"EFFECTS OF *MONDIA WHYTEI* AQUEOUS ROOT EXTRACT ON ISOLATED RABBIT HEART AND JEJUNUM PREPARATIONS"

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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ABSTRACT

Mondia Whytei is a forest floor plant with aromatic rhizomatous roots. It belongs to the *Asclepiadaceae* Family. Its roots are rich in bioactive phytochemicals, essential minerals and vitamins. Among the bioactive phytochemicals that have been isolated so far, 2-hydroxy-4-methoxybenzaldehyde is known to be responsible for the taste characteristics of *Mondia Whytei* root extract.

Mondia Whytei roots were harvested, dried and powdered. 100 mg of this powder was extracted with 500 ml water and subsequently freeze-dried. The freeze-dried extract was then reconstituted with physiological salt solutions immediately before carrying out the experiment. Fifteen California white rabbits were sacrificed with the exclusion of the pregnant ones.

The objective of the study was to evaluate the overall effects of *Mondia Whytei* aqueous root extract on the three kind of muscles in vitro namely skeletal, smooth and cardiac muscles. The set-ups involved isolated rabbit heart and jejunum preparations that were mounted in an organ bath.

Previous preliminary experiments with phrenic nerve diaphragm had showed that the extract have no effect on the skeletal muscle neuromuscular junction.

On the isolated rabbit heart preparation the size (height) of the twitch represented the heart force whereas the frequency of the twitches represented the heart rate.

Mondia Whytei root extract was found to reduce both the heart force and the heart rate. The data obtained was used to plot the graph of: -

(i) Percentage drop in the heart force versus concentration of *Mondia Whytei.*

(ii) Percentage drop in the heart rate versus concentration of *Mondia Whytei.*

The 'best line of fit' was preferable in data analysis since the data collected formed a scatter diagram.

Above the dose of 4.8 mg *Mondia Whytei* aqueous root extract caused ceasation of myocardial contractility of the heart but at low doses it exhibited both negative chronotropic and ionotropic effects. Conclusively, the overall effect on both the heart and the jejunum were parasympathomimetic effects

The amount of *Mondia Whytei* that effectively reduced the heart force by half the original force (ED_{50}) was calculated and determined to be 3.6 mg whereas the ED_{50} for heart rate was calculated to be 5.25 mg of freeze dried *Mondia Whytei* extract.

On isolated rabbit jejunum, *Mondia Whytei* aqueous root extract caused contraction but the organ recovered its intrinsic activity over a period of ten minutes. It was also observed that the jejunum recovered from the effects (contraction) of *Mondia Whytei* even without washing it suggesting the probability of having some degrading enzymes that rendered the extract inactive.

Further scientific work therefore ought to be done in order to isolate pure compounds and elucidate mechanisms through which *Mondia Whytei* aqueous root extract mediates the effects presented here and also analyze its toxicological profile.

KEY WORDS: Mondia Whytei, 2-Hydroxy-4-methoxybenzaldehyde, Chronotropic and Ionotropic effects.

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1.0 INTRODUCTION

Mondia Whytei is a forest floor plant with aromatic rhizomatous roots which belongs to the *Asclepiadaceae* family. There are two species in the genus *Mondia;* namely *Mondia Whytei* and *Mondia Econata*. The two species are differentiated by their morphological characteristics. *Mondia Econata* has a corona with long appendages, while *Mondia Whytei* is a climber, which grows up to 3-6 m high. The leaves are broadly ovate with a base cordate, apex acuminate and corona of 11-12 mm long. The fruit follicles are ovoid with dimensions of 7-8 by 1.5 – 2.0 mm (Kokwaro, J.O. 1994).

Asclepiadaceae has more than 300 genera and 2000 species (Van Heerden, 1999). However there are about 104 species in the family that have been identified in East Africa (Agnew and Agnew, 1994). *Asclepiadaceae* species are closely related to *Aporcynaceae* and *Rubiaceae*. *Asclepiadaceae* are characterized by milky latex, leaves opposite with minute stipules, numerous flowers (usually with a corona) and anthers with pollen or sticky pollinia. Their fruits have two follicles while the seeds have a sticky corona. The family is mostly found in the tropics and subtropical regions.

