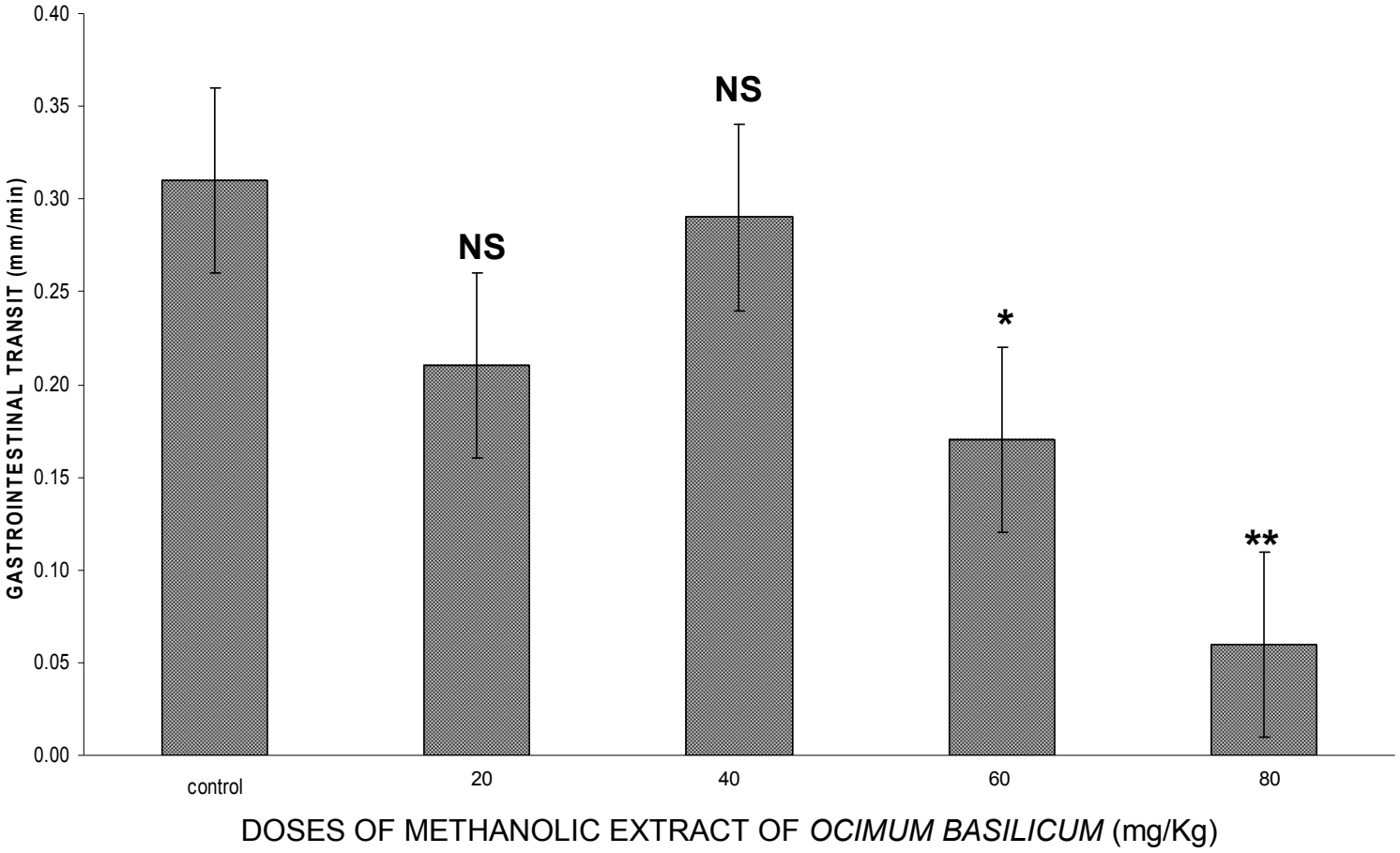


Figure 4.3: Effect of oral administration of methanolic extract of *Ocimum basilicum* on gastrointestinal transit time (mm/min). Data indicate Mean ± SEM *P<0.05 (significant difference), **P<0.001 (highly significant difference) and ^{NS}P>0.05 (non significant difference) as compared with control group (n = 5).

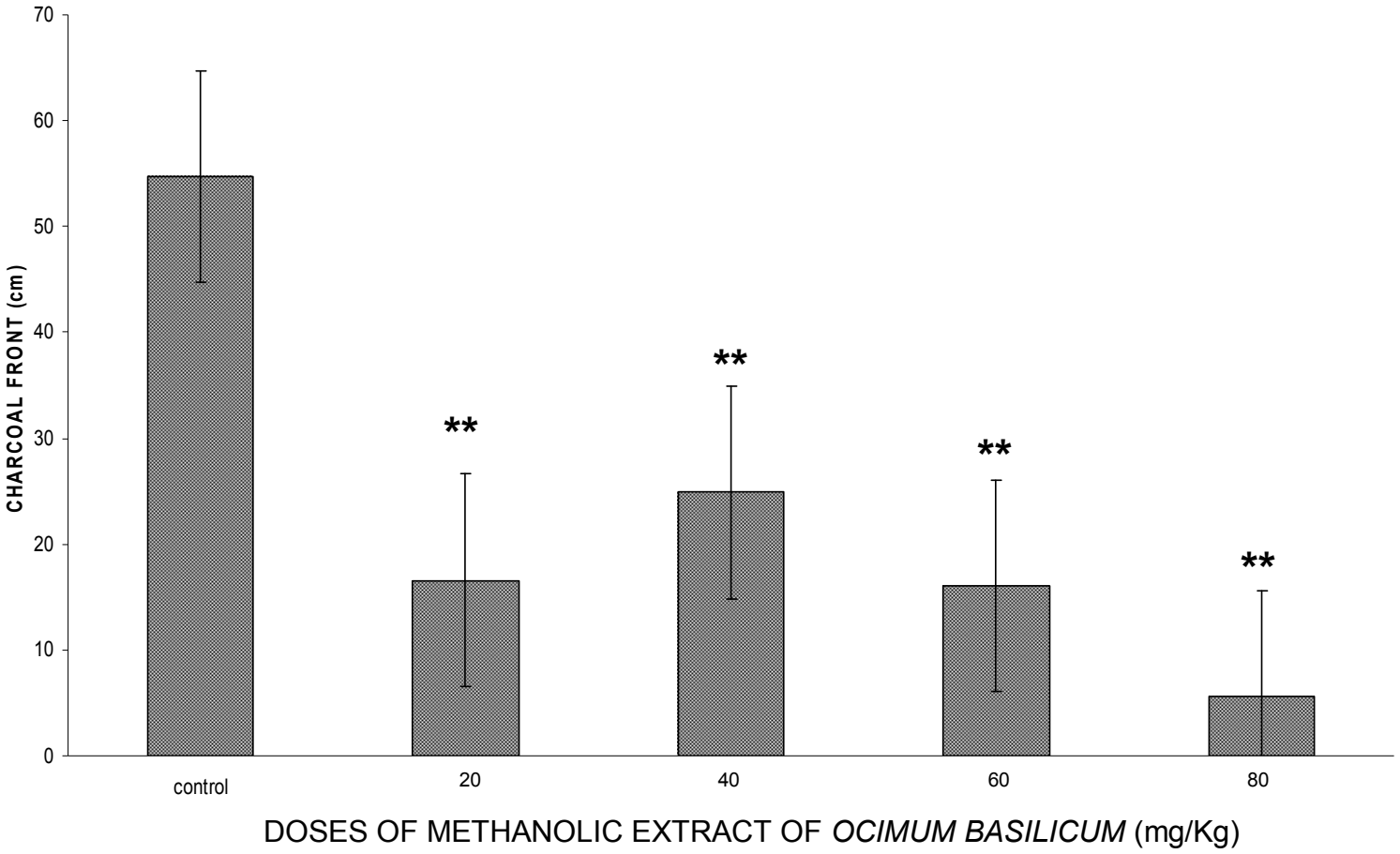


Figure 4.4: Effect of oral administration of methanolic extract of *Ocimum basilicum* on length of charcoal Front (cm). Data indicate Mean ± SEM. *P<0.05 (significant difference), **P<0.001 (highly significant difference) and ^{NS} P>0.05 (non significant difference) as compared with control group (n = 5).

4.3.0 IN VITRO STUDIES OF EXTRACTS OF *OCIMUM BASILICUM* ON RABBIT ILEUM

Segments of rabbit ileum exhibited rhythmic and cyclic spontaneous contractions when suspended in the organ bath containing tyrode solution. Normal contractions were established with an average frequency of contractions at 12.60 $\pm$ 0.42 contractions/minute and amplitude of contraction at 29.60 $\pm$ 0.79 mm (n=9) (appendix 42).

4.3.1 EFFECTS OF ACETYLCHOLINE ON RABBIT ILEUM

Tissues (n=9) (see appendix 12) showed an increase in spontaneous contraction with a threshold concentration of 1.6 X 10^{-9} g/ml and maximum response was attained at 1.024 X 10^{-7} g/ml (Figure 4.5). At concentrations lower than 1.60 X 10^{-9} g/ml, increase in spontaneous contractions (34.23 $\pm$ 0.09 to 34.59 $\pm$ 0.13 mm) were not significant ($P>0.05$). At concentrations higher than 1.60 X 10^{-9} g/ml, increase in spontaneous contraction (33.88 $\pm$ 0.25 to 65.68 $\pm$ 0.21 mm) were highly significant ($P<0.001$) (Table 4.7). Chart in Figure 4.7 illustrates the increase in contractions.

There was no significant increase ($P>0.05$) in the frequency of contraction (Table 4.8). At 1.6 X 10^{-9} g/ml, frequency was 12.68 $\pm$ 0.09 contractions/minute (control group) and 13.80 $\pm$ 0.20 contractions/minute (test group) and at 1.024 X 10^{-7} g/ml, frequency was 13.39 $\pm$ 0.14 contractions/minute (control group) and 13.46 $\pm$ 0.11 contractions/minute (test group). Figure 4.6 illustrates response in frequency.

Table 4.7: Effect of acetylcholine on amplitude (mm) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of acetylcholine (g/ml)	Amplitude of contractions (mm)		T-value	P- value
	Control	Test		
$4X10^{-10}$	34.61 ± 0.39	35.27 ± 0.20	-1.51	0.152
$8X10^{-10}$	34.33 ± 0.28	34.44 ± 0.14	-0.35	0.730
$1.6X10^{-9}$	34.23 ± 0.09	34.59 ± 0.13	-2.28	0.037
$3.2X10^{-9}$	34.41 ± 0.12	38.97 ± 0.62	-7.22	<0.001
$6.4X10^{-9}$	33.31 ± 0.81	43.74 ± 0.15	-12.66	<0.001
$1.28X10^{-8}$	33.87 ± 0.44	62.37 ± 0.40	-47.93	<0.001
$2.56X10^{-8}$	34.23 ± 0.50	62.42 ± 0.44	-42.33	<0.001
$5.12X10^{-8}$	33.68 ± 0.22	64.77 ± 0.43	-64.37	<0.001
$1.024X10^{-7}$	33.88 ± 0.25	65.68 ± 0.21	-97.40	<0.001

- Test indicates amplitude of spontaneous contraction of rabbit ileum when acetylcholine was added into organ bath containing tyrode solution
- Control indicates amplitude of spontaneous contraction of rabbit ileum when acetylcholine was washed out of organ bath and replaced with tyrode solution only
- Increase in spontaneous contraction to acetylcholine of rabbit ileum was dose dependent
- **g/ml**- [gram(s) per milliliter] and **mm**-[millimeters]

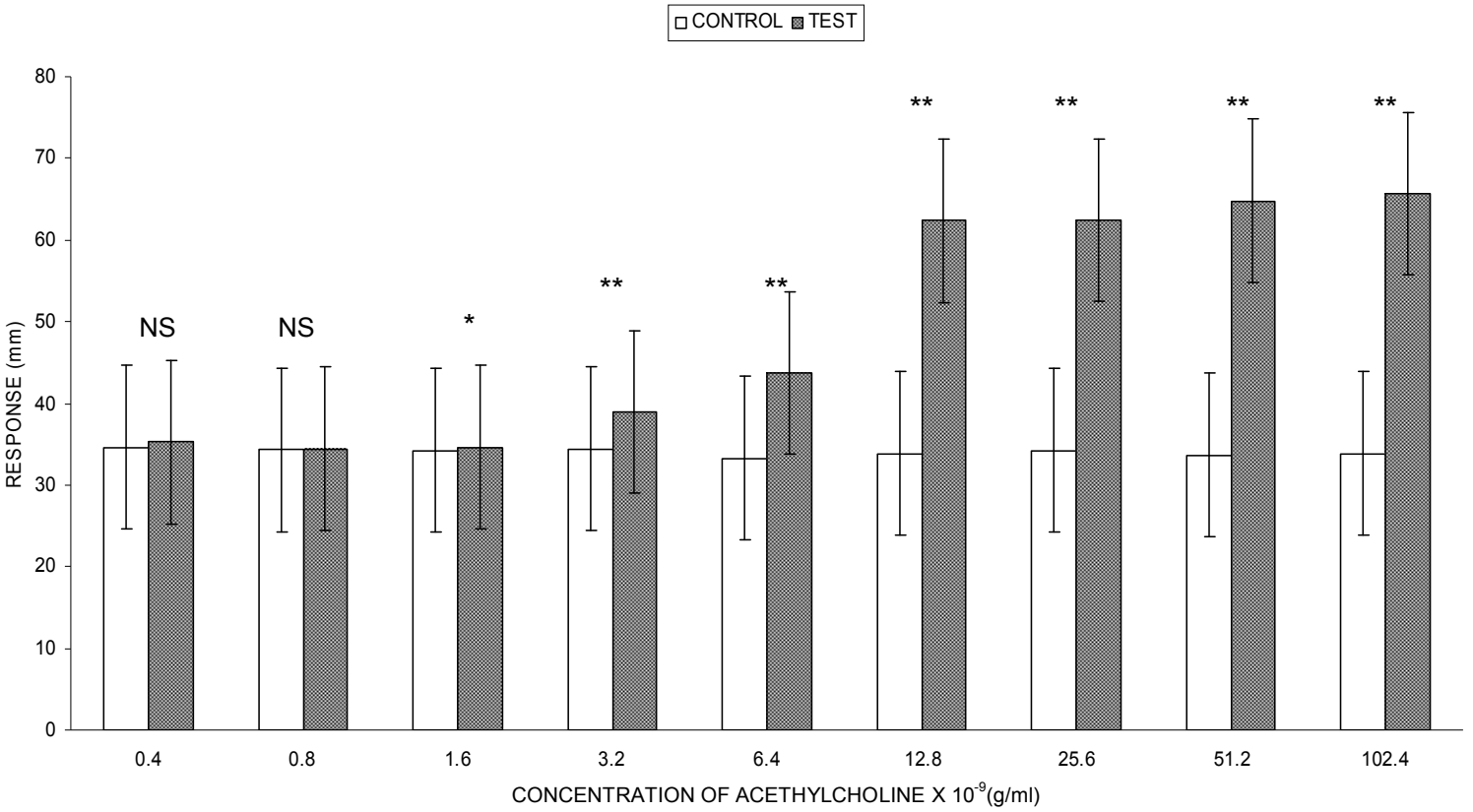


Figure 4.5: Chart represents the amplitude of spontaneous contraction of rabbit ileum after the administration of acetylcholine into organ bath containing tyrode solution. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the amplitude of spontaneous contractions after administration of acetylcholine into the organ bath and control bars indicate spontaneous contraction after acetylcholine was washed out of the organ bath and replaced with tyrode solution.

Table 4.8: Effect of acetylcholine on frequency (contractions/minute) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of acetylcholine (g/ml)	Frequency (cpm)		T- value	P- value
	Control	Test		
$4X10^{-10}$	12.17 ± 0.16	12.04 ± 0.15	0.59	0.562
$8X10^{-10}$	12.93 ± 0.18	12.80 ± 0.24	0.43	0.671
$1.6X10^{-9}$	12.68 ± 0.09	13.80 ± 0.20	-5.11	<0.001
$3.2X10^{-9}$	13.10 ± 0.14	14.07 ± 0.18	-4.25	<0.001
$6.4X10^{-9}$	13.77 ± 0.14	13.40 ± 0.10	2.15	0.047
$1.28X10^{-8}$	13.41 ± 0.12	13.54 ± 0.14	-0.71	0.419
$2.56X10^{-8}$	13.42 ± 0.16	13.33 ± 0.12	0.45	0.659
$5.12X10^{-8}$	13.38 ± 0.13	13.47 ± 0.15	-0.45	0.656
$1.024X10^{-7}$	13.39 ± 0.14	13.46 ± 0.11	-0.39	0.699

- Test indicates frequency of spontaneous contraction of rabbit ileum when acetylcholine was added into organ bath containing tyrode solution
- Control indicates frequency of spontaneous contraction of rabbit ileum when acetylcholine was washed out of organ bath and replaced with tyrode solution only.
- **g/ml**- [gram(s) per milliliter] and **cpm**- contractions per minute

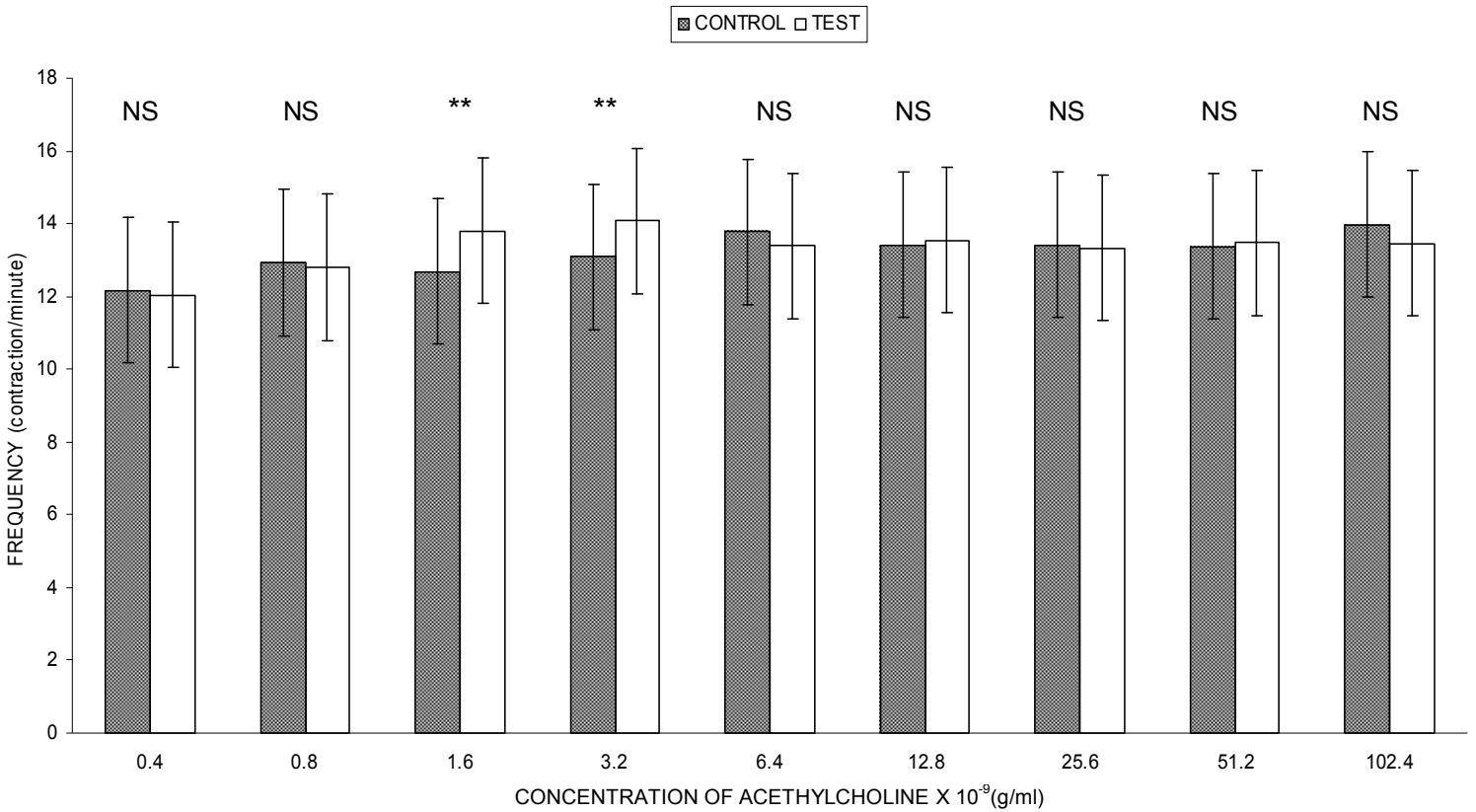


Figure 4.6: Chart represents the frequency of spontaneous contraction of rabbit ileum after the administration of acetylcholine into organ bath containing tyrode solution. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the frequency of spontaneous contractions after administration of acetylcholine into the organ bath and control bars indicate frequency of spontaneous contraction after acetylcholine was washed out of the organ bath.

4.3.2 EFFECTS OF HISTAMINE ON RABBIT ILEUM

An increase in amplitude of spontaneous contraction in tissues (n=9) (appendix 29) was observed with threshold response at the concentration (Figure 4.7) of 8.0 X 10^{-10} g/ml (26.46 ± 0.64 and 35.09 ± 0.80 mm for control and test groups respectively) and a maximum response at the concentration of 1.024 X 10^{-7} g/ml (26.47 ± 0.87 and 62.93 ± 0.79 mm for control and test groups respectively) which were considered highly significant (P<0.001) (Table 4.9).

Tissues (n=9) (appendix 30) showed no significant difference (P>0.05) in the frequency of contractions (Figures 4.8) when histamine was administered. At a maximum concentration of 1.024 X 10^{-7} g/ml, frequency was 16.33 ± 1.13 contractions/minute in control group and 16.67± 1.13 contractions/minute in test group (Table 4.10).

4.3.3 EFFECTS OF SEROTONIN (5-HT) ON RABBIT ILEUM

Threshold response (Figure 4.9) in amplitude of tissues (n=9) (appendix 31) were established at 1.6 X 10^{-9} g/ml (26.79 ± 0.80 and 34.30 ± 0.94 mm for control and test groups respectively) and the maximum response was obtained at a concentration of 1.024 X 10^{-7} g/ml (26.73 ± 0.76 and 62.84 ± 0.51 mm for control and test groups respectively). At these concentrations, increase in amplitude were considered highly significant (p<0.001) (Table 4.11).

There was no significant difference (P>0.05) in the frequency (Figure 4.10) of spontaneous contractions of tissues (n=9)(appendix 32) at a maximum concentration of 1.024 X 10^{-7} g/ml, frequency was 16.33 ± 1.42 to 16.67 ± 1.24 contractions/minute in control and test groups respectively) (Table 4.12).

Table 4.9: Effect of histamine on amplitude (mm) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of histamine (g/ml)	Amplitude (mm)		T- value	P- value
	Control	Test		
$4X10^{-10}$	26.47 ± 0.87	6.57 ± 0.92	-0.08	0.938
$8X10^{-10}$	26.46 ± 0.64	5.09 ± 0.80	-8.42	<0.001
$1.6X10^{-9}$	25.18 ± 0.96	0.63 ± 0.90	-11.74	<0.001
$3.2X10^{-9}$	25.48 ± 0.79	1.20 ± 0.81	-22.73	<0.001
$6.4X10^{-9}$	26.24 ± 0.74	3.02 ± 0.88	-23.29	<0.001
$1.28X10^{-8}$	26.96 ± 0.87	6.42 ± 1.00	-22.23	<0.001
$2.56X10^{-8}$	26.16 ± 0.73	8.48 ± 0.99	-26.28	<0.001
$5.12X10^{-8}$	26.79 ± 0.74	0.59 ± 0.85	-29.99	<0.001
$1.024X10^{-7}$	26.47 ± 0.87	2.93 ± 0.79	-28.79	<0.001

- Test indicates amplitude of spontaneous contraction of rabbit ileum when histamine was added into organ bath containing tyrode solution
- Control indicates amplitude of spontaneous contraction of rabbit ileum when histamine was washed out of organ bath and replaced with tyrode solution only
- Increase in spontaneous contraction to histamine of rabbit ileum was dose dependent
- **g/ml**- [gram(s) per milliliter] and **mm**-[millimeters]

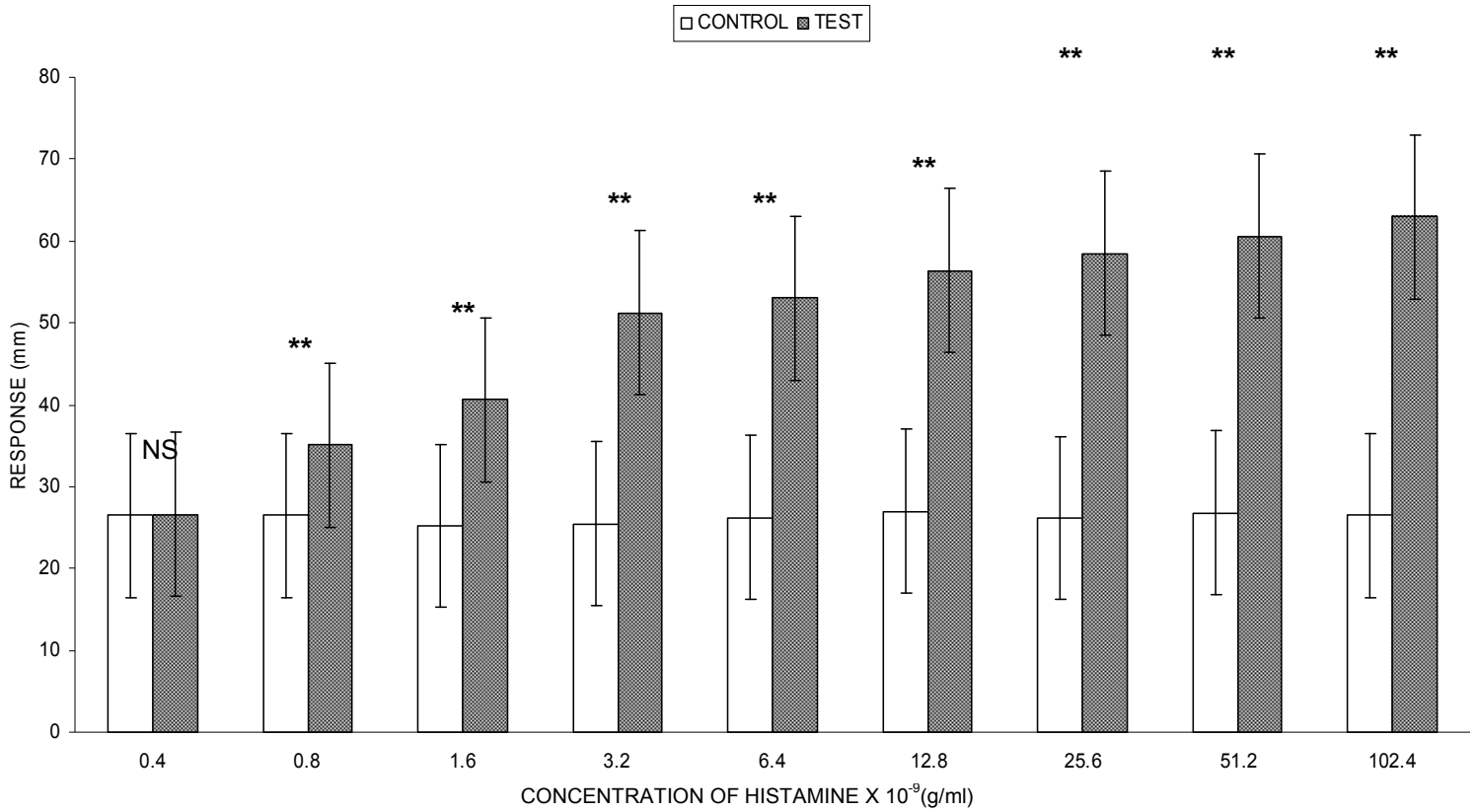


Figure 4.7: Chart represents the amplitude of spontaneous contraction of rabbit ileum after the administration of histamine into organ bath containing tyrode solution. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the amplitude of spontaneous contractions after administration of histamine into the organ bath and the control bars indicate the amplitude of spontaneous contraction after histamine was washed out of the organ bath and replaced with tyrode solution.

Table 4.10: Effect of histamine on frequency (contractions/minute) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration (g/ml)	Frequency (cpm)		T- value	P- value
	Control	Test		
$4X10^{-10}$	16.33 ± 1.13	16.67 ± 1.13	-0.21	0.834
$8X10^{-10}$	17.00 ± 1.32	17.33 ± 1.39	-0.17	0.865
$1.6X10^{-9}$	16.33 ± 1.13	16.67 ± 1.13	-0.21	0.834
$3.2X10^{-9}$	17.67 ± 1.27	16.67 ± 1.33	0.54	0.594
$6.4X10^{-9}$	16.33 ± 1.13	17.67 ± 1.36	-0.76	0.460
$1.28X10^{-8}$	16.67 ± 1.13	17.00 ± 1.00	-0.33	0.830
$2.56X10^{-8}$	16.33 ± 1.13	16.67 ± 1.67	-0.17	0.868
$5.12X10^{-8}$	17.00 ± 1.22	16.67 ± 1.13	0.20	0.845
$1.024X10^{-7}$	16.33 ± 1.13	16.67 ± 1.13	-0.21	0.834

- Test indicates frequency of spontaneous contraction of rabbit ileum when histamine was added into organ bath containing tyrode solution
- Control indicates frequency of spontaneous contraction of rabbit ileum when histamine was washed out of organ bath and replaced with tyrode solution only.
- **g/ml**- [gram(s) per milliliter] and **cpm**- contractions per minute

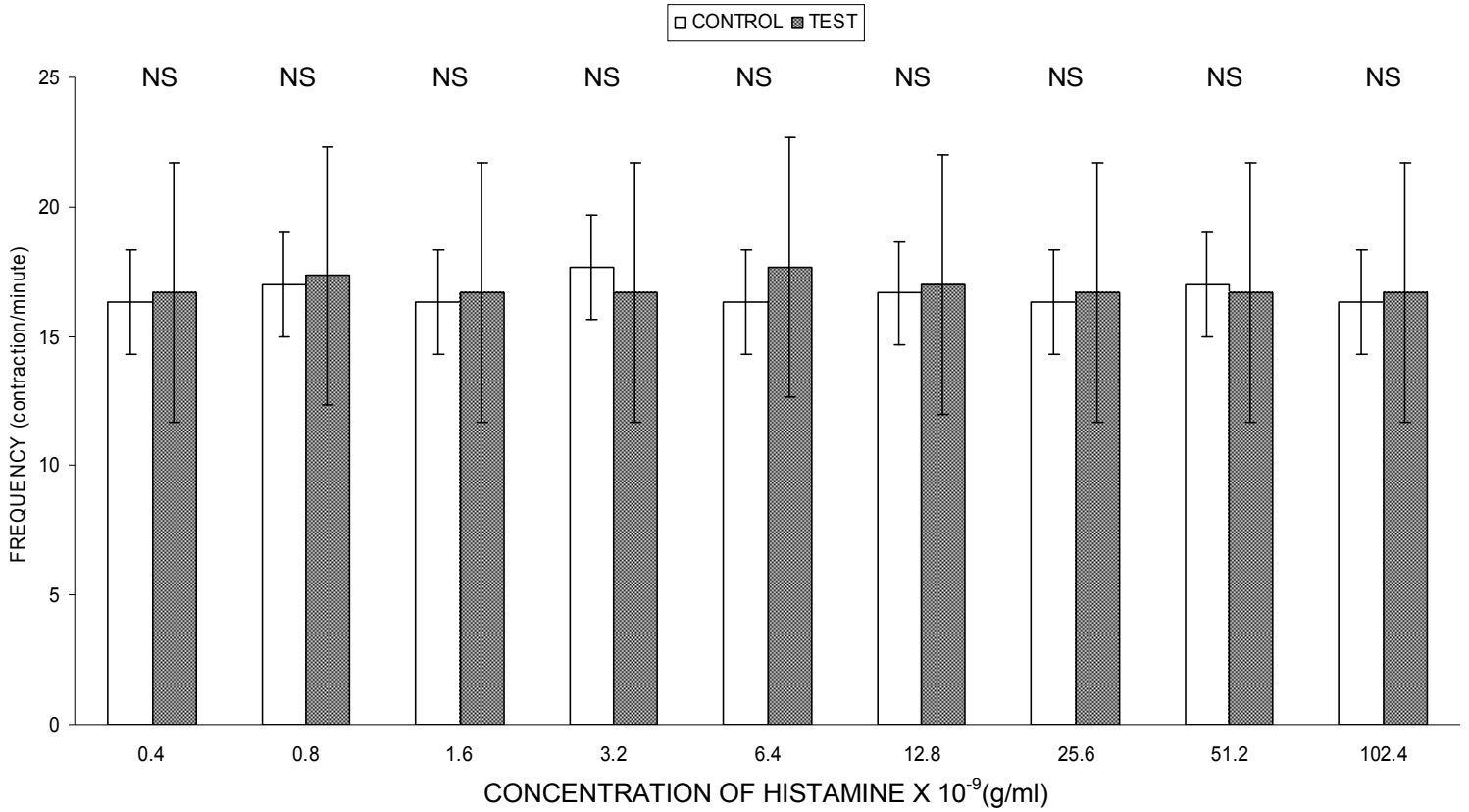


FIGURE 4.8 Chart represents the frequency of spontaneous contraction of rabbit ileum after the administration of histamine into organ bath containing tyrode solution. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the frequency of spontaneous contractions after administration of histamine into the organ bath and control bars indicate frequency of spontaneous contraction after histamine was washed out of the organ bath and replaced with tyrode solution.