Mondia Whytei is locally known as Mukombela (Luhya), Ogomba (Luo) , Olmkonkora (Maasai), Mkonkora (Kamba) and Muhukura (Kikuyu). Outside Kenya the plant is known as Omurondwa (Lunyore- Uganda), Ilivi (Sudan), Omondi (Zulu) and Mbombogazi (Swahili of Tanzania) Kokwaro, J.O, (1994).

1.1.1 DISTRIBUTION OF *MONDIA WHYTEI*

Mondia Whytei is widely spread in Africa. It is found in Guinea in West Africa through Sudan, Uganda, Kenya, Tanzania, Malawi, South Africa and westwards to Angola (Beentje, J. 1994). In Kenya, *Mondia Whytei* is common in wet and humid areas. It is particularly common in western Kenya. The plant grows widely in undisturbed forest or fallow lands. It is a primary colonizer and does well in savannah thickets, but it is mostly found on the outskirts of forests. Sometimes *Mondia Whytei* is found in the interior of the forest where conditions are favorable. The species does well in agroforestry cultivation. Currently, it is found in the Kakamega forest and outlying areas such as Kisero, Malava and Bunyala forest blocks. The species is also found on the main hill top ranges of western Kenya (Beentje, J. 1994).

1.1.2 UTILIZATION OF *MONDIA WHYTEI*

Mondia Whytei is widely used in traditional medicine and spiritually revered by local communities. It is also believed to be a verdic medicine, that means it gives good health, long life and protection from death (Agnew and Agnew 1994).

In addition to spiritual values, *Mondia Whytei* is widely used in the treatment of stomach ulcers, diabetes mellitus, hypertension, infertility, leprosy and various skin diseases. It has also been shown to have some anti- inflammatory, anti- pyretic and anti- microbial activity (Jain *et al.*, 1996).

In East Africa, *Mondia Whytei* is used to enhance milk production in both humans and livestock, as an antidote for snakebites and as an aphrodisiac (Kokwaro J.O, 1994).

Phytochemical investigations on various *Asclepiadaceae* plants has revealed different compounds with varied bioactivities. Cardenolides from *Calotropis Gigantea* (*Asclepiadaceae*) have been shown to have spasmolytic activities (Dao *et al.*, 1992), while other members of *Asclepiadaceae* family have been found to contain compounds with potential flavoring and taste modifying qualities that are needed by food and pharmaceutical industries (Yoshikawa *et al.*, 1993).

1.1.3 CHEMISTRY OF *MONDIA WHYTEI*

Intensive analytical work has been and is still being carried out in order to isolate compounds from extracts of *Mondia Whytei*. So far, 2-hydroxy-4-methoxybenzaldehyde and 3-hydroxy-4-methoxybenzaldehyde glycoside has been isolated and identified (Msonthi, 1994; Mukonyi and Ndiege, 1999). Another eight compounds have been isolated using modified National cancer Institute (NCI) of America protocol but their identification is still in progress (Msonthi, 1994).

Fig 1.1a

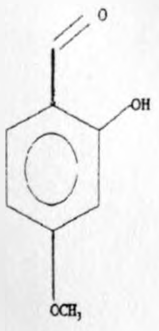


Fig 1.1b

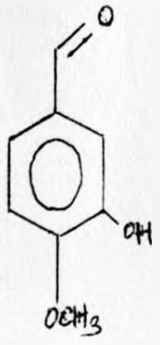


Figure 1.1a: Structure of 2-hydroxy -4-methoxybenzaldehyde

Figure 1.1b: Structure of 3-hydroxy-4-methoxybenzaldehyde

1.1.4 PHARMACOLOGICAL CHARACTERIZATION OF CHEMICAL COMPOUNDS ISOLATED FROM *MONDIA WHYTEI*

Apart from analytical chemistry work done on *Mondia Whytei.* other aspect of the plant that has been researched on are as follows. 2-hydroxy -4-methoxybenzaldehyde was found to be a tyrosinase inhibitor (Kubo and Ikuyo. 1999). Tyrosinase is a key enzyme in the process of insect moulting and therefore its inhibitors could provide an alternative mechanism for insect control.

3-hydroxy-4-methoxybenzaldehyde has been shown to have keratolytic activities similar to that of salicylic acid (Sakuma *et al.*, 1968). Kubo and Ikunyo (1999) showed that 3-hydroxy-4-methoxybenzaldehyde has strong keratolytic properties than salicylic acid.

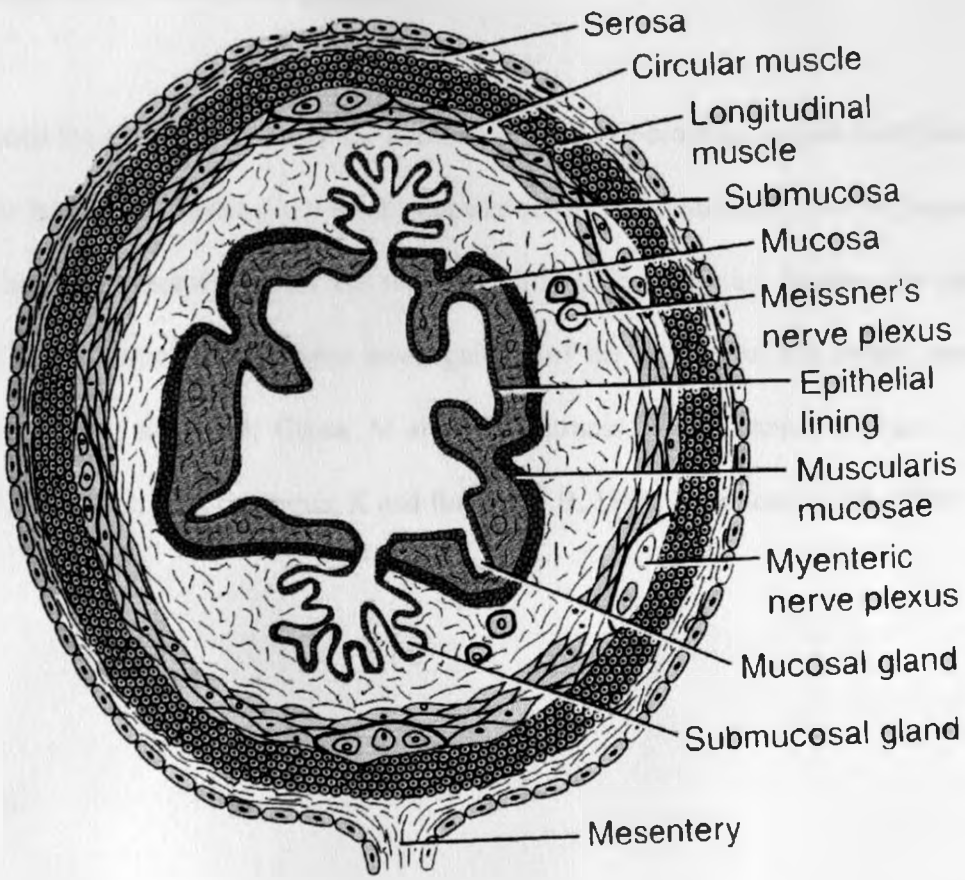
1.2.0 GASTROINTESTINAL MOTOR STATE

The motor function of the gastrointestinal tract, as accomplished by the co-ordination of its muscle layers into stereotyped motility patterns, is of fundamental importance for the digestive process as well as for maintaining the mucosal barrier against the external world (i.e., the luminal contents). The intrinsic inhibitory innervation of gut is of utmost significance for the generation of such motility patterns (Wood, J.D, 1975 and 1994).

The opinion that the intrinsic nerves of the gut, independent from the central nervous system, can process sensory information and control gastrointestinal motility was established when Langley, in 1921, classified enteric nerves as being a subdivision of the autonomic nervous system. The basis for this view consisted mainly of morphological descriptions of the intramural nerves made during the 19th century, as well as observations from physiological experiments, in vivo and in vitro, commencing at the later half of that century.