Table 4.11: Effect of serotonin (5-HT) on amplitude (mm) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of serotonin (g/ml)	Amplitude of contractions (mm)		T-value	P- value
	Control	Test		
$4X10^{-10}$	25.74 ± 0.79	26.11 ± 0.94	-0.30	0.767
$8X10^{-10}$	26.73 ± 0.76	26.57 ± 0.66	0.16	0.876
$1.6X10^{-9}$	26.79 ± 0.80	34.30 ± 0.94	-6.08	<0.001
$3.2X10^{-9}$	26.47 ± 0.61	51.27 ± 1.05	-20.42	<0.001
$6.4X10^{-9}$	27.72 ± 0.64	52.78 ± 1.05	-20.38	<0.001
$1.28X10^{-8}$	26.97 ± 0.73	57.66 ± 0.96	-25.45	<0.001
$2.56X10^{-8}$	27.40 ± 0.64	61.21 ± 0.82	-32.50	<0.001
$5.12X10^{-8}$	25.74 ± 0.79	61.82 ± 0.48	-39.03	<0.001
$1.024X10^{-7}$	26.73 ± 0.76	62.84 ± 0.51	-39.45	<0.001

- Test indicates amplitude of spontaneous contraction of rabbit ileum when serotonin was added into organ bath containing tyrode solution
- Control indicates amplitude of spontaneous contraction of rabbit ileum when serotonin was washed out of organ bath and replaced with tyrode solution only
- Increase in spontaneous contraction to serotonin of rabbit ileum was dose dependent
- **g/ml**- [gram(s) per milliliter] and **mm**-[millimeters]

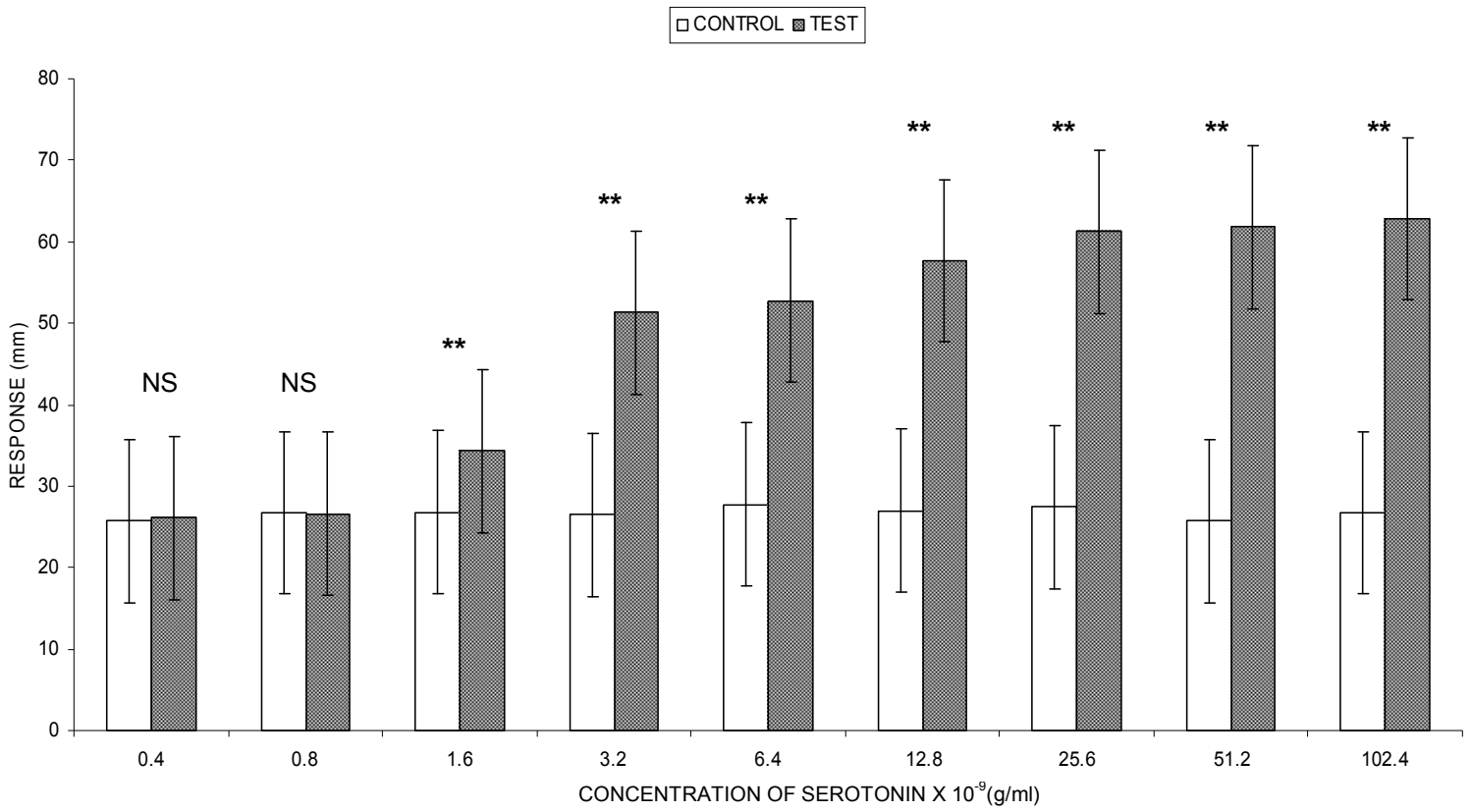


Figure 4.9: Chart represents the amplitude of spontaneous contraction of rabbit ileum (n=9) after the administration of serotonin into the organ bath containing tyrode solution. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the amplitude of spontaneous contractions after administration of serotonin into the organ bath and control bars indicate amplitude of spontaneous contraction after serotonin was washed out of the organ bath and replaced with tyrode solution.

Table 4.12: Effect of serotonin (5-HT) on frequency (contractions/minute) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of serotonin (g/ml)	Frequency of contractions (cpm)		T-value	P-value
	Control	Test		
$4X10^{-10}$	16.33 ± 1.42	16.67 ± 1.24	-0.18	0.859
$8X10^{-10}$	16.33 ± 1.59	17.33 ± 1.20	-0.50	0.623
$1.6X10^{-9}$	16.67 ± 1.51	17.67 ± 1.54	-0.46	0.649
$3.2X10^{-9}$	16.33 ± 1.24	16.67 ± 1.13	-0.20	0.842
$6.4X10^{-9}$	17.33 ± 1.39	17.00 ± 1.32	0.17	0.865
$1.28X10^{-8}$	16.33 ± 1.42	17.67 ± 1.05	-0.76	0.459
$2.56X10^{-8}$	16.67 ± 1.33	16.33 ± 1.59	0.16	0.872
$5.12X10^{-8}$	16.33 ± 1.42	16.67 ± 1.24	-0.18	0.859
$1.024X10^{-7}$	16.33 ± 1.42	16.67 ± 1.24	-0.18	0.859

- Test indicates frequency of spontaneous contraction of rabbit ileum when serotonin was added into organ bath containing tyrode solution
- Control indicates frequency of spontaneous contraction of rabbit ileum when serotonin was washed out of organ bath and replaced with tyrode solution only.
- **g/ml**- [gram(s) per milliliter] and **cpm**- contractions per minute

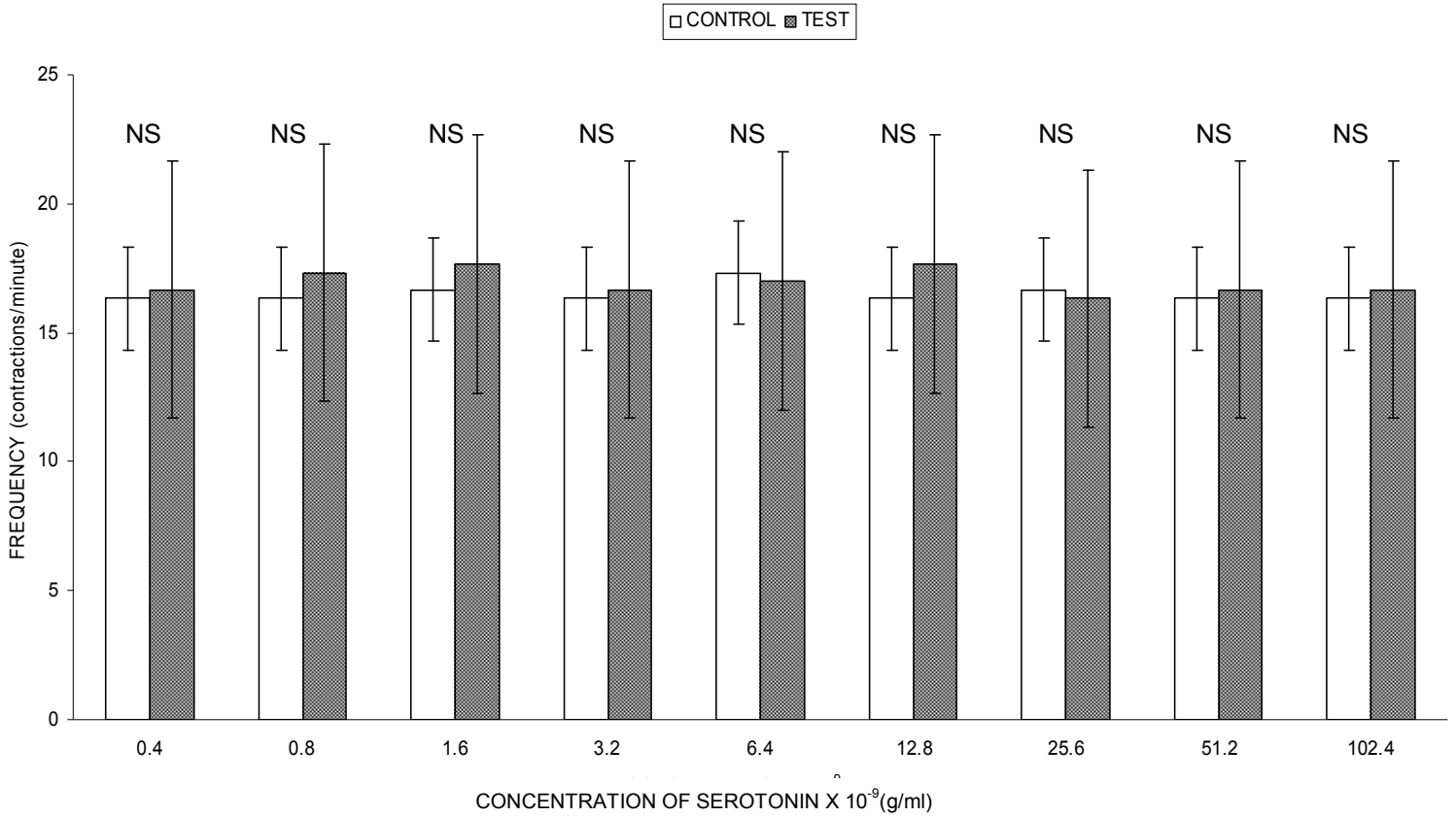


Figure 4.10: Chart represents the frequency of spontaneous contraction of rabbit ileum (n=9) after the administration of serotonin into the organ bath containing tyrode solution. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the frequency of spontaneous contractions after administration of serotonin into the organ bath and control bars indicate frequency of spontaneous contraction after serotonin was washed out of the organ bath and replaced with tyrode solution.

4.3.4 EFFECTS OF EPINEPHRINE ON RABBIT ILEUM

Decrease in amplitude of spontaneous contractions (Figure 4.11) were established in tissues (n=9) (appendix 19) at a threshold concentration of 6.4 X 10^{-9} g/ml (35.14 $\pm$ 0.84 mm and 25.27 $\pm$ 1.29 mm for control and test groups respectively) which was highly significant (P<0.001). At concentration of 4.096 X 10^{-7} g/ml, maximum response was recorded for control (37.56 $\pm$ 1.04 mm) and test (0.54 $\pm$ 0.11 mm) (Table 4.13) which was considered as highly significant (P<0.001).

There was no significant difference (P>0.05) in the frequency of spontaneous contractions (Figure 4.12) of tissues (n=9) (appendix 20). At a maximum concentration of 4.096 X 10^{-7} g/ml, frequency recorded was 15.00 $\pm$ 1.58 for control group and 14.00 $\pm$ 1.66 for test group (Table 4.14).

4.3.5 EFFECTS OF NOREPINEPHRINE ON RABBIT ILEUM

When 1.6 X 10^{-9} g/ml of norepinephrine was administered, tissues (n=9) (appendix 17) showed a decrease in amplitude of spontaneous contraction (Figure 4.13) which was considered highly significant (P<0.001) with a threshold response of 39.02 $\pm$ 0.72 mm (control) and 31.64 $\pm$ 1.08 mm (Test). Maximum response was obtained at the concentration of 4.096 X 10^{-7} g/ml (31.51 $\pm$ 1.59 mm and 2.03 $\pm$ 0.19 mm for control and test groups respectively) which was considered highly significant (P<0.001) (Table 4.15).

No significant difference (P>0.05) in the frequency of spontaneous contractions illustrated in Figure 4.14 in tissue (n=9) (appendix 18) even at a maximum concentration of 4.096 X 10^{-7} g/ml (16.33 ± 1.13 and 16.67 ± 1.13 contractions/minute for control and test groups) (Table 4.16).

4.3.6 EFFECTS OF ISOPRENALINE ON RABBIT ILEUM

A decrease (Figure 4.15) in amplitude of spontaneous contraction in tissues (n = 9) (appendix 33) was observed at a threshold concentration of 3.2 X 10^{-9} g/ml with amplitude of 29.12 ± 0.54 mm and 25.84 ± 0.53 mm for control and test groups respectively which was considered highly significant (P<0.001)(Table 4.17). Maximum response was recorded at the concentration of 4.096 X 10^{-7} g/ml with amplitude of 29.18 ± 0.41 mm and 1.82 ± 0.20 mm for control and test groups respectively. This was considered highly significant (P<0.001) (Table 4.17).

Frequency of spontaneous contractions in Figure 4.16 of tissues (n=9) (appendix 34) showed no significant difference (P>0.05) even at a concentration of 4.096 X 10^{-7} g/ml (17.33 ± 1.56 contractions/minute and 16.33 ± 1.82 contractions/minute respectively) (Table 4.18).

Table 4.13: Effect of epinephrine on amplitude (mm) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of epinephrine (g/ml)	Amplitude of contractions (mm)		T-value	P-value
	Control	Test		
$4X10^{-10}$	22.86 ± 0.99	23.58 ± 0.92	-0.53	0.602
$8X10^{-10}$	24.02 ± 0.71	23.90 ± 0.99	0.76	0.456
$1.6X10^{-9}$	32.34 ± 1.03	29.31 ± 1.33	1.80	0.091
$3.2X10^{-9}$	34.24 ± 1.01	29.43 ± 1.62	2.52	0.023
$6.4X10^{-9}$	35.14 ± 0.84	25.27 ± 1.29	6.41	<0.001
$1.28X10^{-8}$	27.23 ± 1.65	18.77 ± 0.90	4.50	<0.001
$2.56X10^{-8}$	33.86 ± 1.26	14.99 ± 1.20	10.85	<0.001
$5.12X10^{-8}$	37.02 ± 0.91	12.13 ± 0.68	21.91	<0.001
$1.024X10^{-7}$	34.91 ± 1.19	5.92 ± 0.53	22.25	<0.001
$2.048X10^{-7}$	37.68 ± 0.80	1.18 ± 0.20	44.26	<0.001
$4.096X10^{-7}$	37.56 ± 1.04	0.54 ± 0.11	35.40	<0.001

- Test indicates amplitude of spontaneous contraction of rabbit ileum when epinephrine was added into organ bath containing tyrode solution
- Control indicates amplitude of spontaneous contraction of rabbit ileum when epinephrine was washed out of organ bath and replaced with tyrode solution only
- decrease in spontaneous contraction to epinephrine of rabbit ileum was dose dependent
- **g/ml**- [gram(s) per milliliter] and **mm**-[millimeters]

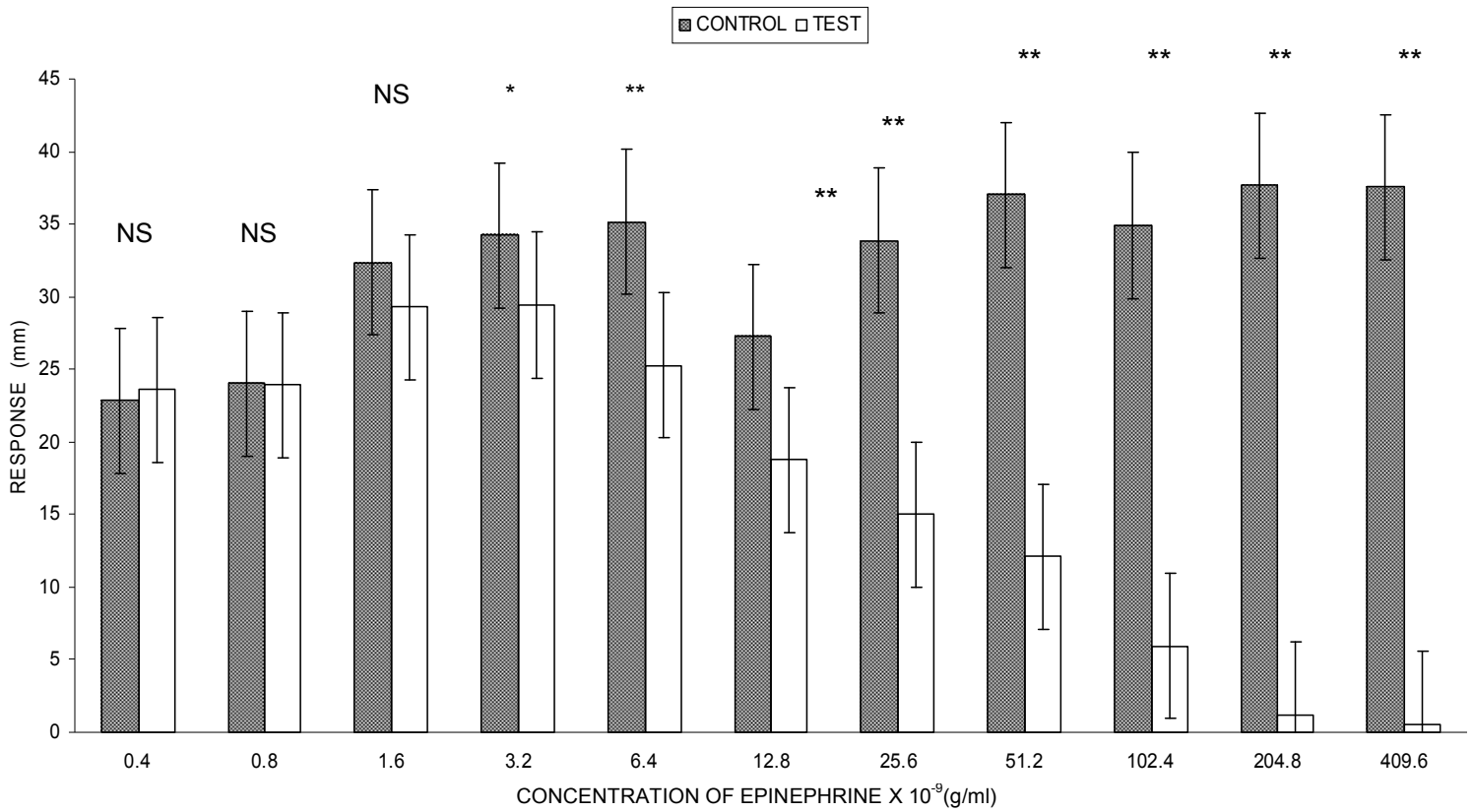


FIGURE 4.11: Chart represents the amplitude of spontaneous contraction of rabbit ileum (n=9) after the administration of epinephrine into the organ bath containing tyrode solution. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the amplitude of spontaneous contractions after administration of epinephrine into the organ bath and control bars indicate amplitude of spontaneous contraction after epinephrine was washed out of the organ bath and replaced with tyrode solution.

Table 4.14: Effect of epinephrine on frequency (contractions/minute) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of epinephrine (g/ml)	Frequency of contraction (cpm)		T-value	P-value
	Control	Test		
$4X10^{-10}$	15.00 ± 1.12	12.67 ± 0.90	1.62	0.124
$8X10^{-10}$	14.67 ± 1.45	14.00 ± 1.41	0.33	0.745
$1.6X10^{-9}$	14.67 ± 1.27	15.00 ± 1.41	-0.17	0.864
$3.2X10^{-9}$	14.67 ± 1.45	14.00 ± 1.22	0.35	0.728
$6.4X10^{-9}$	14.33 ± 1.20	14.00 ± 1.66	0.16	0.874
$1.28X10^{-8}$	14.33 ± 1.30	14.00 ± 1.41	0.17	0.866
$2.56X10^{-8}$	14.67 ± 1.45	13.67 ± 1.01	0.57	0.579
$5.12X10^{-8}$	14.67 ± 1.54	13.33 ± 1.59	0.61	0.553
$1.024X10^{-7}$	15.33 ± 1.45	14.67 ± 1.96	0.27	0.790
$2.048X10^{-7}$	14.67 ± 1.27	14.67 ± 1.54	0.00	1.000
$4.096X10^{-7}$	15.00 ± 1.58	14.00 ± 1.66	0.44	0.668

- Test indicates frequency of spontaneous contraction of rabbit ileum when epinephrine was added into organ bath containing tyrode solution
- Control indicates frequency of spontaneous contraction of rabbit ileum when epinephrine was washed out of organ bath and replaced with tyrode solution only.
- **g/ml**- [gram(s) per milliliter] and **cpm**- contractions per minute

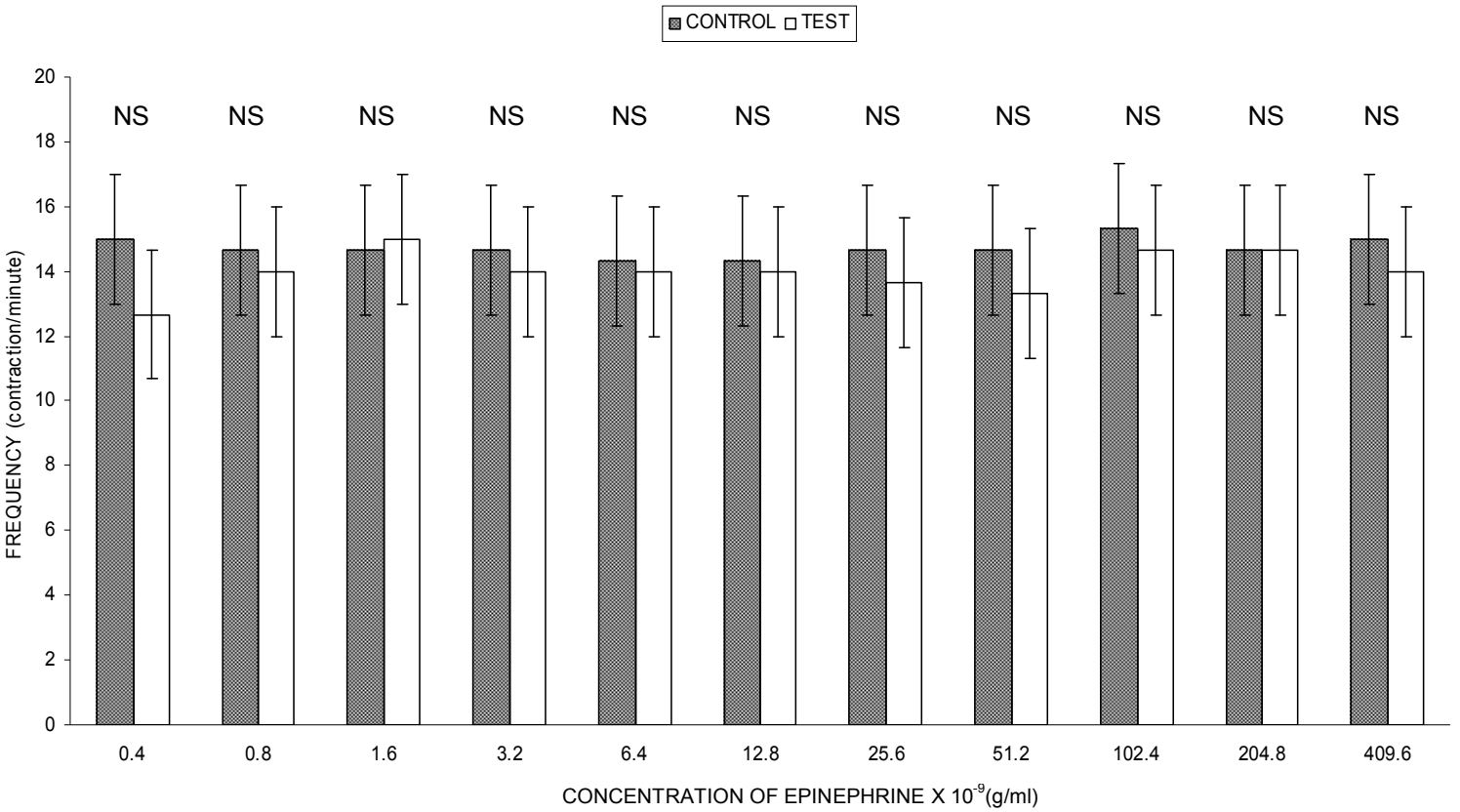


FIGURE 4.12: Chart represents the frequency of spontaneous contraction of rabbit ileum (n=9) after the administration of epinephrine into the organ bath containing tyrode solution. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the frequency of spontaneous contractions after administration of epinephrine into the organ bath and control bars indicate frequency of spontaneous contraction after epinephrine was washed out of the organ bath and replaced with tyrode solution

Table 4.15: Effect of norepinephrine on amplitude (mm) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of norepinephrine (g/ml)	Amplitude of contraction (mm)		T-value	P-value
	Control	Test		
$4X10^{-10}$	39.11 ± 0.45	38.60 ± 0.85	0.53	0.603
$8X10^{-10}$	38.76 ± 0.70	39.14 ± 0.77	-0.37	0.720
$1.6X10^{-9}$	39.02 ± 0.72	31.64 ± 1.08	5.69	<0.001
$3.2X10^{-9}$	37.97 ± 0.87	27.88 ± 0.50	10.06	<0.001
$6.4X10^{-9}$	33.12 ± 0.90	20.58 ± 0.91	9.80	<0.001
$1.28X10^{-8}$	31.72 ± 0.91	20.29 ± 1.00	8.45	<0.001
$2.56X10^{-8}$	32.30 ± 0.95	20.22 ± 1.32	7.43	<0.001
$5.12X10^{-8}$	32.88 ± 1.25	18.36 ± 1.33	7.96	<0.001
$1.024X10^{-7}$	33.88 ± 1.32	8.87 ± 0.71	16.69	<0.001
$2.048X10^{-7}$	35.09 ± 0.73	5.81 ± 0.79	27.22	<0.001
$4.096X10^{-7}$	31.51 ± 1.59	2.03 ± 0.19	18.41	<0.001

- Test indicates amplitude of spontaneous contraction of rabbit ileum when norepinephrine was added into organ bath containing tyrode solution
- Control indicates amplitude of spontaneous contraction of rabbit ileum when norepinephrine was washed out of organ bath and replaced with tyrode solution only
- decrease in spontaneous contraction to norepinephrine of rabbit ileum was dose dependent
- **g/ml**- [gram(s) per milliliter] and **mm**-[millimeters]

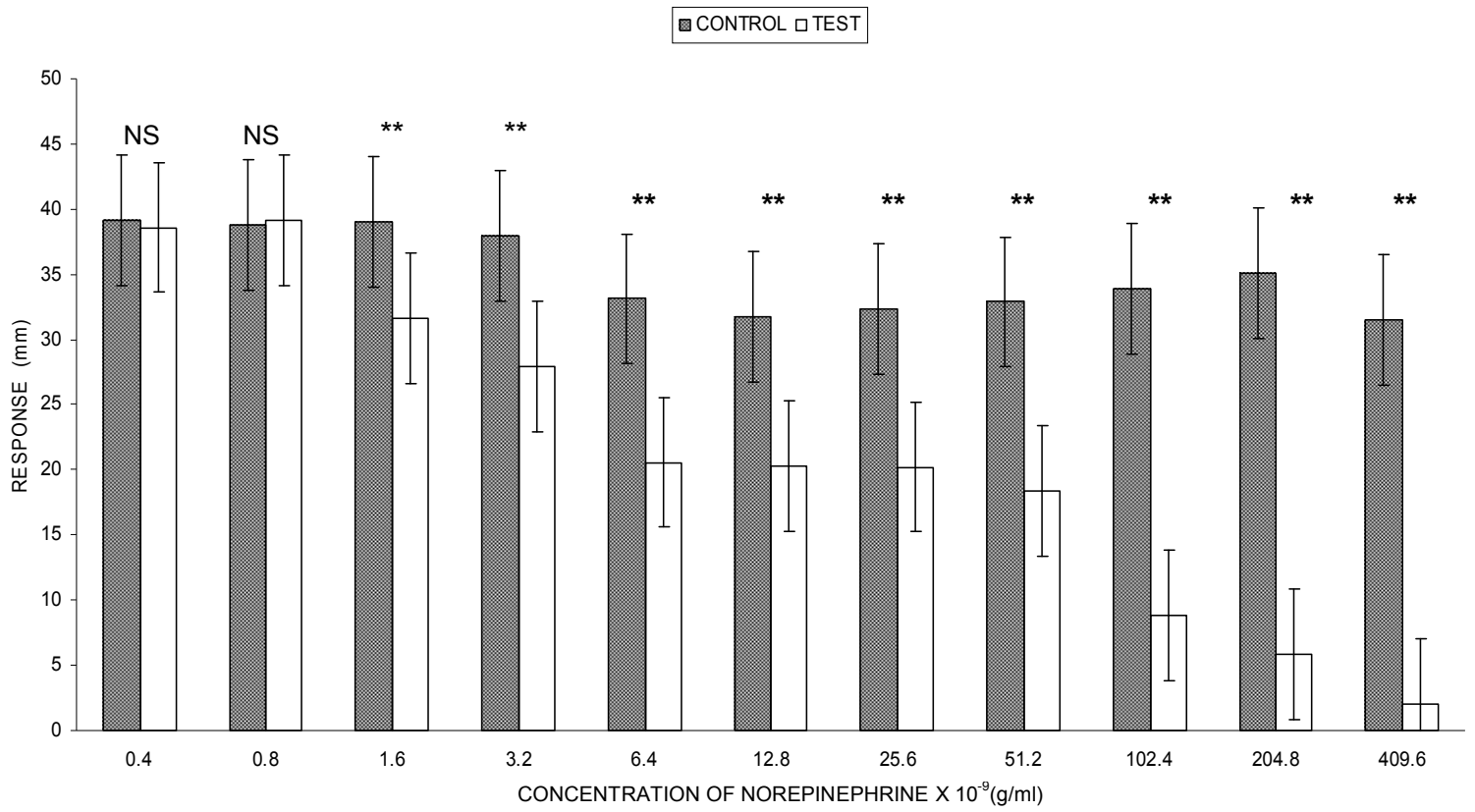


FIGURE 4.13: Chart represents the amplitude of spontaneous contraction of rabbit ileum (n=9) after the administration of norepinephrine into the organ bath containing tyrode solution. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the amplitude of spontaneous contractions after administration of norepinephrine into the organ bath and control bars indicate amplitude of spontaneous contraction after norepinephrine was washed out of the organ bath and replaced with tyrode solution.

Table 4.16: Effect of norepinephrine on frequency (contractions/minute) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of norepinephrine (g/ml)	Frequency of contractions (cpm)		T-value	P-value
	Control	Test		
$4X10^{-10}$	14.33 ± 0.83	15.00 ± 1.12	-0.48	0.637
$8X10^{-10}$	14.67 ± 0.93	15.00 ± 0.87	-0.26	0.799
$1.6X10^{-9}$	15.67 ± 1.09	15.33 ± 0.78	0.25	0.803
$3.2X10^{-9}$	14.33 ± 0.83	15.33 ± 1.05	-0.75	0.466
$6.4X10^{-9}$	15.00 ± 1.12	15.67 ± 1.09	-0.43	0.674
$1.28X10^{-8}$	14.67 ± 1.76	14.33 ± 1.56	0.15	0.887
$2.56X10^{-8}$	16.33 ± 1.67	15.33 ± 0.93	0.52	0.608
$5.12X10^{-8}$	16.33 ± 1.67	16.67 ± 1.13	-0.17	0.868
$1.024X10^{-7}$	16.33 ± 2.07	16.67 ± 1.67	-0.13	0.900
$2.048X10^{-7}$	14.33 ± 1.09	16.67 ± 1.13	-1.49	0.156
$4.096X10^{-7}$	16.33 ± 1.13	16.67 ± 1.13	-0.21	0.834

- Test indicates frequency of spontaneous contraction of rabbit ileum when norepinephrine was added into organ bath containing tyrode solution
- Control indicates frequency of spontaneous contraction of rabbit ileum when norepinephrine was washed out of organ bath and replaced with tyrode solution only.
- **g/ml**- [gram(s) per milliliter] and **cpm**- contractions per minute

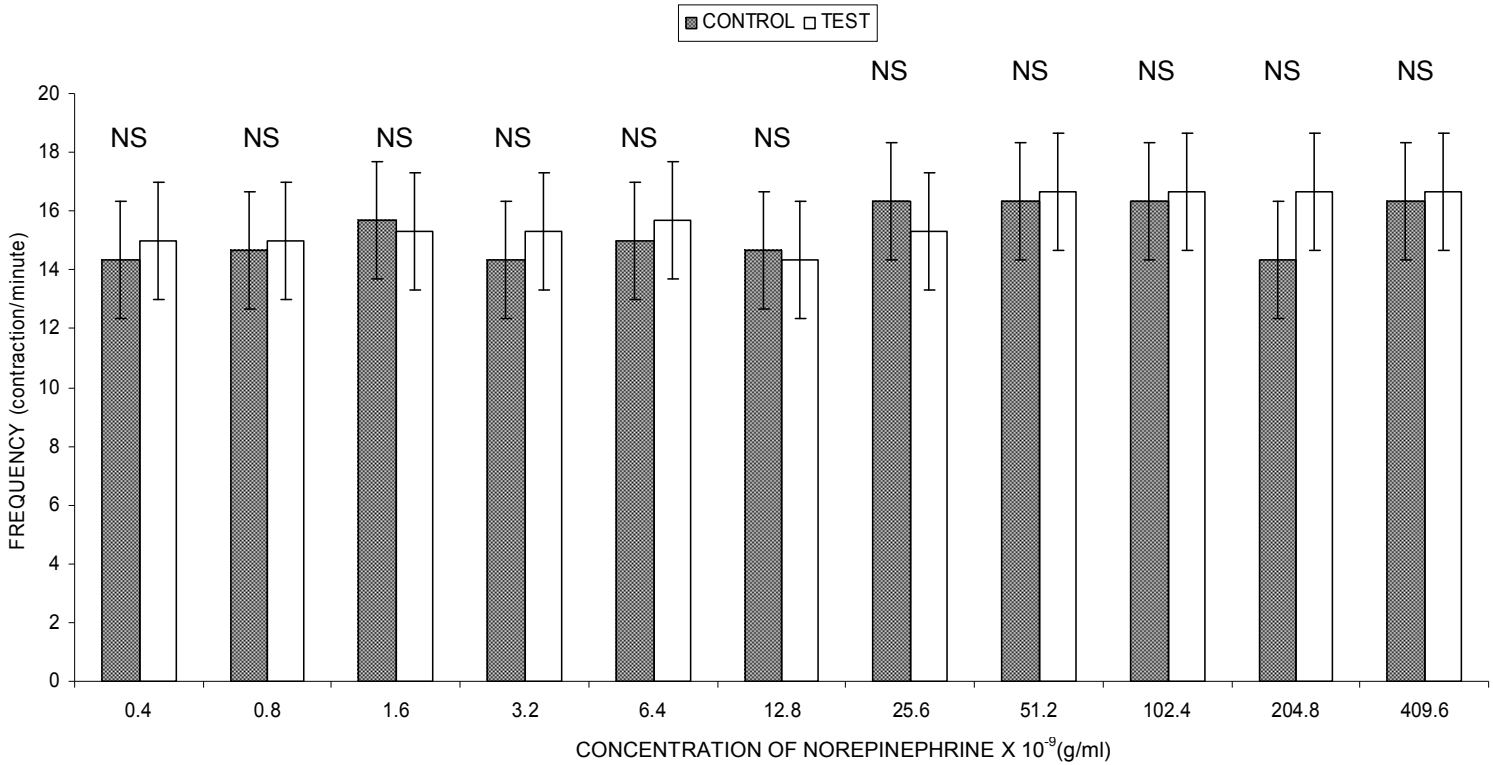


FIGURE 4.14: Chart represents the frequency of spontaneous contraction of rabbit ileum (n=9) after the administration of norepinephrine into the organ bath containing tyrode solution. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the frequency of spontaneous contractions after administration of norepinephrine into the organ bath and control bars indicate amplitude of spontaneous contraction after norepinephrine was washed out of the organ bath and replaced with tyrode solution

Table 4.17: Effect of isoprenaline on amplitude (mm) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of isoprenaline (g/ml)	Amplitude of contractions (mm)		T-value	P-value
	Control	Test		
$4X10^{-10}$	28.92 ± 0.56	29.01 ± 0.68	-0.10	0.920
$8X10^{-10}$	29.29 ± 0.61	28.80 ± 0.53	0.61	0.553
$1.6X10^{-9}$	29.39 ± 0.53	26.87 ± 0.72	2.82	0.012
$3.2X10^{-9}$	29.12 ± 0.54	25.84 ± 0.53	4.34	<0.001
$6.4X10^{-9}$	29.03 ± 0.63	22.86 ± 0.65	6.82	<0.001
$1.28X10^{-8}$	29.02 ± 0.47	20.40 ± 0.57	11.67	<0.001
$2.56X10^{-8}$	29.09 ± 0.53	16.38 ± 0.75	13.84	<0.001
$5.12X10^{-8}$	29.17 ± 0.42	10.44 ± 0.47	29.72	<0.001
$1.024X10^{-7}$	29.08 ± 0.35	7.86 ± 0.49	35.24	<0.001
$2.048 X10^{-7}$	29.76 ± 0.23	5.60 ± 0.37	55.46	<0.001
$4.096 X10^{-7}$	29.18 ± 0.41	1.82 ± 0.20	59.98	<0.001

- Test indicates amplitude of spontaneous contraction of rabbit ileum when isoprenaline was added into organ bath containing tyrode solution
- Control indicates amplitude of spontaneous contraction of rabbit ileum when isoprenaline was washed out of organ bath and replaced with tyrode solution only
- decrease in spontaneous contraction to isoprenaline of rabbit ileum was dose dependent
- **g/ml**- [gram(s) per milliliter] and **mm**-[millimeters]

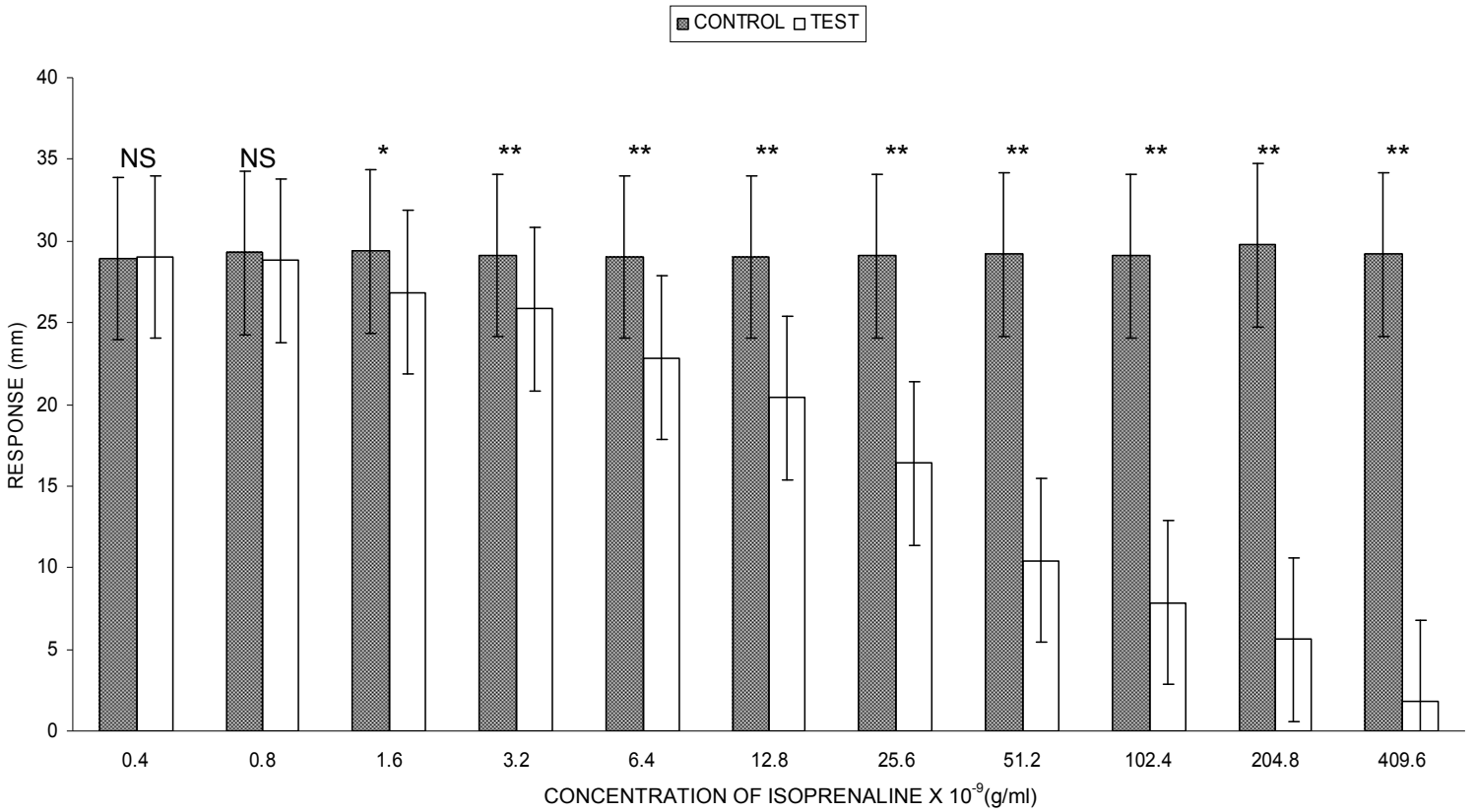


Figure 4.15: Chart represents the amplitude of spontaneous contraction of rabbit ileum (n=9) after the administration of isoprenaline into the organ bath containing tyrode solution. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the amplitude of spontaneous contractions after administration of isoprenaline into the organ bath and control bars indicate amplitude of spontaneous contraction after isoprenaline was washed out of the organ bath and replaced with tyrode solution.

Table 4.18: Effect of isoprenaline on frequency (contractions/minute) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of isoprenaline (g/ml)	Frequency of isoprenaline (cpm)		T-value	P-value
	Control	Test		
$4X10^{-10}$	16.33 ± 1.01	15.00 ± 1.12	0.88	0.391
$8X10^{-10}$	16.33 ± 1.24	17.00 ± 1.22	-0.39	0.705
$1.6X10^{-9}$	16.33 ± 1.01	16.67 ± 1.67	-0.74	0.864
$3.2X10^{-9}$	16.33 ± 1.13	16.67 ± 1.13	-0.21	0.834
$6.4X10^{-9}$	16.33 ± 1.59	15.67 ± 1.39	0.31	0.759
$1.28X10^{-8}$	15.33 ± 1.45	15.67 ± 1.64	-0.16	0.879
$2.56X10^{-8}$	16.00 ± 1.58	16.33 ± 1.24	-0.16	0.872
$5.12X10^{-8}$	16.67 ± 1.42	15.33 ± 1.45	0.66	0.518
$1.024X10^{-7}$	15.67 ± 1.30	16.33 ± 1.81	-0.30	0.771
$2.048\ X10^{-7}$	16.33 ± 1.42	16.67 ± 1.51	-0.16	0.872
$4.096\ X10^{-7}$	17.33 ± 1.56	16.33 ± 1.82	0.42	0.682

- Test indicates frequency of spontaneous contraction of rabbit ileum when isoprenaline was added into organ bath containing tyrode solution
- Control indicates frequency of spontaneous contraction of rabbit ileum when isoprenaline was washed out of organ bath and replaced with tyrode solution only.
- **g/ml**- [gram(s) per milliliter] and **cpm**- contractions per minute

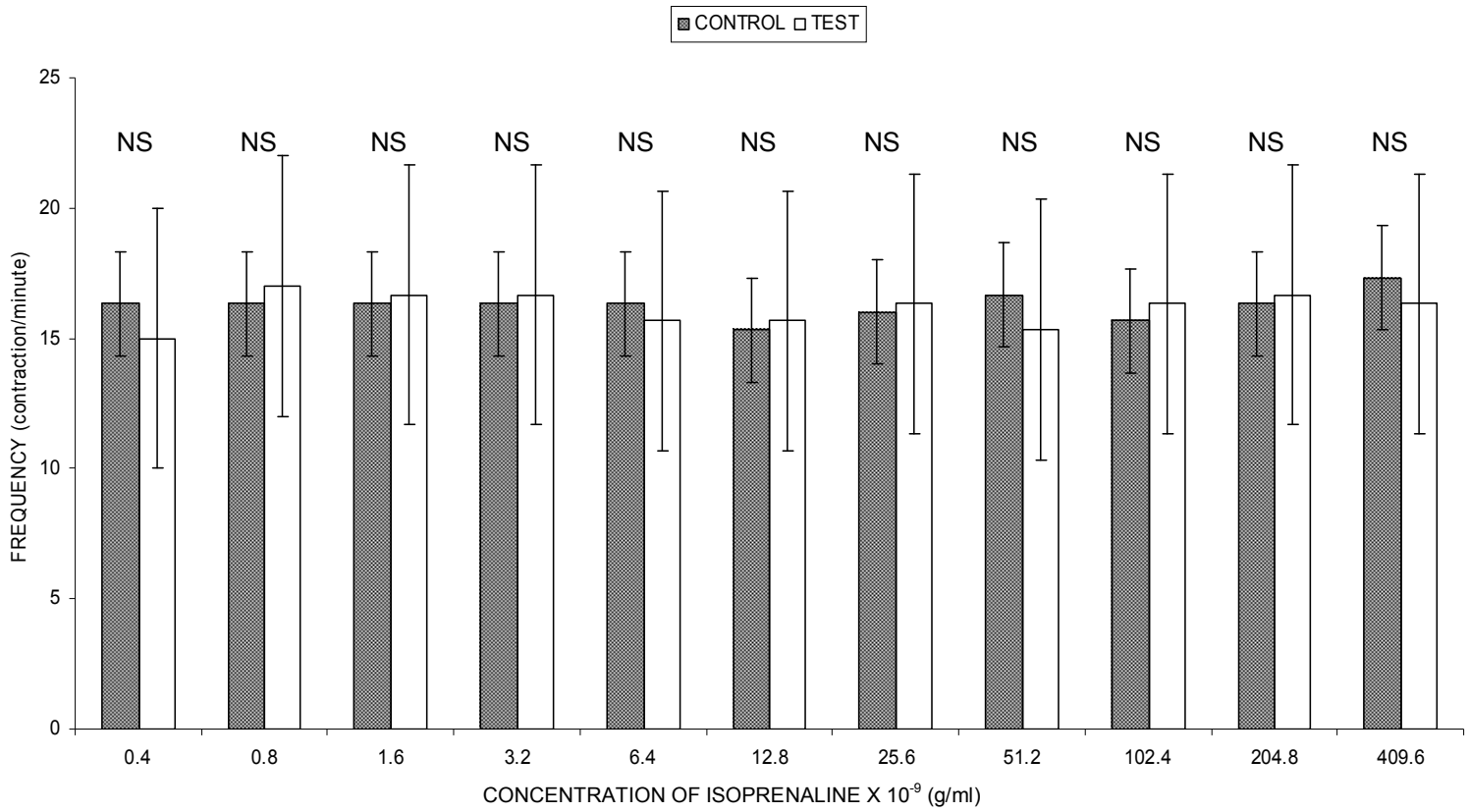


FIGURE 4.16 Chart represents the frequency of spontaneous contraction of rabbit ileum (n=9) after the administration of isoprenaline into the organ bath containing tyrode solution. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the frequency of spontaneous contractions after administration of isoprenaline into the organ bath and control bars indicate frequency of spontaneous contraction after isoprenaline was washed out of the organ bath and replaced with tyrode solution.

4.3.7 EFFECTS OF AQUEOUS EXTRACT ON RABBIT ILEUM

A decrease (Figure 4.17) in spontaneous contraction (n=9) (appendix 15) was observed at a threshold concentration of 1.6 X 10^{-5} g/ml with amplitude of 34.43 ± 0.97 mm and 28.61 ± 0.49 mm for control and test groups respectively (Table 4.19). At a concentration of 2.048 X 10^{-3} g/ml, maximum response was recorded with amplitude of 36.61 ± 0.83 mm and 1.18 ± 0.13 mm for control and test groups respectively. These values were considered highly significant ($P<0.001$) (Table 4.19).

No significant difference ($P>0.05$) was observed in the frequency of spontaneous contractions in tissues (n=9) (appendix 16) as illustrated in Figure 4.18 even at a concentration of 2.048 X 10^{-3} g/ml (14.67 ± 0.93 contractions/minute and 12.67 ± 1.09 contractions/minute for control and test groups respectively) (Table 4.20).

4.3.8 EFFECTS OF METHANOLIC EXTRACT ON RABBIT ILEUM

A decrease in amplitude (Figure 4.19) of spontaneous contraction (n=9) (appendix 13) was recorded. At concentration of 1.6 X 10^{-5} g/ml (27.08 ± 0.76mm and 23.37 ± 0.82mm for control and test groups respectively) a threshold response was recorded. At 2.048 X 10^{-3} g/ml, amplitude recorded was 26.19 ± 0.44 mm and 2.39 ± 0.27 mm for control and test groups respectively. Decrease in amplitude of contraction was considered highly significant ($P<0.001$) (Table 4.21).

No significant difference ($P>0.05$) was observed in the frequency of spontaneous contractions as illustrated in Figure 4.20 in tissues (n=9) (appendix 14) in both control and test groups (Table 4.22).

Table 4.19: Effect of aqueous extract on amplitude (mm) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of aqueous extract (g/ml)	Amplitude of contraction (mm)		T-value	P-value
	Control	Test		
$4X10^{-6}$	28.08 ± 0.72	28.10 ± 1.01	-0.12	0.99
$8X10^{-6}$	34.09 ± 0.94	34.56 ± 0.79	-0.38	0.71
$1.6X10^{-5}$	34.43 ± 0.97	28.61 ± 0.49	5.36	<0.001
$3.2X10^{-5}$	34.84 ± 0.84	27.68 ± 0.39	7.73	<0.001
$6.4X10^{-5}$	37.11 ± 1.21	28.43 ± 0.48	6.67	<0.001
$1.28X10^{-4}$	32.29 ± 0.63	21.79 ± 0.72	10.98	<0.001
$2.56X10^{-4}$	34.79 ± 0.97	10.50 ± 0.53	21.98	<0.001
$5.12X10^{-4}$	35.77 ± 0.70	6.23 ± 0.35	37.75	<0.001
$1.024X10^{-3}$	36.16 ± 0.87	2.58 ± 0.41	36.99	<0.001
$2.048X10^{-3}$	36.61 ± 0.83	1.18 ± 0.13	42.17	<0.001

- Test indicates amplitude of spontaneous contraction of rabbit ileum when aqueous extract was added into organ bath containing tyrode solution
- Control indicates amplitude of spontaneous contraction of rabbit ileum when acetylcholine was washed out of organ bath and replaced with tyrode solution only
- decrease in spontaneous contraction to aqueous extract of rabbit ileum was dose dependent
- **g/ml**- [gram(s) per milliliter] and **mm**-[millimeters]

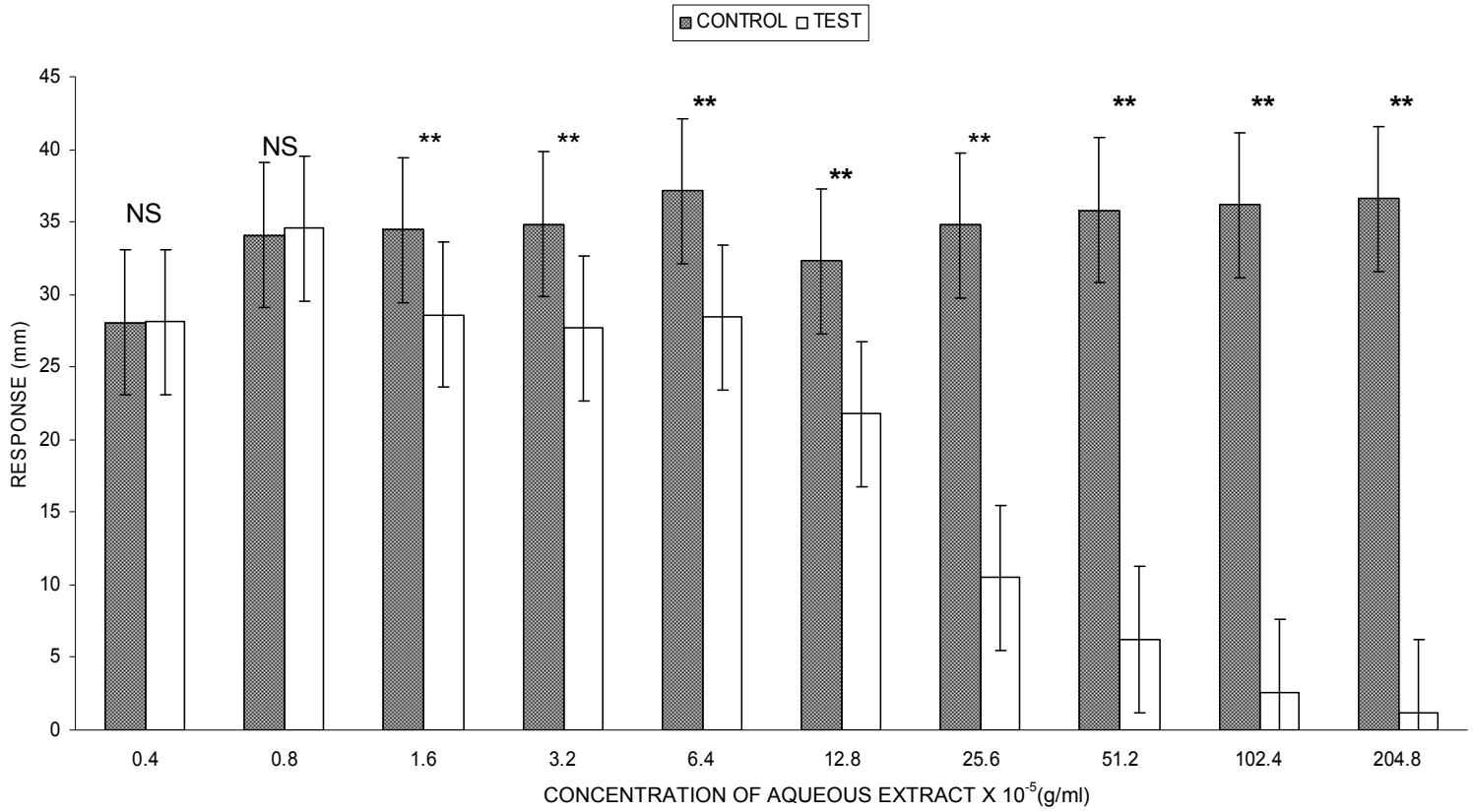


FIGURE 4.17 Chart represents the amplitude of spontaneous contraction of rabbit ileum (n=9) after the administration of aqueous extract into the organ bath containing tyrode solution. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the amplitude of spontaneous contractions after administration of isoprenaline into the organ bath and control bars indicate amplitude of spontaneous contraction after isoprenaline was washed out of the organ bath and replaced with tyrode solution.

Table 4.20: Effect of aqueous extract on frequency (contractions/minute) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of aqueous extract (g/ml)	Frequency of contraction (cpm)		T-value	P-value
	Control	Test		
$4X10^{-6}$	16.67 ± 1.13	16.33 ± 1.13	0.21	0.834
$8X10^{-6}$	12.56 ± 1.38	13.00 ± 1.12	-0.25	0.808
$1.6X10^{-5}$	12.00 ± 0.87	12.33 ± 1.53	-0.19	0.854
$3.2X10^{-5}$	14.67 ± 1.36	13.78 ± 1.89	0.38	0.707
$6.4X10^{-5}$	13.00 ± 1.00	12.67 ± 0.97	0.24	0.816
$1.28X10^{-4}$	12.00 ± 0.87	12.33 ± 0.78	-0.28	0.781
$2.56X10^{-4}$	12.67 ± 0.97	12.33 ± 0.78	0.27	0.788
$5.12X10^{-4}$	13.33 ± 0.88	12.67 ± 0.97	0.50	0.621
$1.024X10^{-3}$	12.00 ± 0.71	12.67 ± 0.97	-0.56	0.585
$2.048X10^{-3}$	14.67 ± 0.93	12.67 ± 1.09	1.40	0.182

- Test indicates frequency of spontaneous contraction of rabbit ileum when aqueous extract was added into organ bath containing tyrode solution
- Control indicates frequency of spontaneous contraction of rabbit ileum when aqueous extract was washed out of organ bath and replaced with tyrode solution only.
- **g/ml**- [gram(s) per milliliter] and **cpm**- contractions per minute

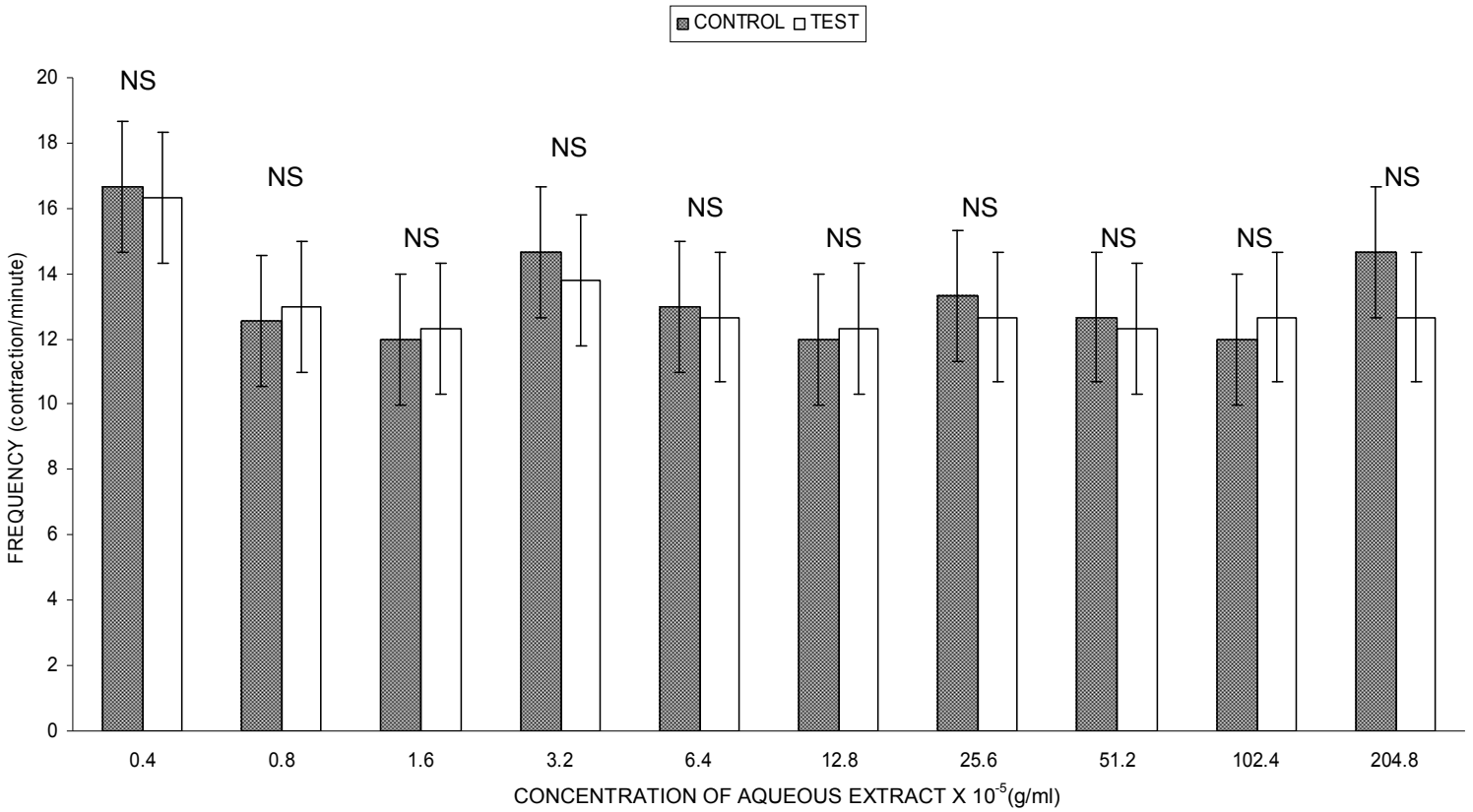


FIGURE 4.18: Chart represents the frequency of spontaneous contraction of rabbit ileum (n=9) after the administration of aqueous extract into the organ bath containing tyrode solution. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the frequency of spontaneous contractions after administration of aqueous extract into the organ bath and control bars indicate frequency of spontaneous contraction after aqueous extract was washed out of the organ bath and replaced with tyrode solution.

Table 4.21: Effect of methanolic extract on amplitude (mm) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of methanolic extract (g/ml)	Amplitude of contraction (mm)		T-value	P-value
	Control	Test		
$4X10^{-6}$	26.26 ± 0.70	25.72 ± 1.01	0.44	0.67
$8X10^{-6}$	26.48 ± 0.89	23.53 ± 0.82	2.44	0.027
$1.6X10^{-5}$	27.08 ± 0.76	23.37 ± 0.82	3.32	0.004
$3.2X10^{-5}$	26.41 ± 0.64	23.87 ± 1.25	1.81	0.009
$6.4X10^{-5}$	26.37 ± 0.45	22.04 ± 1.01	3.92	0.001
$1.28X10^{-4}$	26.16 ± 0.59	15.47 ± 0.88	10.09	<0.001
$2.56X10^{-4}$	26.80 ± 0.53	7.64 ± 0.68	22.22	<0.001
$5.12X10^{-4}$	26.27 ± 0.53	4.47 ± 0.46	31.06	<0.001
$1.024X10^{-3}$	26.71 ± 0.59	4.09 ± 0.71	26.05	<0.001
$2.048X10^{-3}$	26.19 ± 0.44	2.39 ± 0.27	24.50	<0.001

- Test indicates amplitude of spontaneous contraction of rabbit ileum when methanolic extract was added into organ bath containing tyrode solution
- Control indicates amplitude of spontaneous contraction of rabbit ileum when methanolic extract was washed out of organ bath and replaced with tyrode solution only
- decrease in spontaneous contraction to methanolic extract of rabbit ileum was dose dependent
- **g/ml**- [gram(s) per milliliter] and **mm**-[millimeters]

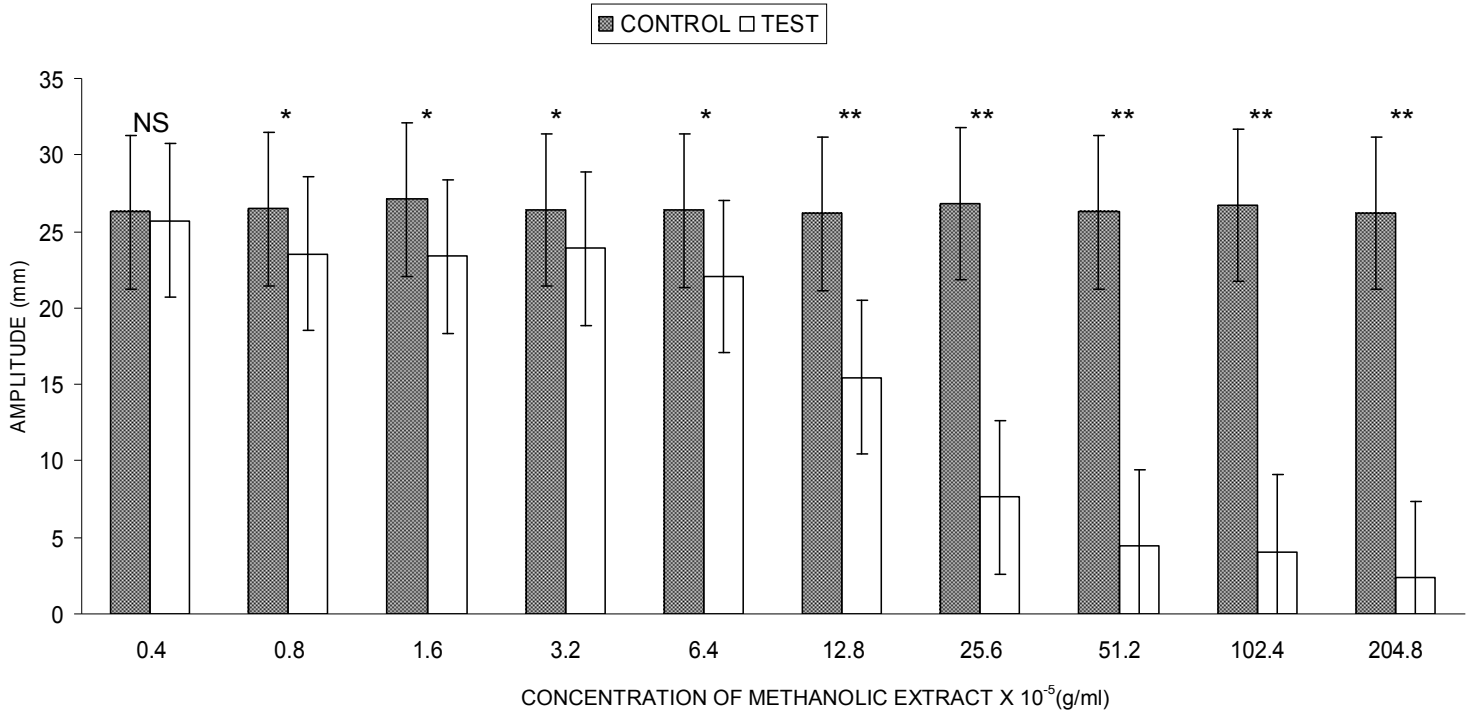


FIGURE 4.19 Chart represents the amplitude of spontaneous contraction of rabbit ileum (n=9) after the administration of methanolic extract into the organ bath containing tyrode solution. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the amplitude of spontaneous contractions after administration of methanolic extract into the organ bath and control bars indicate amplitude of spontaneous contraction after methanolic extract was washed out of the organ bath and replaced with tyrode solution.