Figure 1.2

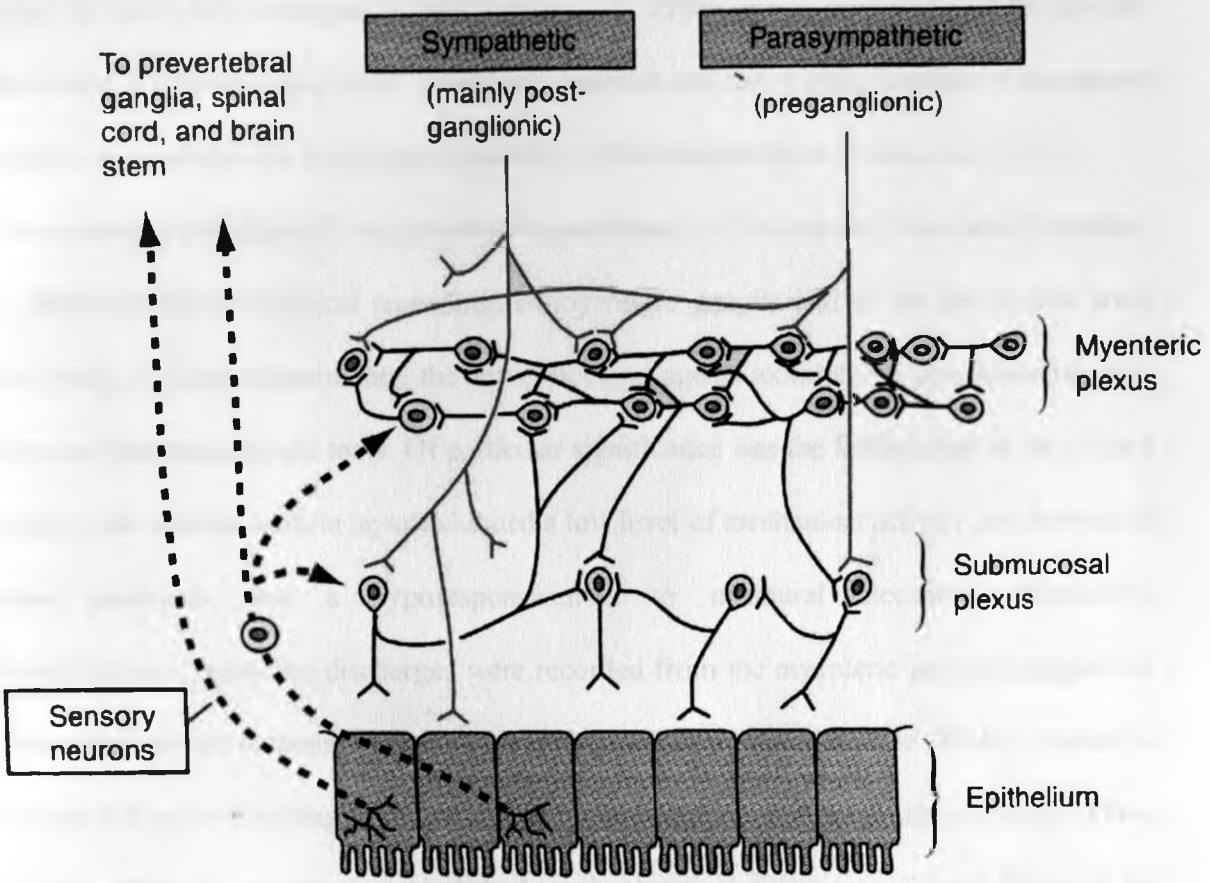
Typical cross section of the gut (Guyton and Hall, 2000).



Neurotransmission mechanisms were explored with pharmacological tools such as atropine and nicotine. Intrinsic excitatory (atropine sensitive and atropine resistant) and inhibitory motor nerves were well known. It was assumed that the inhibitory fibers constituted a population of sympathetic (postganglionic) nerves that were distinct from those that have their cell bodies in the prevertebral ganglia.

It was not until the early 1960s, when the noradrenergic nerve-blocking agents bretylium and guanethidine had become available, that the nonadrenergic noncholinergic (NANC) nature of the enteric inhibitory motor neurons was revealed. This major discovery became the starting point for a large number of extensive investigations of the biology of the enteric nervous system (Bornstein *et al.*, 1994; Costa, M and J.B. Furness, 1994; Daniel, E.E and Fox – Threlkeld, J.E.T, 1992; McConalogue, K and furness, J.B, 1994; Makhlouf, G.M, 1994).

Figure 1.3



Neural control of the gut wall, showing the myenteric and submucosal plexus (black fibres); extrinsic control of these plexuses by the sympathetic and parasympathetic nervous systems (red fibres); and sensory fibres passing from the luminal epithelium and gut wall to the enteric plexuses and from there to the prevertebral ganglia of the spinal cord and directly to the spinal cord and brain stem (dashed fibres), (Guyton and Hall, 2000).