Table 4.22: Effect of methanolic extract on frequency (contractions/minute) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of methanolic extract (g/ml)	Frequency of contraction (cpm)		T-value	P-value
	Control	Test		
$4X10^{-6}$	16.00 ± 1.00	16.00 ± 0.71	0.00	1.000
$8X10^{-6}$	20.44 ± 1.03	19.67 ± 1.13	0.50	0.621
$1.6X10^{-5}$	18.89 ± 0.68	17.67 ± 1.36	0.80	0.434
$3.2X10^{-5}$	19.00 ± 1.22	17.33 ± 1.39	0.90	0.380
$6.4X10^{-5}$	18.67 ± 1.20	19.33 ± 1.33	-0. 37	0.717
$1.28X10^{-4}$	19.33 ± 1.24	19.33 ± 0.88	0.00	1.000
$2.56X10^{-4}$	20.00 ± 1.12	19.00 ± 1.41	0.56	0.586
$5.12X10^{-4}$	19.33 ± 1.13	19.67 ± 1.13	-0.26	0.834
$1.024X10^{-3}$	18.33 ± 1.05	19.33 ± 1.13	-0.65	0.526
$2.048X10^{-3}$	18.67 ± 1.39	18.00 ± 1.22	0.36	0.722

- Test indicates frequency of spontaneous contraction of rabbit ileum when methanolic extract was added into organ bath containing tyrode solution
- Control indicates frequency of spontaneous contraction of rabbit ileum when methanolic extract was washed out of organ bath and replaced with tyrode solution only.
- **g/ml**- [gram(s) per milliliter] and **cpm**- contractions per minute

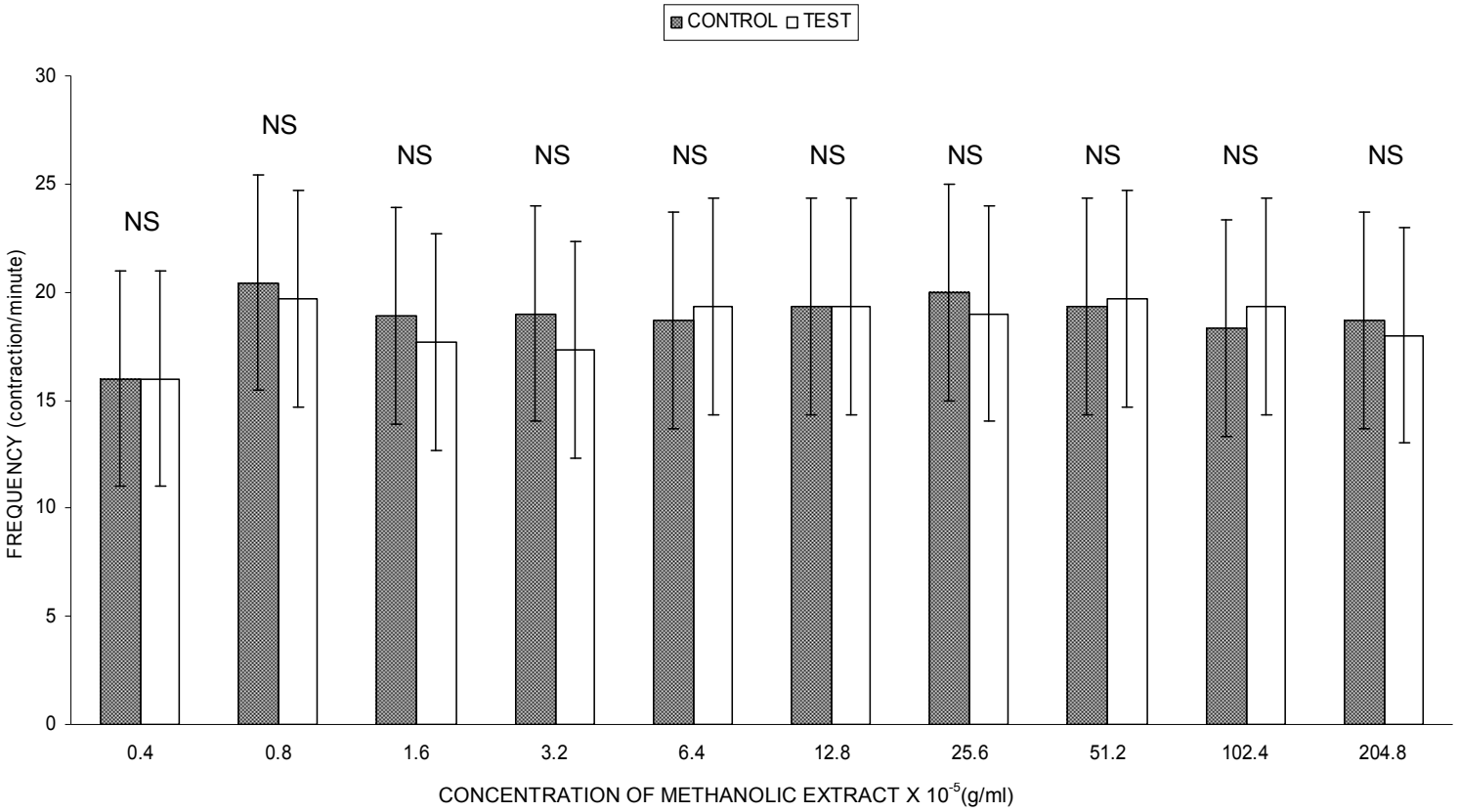


Figure 4.20: Chart represents the frequency of spontaneous contraction of rabbit ileum (n=9) after the administration of methanolic extract into the organ bath containing tyrode solution. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the frequency of spontaneous contractions after administration of methanolic extract into the organ bath and control bars indicate frequency of spontaneous contraction after methanolic extract was washed out of the organ bath and replaced with tyrode solution.

4.5.0 TO INVESTIGATE THE POSSIBLE MECHANISMS OF ACTION

Results from sections 4.4.7 and 4.4.8 showed that the extracts (aqueous and methanolic extracts of *Ocimum basilicum*) decreased the amplitude of spontaneous contraction.

4.5.1 EFFECTS OF AQUEOUS EXTRACT OF *OCIMUM BASILICUM* IN THE PRESENCE OF PHENTOLAMINE USING RABBIT ILEUM

Administration of the aqueous extract of *Ocimum basilicum* produced dose dependent decrease contraction of rabbit ileum with its maximum response at 2.048 X 10^{-3} g/ml (E_{max}). This effect was blocked by phentolamine (6.4 X 10^{-9} g/ml) (Figure 4.21 and Table 4.23). Appendix 21 (n=9), further illustrates phentolamine blocking the effect of aqueous extract (Figure 4.21). Aqueous extract (E_{max}) decreased spontaneous contraction (Table 4.23) from 28.87 $\pm$ 0.88 mm to 5.63 $\pm$ 0.62 mm which was considered highly significant ($P<0.001$) after Phentolamine (4 X 10^{-10} g/ml) administration while a non significant ($P>0.05$) decrease in amplitude of spontaneous contraction, after phentolamine at 6.4 X 10^{-9} g/ml (decrease from 30.60 $\pm$ 0.92 mm to 28.81 $\pm$ 1.17 mm) and 2.56 X 10^{-8} g/ml (amplitude decrease from 28.11 $\pm$ 0.72 mm and 28.12 $\pm$ 0.51 mm) administration (Table 4.19).

There was no significant difference ($P>0.05$) in the frequency of spontaneous contraction (Table 4.24) in tissues (n = 9) (appendix 22). Figure

4.22 illustrates the effect of aqueous extract on frequency after phentolamine administration.

4.5.2 EFFECTS OF METHANOLIC EXTRACT OF *OCIMUM BASILICUM* IN THE PRESENCE OF PHENTOLAMINE USING RABBIT ILEUM

Administration of the methanolic extract of *Ocimum basilicum* produced dose dependent decreased contraction of rabbit ileum with its maximum response at 2.048 X 10^{-3} g/ml (E_{max}). Phentolamine was able to antagonize this effect. Methanolic extract decreased amplitude of contraction (n=9) (appendix 23) after phentolamine (4 X 10^{-10} g/ml) administration (Table 4.25). An amplitude of 29.20 $\pm$ 0.60 mm and 6.31 $\pm$ 0.55 mm for control and test groups respectively was recorded (at 4 X 10^{-10} g/ml phentolamine) which was considered highly significant ($P<0.001$). In Figure 4.23, the concentration of phentolamine was increased to 1.28 X 10^{-7} g/ml, amplitude decreased from 29.44 $\pm$ 0.67mm to 27.73 $\pm$ 0.51 mm which was not significant ($P>0.05$) (Table 4.25).

The effect of methanolic extract in tissues (n=9) (appendix 24) after an initial administration of phentolamine (Figure 4.24) showed no significant difference in the frequency of spontaneous contractions ($P>0.05$) (Table 4.26).

Table 4.23: Effect of aqueous extract in the presence of phentolamine on amplitude (mm) of spontaneous contractions (n=9) in rabbit ileum. Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of phentolamine (g/ml)	Amplitude of contraction (mm)		T-value	P-value
	Control	Test		
$4X10^{-10}$	28.87 ± 0.88	5.63 ± 0.62	21.59	<0.001
$8X10^{-10}$	29.34 ± 0.96	12.39 ± 0.68	14.41	<0.001
$1.6X10^{-9}$	30.07 ± 1.06	21.08 ± 1.03	6.08	<0.001
$3.2X10^{-9}$	30.81 ± 0.89	25.34 ± 0.72	4.78	<0.001
$6.4X10^{-9}$	30.60 ± 0.92	28.81 ± 1.17	1.20	0.247
$1.28X10^{-8}$	26.26 ± 0.89	29.40 ± 0.87	-2.52	0.023
$2.56X10^{-8}$	28.11 ± 0.72	28.12 ± 0.51	-0.01	0.991

- Test indicates the effect of aqueous extract on amplitude of spontaneous contraction of rabbit ileum after an initial phentolamine administration in organ bath containing tyrode solution
- Control indicates amplitude of spontaneous contraction of rabbit ileum when aqueous extract and phentolamine was washed out of organ bath and replaced with tyrode solution only
- **g/ml**- [gram(s) per milliliter] and **mm**-[millimeters]

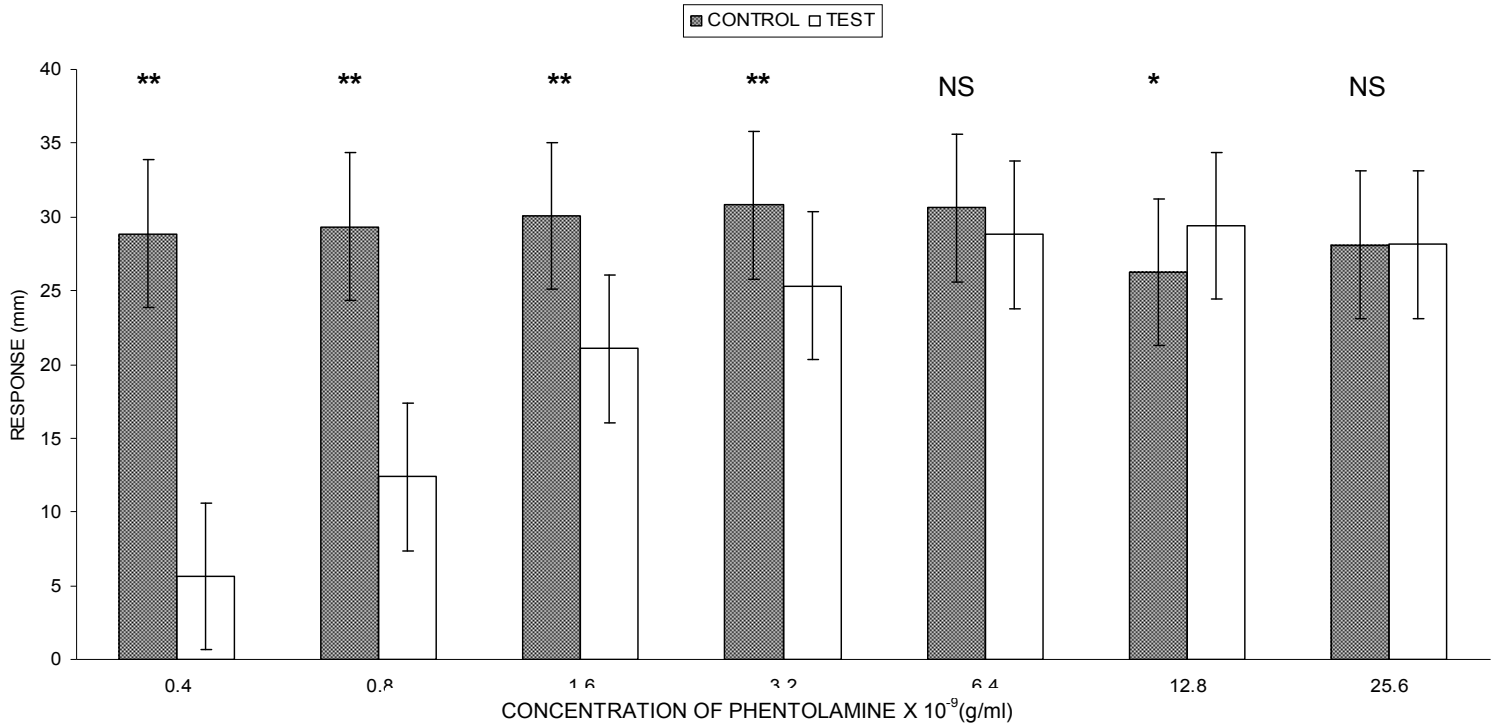


Figure 4.21 Chart represents the amplitude of spontaneous contraction of rabbit ileum (n=9) to aqueous extract in the presence of phentolamine. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the amplitude of spontaneous contractions after administration of test drugs into the organ bath and control bars indicate amplitude of spontaneous contraction after test drugs were washed out of the organ bath and replaced with tyrode solution.

Table 4.24: Effect of aqueous extract in the presence of phentolamine on frequency (contractions/minute) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of phentolamine (g/ml)	Frequency of contractions (cpm)		T-value	P-value
	Control	Test		
$4X10^{-10}$	12.67 ± 0.97	13.00 ± 0.71	-0.28	0.787
$8X10^{-10}$	15.00 ± 1.22	13.67 ± 1.01	0.84	0.413
$1.6X10^{-9}$	13.00 ± 0.87	13.33 ± 1.13	-0.23	0.82
$3.2X10^{-9}$	13.33 ± 0.88	13.67 ± 0.88	-0.27	0.788
$6.4X10^{-9}$	13.33 ± 1.33	13.67 ± 1.24	-0.19	0.854
$1.28X10^{-8}$	13.67 ± 1.13	13.33 ± 0.88	0.24	0.815
$2.56X10^{-8}$	13.67 ± 1.33	12.67 ± 0.97	0.61	0.552

- Test indicates the effect of aqueous extract on frequency of spontaneous contraction of rabbit ileum after an initial phentolamine administration in organ bath containing tyrode solution
- Control indicates frequency of spontaneous contraction of rabbit ileum when aqueous extract and phentolamine was washed out of organ bath and replaced with tyrode solution only
- **g/ml**- [gram(s) per milliliter] and **mm**-[millimeters]

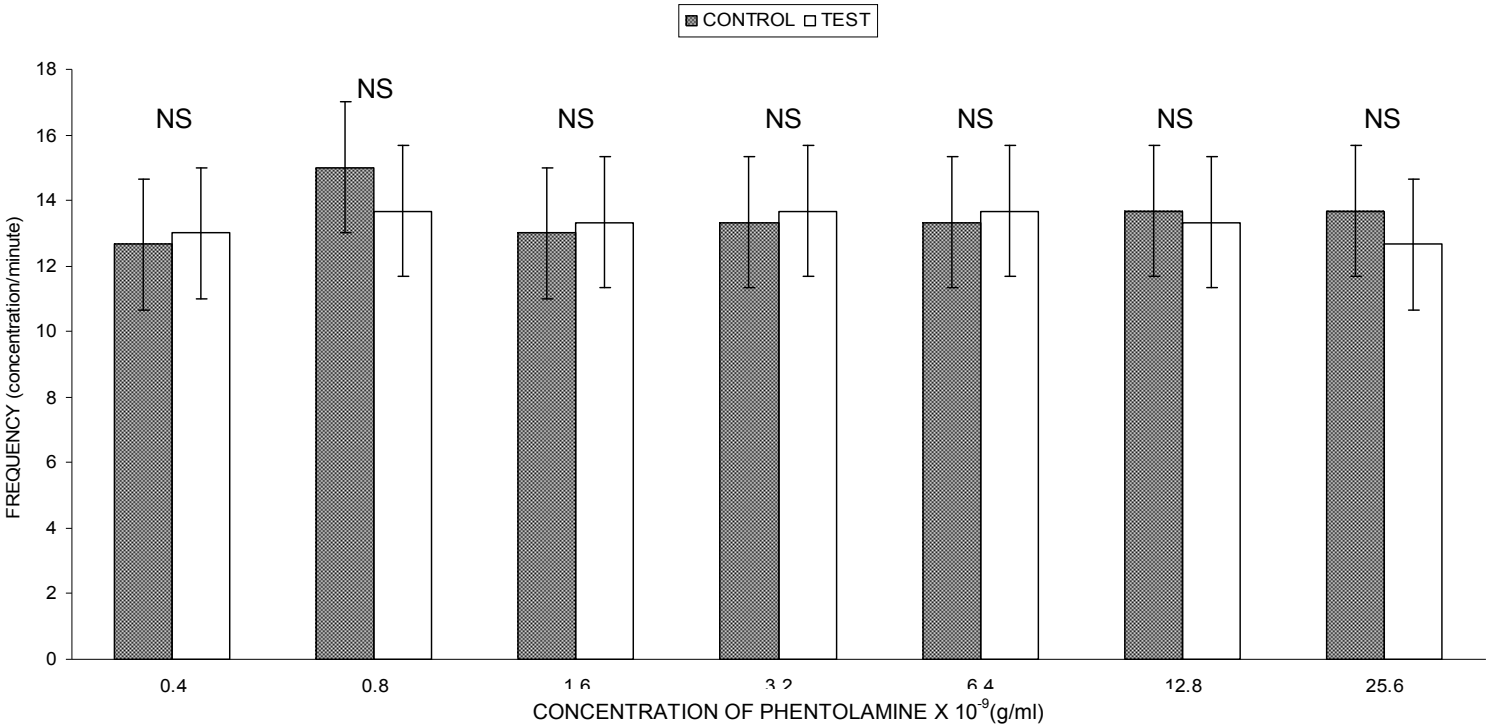


Figure 4.22 Chart represents the effect on frequency of spontaneous contraction of rabbit ileum (n=9) to aqueous extract in the presence of phentolamine. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the frequency of spontaneous contractions after administration of test drugs into the organ bath and control bars indicate frequency of spontaneous contraction after test drugs were washed out of the organ bath and replaced with tyrode solution.

Table 4.25: Effect of methanolic extract in the presence of phentolamine on amplitude (mm) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of methanolic extract (g/ml)	Amplitude of contraction (mm)		T-value	P-value
	Control	Test		
$4X10^{-10}$	29.20 ± 0.60	6.31 ± 0.55	28.12	<0.001
$8X10^{-10}$	29.04 ± 0.79	11.43 ± 0.48	19.05	<0.001
$1.6X10^{-9}$	29.07 ± 0.42	19.86 ± 0.61	12.44	<0.001
$3.2X10^{-9}$	30.29 ± 0.63	24.96 ± 0.78	5.32	<0.001
$6.4X10^{-9}$	29.63 ± 0.31	25.52 ± 0.79	4.84	<0.001
$1.28X10^{-8}$	29.44 ± 0.67	27.73 ± 0.51	2.03	0.059
$2.56X10^{-8}$	29.83 ± 0.49	29.84 ± 0.69	-.001	0.991

- Test indicates the effect of methanolic extract on amplitude of spontaneous contraction of rabbit ileum after an initial phentolamine administration in organ bath containing tyrode solution
- Control indicates amplitude of spontaneous contraction of rabbit ileum when methanolic extract and phentolamine was washed out of organ bath and replaced with tyrode solution only
- **g/ml**- [gram(s) per milliliter] and **mm**-[millimeters]

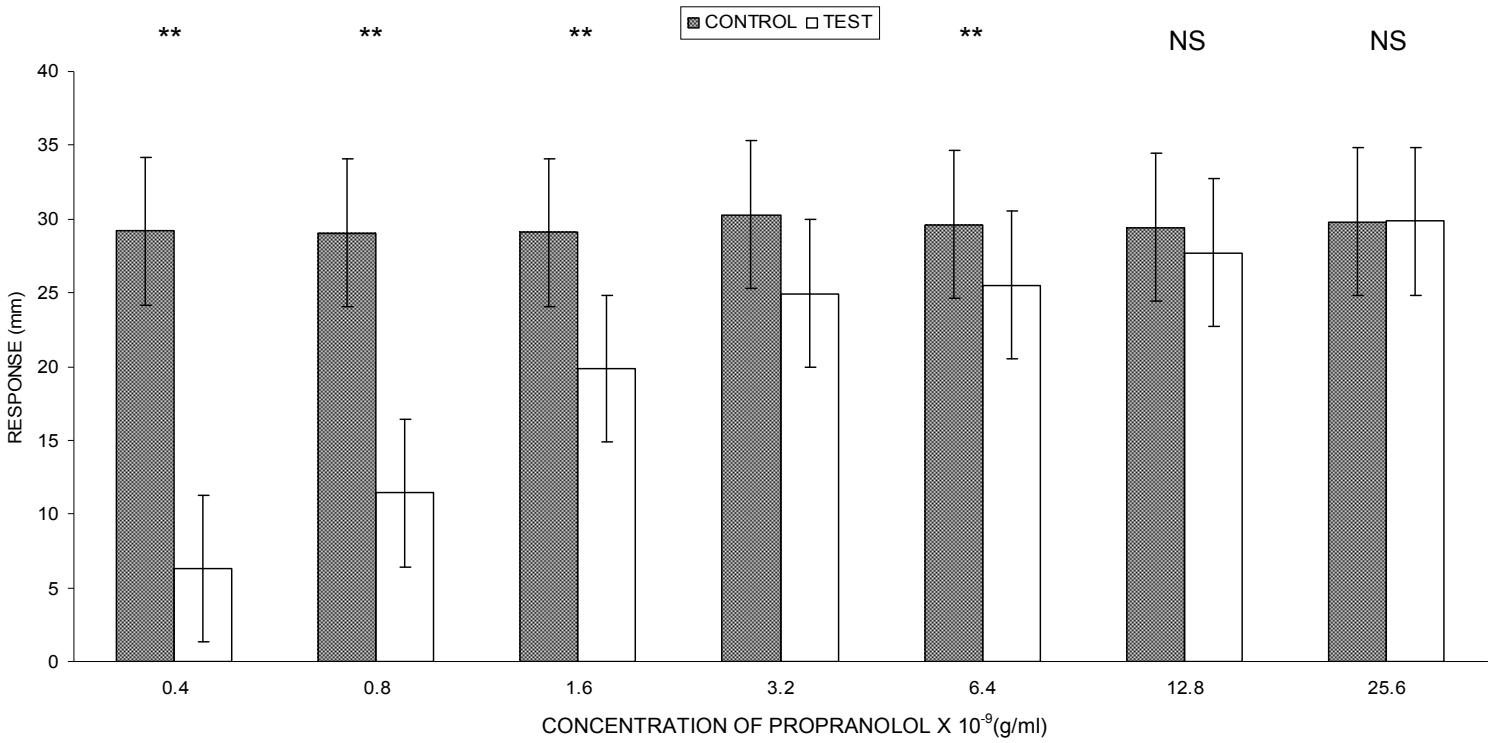


Figure 4.23: Chart represents the effect on amplitude of spontaneous contraction of rabbit ileum (n=9) to methanolic extract in the presence of phentolamine. [$^{NS}P>0.05$, *$P<0.05$, **$P<0.001$ compared with control]. Test bars indicate the amplitude of spontaneous contractions after administration of test drugs into the organ bath and control bars indicate amplitude of spontaneous contraction after test drugs were washed out of the organ bath and replaced with tyrode solution.

Table 4.26: Effect of methanolic extract in the presence of phentolamine on frequency (contraction/minute) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of methanolic extract (g/ml)	Frequency of contraction (cpm)		T-value	P-value
	Control	Test		
$4X10^{-10}$	12.67 ± 0.97	12.33 ± 0.78	0.27	0.788
$8X10^{-10}$	13.33 ± 1.33	12.33 ± 0.78	0.65	0.526
$1.6X10^{-9}$	12.67 ± 0.97	12.33 ± 0.78	0.27	0.788
$3.2X10^{-9}$	13.00 ± 1.41	13.67 ± 1.01	-0.39	0.704
$6.4X10^{-9}$	12.00 ± 1.00	13.33 ± 1.42	-0.77	0.455
$1.28X10^{-8}$	13.00 ± 1.22	12.67 ± 0.97	0.21	0.835
$2.56X10^{-8}$	13.00 ± 1.00	13.33 ± 0.88	-0.25	0.807

- Test indicates the effect of methanolic extract on frequency of spontaneous contraction of rabbit ileum after an initial phentolamine administration in organ bath containing tyrode solution
- Control indicates frequency of spontaneous contraction of rabbit ileum when methanolic extract and phentolamine was washed out of organ bath and replaced with tyrode solution only
- **g/ml**- [gram(s) per milliliter] and **mm**-[millimeters]

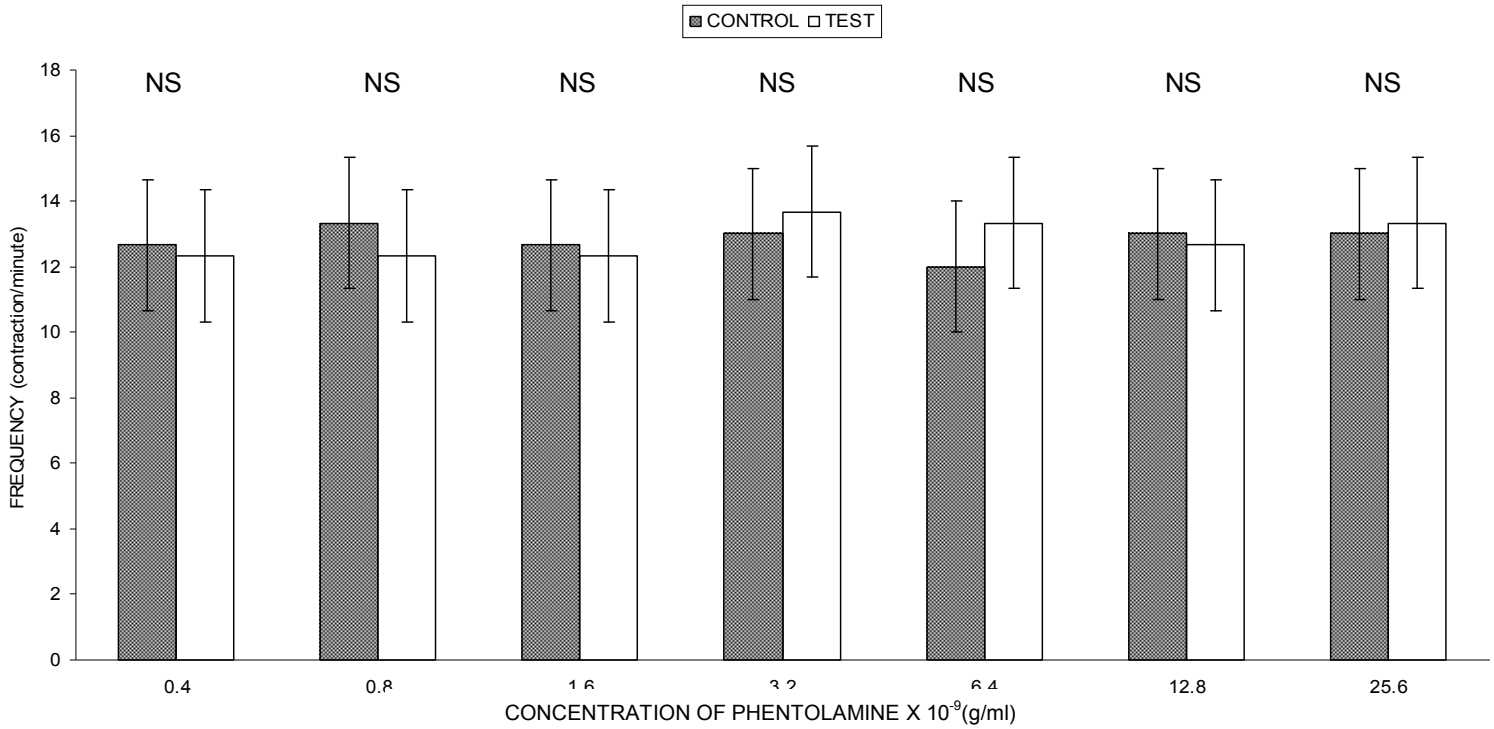


Figure 4.24: Chart represents the effect on frequency of spontaneous contraction of rabbit ileum (n=9) to methanolic extract in the presence of phentolamine. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the frequency of spontaneous contractions after administration of test drugs into the organ bath and control bars indicate frequency of spontaneous contraction after test drugs were washed out of the organ bath and replaced with tyrode solution.

4.5.3 EFFECTS OF AQUEOUS EXTRACT OF *OCIMUM BASILICUM* IN THE PRESENCE OF PROPRANOLOL USING RABBIT ILEUM

Aqueous extract (E_{max}) decreased (Figure 4.25) the amplitude of spontaneous contraction (n=9) (appendix 25) at all concentrations of propranolol which were highly significant (P<0.001) (Table 4.27) even at a maximum propranolol concentration of 2.56 X 10^{-7} g/ml (26.37 $\pm$ 1.11 mm and 6.14 $\pm$ 0.21 mm for control and test groups respectively).

Appendix 26 (n=9) illustrates the effect of aqueous extract (E_{max}) on frequency of contraction after initial propranolol administration. No significant difference (P>0.05) was observed in the frequency of contraction (Table 4.28) and illustrated in Figure 4.26.

4.5.4 EFFECTS OF METHANOLIC EXTRACT OF *OCIMUM BASILICUM* IN THE PRESENCE OF PROPRANOLOL USING RABBIT ILEUM

Methanolic extract (E_{max}) causes significant decrease in the amplitude of spontaneous contraction (n=9) (appendix 27) after the initial administration of propranolol (Figure 4.27) at all concentrations which were considered highly significant (P<0.001)(Table 4.29). At a maximum contraction of 2.56 X 10^{-7} g/ml (propranolol), methanolic extract (E_{max}) decreased the amplitude from 28.36 $\pm$ 0.72 mm to 7.2 $\pm$ 0.48 mm for control and test groups respectively.

Methanolic extract (E_{max}) had no effect on frequency (n=9) (appendix 28) of contraction (Figure 4.28). No significant difference (P>0.05) in the frequency of spontaneous contractions was recorded (Table 4.30).

Table 4.27: Effect of aqueous extract in the presence of propranolol on amplitude (mm) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of aqueous extract (g/ml)	Amplitude of contraction (mm)		T-value	P-value
	Control	Test		
$4X10^{-10}$	26.37 ± 1.11	6.14 ± 0.21	17.91	<0.001
$8X10^{-10}$	27.67 ± 0.48	6.11 ± 0.49	31.43	<0.001
$1.6X10^{-9}$	25.60 ± 0.65	6.04 ± 0.39	25.80	<0.001
$3.2X10^{-9}$	25.60 ± 0.55	6.12 ± 0.38	29.14	<0.001
$6.4X10^{-9}$	26.19 ± 0.62	6.16 ± 0.31	28.90	<0.001
$1.28X10^{-8}$	25.63 ± 1.03	6.28 ± 0.49	16.97	<0.001
$2.56X10^{-8}$	26.37 ± 1.11	6.14 ± 0.21	17.91	<0.001

- Test indicates the effect of aqueous extract on amplitude of spontaneous contraction of rabbit ileum after an initial propranolol administration in the organ bath containing tyrode solution
- Control indicates amplitude of spontaneous contraction of rabbit ileum when aqueous extract and propranolol was washed out of organ bath and replaced with tyrode solution only
- **g/ml**- [gram(s) per milliliter] and **mm**-[millimeters]

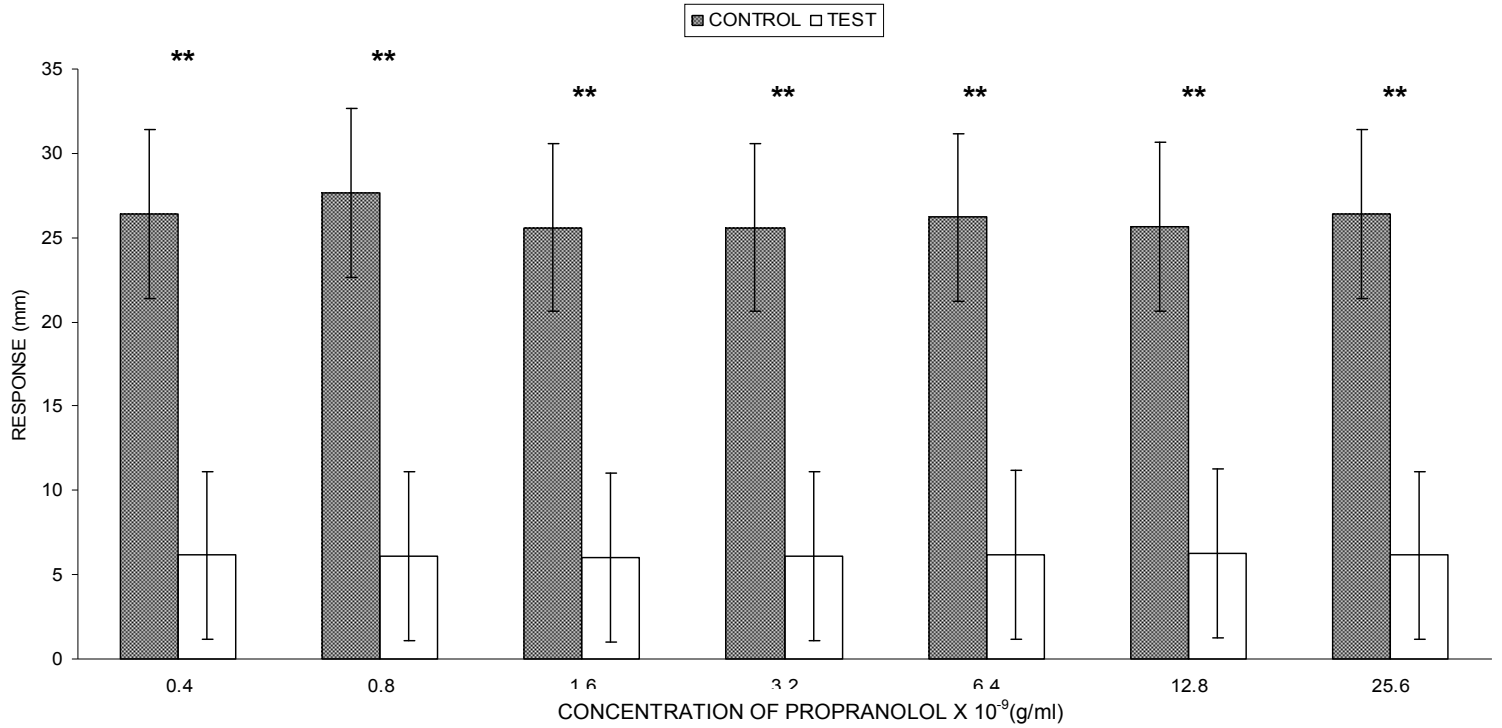


Figure 4.25 Chart represents the effect on amplitude of spontaneous contraction of rabbit ileum (n=9) to aqueous extract in the presence of propranolol. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the amplitude of spontaneous contractions after administration of test drugs into the organ bath and control bars indicate amplitude of spontaneous contraction after test drugs were washed out of the organ bath and replaced with tyrode solution.

Table 4.28: Effect of aqueous extract in the presence of propranolol on frequency (contractions/minute) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of aqueous extract (g/ml)	Frequency of contraction (cpm)		T-value	P-value
	Control	Test		
$4X10^{-10}$	17.33 ± 1.20	18.00 ± 1.41	-0.36	0.722
$8X10^{-10}$	18.67 ± 1.39	18.00 ± 1.41	0.34	0.739
$1.6X10^{-9}$	17.33 ± 1.09	16.33 ± 1.51	0.54	0.599
$3.2X10^{-9}$	16.67 ± 1.51	17.33 ± 1.56	-0.31	0.765
$6.4X10^{-9}$	16.67 ± 1.51	17.67 ± 1.90	-0.41	0.686
$1.28X10^{-8}$	16.67 ± 1.51	16.33 ± 1.24	0.17	0.864
$2.56X10^{-8}$	17.00 ± 1.50	16.67 ± 1.33	0.17	0.871

- Test indicates the effect of aqueous extract on amplitude of spontaneous contraction of rabbit ileum after an initial propranolol administration in organ bath containing tyrode solution
- Control indicates amplitude of spontaneous contraction of rabbit ileum when aqueous extract and propranolol was washed out of organ bath and replaced with tyrode solution only
- **g/ml**- [gram(s) per milliliter] and **mm**-[millimeters]

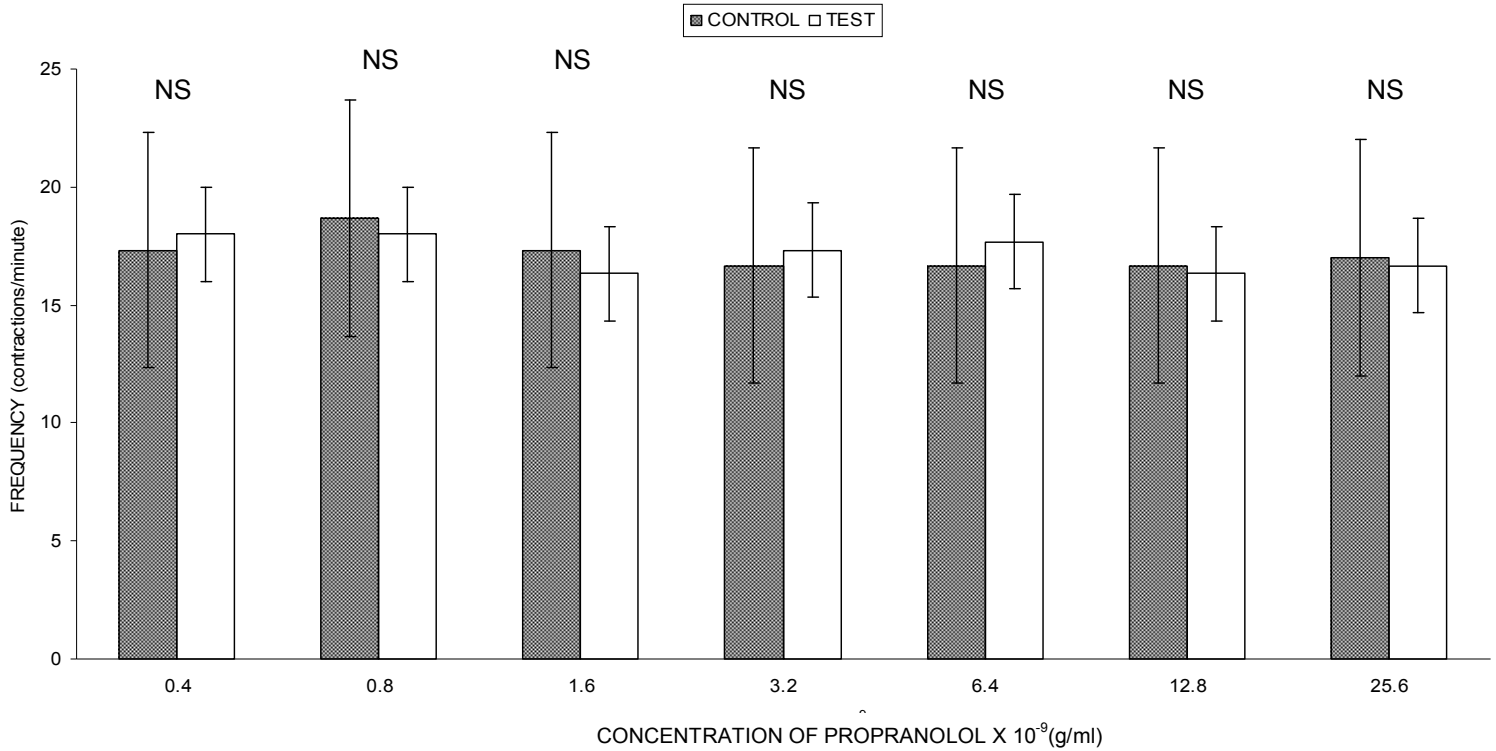


Figure 4.26: Chart represents the effect on frequency of spontaneous contraction of rabbit ileum (n=9) to aqueous extract in the presence of propranolol. [$^{NS}P>0.05$, *$P<0.05$, **$P<0.001$ compared with control]. Test bars indicate the frequency of spontaneous contractions after administration of test drugs into the organ bath and control bars indicate amplitude of spontaneous contraction after test drugs were washed out of the organ bath and replaced with tyrode solution.

Table 4.29: Effect of methanolic extract in the presence of propranolol on amplitude (mm) of spontaneous contractions in rabbit ileum (n=9. Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of methanolic extract (g/ml)	Frequency of contraction (cpm)		T-value	P-value
	Control	Test		
$4X10^{-10}$	29.34 ± 0.76	7.34 ± 0.55	23.45	<0.001
$8X10^{-10}$	28.46 ± 0.68	7.53 ± 0.38	26.87	<0.001
$1.6X10^{-9}$	29.70 ± 0.37	7.92 ± 0.36	42.19	<0.001
$3.2X10^{-9}$	28.63 ± 1.02	7.51 ± 0.43	1.08	<0.001
$6.4X10^{-9}$	28.78 ± 0.54	7.30 ± 0.52	28.65	<0.001
$1.28X10^{-8}$	28.58 ± 0.40	7.11 ± 0.43	36.56	<0.001
$2.56X10^{-8}$	28.36 ± 0.72	7.29 ± 0.48	24.35	<0.001

- Test indicates the effect of methanolic extract on amplitude of spontaneous contraction of rabbit ileum after an initial propranolol administration in organ bath containing tyrode solution
- Control indicates amplitude of spontaneous contraction of rabbit ileum when methanolic extract and propranolol was washed out of organ bath and replaced with tyrode solution only
- **g/ml**- [gram(s) per milliliter] and **mm**-[millimeters]

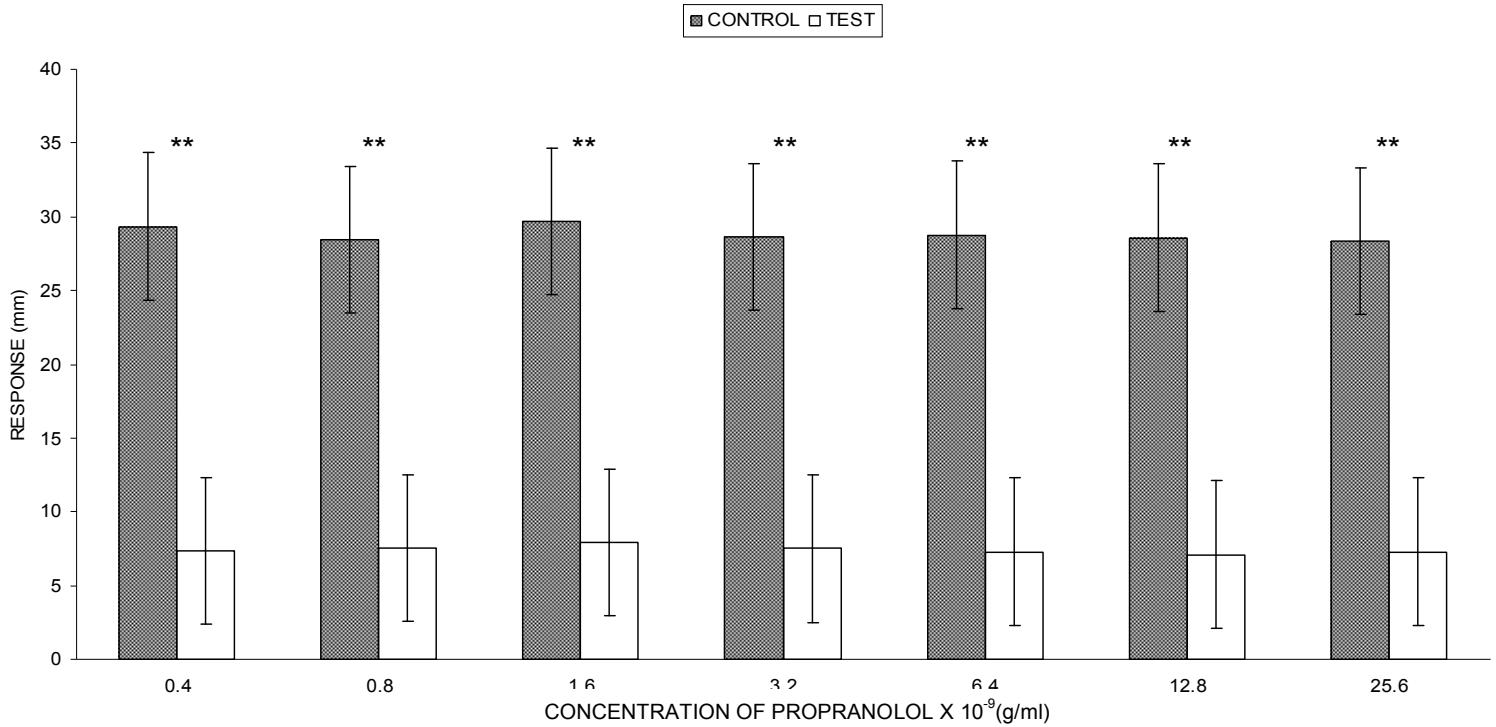


Figure 4.27: Chart represents the effect on amplitude of spontaneous contraction of rabbit ileum (n=9) to methanolic extract in the presence of propranolol. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the amplitude of spontaneous contractions after administration of test drugs into the organ bath and control bars indicate amplitude of spontaneous contraction after test drugs were washed out of the organ bath and replaced with tyrode solution.

Table 4.30: Effect of methanolic extract in the presence of propranolol on frequency (contractions/minute) of spontaneous contractions in rabbit ileum (n=9). Data expressed as Mean ± SEM [P>0.05, P<0.05, P<0.001 compared with control].

Concentration of methanolic extract (g/ml)	Frequency of contraction (cpm)		T-value	P-value
	Control	Test		
$4X10^{-10}$	17.67 ± 1.27	17.33 ± 1.56	0.17	0.868
$8X10^{-10}$	17.00 ± 1.22	17.67 ± 0.78	-0.46	0.650
$1.6X10^{-9}$	17.33 ± 0.97	17.67 ± 1.05	-0.24	0.815
$3.2X10^{-9}$	17.33 ± 1.20	17.67 ± 0.93	-0.24	0.809
$6.4X10^{-9}$	18.00 ± 1.32	17.67 ± 1.54	0.16	0.873
$1.28X10^{-8}$	17.33 ± 1.48	16.67 ± 1.13	0.35	0.728
$2.56X10^{-8}$	17.33 ± 0.97	17.00 ± 1.50	0.19	0.856

- Test indicates the effect of methanolic extract on frequency of spontaneous contraction of rabbit ileum after an initial propranolol administration in organ bath containing tyrode solution
- Control indicates frequency of spontaneous contraction of rabbit ileum when methanolic extract and propranolol was washed out of organ bath and replaced with tyrode solution only
- **g/ml**- [gram(s) per milliliter] and **mm**-[millimeters]

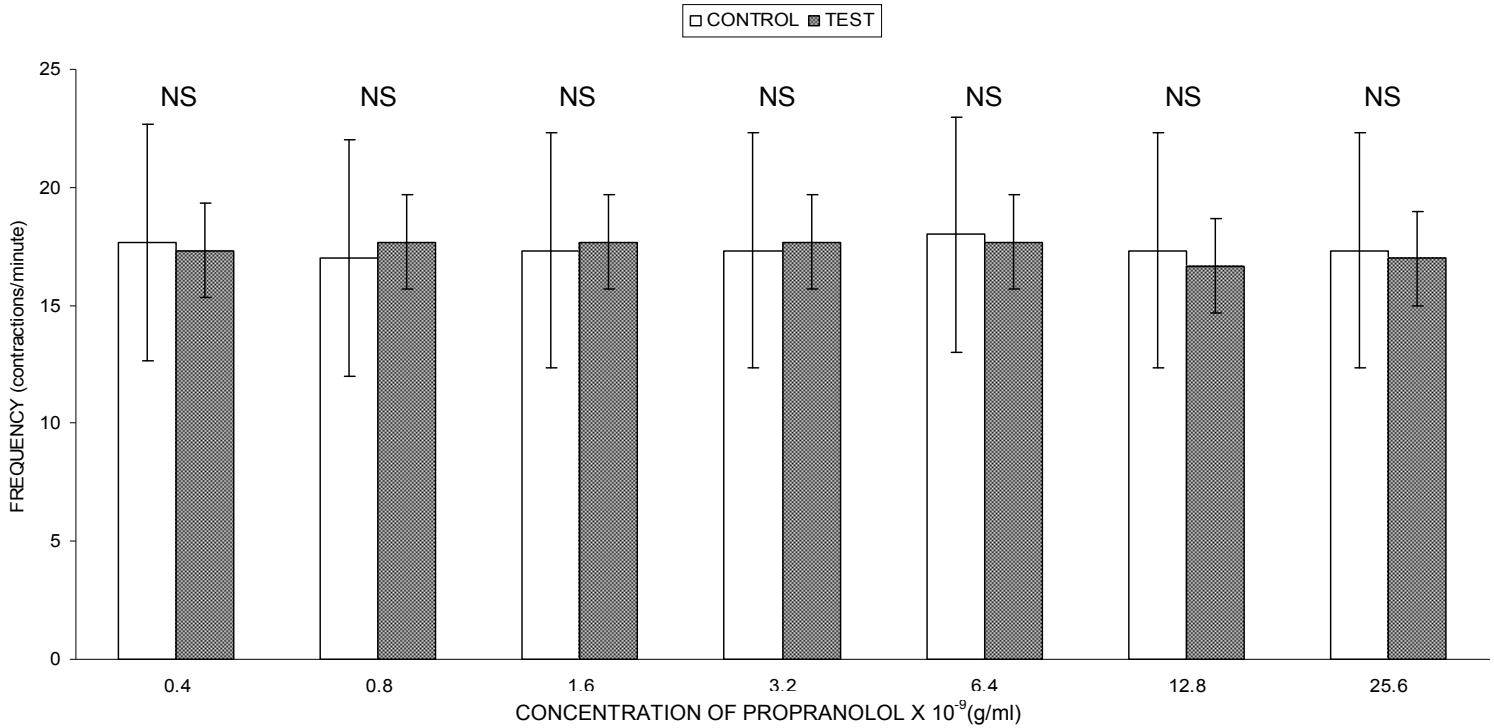


Figure 4.28: Chart represents the effect on frequency of spontaneous contraction of rabbit ileum (n=9) to methanolic in the presence of propranolol. [^{NS}P>0.05, *P<0.05, **P<0.001 compared with control]. Test bars indicate the frequency of spontaneous contractions after administration of test drugs into the organ bath and control bars indicate frequency of spontaneous contraction after test drugs were washed out of the organ bath and replaced with tyrode solution.

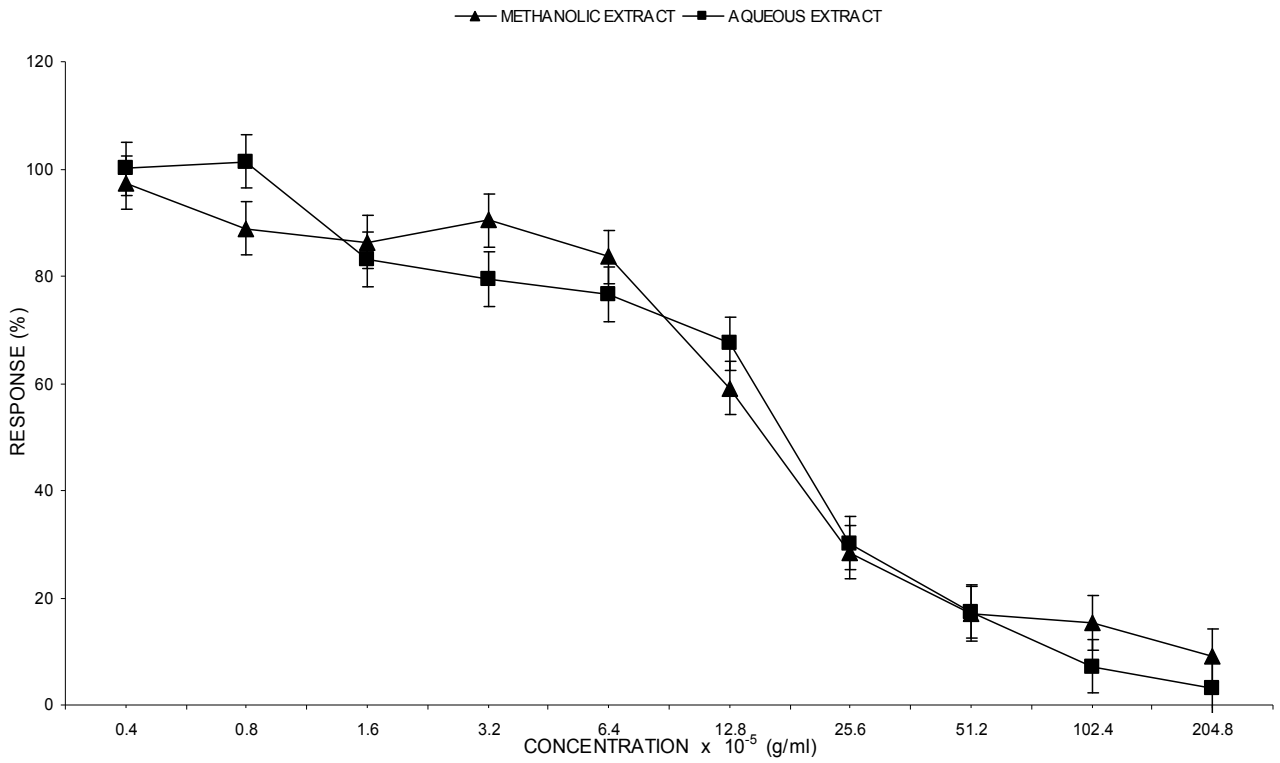


Figure 4.29: Curves illustrate the response (%) of rabbit ileum to methanolic and aqueous extracts. Curves showed decrease in spontaneous contractions as concentration of extracts increased. At maximum concentrations (2.048 X 10^{-7} g/ml), spontaneous contractions were almost abolished. Pattern of curves showed similarities to that of curves of epinephrine, norepinephrine and isoprenaline in figure 5.2. Gradients of curves in figure 5.1 and 5.2 after evaluation were expressed as negative values, which indicate a similarity in effects.

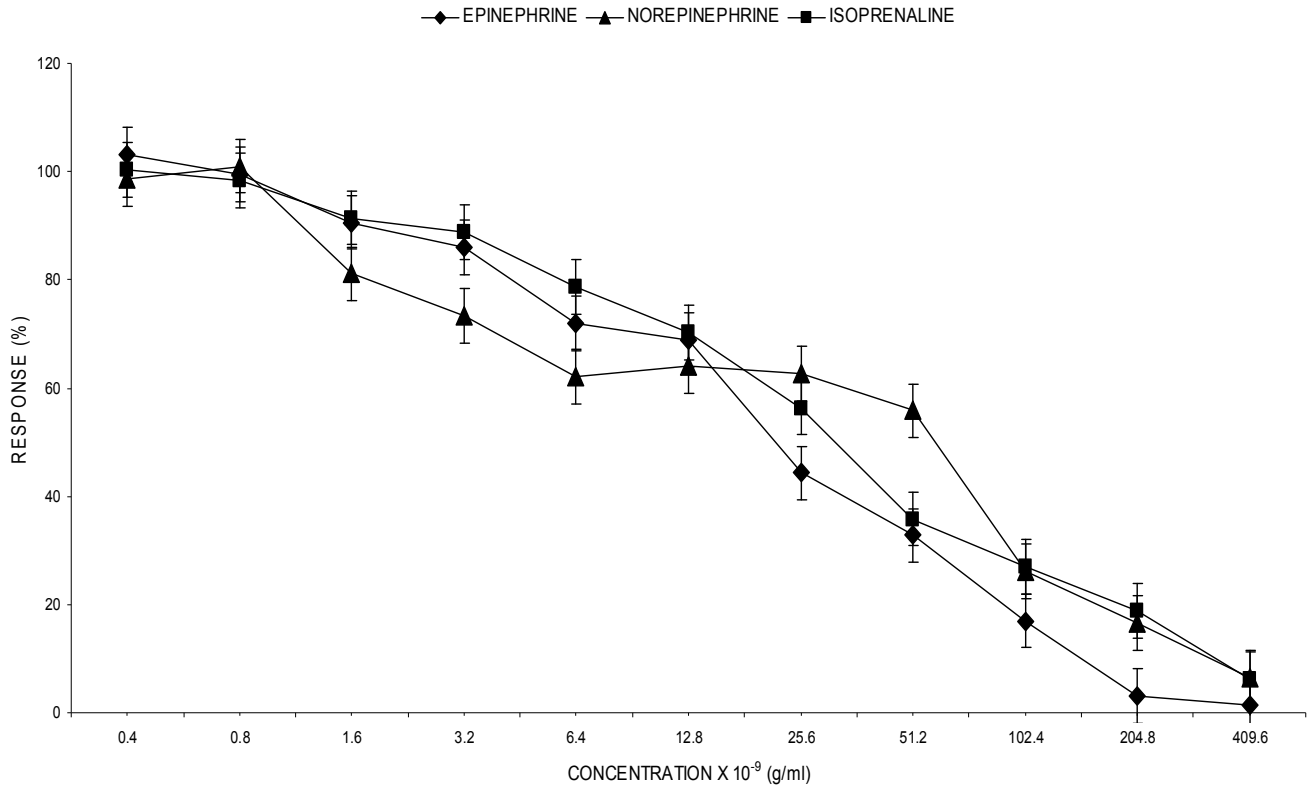


Figure 4.30: charts illustrate the response of tissues to epinephrine, norepinephrine and isoprenaline. Tissue responses (%) of spontaneous contraction decreased as the concentration (g/ml) of test substances increased. When gradients of curves were evaluated, they expressed negative values. Spontaneous contractions were abolished at high concentrations (maximum responses observed at 4.096×10^{-7} g/ml)

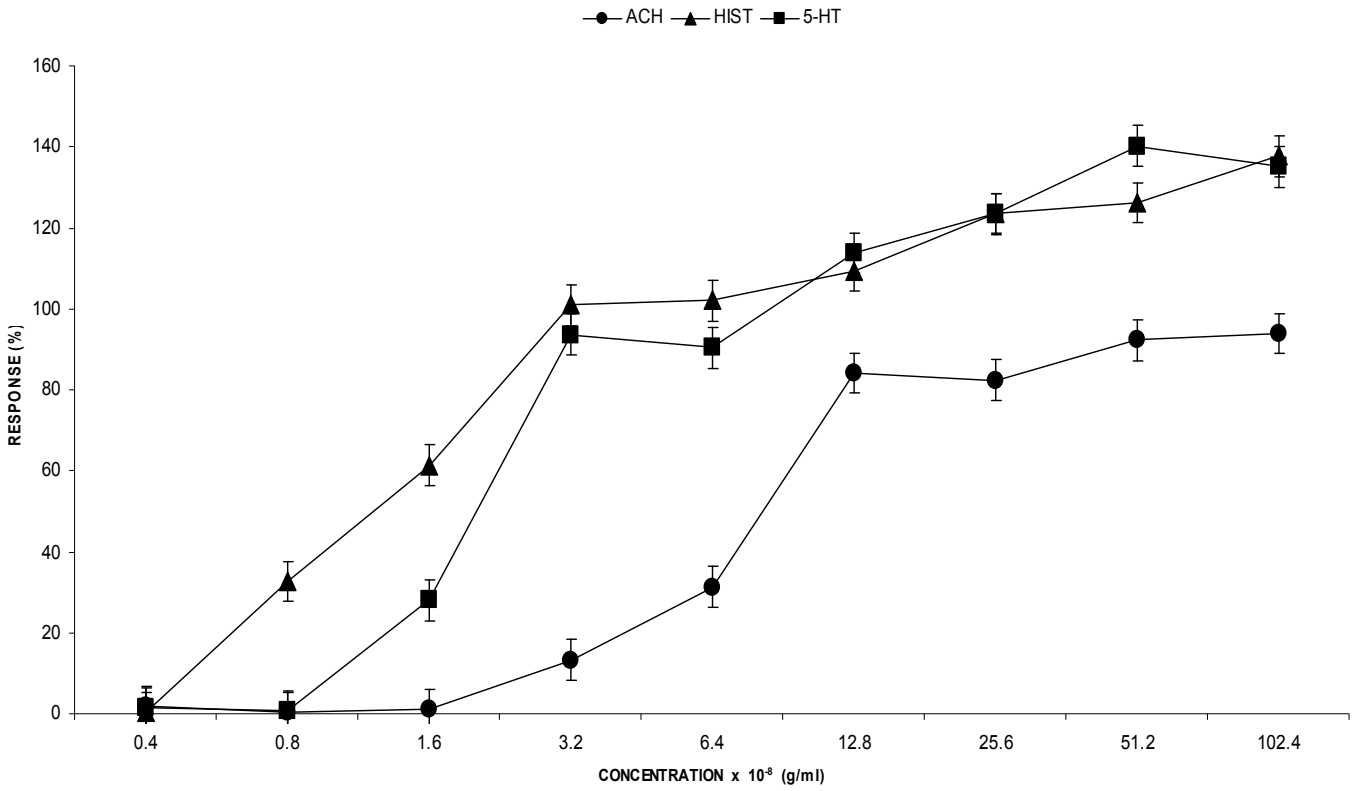


Figure 4.31: Charts represent response of tissues to acetylcholine (ACH), histamine and serotonin (5-HT). Tissues showed an increase in spontaneous contractions as concentrations (g/ml) of test substances increased. Maximum response was observed at a concentration of 1.024 X 10^{-7} g/ml. Gradients of curves after evaluation were expressed as negative values.