In the early 1970s, two reports were published in which a hypothesis was put forward that challenged the traditional concept that gut muscle excitation and inhibition are accomplished by the activity of excitatory and inhibitory motor neurons, respectively (Furness, J.B. and Costa, M, 1987; McConalogue, K and Furness, J.B, 1994). It was proposed that the circular gut muscle is subjected to a tonic, neurogenic restraint and that a main function of the enteric nerves is to modulate the myogenic excitability of this muscle layer (Wood, J.D, 1975).

This view was based mainly on experiments performed in Vitro on the feline small intestine, in which electrophysiological recordings of myenteric ganglia and of the gut muscle were undertaken. In these experiments, the nerve blocking agents tetrodotoxin and lidocaine were important pharmacological tools. Of particular significance was the finding that in the control situation, the circular muscle layer exhibited a low level of mechanical activity, an absence of action potentials, and a hyporesponsiveness to trasmural electrical stimulation. Simultaneously, burst-like discharges were recorded from the myenteric ganglia, suggestive of neuronal activity responsible for the prevailing inhibition of the muscle. When exposed to either of the nerve-blocking agents, the muscle contractions were markedly increased. Thus, after the neuronal activity was abolished, each electrical "slow wave" was followed by muscle action potentials and large-amplitude phasic contractions. Conversely, after 7 days of cold storage (a procedure that preferentially impairs the enteric nerves), the circular muscle was unresponsive to lidocaine.

The various observations, when taken together, suggested that the circular muscle layer was held under tonic suppression, resulting from spontaneously active myenteric neurons that provide a continuous, synaptic drive to NANC, inhibitory motor fibers (Figure 1.4).

When this neurogenic restraint was abolished (e.g., by tetrodotoxin), myogenic excitation resulted as a consequence of disinhibition (Wood, J.D, 1975).

Figure 1.4

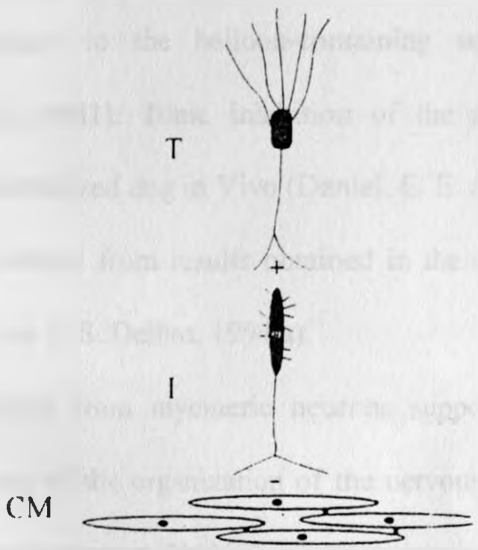


Diagram of an hypothetical neural pathway for regulation of the motility of the circular muscle layer (CM) of the small intestine. The tonically active myenteric neuron (T) provides excitatory input to the inhibitory motor neuron (I), (Wood, J.D, 1975).

An unspecific excitatory action of tetrodotoxin on the smooth muscle could, furthermore, be ruled out, since this compound was inefficient in preparations of the circular muscle that were devoid of ganglion cells and the deep muscular plexus (Wood, J.D. 1975).

Neurogenic inhibition, as revealed by the excitatory effect of tetrodotoxin, was subsequently reported for other isolated preparations of visceral muscle (e.g., rabbit ileum, colon, renal pelvis; guinea pig colon) (Tonini *et al.*, 1989). In the anesthetized cat in which motility of the small intestine was monitored by an intraluminal balloon, the in Vivo intra-arterial administration of tetrodotoxin to the balloon-containing segment resulted in local hypermotility (Wood, J.D. 1981). Tonic inhibition of the small intestines was later demonstrated also in the anesthetized dog in Vivo (Daniel, E. E. and J.E.T. Fox – Threlkeld, 1992) and moreover was inferred from results obtained in the chronically instrumented a wake dog (Gustafsson, B.I and D.S. Delbro, 1994 a).