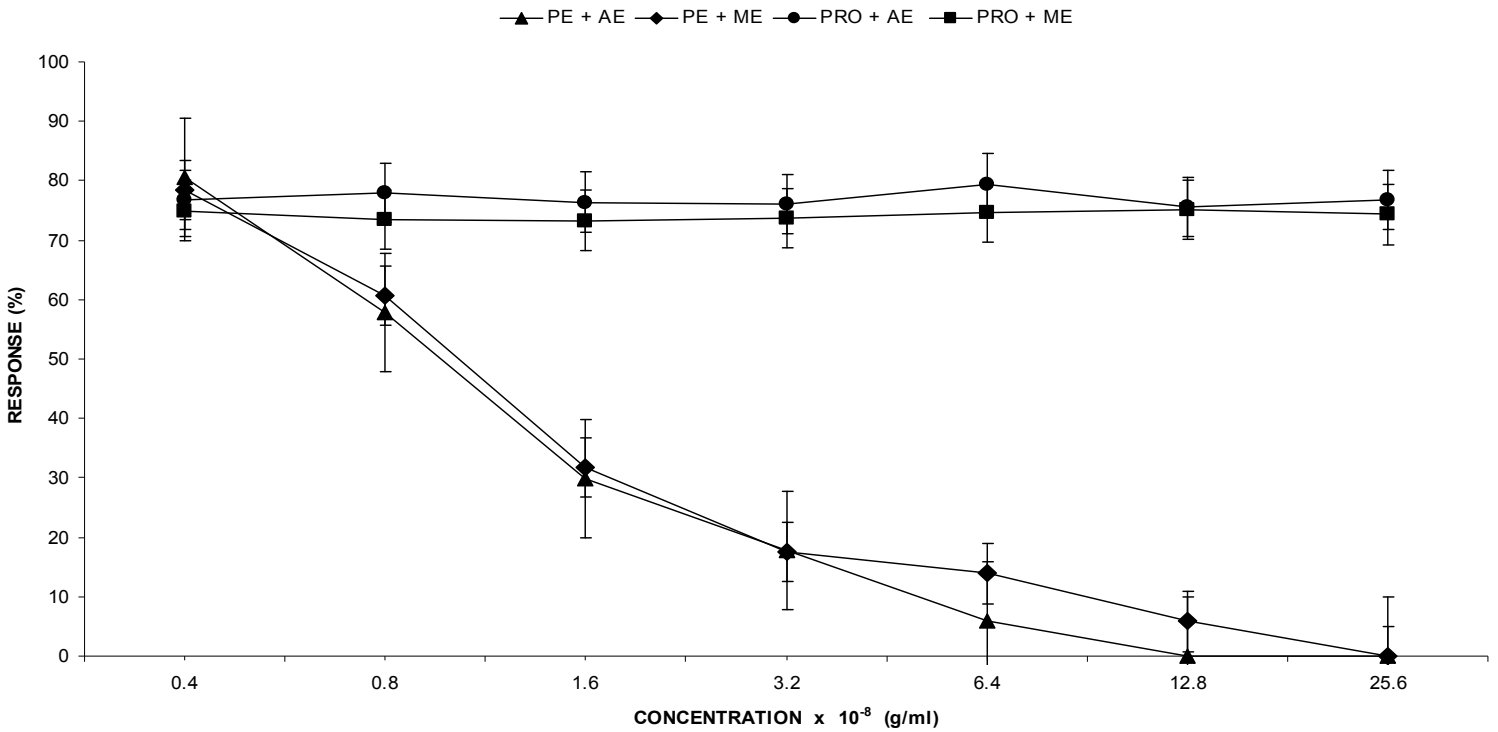


Figure 4.32: Chart illustrates the effect of methanolic and aqueous extracts (at concentrations that produced maximum response) after the initial administration of propranolol (α-adrenergic receptor antagonist) and phentolamine (β-adrenergic antagonist). Effects of extracts were still elicited with the initial administration of propranolol (2.56 X 10^{-8} g/ml of propranolol was unable to block the effect of extracts), while spontaneous contractions were abolished with an initial administration of phentolamine. In chart PE, PRO, AE and ME represent phentolamine, propranolol, aqueous extract and methanolic extract of *Ocimum basilicum.*

CHAPTER FIVE – DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Acute oral toxicity studies showed toxicity of plant extracts in experimental animals (mice) at high doses (tables 4.1, 4.2, 4.3 and 4.4). LD_{50} (median lethal dose) determined from results obtained was 3807.89 and 2154 mg/Kg for aqueous and methanolic extracts respectively (refer to section 4.2.3). Other members of the genus '*Ocimum*' have also shown toxic effects in experimental animals. *Ocimum kenyense, gratissimum,* and *selloli* showed toxic effects on insect pest (Jembere and Ahmed 2001). *Ocimum basilicum* and *gratissimum* was used as an insecticidal fumigant and powder to control Callosobruchus maculates (Sekou et al 2001). Ocimum selloi also showed toxicity and was also used as a mosquito repellant (Josiane et al 2003 and Josiah et al 2005). The toxic nature of the '*Ocimum*' genus probably explains the anti-bacterial effect of *Ocimum basilicum* essential oils (Ndounga and Ouamba 1997, Koga and Takumi 1999, Cimanga et al 2002 and Opalchenova and Obreshkova 2003) and *Ocimum gratissimum* (Orafidiya et al 2002). This property might also explain the anti-fungal (Ndounga and Ouamba 1997) and anti-viral effects (Yamasaki et al 1998, and Nana and Caesar 2003) of *Ocimum gratissimum*. This ability to destroy microorganisms might be due to the presence of flavonoids which are found in *Ocimum basilicum* leaves (Renee et al 2001 and Roberto et al 2001). The toxicity of these plants has shown scientific benefits. Examples are the use of essential oils of *Ocimum gratissimum* in controlling food spoilage from the mycotoxin produced by fungi (Nguefack et al 2004), the anti-diarrhea effect of *Ocimum gratissimum* volatile oil (Ilori and Steteolu 1996, Offiah and Chikwendu 1999, Orafidiya and Elujobi 2000),

the anti-bacterial effect of *Ocimum basilicum* and *Ocimum gratissimum* (Ndounga and Ouamba 1997, Koga and Takumi 1999, Cimanga et al 2002, Opalchenova and Obreshkova 2003, and Orafidiya et al 2002) and the anti-viral activity of *Ocimum gratissimum* (Yamasaki et al 1998, and Nana and Caesar 2003).

Intestinal motility pattern is attributed to rhythmic oscillations in membrane potential of slow waves (Makhlouf 1994) and this activity is generated by the pacemaker cells (interstitial cells of Cajal) in the intestines (Daniel and Berezin 1992, and Sanders 1996). These rhythmic oscillations can be modulated by myogenic, efferent and afferent neural pathways, hormonal control mechanisms (Otterson and Sarr 1993, and Olsson and Holmgren 2001) and the immune system (Collins 1996). Exogenous microorganisms and substances (such as drugs, bacterial, fungi and viruses) trigger the immune system and the impressive network of resident macrophages within the intestinal wall (Mikkelsen 1995), when activated secrete substances including prostaglandins (PG), leukotrienes, nitric oxide (NO) and cytokines (Nathan 1987 and Eskandari et al 1999) which modulate intestinal motility. These factors can modulate intestinal motility and in turn cause diarrhea. Diarrhea is a very important cause of infant mortality and early childhood death in Nigeria and most developing countries (Syder and Merson 1982). The causes of diarrhea are legion but the overall alterations in intestinal function are similar.

Five major mechanisms were enumerated through which diarrhea can occur (Fine et al 1989):

(i) Presence in the gut lumen of unusual amount of poorly absorbable and highly osmotically active solutes

(ii) Intestinal ion secretion

(iii) Outpouring into the gut lumen of mucins, blood and proteins from sites of inflammations

(iv) Inhibition of normal ion absorptive process

(v) Deranged intestinal motility (Fine et al 1989).

It has been established that alteration in intestinal transit can affect the rate of absorption in the gut and can predispose man to diarrhea conditions (Contran et al 1999). Thus drugs used in the treatment of diarrhea are aimed at correcting the derangement in intestinal motility, thereby favouring absorption of water and electrolytes (Katzung 1995 and Bruton 1996). Further deranged intestinal motility caused by toxin producing microorganisms are probably mediated via opiate receptors (Fioramonti et al 1984), platelet activating factor (Pons et al 1991), interleukin-1β [IL-1 β] (Plaza et al 1997), tumoral necrosis factor-α [TNF- α](Lodato et al 1999), interferon-γ (Hewett and Roth 1993), nitric oxide [NO] (Hellstrom et al 1997, Takakura et al 1997, Eskandari et al 1999, Lodato et al 1999 and Cullen et al 1999) and prostaglandins [PG](Bottoms and Adams 1992, Wechsung et al 1996, Hellstrom et al 1997 and Hori et al 2001).

In the present study, extracts of *Ocimum basilicum* decreased intestinal motility in both in-vivo [intestinal transit time experiment] and in-vitro studies [smooth muscle contractility experiment] (figure 4.29). Effects of extracts on rabbit ileum where compared to standard drugs which showed similarities to the effects of epinephrine, norepinephrine and isoprenaline (figure 4.30) on rabbit ileum. However it showed no similarities to the effects of acetylcholine, histamine and serotonin (figure 4.31) on rabbit ileum.

In conclusion results obtained from this study are in agreement with a previous study on the anti-diarrhea effects of *Ocimum gratissimum* (Ilori and Steteolu 1996, Offiah and Chikwendu 1999, Orafidiya and Elujobi 2000). Extracts of *Ocimum basilicum* and *gratissimum* probably decrease intestinal motility by binding to receptors on the cell membrane of smooth muscle cells or by inhibiting the production of substances that increase intestinal motility.

A further study which tries to elucidate possible mechanisms of action of extracts, suggests that extracts might be acting via α-adrenergic receptors (Figure 4.32). In a previous study, *Ocimum gratissimum* was reported to likely elicit its effects via the inhibition of muscarinic receptors (Offiah et al 1999). However conclusions cannot be drawn at this level due to the inclusive nature of the methodology used. Studying the actual mechanisms of action of these plants extracts will require the cloning of receptors at the molecular level.

5.2 CONCLUSIONS

Ocimum basilicum leaves extract exhibits a relaxant effect on intestinal smooth muscles and its possible mechanisms of action might be via α-adrenergic receptors. It also showed mild toxic effect at high concentrations.

5.3 RECOMMENDATION

Further studies are highly recpommended to ascertain the actual mechanisms of action of *Ocimum basilicum* leaf extracts on intestinal smooth muscle contractility, which might require genetic cloning of receptor sub-types.

Ocimum basilicum leaf extracts can be incorporated as a medicinal supplement for gastrointestinal disorders such as diarrhea.

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APPENDIX 1
EFFECTS OF METHANOLIC EXTRACT ON INTESTINAL TRANSIT TIME [GROUP I (CONTROL)] (n=5)

ANIMALS	1	2	3	4	5	MEAN ± SEM
LENGTH OF SMALL INTESTINE (cm)	168.2	192.7	167.6	179.4	183.4	178.26 ± 4.75
CHARCOAL FRONT (cm)	43.6	50.8	62.7	53.4	63.2	54.74 ± 3.72

APPENDIX 2
EFFECTS OF METHANOLIC EXTRACT ON INTESTINAL TRANSIT TIME (GROUP II) (n=5)

ANIMALS	1	2	3	4	5	MEAN ± SEM
LENGTH OF SMALL INTESTINE (cm)	74.6	86.2	76.5	84.4	70.6	78.46 ± 3.00
CHARCOAL FRONT (cm)	14.3	18.4	17.5	16.6	16.2	16.60 ± 0.69

APPENDIX 3
EFFECTS OF METHANOLIC EXTRACT ON INTESTINAL TRANSIT TIME (GROUP III) (n=5)

ANIMALS	1	2	3	4	5	MEAN ± SEM
LENGTH OF SMALL INTESTINE (cm)	86.4	74.3	95.6	98.6	76.8	86.34 ± 0.38
CHARCOAL FRONT (cm)	15.8	30.4	26.4	27.5	24.3	24.88 ± 2.47

APPENDIX 4
EFFECTS OF METHANOLIC EXTRACT ON INTESTINAL TRANSIT TIME (GROUP IV) (n=5)

ANIMALS	1	2	3	4	5	MEAN ± SEM
LENGTH OF SMALL INTESTINE (cm)	86.2	95.6	95.2	88.7	98.1	92.76 ± 2.26
CHARCOAL FRONT (cm)	12.9	15.3	17.2	16.9	18.1	16.08 ± 0.91

APPENDIX 5
EFFECTS OF METHANOLIC EXTRACT ON INTESTINAL TRANSIT TIME (GROUP V) (n=5)

ANIMALS	1	2	3	4	5	MEAN ± SEM
LENGTH OF SMALL INTESTINE (cm)	86.4	99.1	90.4	85.9	89.2	90.20 ± 2.38
CHARCOAL FRONT (cm)	5.8	3.6	6.2	7.4	4.8	5.56 ± 2.24

APPENDIX 6
EFEFECT OF AQUEOUS EXTRACT ON INTESTINAL TRANSIT TIME [GROUP I (CONTROL)] (n=5)

ANIMALS	1	2	3	4	5	MEAN ± SEM
LENGTH OF SMALL INTESTINE (cm)	123.4	104.6	138.2	96.4	134.5	119.42 ± 8.19
CHARCOAL FRONT (cm)	52.6	50.7	45.3	53.5	60.2	52.46 ± 2.39

APPENDIX 7
EFFECT OF AQUEOUS EXTRACT ON INTESTINAL TRANSIT TIME (GROUP II) (n=5)

ANIMALS	1	2	3	4	5	MEAN ± SEM
LENGTH OF SMALL INTESTINE (cm)	124.6	88.1	96.3	134.2	105.6	109.76 ± 8.60
CHARCOAL FRONT (cm)	38.6	18.4	15.3	44.9	34.7	30.38 ± 5.77

APPENDIX 8
EFFECT OF AQUEOUS EXTRACT ON INTESTINAL TRANSIT TIME (GROUP III) (n=5)

ANIMALS	1	2	3	4	5	MEAN ± SEM
LENGTH OF SMALL INTESTINE (cm)	102.1	95.4	88.7	113.4	86.2	97.16 ± 4.91
CHARCOAL FRONT (cm)	28.7	18.4	25.6	34.8	41.2	29.74 ± 3.89

APPENDIX 9
EFFECT OF AQUEOUS EXTRACT ON INTESTINAL TRANSIT TIME (GROUP IV) (n=5)

ANIMALS	1	2	3	4	5	MEAN ± SEM
LENGTH OF SMALL INTESTINE (cm)	89.7	102.4	98.5	123.8	143.6	111.60 ± 9.75
CHARCOAL FRONT (cm)	25.4	12.5	26.1	14.3	27.4	21.14 ± 3.18

APPENDIX 10
EFFECT OF AQUEOUS EXTRACT ON INTESTINAL TRANSIT TIME (GROUP V) (n=5)

ANIMALS	1	2	3	4	5	MEAN ± SEM
LENGTH OF SMALL INTESTINE (cm)	134.2	86.4	97.4	125.4	94.6	107.60 ± 9.33
CHARCOAL FRONT (cm)	4.6	5.6	14.7	2.6	10.7	7.64 ± 2.21

APPENDIX 11

EFFECTS OF ACETYLCHOLINE ON AMPLITUDE OF SPONTANEOUS CONTRACTIONS IN ISOLATED RABBIT ILEUM (n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-10}$	C	34.5	36.4	32.6	35.8	33.6	34.7	35.2	34.9	33.8	34.61±0.39
	T	34.9	35.1	34.7	36.2	35.8	34.6	36.1	34.8	35.2	35.27±0.20
$8X10^{-10}$	C	34.8	35.6	34.3	33.7	34.5	35.1	34.6	32.8	33.6	34.33±0.28
	T	34.9	35.1	33.9	34.2	34.2	34.8	34.6	34.4	33.9	34.44±0.14
$1.6X10^{-9}$	C	34.2	33.9	34.6	34.5	34.2	34.3	34.1	34.5	33.8	34.23±0.09
	T	34.5	34.2	34.9	35.1	34.9	34.8	34.7	34.3	33.9	34.59±0.13
$3.2X10^{-9}$	C	34.7	33.9	34.5	34.8	34.6	34.7	33.8	34.2	34.5	34.41±0.12
	T	40.1	39.3	35.6	37.8	40.4	41.8	37.2	39.2	39.3	38.97±0.62
$6.4X10^{-9}$	C	28.9	34.1	34.8	29.8	32.4	34.8	35.7	34.4	34.9	33.31±0.81
	T	43.5	42.9	43.8	43.7	44.1	44.5	43.7	43.6	43.9	43.74±0.15
$1.28X10^{-8}$	C	33.8	34.1	34.9	33.8	34.6	34.5	34.7	33.9	30.5	33.87±0.44
	T	60.9	61.5	63.4	62.7	63.8	60.3	63.1	62.7	62.9	62.37±0.40
$2.56X10^{-8}$	C	31.9	32.8	34.5	36.8	35.9	34.3	34.7	33.8	33.4	34.23±0.50
	T	60.4	64.5	63.7	62.8	61.6	62.8	62.8	62.5	60.7	62.42±0.44
$5.12X10^{-8}$	C	34.2	33.9	32.7	33.6	34.8	33.2	34.1	32.9	33.7	33.68±0.22
	T	65.2	64.7	66.7	65.4	65.7	65.1	62.4	63.4	64.3	64.77±0.43
$1.024X10^{-7}$	C	32.5	34.5	33.9	32.9	34.2	34.1	34.8	33.8	34.2	33.88±0.25
	T	65.9	66.3	65.9	64.6	65.8	66.1	66.3	65.4	64.8	65.68±0.21

APPENDIX 12

EFFECTS OF ACETYLCHOLINE ON FREQUENCY OF SPONTANEOUS CONTRACTIONS IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ±SEM
$4X10^{-10}$	C	12	15	12	12	18	15	12	12	12	13.33±0.73
	T	9	12	12	15	18	12	15	12	12	13.00±0.87
$8X10^{-10}$	C	15	12	12	9	18	12	12	18	18	14.12±1.09
	T	15	12	9	15	12	15	12	18	12	13.33±0.88
$1.6X10^{-9}$	C	12	12	15	18	21	12	15	12	18	15.07±1.10
	T	21	12	12	15	15	9	12	12	12	13.37±1.13
$3.2X10^{-9}$	C	15	12	15	12	15	12	15	12	12	13.33±0.53
	T	12	18	15	12	21	12	9	15	15	14.27±1.20
$6.4X10^{-9}$	C	12	15	18	12	9	15	12	15	12	13.28±0.87
	T	12	15	12	12	15	12	12	18	12	13.33±0.73
$1.28X10^{-8}$	C	12	15	12	9	21	12	15	12	15	13.67±1.13
	T	15	12	15	12	15	12	18	12	12	13.67±0.73
$2.56X10^{-8}$	C	12	15	12	9	12	18	12	15	21	14.00±1.22
	T	12	15	12	12	9	18	12	18	15	13.67±1.01
$5.12X10^{-8}$	C	15	21	9	12	12	21	12	18	12	14.67±1.45
	T	21	12	15	12	9	12	18	9	15	13.67±1.33
$1.024X10^{-7}$	C	12	15	12	18	21	12	9	18	12	14.33±1.30
	T	15	12	12	15	15	18	9	12	12	13.33±0.88

APPENDIX 13

EFFECTS OF METHANOLIC EXTRACT ON AMPLITUDE OF SPONTANEOUS CONTRACTIONS IN ISOLATED RABBIT ILEUM

(n=9)

Concentrations (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-9}$	C	25.4	26.8	27.3	28.2	24.3	23.1	26.9	29.8	24.5	26.26±0.70
	T	20.2	25.3	26.9	25.4	22.8	29.2	30.3	25.3	26.1	25.72±1.01
$8X10^{-9}$	C	28.4	29.3	22.7	25.5	24.8	28.2	25.4	23.6	30.4	26.48±0.89
	T	20.4	22.9	25.2	21.8	27.3	23.8	26.2	20.3	23.9	23.53±0.82
$1.6X10^{-8}$	C	30.5	28.2	26.4	24.9	27.6	26.5	26.6	23.2	29.8	27.08±0.76
	T	21.1	22.9	28.7	24.6	25.2	23.1	21.7	21.2	21.8	23.37±0.82
$3.2X10^{-8}$	C	30.1	27.9	26.3	27.8	24.8	23.7	26.1	25.7	25.3	26.41±0.64
	T	14.9	22.1	27.8	26.8	23.9	24.7	25.6	25.2	23.8	23.87±1.25
$6.4X10^{-8}$	C	27.4	26.8	28.9	26.1	25.3	27.2	24.7	24.1	26.8	26.37±0.45
	T	16.2	18.9	24.2	25.3	23.1	20.3	22.3	24.9	23.2	22.04±1.01
$1.28X10^{-7}$	C	28.2	27.3	27.8	24.9	28.1	26.2	23.5	24.6	24.8	26.16±0.59
	T	12.1	10.8	17.9	18.7	16.2	15.2	17.8	14.8	15.7	15.47±0.88
$2.56X10^{-7}$	C	28.1	25.7	28.3	28.7	24.2	26.5	26.3	25.2	28.2	26.80±0.53
	T	4.3	7.8	9.7	5.3	9.2	6.5	10.4	8.4	7.2	7.64±0.68
$5.12X10^{-7}$	C	26.3	28.4	28.8	24.7	26.2	24.8	24.3	27.1	25.8	26.27±0.53
	T	5.2	3.5	3.8	4.3	2.3	6.1	4.3	6.8	3.9	4.47±0.46
$1.024X10^{-6}$	C	29.1	27.8	25.3	26.1	24.7	28.3	25.6	28.7	24.8	26.71±0.59
	T	6.2	8.9	4.2	2.5	3.2	2.8	2.4	3.5	3.1	4.09±0.71
$2.048X10^{-6}$	C	28.3	27.1	24.8	27.3	25.7	25.3	24.2	26.8	26.2	26.19±0.44
	T	2.2	3.8	1.3	3.2	2.4	1.2	2.5	2.3	2.6	2.39±0.27

APPENDIX 14

EFFECTS OF METHANOLIC EXTRACT ON FREQUENCY OF SPONTANEOUS CONTRACTIONS IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-9}$	C	15	12	18	12	15	18	21	15	18	16.00±1.00
	T	18	15	15	15	12	18	18	15	18	16.00±0.71
$8X10^{-9}$	C	18	21	24	18	16	24	24	21	18	20.44±1.03
	T	15	15	18	21	21	18	24	24	21	19.67±1.13
$1.6X10^{-8}$	C	20	21	21	18	18	15	18	21	18	18.89±0.68
	T	15	18	12	21	24	18	12	18	21	17.67±1.36
$3.2X10^{-8}$	C	21	15	21	24	21	18	12	18	21	19.00±1.22
	T	15	12	18	21	24	12	15	18	21	17.33±1.39
$6.4X10^{-8}$	C	21	18	12	15	18	18	21	24	21	18.67±1.20
	T	18	12	15	18	21	24	21	24	21	19.33±1.33
$1.28X10^{-7}$	C	18	24	18	24	12	21	18	18	21	19.33±1.24
	T	18	21	24	21	18	21	15	18	18	19.33±0.88
$2.56X10^{-7}$	C	21	18	15	21	24	21	21	24	15	20.00±1.12
	T	24	21	21	15	12	15	18	21	24	19.00±1.41
$5.12X10^{-7}$	C	18	15	21	18	24	21	18	15	24	19.33±1.13
	T	15	18	21	24	21	18	21	24	15	19.67±1.13
$1.024X10^{-6}$	C	15	18	21	24	21	18	15	18	15	18.33±1.05
	T	18	21	24	21	18	15	15	18	24	19.33±1.13
$2.048X10^{-6}$	C	18	21	24	15	12	24	21	18	15	18.67±1.39
	T	15	18	21	24	21	18	15	18	12	18.00±1.22

APPENDIX 15

EFFECTS OF AQUEOUS EXTRACT ON AMPLITUDE OF SPONTANEOUS CONTRACTIONS IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-9}$	C	28.1	28.8	30.2	25.1	25.7	29.8	29.6	30.2	25.2	28.08±0.72
	T	28.5	29.7	28.7	28.7	28.2	29.3	29.3	20.2	30.3	28.10±1.01
$8X10^{-9}$	C	38.1	36.2	36.8	34.2	32.3	29.7	34.2	30.5	34.8	34.09±0.94
	T	34.1	35.8	33.2	37.2	38.1	32.1	35.8	33.8	30.9	34.56±0.79
$1.6X10^{-8}$	C	36.1	33.9	38.3	38.7	34.8	31.9	30.1	34.2	31.9	34.43±0.97
	T	29.9	27.3	28.9	28.2	27.1	29.8	26.8	31.2	28.3	28.61±0.49
$3.2X10^{-8}$	C	37.2	32.1	38.9	36.2	31.8	34.3	36.6	32.3	34.2	34.84±0.84
	T	26.2	27.1	27.8	28.1	26.7	28.7	26.5	28.2	29.8	27.68±0.39
$6.4X10^{-8}$	C	28.1	36.3	39.8	38.7	37.8	36.7	40.2	39.2	37.2	37.11±1.21
	T	27.2	30.4	28.7	27.6	30.2	26.3	29.2	27.1	29.2	28.43±0.48
$1.28X10^{-7}$	C	35.2	31.1	33.8	30.2	31.4	33.5	30.4	30.8	34.2	32.29±0.63
	T	24.2	21.1	21.7	20.3	19.8	26.2	20.2	22.3	20.3	21.79±0.72
$2.56X10^{-7}$	C	34.9	37.2	32.3	37.8	36.2	34.7	38.1	32.3	29.6	34.79±0.97
	T	10.8	9.2	13.8	9.7	11.5	9.6	8.6	9.8	11.5	10.50±0.53
$5.12X10^{-7}$	C	37.2	34.5	38.4	38.7	32.2	35.4	34.2	36.1	35.2	35.77±0.70
	T	6.2	5.3	7.1	4.8	8.3	6.7	6.2	5.3	6.2	6.23±0.35
$1.024X10^{-6}$	C	36.8	39.2	35.1	37.2	33.9	31.1	37.6	35.3	39.2	36.16±0.87
	T	2.3	5.4	2.3	1.4	3.1	2.9	2.7	1.9	1.2	2.58±0.41
$2.048X10^{-6}$	C	39.2	32.2	38.1	34.8	35.7	40.3	35.3	36.1	37.8	36.61±0.83
	T	0.8	1.1	1.9	0.7	1.2	1.4	0.7	1.3	1.5	1.18±0.13

APPENDIX 16

EFFECTS OF AQUEOUS EXTRACT ON FREQUENCY OF SPONTANEOUS CONTRACTIONS IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-9}$	C	12	18	15	18	21	15	12	18	21	16.67±1.13
	T	12	15	18	21	21	18	15	12	15	16.33±1.13
$8X10^{-9}$	C	12	18	15	12	18	12	9	12	5	12.56±1.38
	T	12	18	12	9	12	9	15	12	18	13.00±1.12
$1.6X10^{-8}$	C	12	15	9	12	9	15	12	9	15	12.00±0.87
	T	15	12	15	9	18	9	12	9	12	12.33±1.53
$3.2X10^{-8}$	C	15	9	12	15	18	21	15	18	9	14.67±1.36
	T	12	15	21	9	12	9	12	9	25	13.78±1.89
$6.4X10^{-8}$	C	15	12	9	15	12	12	15	9	18	13.00±1.00
	T	12	15	18	9	9	12	12	15	12	12.67±0.97
$1.28X10^{-7}$	C	12	15	15	9	12	9	15	9	12	12.00±0.87
	T	15	12	9	12	15	12	9	12	15	12.33±0.78
$2.56X10^{-7}$	C	15	12	18	15	12	15	12	9	12	13.33±0.88
	T	12	15	18	12	9	12	9	15	12	12.67±0.97
$5.12X10^{-7}$	C	9	12	18	15	12	15	12	9	12	12.67±0.97
	T	12	15	15	12	9	12	9	15	12	12.33±0.78
$1.024X10^{-6}$	C	12	9	12	9	12	15	12	15	12	12.00±0.71
	T	15	12	18	12	9	12	15	12	9	12.67±0.97
$2.048X10^{-6}$	C	12	15	18	12	12	18	12	15	18	14.67±0.93
	T	15	9	15	12	9	18	9	15	12	12.67±1.09

APPENDIX 17

EFFECTS OF NOREPINEPHRINE ON AMPLITUDE OF SPONTANEOUS CONTRACTIONS IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-10}$	C	39.8	39.1	36.2	40.7	39.8	37.9	39.2	40.1	39.2	39.11±0.45
	T	42.2	40.1	39.8	35.5	39.2	37.5	41.5	35.3	36.3	38.60±0.85
$8X10^{-10}$	C	40.3	38.8	37.3	36.2	38.2	39.8	39.2	36.2	42.8	38.76±0.70
	T	43.2	37.3	35.5	39.4	40.7	38.3	38.8	41.2	37.9	39.14±0.77
$1.6X10^{-9}$	C	39.2	42.6	37.3	40.8	39.2	36.3	38.7	40.8	36.3	39.02±0.72
	T	31.2	36.2	33.7	35.8	26.2	29.1	29.8	32.4	30.4	31.64±1.08
$3.2X10^{-9}$	C	37.3	39.8	41.3	36.5	38.3	32.3	39.2	39.8	37.2	37.97±0.87
	T	30.2	27.8	26.3	27.2	28.9	27.2	29.2	25.5	28.6	27.88±0.50
$6.4X10^{-9}$	C	30.2	32.1	35.6	29.3	31.7	36.2	37.1	33.7	32.2	33.12±0.90
	T	19.2	17.5	18.6	23.2	21.9	22.7	25.2	19.1	17.8	20.58±0.91
$1.28X10^{-8}$	C	34.2	30.9	27.9	34.9	31.1	34.2	29.8	28.3	34.2	31.72±0.91
	T	19.8	17.8	17.3	21.3	25.2	16.5	19.3	21.2	24.2	20.29±1.00
$2.56X10^{-8}$	C	34.2	30.5	36.2	33.4	29.5	28.3	30.3	32.2	36.1	32.30±0.95
	T	23.1	29.3	17.5	20.6	18.4	16.3	18.6	20.4	17.8	20.22±1.32
$5.12X10^{-8}$	C	28.4	26.7	32.2	36.3	37.8	31.2	35.7	32.2	35.4	32.88±1.25
	T	15.2	18.3	24.8	19.7	24.4	16.4	14.8	17.8	13.8	18.36±1.33
$1.024X10^{-7}$	C	31.7	28.9	27.9	35.2	38.7	36.8	32.2	38.7	34.8	33.88±1.32
	T	9.2	7.3	9.8	12.7	6.3	9.3	8.2	10.8	6.2	8.87±0.71
$2.048X10^{-7}$	C	34.2	36.5	35.5	38.2	31.8	35.3	35.4	37.1	31.8	35.09±0.73
	T	2.8	5.3	8.2	4.5	2.7	7.8	4.6	7.2	9.2	5.81±0.79
$4.096X10^{-7}$	C	35.2	32.1	38.8	29.2	31.8	36.8	28.8	25.7	25.2	31.51±1.59
	T	2.2	1.3	2.5	2.8	1.2	2.4	2.2	2.3	1.4	2.03±0.19

APPENDIX 18

EFFECTS OF NOREPINEPHRINE ON FREQUENCY OF SPONTANEOUS CONTRACTIONS IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-10}$	C	18	15	12	12	15	12	15	12	18	14.33±0.83
	T	18	15	12	18	12	15	21	12	12	15.00±1.12
$8X10^{-10}$	C	12	15	12	18	12	15	18	12	18	14.67±0.93
	T	15	18	12	15	18	12	15	12	18	15.00±0.87
$1.6X10^{-9}$	C	12	15	18	12	15	18	12	21	18	15.67±1.09
	T	12	15	18	12	18	15	15	15	18	15.33±0.78
$3.2X10^{-9}$	C	15	12	18	12	15	12	18	12	15	14.33±0.83
	T	12	18	15	12	15	18	21	12	15	15.33±1.05
$6.4X10^{-9}$	C	15	12	18	12	15	18	12	21	12	15.00±1.12
	T	12	18	21	15	12	18	12	18	15	15.67±1.09
$1.28X10^{-8}$	C	12	18	15	12	9	21	24	12	9	14.67±1.76
	T	18	21	9	21	12	9	15	12	12	14.33±1.56
$2.56X10^{-8}$	C	18	15	9	24	21	21	12	15	12	16.33±1.67
	T	12	15	18	15	18	12	18	12	18	15.33±0.93
$5.12X10^{-8}$	C	15	12	18	21	24	21	12	15	9	16.33±1.67
	T	12	15	18	12	15	21	18	21	18	16.67±1.13
$1.024X10^{-7}$	C	15	12	9	24	21	24	9	12	21	16.33±2.07
	T	12	15	18	21	24	21	12	9	18	16.67±1.67
$2.048X10^{-7}$	C	15	12	9	12	18	12	15	18	18	14.33±1.09
	T	12	15	18	21	21	18	18	12	15	16.67±1.13
$4.096X10^{-7}$	C	21	18	12	15	12	15	18	21	15	16.33±1.13
	T	12	15	21	18	15	18	18	12	21	16.67±1.13

APPENDIX 19

EFFECTS OF EPINEPHRINE ON AMPLITUDE OF SPONTANEOUS CONTRACTIONS IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-10}$	C	21.5	23.8	18.3	18.1	24.3	26.5	23.3	24.7	25.2	22.86±0.99
	T	23.4	26.5	23.8	25.2	20.6	20.2	19.8	26.2	26.5	23.58±0.92
$8X10^{-10}$	C	24.5	26.8	24.8	21.4	26.5	23.6	22.3	20.9	25.4	24.02±0.71
	T	24.2	21.2	19.7	23.5	27.5	21.7	28.4	22.6	26.3	23.90±0.99
$1.6X10^{-9}$	C	35.2	36.5	32.3	29.1	26.5	32.5	31.2	33.6	34.2	32.34±1.03
	T	29.3	23.1	25.3	32.5	36.3	31.2	30.2	29.5	26.4	29.31±1.33
$3.2X10^{-9}$	C	36.1	32.2	28.4	35.5	36.3	35.4	32.4	38.7	33.2	34.24±1.01
	T	36.2	34.5	24.2	22.3	34.2	29.1	30.2	25.5	28.7	29.43±1.62
$6.4X10^{-9}$	C	34.5	38.1	36.4	34.7	36.9	38.6	31.7	33.6	31.8	35.14±0.84
	T	23.3	28.2	21.4	20.1	22.5	27.8	31.9	27.8	24.4	25.27±1.29
$1.28X10^{-8}$	C	27.2	20.9	27.8	23.1	28.8	20.7	35.5	31.9	29.2	27.23±1.65
	T	16.5	19.2	23.1	21.5	17.2	15.5	20.8	19.3	15.8	18.77±0.90
$2.56X10^{-8}$	C	34.2	37.8	33.6	28.3	30.2	38.7	29.6	37.1	35.2	33.86±1.26
	T	13.6	12.4	16.2	14.4	11.8	11.6	23.4	16.2	15.3	14.99±1.20
$5.12X10^{-8}$	C	34.2	32.1	36.7	37.5	38.7	41.2	39.4	37.1	36.3	37.02±0.91
	T	12.2	9.1	14.7	12.2	12.5	12.7	9.5	11.2	15.1	12.13±0.68
$1.024X10^{-7}$	C	37.3	32.5	31.2	37.5	31.6	37.5	29.8	37.6	39.2	34.91±1.19
	T	4.2	5.6	5.2	4.7	7.8	6.3	4.1	8.6	6.8	5.92±0.53
$2.048X10^{-7}$	C	38.2	36.2	41.1	39.3	35.5	33.8	38.7	40.2	36.1	37.68±0.80
	T	1.4	2.1	0.3	1.7	1.3	0.4	0.7	1.2	1.5	1.18±0.20
$4.096X10^{-7}$	C	38.2	33.5	39.3	40.7	36.4	39.2	38.1	31.8	40.8	37.56±1.04
	T	0.5	0.3	0.8	0.5	0.3	0.6	0.4	0.2	1.3	0.54±0.11