Electrophysiological recordings from myenteric neurons support the existence of tonic inhibition. In an early scheme of the organization of the nervous control of gut motility, it was suggested that the nonspontaneous (NANC) inhibitory motor fibers are driven by tonic myenteric neurons of the steady – burster category, as observed by extracellular recordings (Wood, J.D. 1975: Figure 1.4). Subsequent studies utilizing intracellular electrodes however,did not detect burst-type cells initially (Wood, J.D. 1981). Intracellular recordings have detected spontaneously active myenteric neurons, suggestive of 'driver nerve cells' that provide a continuous synaptic input to the motor neurons. These driver cells belong to the AH/type 2 category and the burstlike electrical activity appears to emanate from neurites distal to the soma (Wood, J.D, 1994).

It should be pointed out that this view of the AH cells is at variance with the suggestion that these cells in fact constitute sensory neurons (Bornstein *et al.*, 1994; McConalogue, K and Furness, J.B, 1994).

The question arises as to why myogenic excitation of circular gut muscle is not observed in every study in which tetrodotoxin is utilized to block neural transmission. This could possibly be due to species differences, but another reason may be that the tonic inhibition is expressed with a functional heterogeneity in the gastrointestinal tract. Thus it was recently found that the most proximal part of the rat colon, which exhibits a high degree of spontaneous activity, is unresponsive to tetrodotoxin, indicating absence of ongoing inhibition (Makhlouf, G.M, 1994).

A further reason why tetrodotoxin sometimes fails to excite circular gut muscle may be that too extensive a dissection of the tissue under study was undertaken (Wood, J.D, 1975). Experimental procedures that cause an ablation of the muscle from its pacemaker region (i.e. the interstitial cells of Cajal) will abolish the slow waves, which are a prerequisite for myogenic contractile activity (Makhlouf, G.M, 1994).

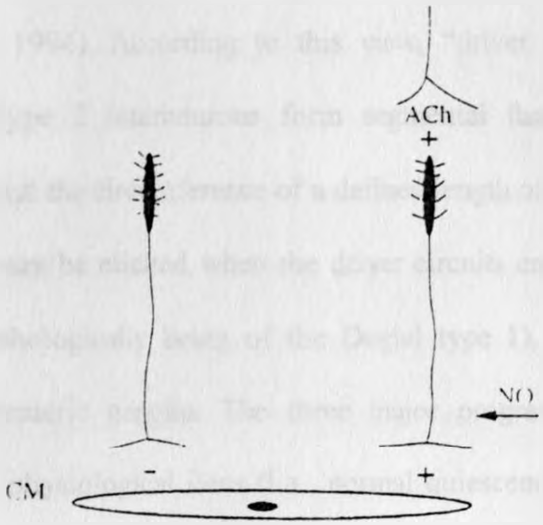
With respect to the NANC inhibitory neurotransmitter, Adenosine tri-phosphate (ATP) and vasoactive intestinal polypeptide (VIP) have been suggested to inhibit the muscle in different parts of the gastrointestinal tract (Costa, M. and Furness, J.B, 1994; McConalogue, K., and Furness, J.B, 1994; Makhlouf, G.M, 1994; Wood, J.D, 1994). Moreover strong evidence for nitric oxide (NO) being a major transmitter released from inhibitory motor neurons in the gut has been presented (McConalogue, K. and Furness, J.B, 1994).

Both VIP and NO appear to be transmitter candidates for tonic inhibition. Thus, in the in-vitro rat mid colon, a specific VIP antiserum mimicked the excitatory effect of tetrodotoxin, suggesting that VIP is responsible for the prevailing neurogenic suppression (Makhlouf, G.M, 1994).

It was suggested that NO may be the tonically released mediator because pharmacological blockade of the synthesis of NO resulted in gut hypermotility (Daniel, E.E and Fox-Threlkeld, J.E.T, 1992).

NO appears to be a final inhibitory transmitter in the feline small intestine (Gustafsson and Delbro, 1994 b; Figure 1.5).

Figure 1.5



Tentative arrangement of the nonadrenergic noncholinergic (NANC) motor neurons that determine small intestinal motility regulation in the rat. Tonically active inhibitory motor neurons suppress contractile activity of the circular muscle layer (CM).The transmitter for this effect has not been identified. Moreover, excitatory NANC motor neurons elicit contractile effects. These neurons are dependent on a synaptic input, i.e. stimulating of nicotinic ganglionic receptors by acetylchloline (ACh). The Excitatory circuit can be inhibited by nitric oxide (NO). + and -, Excitatory and inhibitory effects, respectively, (Gustafsson and Delbro, 1994 b.).