APPENDIX 20

EFFECTS OF EPINEPHRINE ON FREQUENCY OF SPONTANEOUS CONTRACTIONS IN ISOLATED RABBIT ILEUM (n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-10}$	C	12	15	18	12	21	18	12	12	15	15.00±1.12
	T	12	9	12	15	18	12	9	12	15	12.67±0.9
$8X10^{-10}$	C	15	18	12	9	21	18	12	18	9	14.67±1.45
	T	12	18	9	12	18	21	9	12	15	14.00±1.41
$1.6X10^{-9}$	C	15	12	18	9	21	12	18	15	12	14.67±1.27
	T	12	15	9	12	21	12	21	18	15	15.00±1.41
$3.2X10^{-9}$	C	15	12	18	9	18	21	9	12	18	14.67±1.45
	T	12	15	18	12	9	12	15	12	21	14.00±1.22
$6.4X10^{-9}$	C	15	12	21	18	12	15	12	9	15	14.33±1.20
	T	12	9	12	21	18	9	15	21	9	14.00±1.66
$1.28X10^{-8}$	C	15	12	18	12	21	9	12	12	18	14.33±1.30
	T	12	21	12	21	12	9	15	12	12	14.00±1.41
$2.56X10^{-8}$	C	9	12	21	18	15	12	12	21	12	14.67±1.45
	T	12	15	12	18	18	12	9	12	15	13.67±1.01
$5.12X10^{-8}$	C	15	12	15	18	12	9	21	9	21	14.67±1.54
	T	12	9	21	12	15	12	9	21	9	13.33±1.59
$1.024X10^{-7}$	C	15	18	12	9	12	21	12	18	21	15.33±1.45
	T	9	12	15	21	9	12	21	24	9	14.67±1.96
$2.048X10^{-7}$	C	12	15	18	12	12	21	18	9	15	14.67±1.27
	T	15	12	9	21	15	12	9	18	21	14.67±1.54
$4.096X10^{-7}$	C	9	18	21	9	12	15	18	12	21	15.00±1.58
	T	21	9	12	15	12	18	21	9	9	14.00±1.66

APPENDIX 21

EFFECTS OF MAXIMUM EFFECTIVE CONCENTRATION OF AQUEOUS EXTRACT AFTER PHENTOLAMINE ADMINISTRATION ON AMPLITUDE IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-9}$	C	31.2	25.1	30.5	24.5	28.2	32.1	30.8	28.3	29.1	28.87±0.88
	T	4.1	6.2	3.3	7.8	4.3	8.7	5.2	6.8	4.3	5.63±0.62
$8 X10^{-9}$	C	27.3	28.5	31.4	26.2	32.1	27.3	30.9	34.2	26.2	29.34±0.96
	T	12.1	15.3	13.9	11.2	11.8	10.4	12.2	9.4	15.2	12.39±0.68
$1.6 X10^{-8}$	C	30.3	25.2	34.5	31.6	27.8	31.4	34.2	27.1	28.5	30.07±1.06
	T	21.3	24.3	16.2	17.5	19.4	20.7	23.4	25.7	21.2	21.08±1.03
$3.2 X10^{-8}$	C	30.4	34.5	31.4	29.2	27.8	30.7	35.6	28.4	29.3	30.81±0.89
	T	25.3	23.1	27.4	25.5	27.6	26.5	27.2	24.3	21.2	25.34±0.72
$6.4 X10^{-8}$	C	30.9	34.7	29.3	26.2	30.8	34.4	28.3	29.1	31.7	30.60±0.92
	T	28.3	21.2	29.1	29.8	26.7	31.3	34.1	29.5	29.3	28.81±1.17
$1.28 X10^{-7}$	C	26.1	23.4	28.2	21.3	27.6	24.8	29.6	28.5	26.8	26.26±0.89
	T	28.7	27.3	25.5	29.6	33.6	28.4	29.7	28.6	33.2	29.40±0.87
$2.56 X10^{-7}$	C	26.3	25.7	31.2	30.3	27.9	25.8	30.7	28.3	26.8	28.11±0.72
	T	27.9	30.7	29.8	29.4	27.3	26.2	27.8	27.6	26.4	28.12±0.51

APPENDIX 22

EFFECTS OF MAXIMUM EFFECTIVE CONCENTRATION OF AQUEOUS EXTRACT AFTER PHENTOLAMINE ADMINISTRATION ON FREQUENCY IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-9}$	C	12	15	12	9	15	12	9	18	12	12.67±0.97
	T	15	12	15	12	15	12	9	15	12	13.00±0.71
$8\ X10^{-9}$	C	12	15	12	18	21	9	15	15	18	15.00±1.22
	T	12	18	15	12	9	18	12	15	12	13.67±1.01
$1.6\ X10^{-8}$	C	12	15	12	18	9	12	15	12	12	13.00±0.87
	T	15	12	9	18	12	15	12	9	18	13.33±1.13
$3.2\ X10^{-8}$	C	12	15	12	9	18	12	15	12	15	13.33±0.88
	T	15	12	18	12	15	12	15	15	9	13.67±0.88
$6.4\ X10^{-8}$	C	12	15	12	18	12	9	21	12	9	13.33±1.33
	T	15	12	18	12	9	18	9	12	18	13.67±1.24
$1.28\ X10^{-7}$	C	12	9	15	18	9	12	15	18	15	13.67±1.13
	T	15	9	15	12	12	15	12	18	12	13.33±0.88
$2.56\ X10^{-7}$	C	12	15	21	18	12	9	15	9	12	13.67±1.33
	T	15	12	9	12	18	12	9	15	12	12.67±0.97

APPENDIX 23

EFFECTS OF MAXIMUM EFFECTIVE CONCENTRATION OF METHANOLIC EXTRACT AFTER PHENTOLAMINE ADMINISTRATION ON AMPLITUDE IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-9}$	C	26.2	28.8	30.7	29.4	27.2	29.3	30.4	32.1	28.7	29.20±0.60
	T	8.2	5.3	5.5	4.3	7.8	8.6	5.4	7.2	4.5	6.31±0.55
$8 X10^{-9}$	C	28.7	24.6	31.2	30.9	30.6	29.5	26.3	28.1	31.5	29.04±0.79
	T	12.3	9.8	12.2	13.4	10.2	11.7	11.3	9.2	12.8	11.43±0.48
$1.6 X10^{-8}$	C	30.2	26.8	28.7	29.2	30.8	28.9	27.7	29.1	30.2	29.07±0.42
	T	19.8	19.3	16.4	22.6	21.2	18.4	20.3	19.3	21.4	19.86±0.61
$3.2 X10^{-8}$	C	30.2	34.1	30.3	29.4	31.2	28.5	27.6	29.8	31.5	30.29±0.63
	T	23.1	26.5	27.6	21.4	24.5	23.4	28.3	26.4	23.4	24.96±0.78
$6.4 X10^{-8}$	C	28.2	30.5	29.4	30.2	29.4	29.8	31.2	29.4	28.6	29.63±0.31
	T	25.8	24.3	21.1	27.6	26.5	24.3	29.2	25.5	25.4	25.52±0.79
$1.28 X10^{-7}$	C	28.3	26.8	29.2	30.7	29.3	27.4	29.2	30.6	33.5	29.44±0.67
	T	26.8	29.3	26.5	29.6	28.5	26.7	28.8	28.3	25.1	27.73±0.51
$2.56 X10^{-7}$	C	28.2	30.9	29.3	32.1	29.3	31.2	28.4	30.9	28.2	29.83±0.49
	T	29.7	33.5	28.4	31.2	28.7	32.4	29.1	27.5	28.1	29.84±0.69

APPENDIX 24

EFFECTS OF MAXIMUM EFFECTIVE CONCENTRATION OF METHANOLIC EXTRACT AFTER PHENTOLAMINE ADMINISTRATION ON FREQUENCY IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-9}$	C	12	15	12	9	12	15	12	9	18	12.67±0.97
	T	15	12	9	12	15	12	15	9	12	12.33±0.78
$8\ X10^{-9}$	C	21	12	9	9	12	18	15	12	12	13.33±1.33
	T	15	12	15	12	9	12	9	15	12	12.33±0.78
$1.6\ X10^{-8}$	C	12	15	12	9	12	15	12	18	9	12.67±0.97
	T	15	9	12	15	12	9	15	12	12	12.33±0.78
$3.2\ X10^{-8}$	C	12	9	15	18	21	9	12	9	12	13.00±1.41
	T	15	12	18	12	15	12	18	12	9	13.67±1.01
$6.4\ X10^{-8}$	C	12	15	12	9	12	9	12	18	9	12.00±1.00
	T	15	18	9	9	12	9	21	12	15	13.33±1.42
$1.28\ X10^{-7}$	C	9	12	9	15	18	12	15	18	9	13.00±1.22
	T	12	18	12	9	15	12	15	12	9	12.67±0.97
$2.56\ X10^{-7}$	C	15	12	15	9	18	9	12	15	12	13.00±1.00
	T	12	15	12	9	12	18	12	15	15	13.33±0.88

APPENDIX 25

EFFECTS OF MAXIMUM EFFECTIVE CONCENTRATIONS OF AQUEOUS EXTRACT AFTER PROPRANOLOL ADMINISTRATION ON AMPLITUDE IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-9}$	C	26.3	23.2	20.4	23.2	28.5	29.6	28.2	29.5	28.4	26.37±1.11
	T	6.2	5.4	5.8	6.4	5.3	6.4	6.1	6.3	7.4	6.14±0.21
$8 X10^{-9}$	C	28.1	25.3	28.7	29.5	26.7	28.8	27.6	28.5	25.8	27.67±0.48
	T	6.2	7.6	6.8	2.9	5.3	7.1	5.3	7.6	6.2	6.11±0.49
$1.6 X10^{-8}$	C	22.9	28.1	24.7	28.3	26.6	24.3	26.5	23.4	25.6	25.60±0.65
	T	5.2	6.5	5.3	8.5	5.1	6.4	5.7	6.9	4.8	6.04±0.39
$3.2 X10^{-8}$	C	25.6	24.5	28.3	23.6	25.5	23.4	27.6	25.6	26.3	25.60±0.55
	T	5.3	5.9	4.2	6.7	6.4	6.4	8.3	5.4	6.5	6.12±0.38
$6.4 X10^{-8}$	C	28.6	27.2	27.9	25.2	26.5	23.4	27.1	26.4	23.4	26.19±0.62
	T	5.3	6.9	5.1	7.3	5.4	7.5	6.2	6.5	5.2	6.16±0.31
$1.28 X10^{-7}$	C	23.8	22.2	21.3	25.5	28.6	24.3	27.5	26.7	30.8	25.63±1.03
	T	6.5	5.8	5.2	6.2	8.6	5.2	6.1	4.3	8.6	6.28±0.49
$2.56 X10^{-7}$	C	26.3	23.2	20.4	23.2	28.5	29.6	28.2	29.5	28.4	26.37±1.11
	T	6.2	5.4	5.8	6.4	5.3	6.4	6.1	6.3	7.4	6.14±0.21

APPENDIX 26

EFFECTS OF MAXIMUM EFFECTIVE CONCENTRATION OF AQUEOUS EXTRACT AFTER PROPRANOLOL ADMINISTRATION ON FREQUENCY IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-9}$	C	21	18	12	21	12	18	21	18	15	17.33±1.20
	T	12	15	12	21	18	21	21	24	18	18.00±1.41
$8\ X10^{-9}$	C	21	12	18	21	18	21	24	21	12	18.67±1.39
	T	12	18	21	24	21	12	15	18	21	18.00±1.41
$1.6\ X10^{-8}$	C	21	15	18	21	18	15	12	15	21	17.33±1.09
	T	12	9	21	18	21	18	12	15	21	16.33±1.51
$3.2\ X10^{-8}$	C	15	12	15	12	21	24	12	21	18	16.67±1.51
	T	21	12	15	18	12	21	24	12	21	17.33±1.56
$6.4\ X10^{-8}$	C	12	18	15	12	21	24	21	12	15	16.67±1.51
	T	21	12	15	21	24	12	21	24	9	17.67±1.90
$1.28\ X10^{-7}$	C	12	15	12	21	24	21	12	18	15	16.67±1.51
	T	21	12	15	18	21	12	18	12	18	16.33±1.24
$2.56\ X10^{-7}$	C	12	21	24	21	12	15	18	18	12	17.00±1.50
	T	15	12	15	18	15	12	18	24	21	16.67±1.33

APPENDIX 27

EFFECTS OF MAXIMUM EFFECTIVE CONCENTRATIONS OF METHANOLIC EXTRACT AFTER PROPRANOLOL ADMINISTRATION ON AMPLITUDE IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-9}$	C	31.2	27.6	26.2	28.3	29.7	33.8	27.6	29.5	30.2	29.34±0.76
	T	5.8	5.2	8.7	5.3	7.8	6.7	8.7	8.2	9.7	7.34±0.55
$8 X10^{-9}$	C	25.2	30.6	28.2	29.3	26.2	31.7	28.6	28.9	27.4	28.46±0.68
	T	6.5	6.2	7.6	8.6	8.1	7.4	6.5	7.2	9.7	7.53±0.38
$1.6 X10^{-8}$	C	29.2	30.8	28.8	27.9	31.2	30.4	28.8	30.7	29.5	29.70±0.37
	T	6.8	7.9	6.2	8.2	9.3	7.8	9.2	7.1	8.8	7.92±0.36
$3.2 X10^{-8}$	C	29.3	30.8	28.7	30.6	28.5	26.4	31.6	21.6	30.2	28.63±1.02
	T	9.8	8.2	6.3	6.5	8.6	7.3	7.4	5.6	7.9	7.51±0.43
$6.4 X10^{-8}$	C	26.3	30.9	29.4	26.9	27.5	29.7	28.5	30.8	29	28.78±0.54
	T	5.2	6.8	5.1	8.5	6.4	9.7	7.4	7.9	8.7	7.30±0.52
$1.28 X10^{-7}$	C	26.4	28.4	29.6	28.3	29.4	27.1	29.8	29.7	28.5	28.58±0.40
	T	6.3	6.8	5.8	8.7	7.9	5.4	8.6	6.2	8.3	7.11±0.43
$2.56 X10^{-7}$	C	24.4	26.5	30.8	29.4	30.9	28.3	29.6	28.7	26.6	28.36±0.72
	T	5.7	7.8	8.9	6.8	9.7	7.6	6.2	5.3	7.6	7.29±0.48

APPENDIX 28

EFFECTS OF MAXIMUM EFFECTIVE CONCENTRATION OF METHANOLIC EXTRACT AFTER PROPRANOLOL ADMINISTRATION ON FREQUENCY IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ± SEM
$4X10^{-9}$	C	15	21	18	12	15	15	24	21	18	17.67±1.27
	T	12	21	18	21	24	21	12	12	15	17.33±1.56
$8\ X10^{-9}$	C	21	12	15	21	18	18	15	12	21	17.00±1.22
	T	18	21	15	18	15	18	21	15	18	17.67±0.78
$1.6\ X10^{-8}$	C	21	12	15	18	18	21	18	18	15	17.33±0.97
	T	12	21	18	21	15	18	21	15	18	17.67±1.05
$3.2\ X10^{-8}$	C	12	18	21	18	21	15	21	18	12	17.33±1.20
	T	21	15	18	15	18	15	21	15	21	17.67±0.93
$6.4\ X10^{-8}$	C	12	21	24	21	15	18	15	21	15	18.00±1.32
	T	18	12	12	21	18	21	12	24	21	17.67±1.54
$1.28\ X10^{-7}$	C	12	21	24	12	18	18	21	18	12	17.33±1.48
	T	15	18	21	12	18	18	21	12	15	16.67±1.13
$2.56\ X10^{-7}$	C	15	18	21	18	21	12	18	15	18	17.33±0.97
	T	18	21	12	21	24	12	12	15	18	17.00±1.50

APPENDIX 29

EFFECTS OF HISTAMINE ON AMPLITUDE OF SPONTANEOUS CONTRACTIONS IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ±SEM
$4X10^{-10}$	C	26.2	28.8	23.8	28.7	21.3	27.9	25.2	27.6	28.7	26.47±0.87
	T	28.8	24.2	20.9	27.6	26.3	27.1	25.5	29.7	29.0	26.57±0.92
$8X10^{-10}$	C	25.3	25.6	23.8	28.7	26.5	28.8	26.8	28.5	24.1	26.46±0.64
	T	33.7	38.2	34.5	30.8	38.5	35.7	34.2	36.4	33.8	35.09±0.80
$1.6X10^{-9}$	C	23.8	24.7	29.1	21.3	22.7	27.8	24.9	29.2	23.1	25.18±0.96
	T	40.8	39.7	46.2	39.3	37.6	38.5	40.5	39.4	43.7	40.63±0.90
$3.2X10^{-9}$	C	23.5	28.5	23.3	28.6	24.9	28.4	24.0	25.2	22.9	25.48±0.79
	T	51.2	49.1	54.6	51.5	50.4	49.1	55.4	51.2	48.3	51.20±0.81
$6.4X10^{-9}$	C	25.8	23.1	27.6	28.7	25.3	28.7	23.2	25.4	28.4	26.24±0.74
	T	55.3	52.1	50.5	51.2	49.2	55.2	51.8	57.1	54.8	53.02±0.88
$1.28X10^{-8}$	C	23.5	28.1	24.7	28.6	24.2	29.7	26.1	31.2	26.5	26.96±0.87
	T	55.2	58.7	52.5	59.7	54.3	56.5	52.2	59.1	59.6	56.42±1.00
$2.56X10^{-8}$	C	27.9	25.4	24.5	29.2	25.4	23.5	28.6	23.4	27.5	26.16±0.73
	T	56.2	60.3	62.7	53.8	58.1	60.5	55.4	57.9	61.4	58.48±0.99
$5.12X10^{-8}$	C	26.3	29.1	24.5	29.5	29.3	26.7	24.6	27.5	23.6	26.79±0.74
	T	65.4	59.8	59.4	60.9	57.3	61.2	59.4	58.3	63.6	60.59±0.85
$1.024X10^{-7}$	C	26.2	28.8	23.8	28.7	21.3	27.9	25.2	27.6	28.7	26.47±0.87
	T	66.2	65.4	61.3	60.8	59.6	64.9	62.2	61.3	64.7	62.93±0.79

APPENDIX 30

EFFECTS OF HISTAMINE ON FREQUENCY OF SPONTANEOUS CONTRACTIONS IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ±SEM
$4X10^{-10}$	C	15	21	18	15	12	15	12	18	21	16.33±1.13
	T	21	12	18	15	18	12	21	18	15	16.67±1.13
$8X10^{-10}$	C	18	12	18	21	24	15	12	18	15	17.00±1.32
	T	18	12	15	12	15	18	21	24	21	17.33±1.39
$1.6X10^{-9}$	C	12	21	15	18	12	21	18	15	15	16.33±1.13
	T	15	12	21	18	12	18	15	21	18	16.67±1.13
$3.2X10^{-9}$	C	15	21	18	15	12	18	24	21	15	17.67±1.27
	T	18	15	12	21	24	12	18	15	15	16.67±1.33
$6.4X10^{-9}$	C	15	12	21	12	15	18	21	18	15	16.33±1.13
	T	18	12	15	18	21	24	21	12	18	17.67±1.36
$1.28X10^{-8}$	C	15	18	21	12	15	18	12	18	21	16.67±1.13
	T	18	15	21	12	18	15	18	21	15	17.00±1.00
$2.56X10^{-8}$	C	15	21	12	15	18	21	12	18	15	16.33±1.13
	T	18	12	15	21	12	9	21	24	18	16.67±1.67
$5.12X10^{-8}$	C	21	12	15	18	21	21	12	15	18	17.00±1.22
	T	15	12	21	15	18	12	21	18	18	16.67±1.13
$1.024X10^{-7}$	C	15	21	18	15	12	15	12	18	21	16.33±1.13
	T	21	12	18	15	18	12	21	18	15	16.67±1.13

APPENDIX 31

EFFECTS OF SEROTONIN ON AMPLITUDE OF SPONTANEOUS CONTRACTIONS IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ±SEM
$4X10^{-10}$	C	26.5	27.8	24.3	27.6	23.2	21.3	27.8	25.4	27.8	25.74±0.79
	T	26.4	28.7	30.1	24.3	21.1	26.6	25.5	23.7	28.6	26.11±0.94
$8X10^{-10}$	C	26.4	23.1	28.5	29.8	25.2	27.6	24.1	29.2	26.7	26.73±0.76
	T	27.2	28.9	24.3	27.6	24.5	28.4	27.6	23.4	27.2	26.57±0.66
$1.6X10^{-9}$	C	25.5	28.6	24.3	22.8	28.7	29.8	27.3	28.7	25.4	26.79±0.80
	T	34.2	37.5	31.4	36.6	32.4	29.3	37.4	35.6	34.3	34.30±0.94
$3.2X10^{-9}$	C	25.3	27.1	25.4	28.5	26.4	24.6	28.9	23.8	28.2	26.47±0.61
	T	49.2	54.3	50.7	48.3	55.4	46.5	52.7	54.7	49.6	51.27±1.05
$6.4X10^{-9}$	C	26.3	29.5	25.4	28.6	29.7	24.4	29.1	28.7	27.8	27.72±0.64
	T	52.3	57.6	53.2	48.4	54.2	56.5	50.9	48.7	53.2	52.78±1.05
$1.28X10^{-8}$	C	24.9	27.5	24.5	27.6	24.6	28.7	30.7	25.6	28.6	26.97±0.73
	T	55.3	59.8	61.4	54.7	53.2	60.7	58.2	56.4	59.2	57.66±0.96
$2.56X10^{-8}$	C	27.4	23.5	27.6	29.8	26.3	29.6	26.4	27.3	28.7	27.40±0.64
	T	65.2	59.1	60.9	61.8	58.7	61.8	64.6	60.5	58.3	61.21±0.82
$5.12X10^{-8}$	C	26.5	27.8	24.3	27.6	23.2	21.3	27.8	25.4	27.8	25.74±0.79
	T	59.6	62.5	61.5	61.9	60.8	64.5	60.5	62.8	62.3	61.82±0.48
$1.024X10^{-7}$	C	26.4	23.1	28.5	29.8	25.2	27.6	24.1	29.2	26.7	26.73±0.76
	T	63.4	65.3	61.2	63.8	64.5	62.6	60.8	61.7	62.3	62.84±0.51

APPENDIX 32

EFFECTS OF SEROTONIN ON FREQUENCY OF SPONTANEOUS CONTRACTIONS IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ±SEM
$4X10^{-10}$	C	9	18	12	18	18	12	21	18	21	16.33±1.42
	T	21	18	12	18	12	18	12	18	21	16.67±1.24
$8X10^{-10}$	C	24	15	24	12	15	12	18	15	12	16.33±1.59
	T	12	15	21	24	18	15	18	15	18	17.33±1.20
$1.6X10^{-9}$	C	12	18	15	21	24	21	12	15	12	16.67±1.51
	T	21	24	18	15	12	15	12	18	24	17.67±1.54
$3.2X10^{-9}$	C	12	21	18	12	18	15	12	18	21	16.33±1.24
	T	21	18	12	15	21	18	15	12	18	16.67±1.13
$6.4X10^{-9}$	C	15	18	12	18	21	24	21	12	15	17.33±1.39
	T	21	12	24	12	18	15	18	15	18	17.00±1.32
$1.28X10^{-8}$	C	12	18	15	12	18	21	24	12	15	16.33±1.42
	T	15	18	21	18	21	15	12	18	21	17.67±1.05
$2.56X10^{-8}$	C	21	24	12	15	12	18	15	18	15	16.67±1.33
	T	12	15	21	24	15	9	15	21	15	16.33±1.59
$5.12X10^{-8}$	C	9	18	12	18	18	12	21	18	21	16.33±1.42
	T	21	18	12	18	12	18	12	18	21	16.67±1.24
$1.024X10^{-7}$	C	24	15	24	12	15	12	18	15	12	16.33±1.59
	T	12	15	21	24	18	15	18	15	18	17.33±1.20

APPENDIX 33

EFFECTS OF ISOPRENALINE ON AMPLITUDE OF SPONTANEOUS CONTRACTIONS IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ±SEM
$4X10^{-10}$	C	29.8	28.6	30.6	27.3	26.5	28.2	31.1	27.5	30.7	28.92
	T	26.3	31.5	29.7	26.5	27.6	30.9	27.8	31.4	29.4	29.01
$8X10^{-10}$	C	28.4	27.8	31.7	30.5	27.3	29.8	26.7	29.8	31.6	29.29
	T	26.8	31	28.9	27.4	26.8	30.8	29.8	28.6	29.1	28.80
$1.6X10^{-9}$	C	27.7	28.4	31.7	28.6	29.5	30.9	28.4	27.8	31.5	29.39
	T	25.8	24.9	29.6	27.6	26.7	28.4	29.8	25.4	23.6	26.87
$3.2X10^{-9}$	C	28.2	25.7	30.8	29.7	28.6	31.2	29.5	28.8	29.6	29.12
	T	26.5	24.3	27.6	25.1	23.9	26.6	25.3	24.7	28.6	25.84
$6.4X10^{-9}$	C	27.4	29.8	31.6	29.7	28.5	29.8	26.7	26.5	31.3	29.03
	T	21.3	20.9	25.6	23.4	21.9	20.7	23.4	26.1	22.4	22.86
$1.28X10^{-8}$	C	28.6	29.3	29.8	30.9	27.5	26.4	30.4	29.7	28.6	29.02
	T	20.3	19.8	18.7	23.5	22.5	19.3	21.4	18.5	19.6	20.40
$2.56X10^{-8}$	C	28.9	27.5	31.9	30.6	28.7	26.8	29.6	27.9	29.9	29.09
	T	15.4	16.6	14.3	17.8	19.6	13.6	19.6	14.3	16.2	16.38
$5.12X10^{-8}$	C	30.8	29.6	26.5	28.4	29.7	30.3	28.8	29.5	28.9	29.17
	T	10.2	9.8	12.1	11.6	9.2	12.6	10.7	8.6	9.2	10.44
$1.024X10^{-7}$	C	29.3	30.1	28.4	30.7	27.6	29.8	28.5	29.4	27.9	29.08
	T	8.7	6.5	9.3	6.6	5.4	9.8	8.6	8.4	7.4	7.86
$2.048X10^{-7}$	C	29.7	30.2	28.7	30.8	28.7	29.7	30.2	29.7	30.1	29.76
	T	5.4	4.7	3.8	6.7	7.2	4.8	5.2	6.1	6.5	5.60
$4.096\ X10^{-7}$	C	28.4	30.8	29.7	30.1	28.7	26.5	29.3	29.7	29.4	29.18
	T	1.8	2.4	1.4	0.8	2.5	1.2	2.4	1.8	2.1	1.82

APPENDIX 34

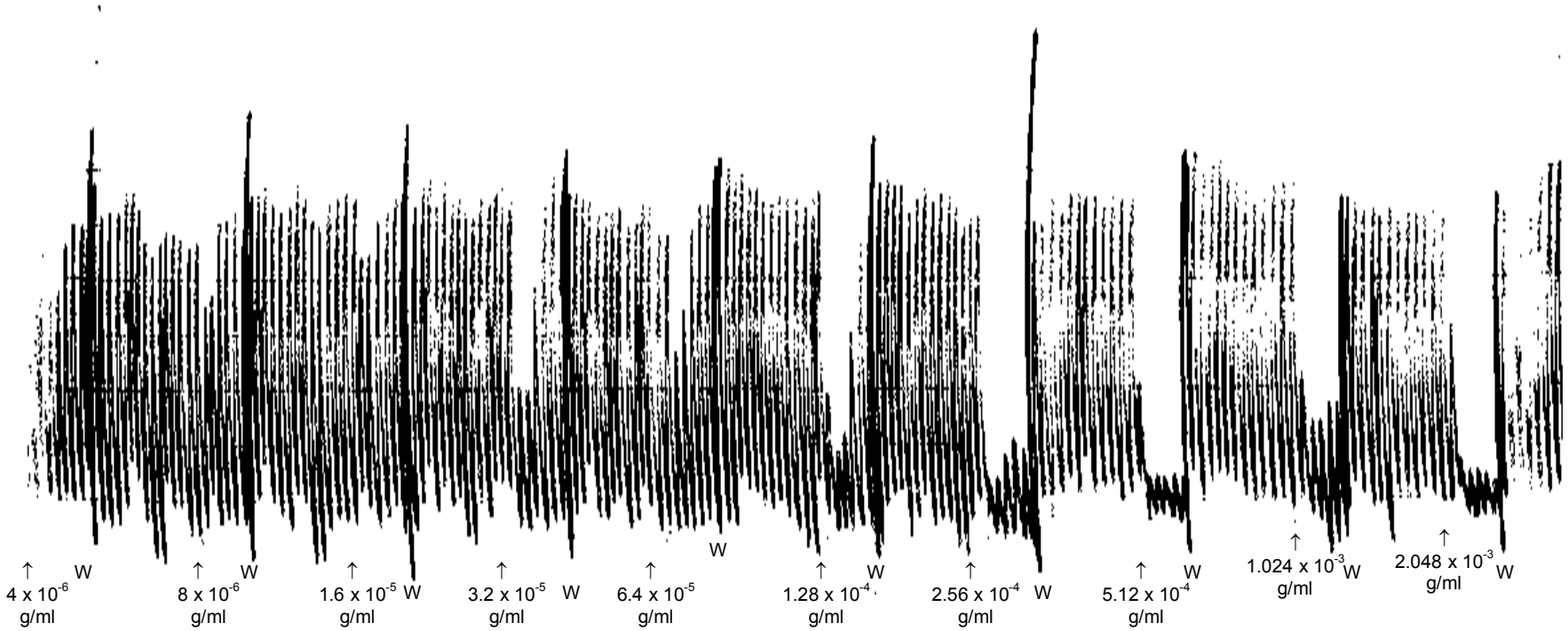
EFFECTS OF ISOPRENALINE ON FREQUENCY OF SPONTANEOUS CONTRACTIONS IN ISOLATED RABBIT ILEUM

(n=9)

Concentration (g/ml)	Tissue	1	2	3	4	5	6	7	8	9	Mean ±SEM
$4X10^{-10}$	C	18	12	15	18	21	15	18	12	18	16.33±1.01
	T	12	21	15	18	12	18	12	15	12	15.00±1.12
$8X10^{-10}$	C	18	12	18	21	12	15	18	12	21	16.33±1.24
	T	12	15	21	21	18	12	15	18	21	17.00±1.22
$1.6X10^{-9}$	C	15	12	18	21	18	12	18	15	18	16.33±1.01
	T	12	21	9	24	12	15	18	21	18	16.67±1.67
$3.2X10^{-9}$	C	15	12	18	15	12	18	15	21	21	16.33±1.13
	T	12	21	18	15	12	18	15	21	18	16.67±1.13
$6.4X10^{-9}$	C	12	15	21	24	15	9	15	21	15	16.33±1.59
	T	21	12	18	15	12	9	15	18	21	15.67±1.39
$1.28X10^{-8}$	C	12	15	12	18	9	12	21	18	21	15.33±1.45
	T	24	12	18	21	12	18	9	12	15	15.67±1.64
$2.56X10^{-8}$	C	12	15	18	21	24	9	12	15	18	16.00±1.58
	T	15	21	18	12	12	18	21	18	12	16.33±1.24
$5.12X10^{-8}$	C	21	18	15	12	18	21	15	21	9	16.67±1.42
	T	12	18	21	12	15	18	12	21	9	15.33±1.45
$1.024X10^{-7}$	C	9	21	18	12	18	12	15	18	18	15.67±1.30
	T	21	15	24	18	21	12	9	18	9	16.33±1.81
$2.048\ X10^{-7}$	C	18	12	18	21	9	18	12	18	21	16.33±1.42
	T	24	21	18	15	12	15	18	9	18	16.67±1.51
$4.096\ X10^{-7}$	C	12	21	18	21	24	15	18	9	18	17.33±1.56
	T	18	12	18	21	9	18	12	18	21	16.33±1.82

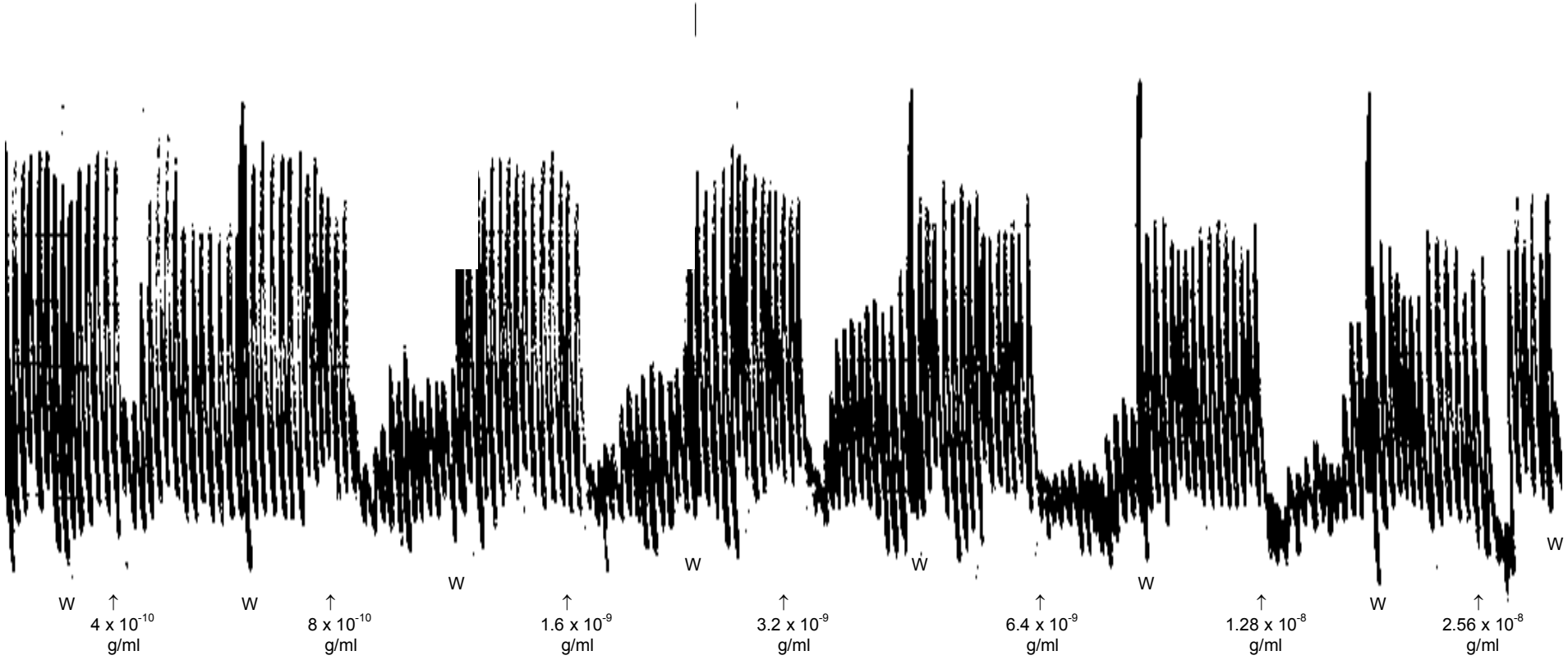
APPENDIX 35

CONTRACTILE RESPONSES IN RABBIT ILEUM TO METHANOLIC EXTRACT



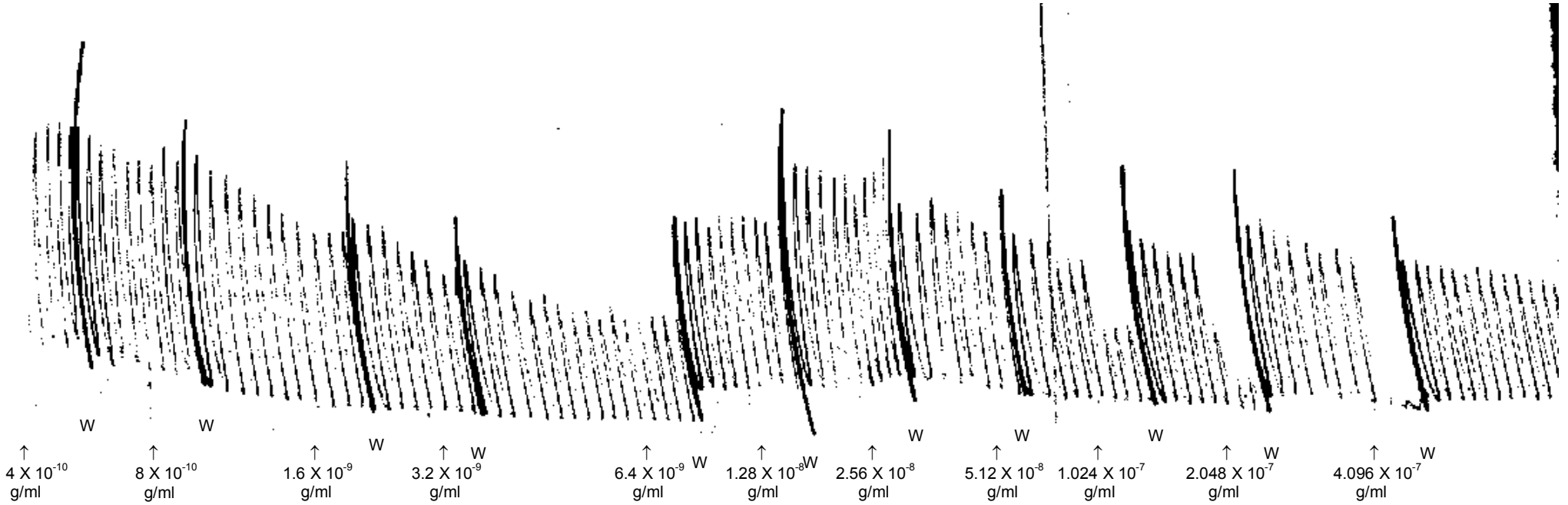
APPENDIX 36

CONTRACTILE RESPONSES TO AQUEOUS EXTRACT (E_{max})
AFTER INITIAL PROPRANOLOL ADMINISTRATION



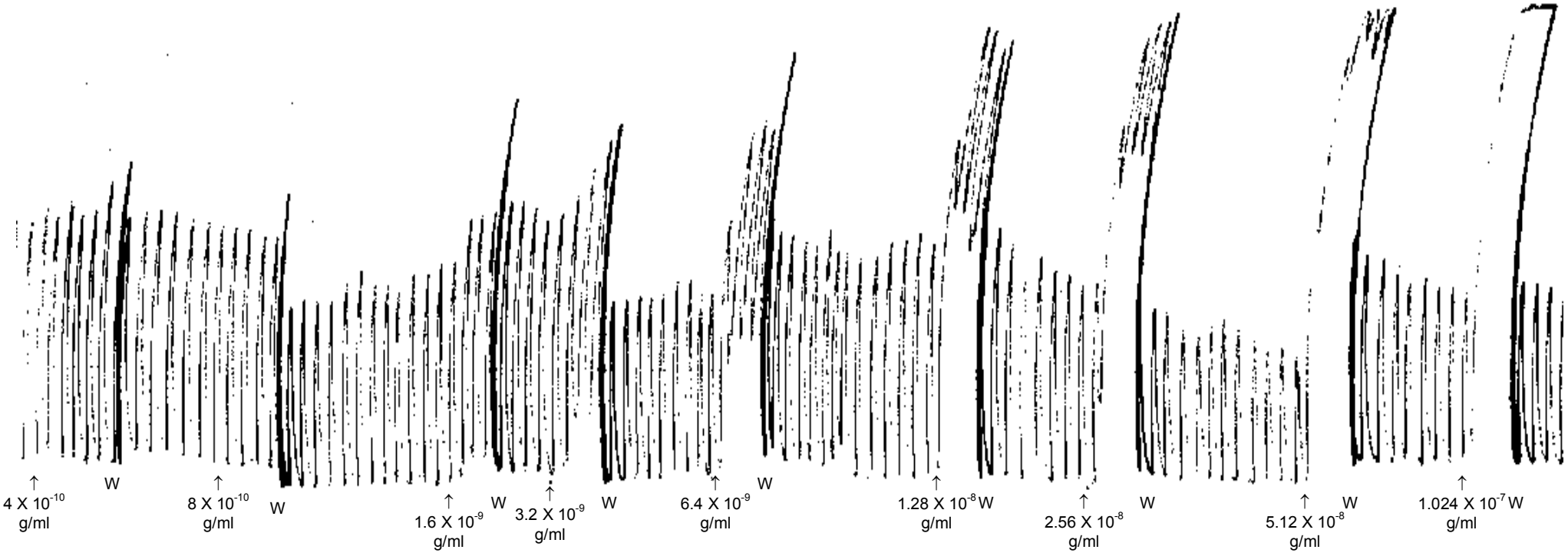
APPENDIX 37

CONTRACTILE RESPONSES IN RABBIT ILEUM TO NOREPINEPHRINE



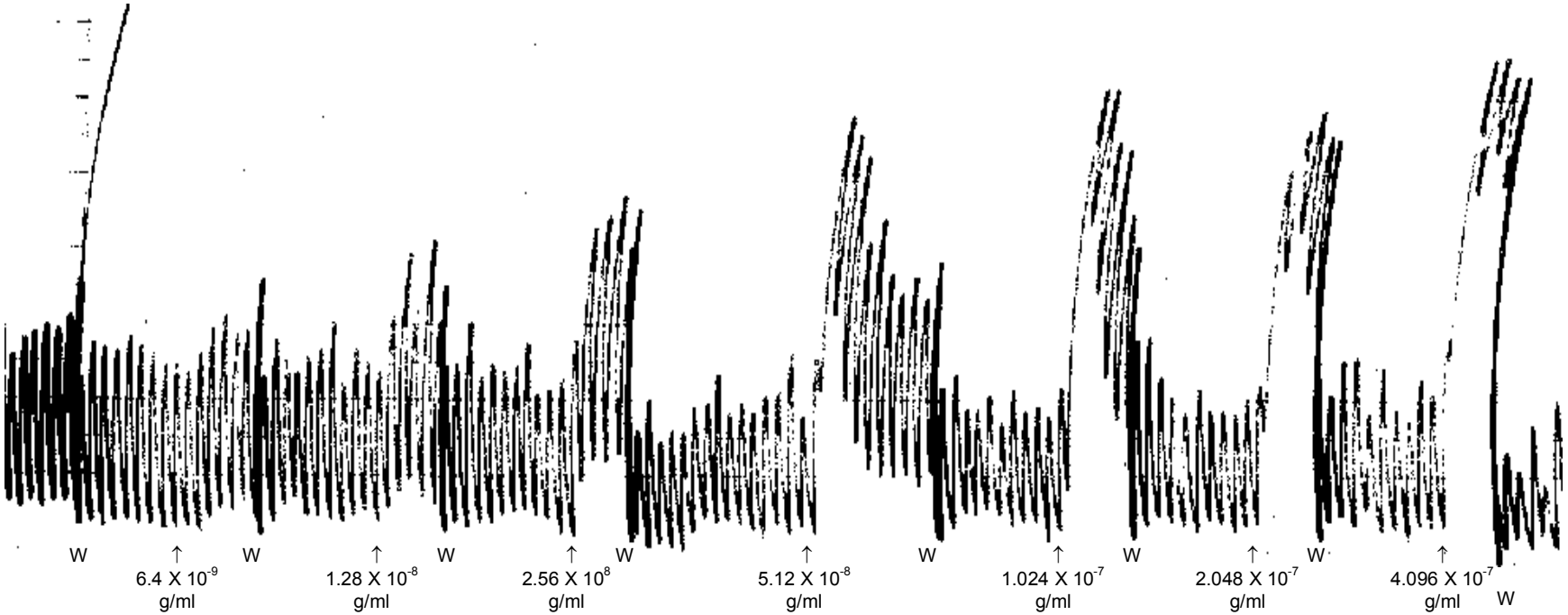
APPENDIX 38

CONTRACTILE RESPONSES IN RABBIT ILEUM TO ACETYLCHOLINE



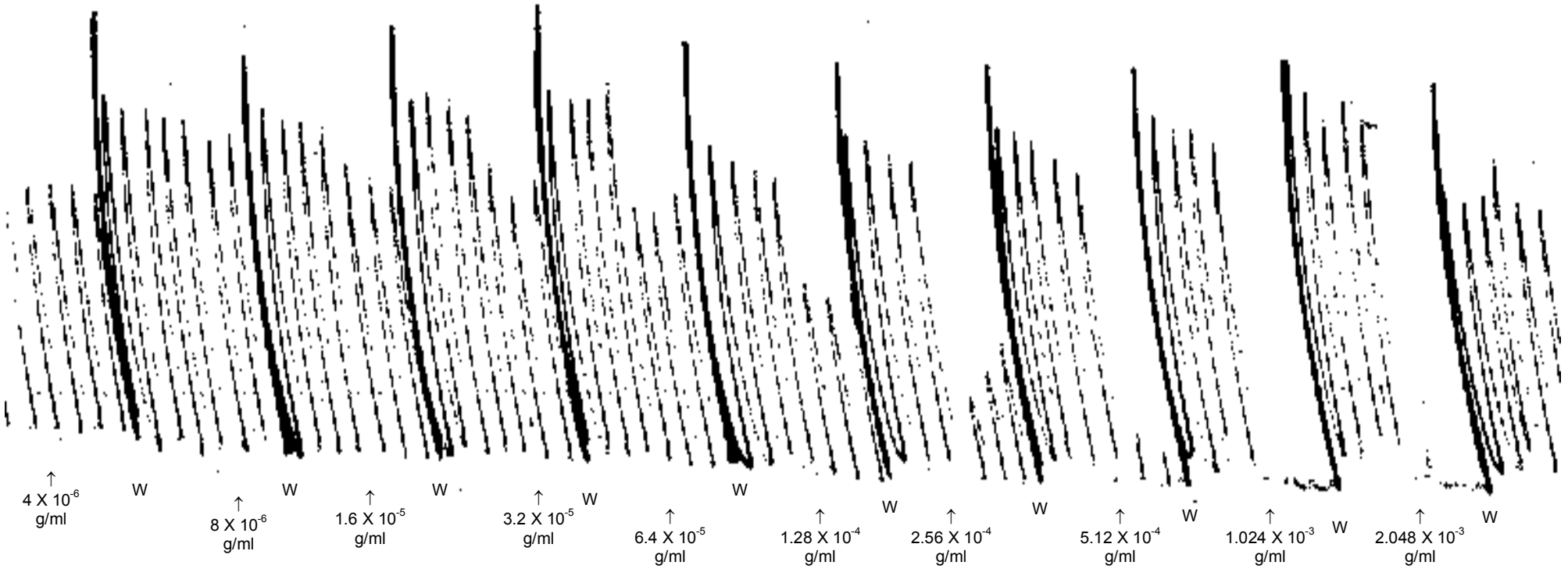
APPENDIX 39

CONTRACTILE RESPONSES IN RABBIT ILEUM TO HISTAMINE

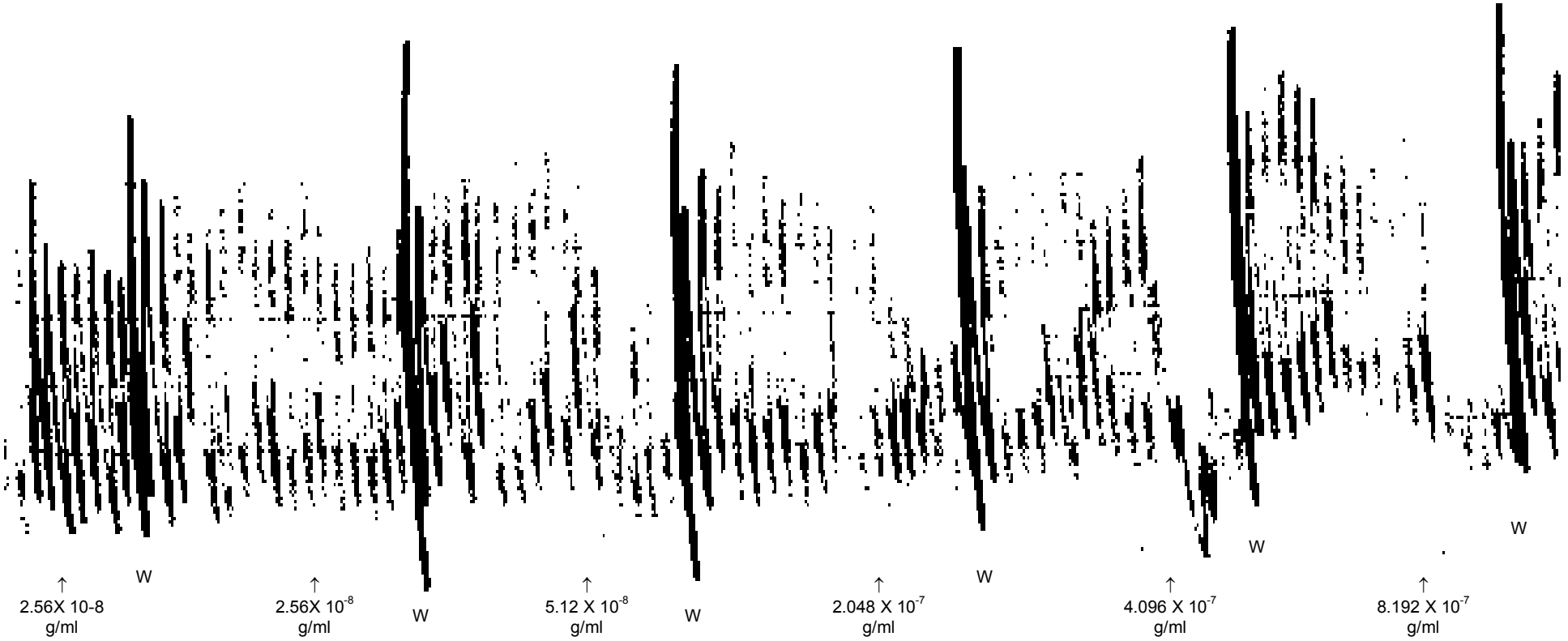


APPENDIX 40

CONTRACTILE RESPONSES IN RABBIT ILEUM TO AQUEOUS EXTRACT



APPENDIX 41
CONTRACTILE RESPONSES IN RABBIT ILEUM TO ISOPRENALINE



APPENDIX 42

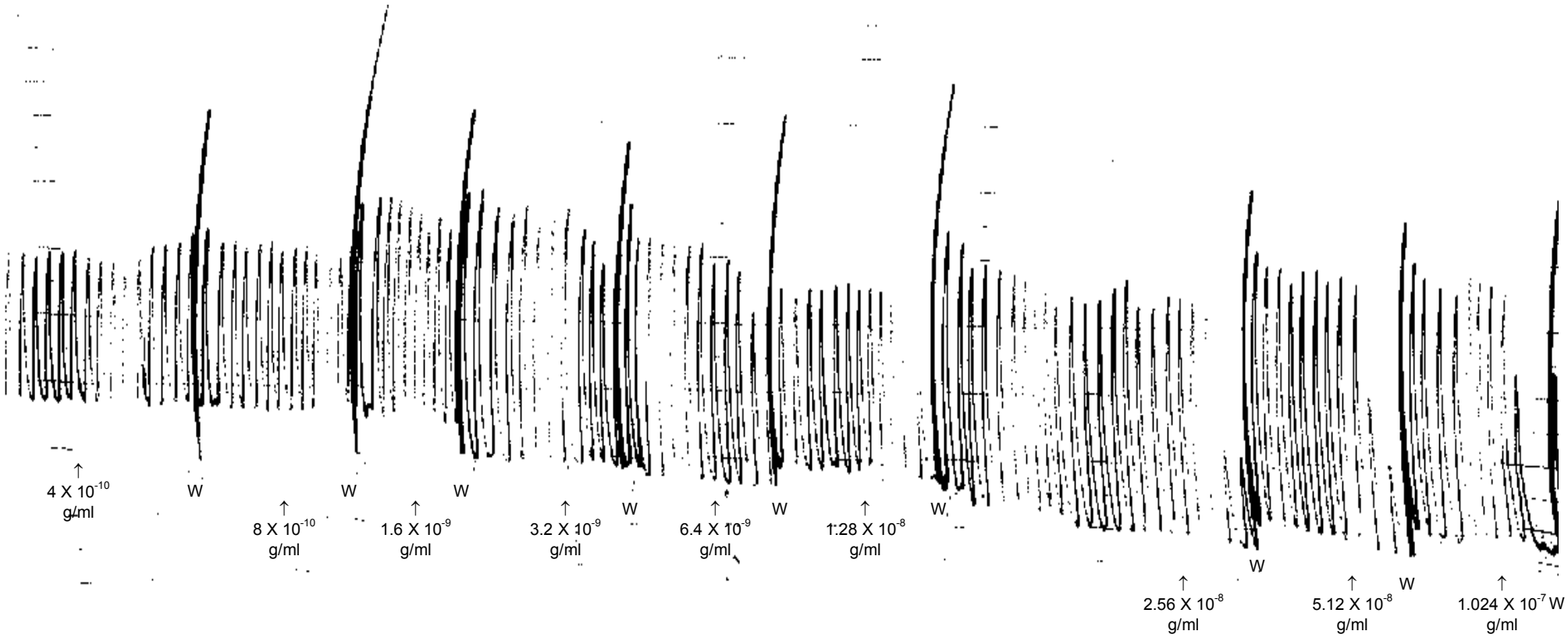
NORMAL SPONTANEOUS CONTRACTION OF RABBIT ILEUM



↑10 mm
↓

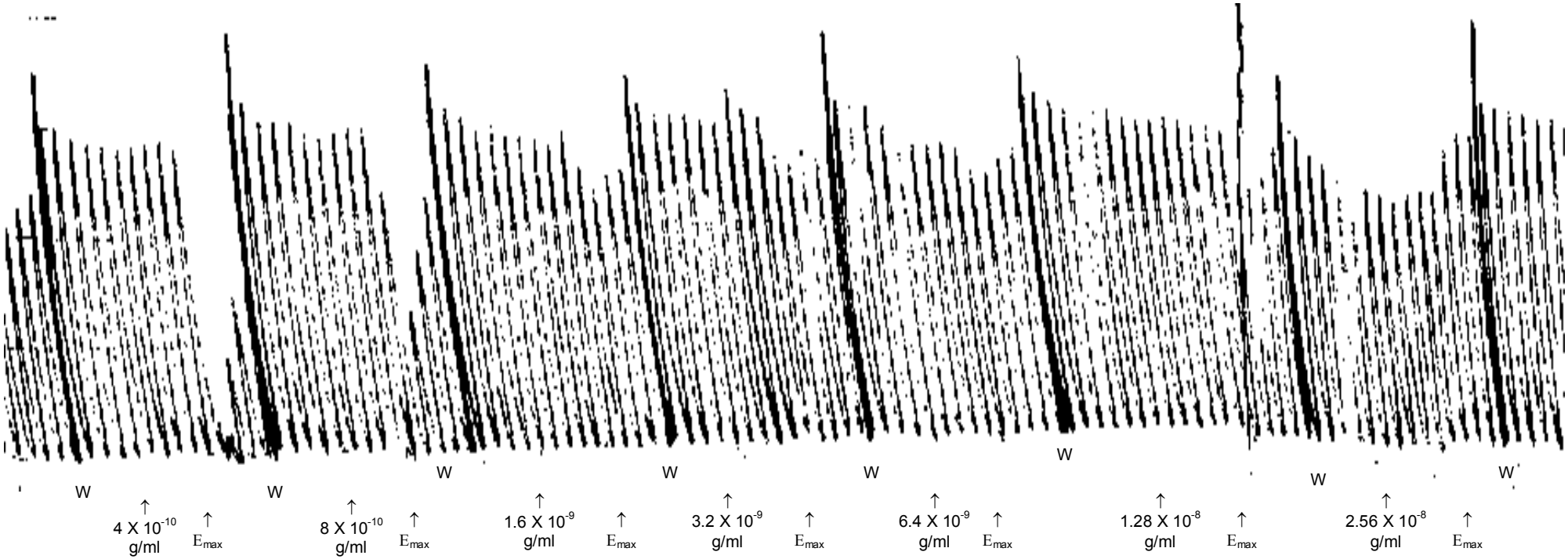
APPENDIX 44

CONTRACTILE RESPONSES IN RABBIT ILEUM TO EPINEPHRINE



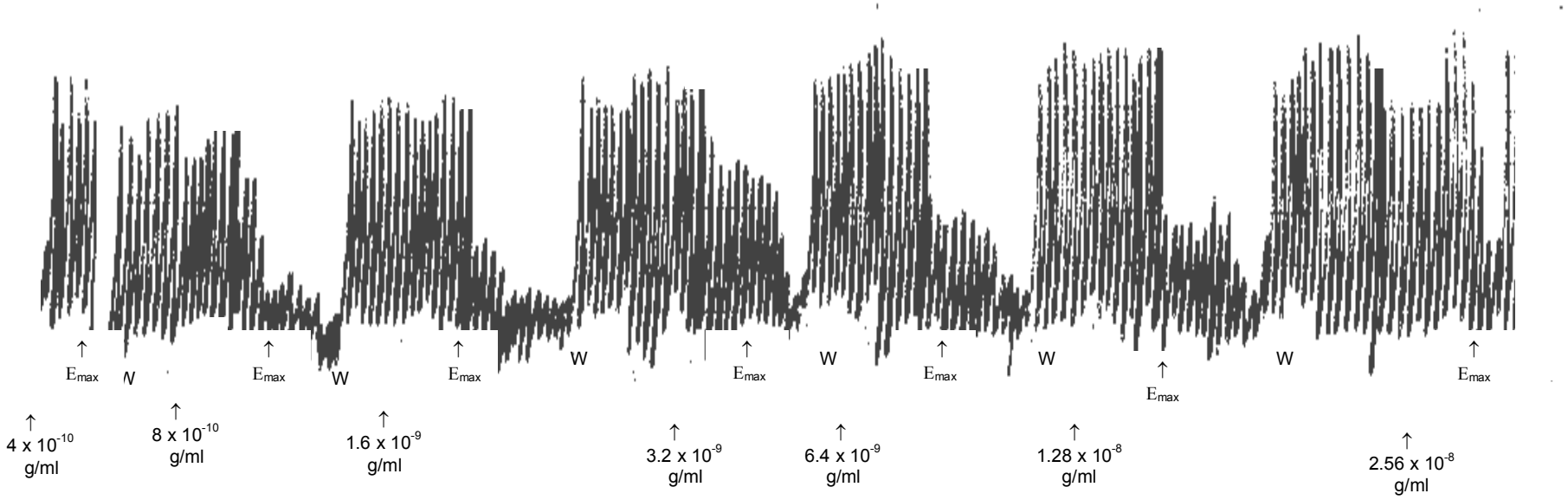
APPENDIX 45

CONTRACTILE RESPONSES TO AQUEOUS EXTRACT (E_{max})
AFTER INITIAL PHENTOLAMINE ADMINISTRATION



APPENDIX 46

CONTRACTILE RESPONSES TO METHANOLIC EXTRACT (E_{max})
AFTER INITIAL PROPRANOLOL ADMINISTRATION



Key
W- wash
E_{max} – maximum effective concentration of extract

APPENDIX 47

DILUTION OF EXTRACTS AND STANDARD DRUGS

Extracts

Initial quantity of extracts measured = 1 gram (g)

Quantity of solvent (deionized water) measured = 10 millilitres (ml)

1 g of extracts dissolved in 10 ml of solvent = $\frac{1}{10}$ g/ml

= 0.1 g/ml

(since 1 g is equivalent to 1000 mg, it implies that 0.1 g/ml is equivalent to 100 mg/ml)

Final concentration of extracts = 100 mg/ml

Standard Drugs

Initial quantity of standard drugs measured = 0.01 grams (g)

Quantity of solvents measured = 100millilitres (ml)

0.01g of drugs in 100ml of water = $\frac{0.01}{100}$ g/ml

= 0.0001 g/ml

Since 1g is equivalent to 10^6μg, this implies that 0.0001g/ml is equivalent to 100μg/ml

Final concentration of drugs = 100 μg/ml

Further dilutions;

Take 1ml of 100mg/ml (same for 100 μg/ml in preparing standard drugs) and dissolve in 10ml of solvent (deionized water) to give 10mg/ml concentration of solution [$^{100}/_{10}$ mg/ml = 10 mg/ml].

Take 1ml of 10mg/ml (same for 10 μg/ml in preparing standard drugs) and dissolve in 10ml of solvent (deionized water) to give 1mg/ml concentration of solution [$^{10}/_{10}$ mg/ml = 1 mg/ml].

Take 1 ml of 1 μg/ml (for standard drugs only) and dissolve in 10 ml of solvent (deionized water) to give 0.1 μg/ml concentration of solution [$^{1}/_{10}$ μg/ml = 0.1 μg/ml]

APPENDIX 48

CALCULATION OF FINAL BATH CONCENTRATIONS

When tests substances were administered to organ bath containing tyrode's solution, they were further diluted and final bath concentrations of organ baths were determined (concentration of substances that interacted with tissue) as follows;

FOR EXTRACTS (AQUEOUS AND METHANOLIC)

0.1 ml of 1 mg/ml contains 0.1 mg/ml of extract. Therefore final bath concentration at

0.1 mg/ml will be $\frac{0.1 \times 1}{25}$ = 0.004 mg/ml = 4.0×10^{-6} g/ml

0.2 ml of 1 mg/ml contains 0.2 mg/ml of extract. Therefore final bath concentration at

0.2 mg/ml will be $\frac{0.2 \times 1}{25}$ = 0.008 mg/ml = 8.0×10^{-6} g/ml

0.4 ml of 1 mg/ml contains 0.4 mg/ml of extract. Therefore final bath concentration at

0.4 mg/ml will be $\frac{0.4 \times 1}{25}$ = 0.016 mg/ml = 1.6×10^{-5} g/ml

0.8 ml of 1 mg/ml contains 0.8 mg/ml of extract. Therefore final bath concentration at

0.8 mg/ml will be $\frac{0.8 \times 1}{25}$ = 0.032 mg/ml = 3.2×10^{-5} g/ml

0.16 ml of 10 mg/ml contains 1.6 mg/ml of extract. Therefore final bath concentration at,

1.6 mg/ml will be $\frac{1.6 \times 1}{25}$ = 0.064 mg/ml = 6.4×10^{-5} g/ml

0.32 ml of 10 mg/ml contains 3.2 mg/ml of extract. Therefore final bath concentration at,

3.2 mg/ml will be $\frac{3.2 \times 1}{25}$ = 0.128 mg/ml = 1.28×10^{-4} g/ml

0.64 ml of 10 mg/ml contains 6.4 mg/ml of extract. Therefore final bath concentration at,

6.4 mg/ml will be $\frac{6.4 \times 1}{25}$ = 0.256 mg/ml = 2.56×10^{-4} g/ml

0.128 ml of 100 mg/ml contains 12.8 mg/ml of extract. Therefore final bath concentration at

12.8 mg/ml will be $\frac{12.8 \times 1}{25}$ = 0.512 mg/ml = 5.12×10^{-4} g/ml

0.256 ml of 100 mg/ml contains 25.6 mg/ml of extract. Therefore final bath concentration at

25.6 mg/ml will be $\frac{25.6 \times 1}{25}$ = 1.024 mg/ml = 1.024×10^{-3} g/ml

0.512 ml of 100 mg/ml contains 51.2 mg/ml of extract. Therefore final bath concentration at

51.2 mg/ml will be $\frac{51.2 \times 1}{25}$ = 2.048 mg/ml = 2.048×10^{-3} g/ml

FOR STANDARD DRUGS

0.1 ml of 0.1 µg/ml contains 0.01 µg/ml of test drug. Therefore final bath concentration at,

0.01 µg/ml will be $\frac{0.01 \times 1}{25}$ = 0.0004 µg/ml = 4.0×10^{-10} g/ml

0.2 ml of 0.1 µg/ml contains 0.02 µg/ml of test drug. Therefore final bath concentration at,

0.02 µg/ml will be $\frac{0.02 \times 1}{25}$ = 0.0008 µg/ml = 8.0×10^{-10} g/ml

0.4 ml of 0.1 µg/ml contains 0.04 µg/ml of test drug. Therefore final bath concentration at,

0.04 µg/ml will be $\frac{0.04 \times 1}{25}$ = 0.0016 µg/ml = 1.6×10^{-9} g/ml

0.8 ml of 0.1 µg/ml contains 0.08 µg/ml of test drug. Therefore final bath concentration at,

0.08 µg/ml will be $\frac{0.08 \times 1}{25}$ = 0.0032 µg/ml = 3.2×10^{-9} g/ml

0.16 ml of 1 µg/ml contains 0.16 µg/ml of test drug. Therefore final bath concentration at,

0.16 µg/ml will be $\frac{0.16 \times 1}{25}$ = 0.0064 µg/ml = 6.4×10^{-9} g/ml

0.32 ml of 1 µg/ml contains 0.32 µg/ml of test drug. Therefore final bath concentration at,

0.32 µg/ml will be $\frac{0.32 \times 1}{25}$ = 0.0128 µg/ml = 1.28×10^{-8} g/ml

0.64 ml of 1 µg/ml contains 0.64 µg/ml of test drug. Therefore final bath concentration at,

0.64 µg/ml will be $\frac{0.64 \times 1}{25}$ = 0.0256 µg/ml = 2.56×10^{-8} g/ml

0.128 ml of 10 µg/ml contains 1.28 µg/ml of test drug. Therefore final bath concentration at,

1.28 µg/ml will be $\frac{1.28 \times 1}{25}$ = 0.0512 µg/ml = 5.12×10^{-8} g/ml

0.256 ml of 10 µg/ml contains 2.56 µg/ml of test drug. Therefore final bath concentration at,

2.56 µg/ml will be $\frac{2.56 \times 1}{25}$ = 0.1024 µg/ml = 1.024×10^{-7} g/ml

0.512 ml of 10 µg/ml contains 5.12 µg/ml of test drug. Therefore final bath concentration at,

5.12 µg/ml will be $\frac{5.12 \times 1}{25}$ = 2.048 µg/ml = 2.048×10^{-7} g/ml

0.1024 ml of 100 µg/ml contains 10.24 µg/ml of test drug. Therefore final bath concentration at,

10.24 µg/ml will be $\frac{1.024 \times 1}{25}$ = 0.4096 µg/ml = 4.096×10^{-7} g/ml