Functionally, the tonic inhibition of the gut muscle was suggested to play an important role for the generation of motility patterns of physiological or pathophysiological significance (Wood, J.D, 1975). An updated view of the organization of the enteric nervous system has been presented (Wood, J.D, 1994). According to this view, "driver circuits" basically consisting of myenteric AH/type 2 interneurons form segmental functional units that syncronize muscle activity around the circumference of a defined length of the gut.

Stereotyped motility patterns may be elicited when the driver circuits engage excitatory or inhibitory motor nerves (morphologically being of the Dogiel type 1), as determined by "motor programs" in the myenteric ganglia. The three major programs are peristalsis, segmentation movements, and physiological ileus (i.e., normal quiescence of the gut). The driver neurons are synaptically interconnected via slow excitatory postsynaptic potentials, which may constitute the most essential synaptic mechanism for neuronal firing within the circuits. Due to on going activity in the circuit, there is a continuous discharge of the inhibitory motor neurons to the circular muscle. Therefore, this tissue will remain relaxed until the inhibitory input is switched off by neuronal mechanisms within the circuits. Thus the inhibitory neurons determine when the omnipresent slow waves elicit a contraction, as well as the direction and the length of propagation of the contraction once it has been initiated (Wood, J.D, 1994).

Opioid peptides and opiates inhibit firing of myenteric (and also submucous) neurons, suggesting that tonic inhibition may be switched off by enteric opioidergic interneurons (Wood, J.D,1994) that could constitute an important modulatory arrangement, e.g., of the peristaltic reflex (Daniel, E.E and Fox-Threlkeld, J.E.T, 1992; Makhlouf, G.M, 1994).

Thus vagal nerve stimulation causes (NANC) contractile responses of the gut by an activation of intrinsic opioidergic neurons which, in turn, elicit myogenic contractions by disinhibition (Daniel, E.E and Fox-Threlkeld, J.E.T, 1992; Makhlouf, G.M, 1994).

The mechanism of tonic inhibition might also play a part in the generation of the cyclical motility pattern of the small intestine, consisting of quiescence and contractile activity that is characteristic for the fasting state in many mammalian species: the migrating motor complex (Furness, J.B and Costa, M. 1987). Thus, in the chronically instrumented awake dog, the intra-arterial administration of tetrodotoxin to a jejunal segment results in the appearance of an "ectopic activity front" immediately distal to the perfused segment. This finding was interpreted as being due to a removal of a neurogenic suppression of a local- activity- front generating mechanism of the gut muscle (Sarna, *et al.*, 1989).

In view of this understanding and extensive review of the of gut motility control, it becomes relevant to test the effects of *Mondia Whytei* root extract in order to scientifically authenticate the folklore claim regarding its role in management of dyspepsia. Dyspepsia (acid indigestion) management requires use of prokinetic agents and therefore if *Mondia Whytei* aqueous root extract causes contraction or increases motility of the rabbit jejunum then the folklore claim in this regard would hold true.

1.3.0 HEART MOTOR STATE

The heart is actually two separate pumps: 'a right pump' that pumps the blood through the lungs and a 'left pump' that pumps the blood through the peripheral organs.

In turn each of these hearts is a pulsatile two- chamber pump composed of an atrium and a ventricle. The atrium functions principally as a weak primer pump for the ventricle, helping to move the blood into the ventricles. The ventricle in turn supplies the main force that propels the blood either through the pulmonary circulation by the right ventricle or through the peripheral circulation by the left ventricle.

Special mechanisms in the heart provide cardiac rythmnicity and transmits an action potential throughout the heart muscle to cause the hearts rythimical beat (Truex, 1961)

The heart beat originates in a specialized cardiac conduction system and spreads via this system to all parts of the myocardium. The structures that make up the conduction systems are the sinoatrial node (SA node), the internodal atrial pathways, the atrioventricular node (AV node), the bundle of His and its branches, and the Purkinje systems (Truex, 1961)

The various parts of the conduction system and under normal conditions, parts of the myocardium, are capable of spontaneous discharge. However, the SA node normally

discharges most rapidly, with depolaralization spreading from it to the other regions before they discharge spontaneously.

The SA node is therefore the normal cardiac pacemaker and its rate of discharge determine the rate at which the heart beats (Truex, 1961).

1.3.1 PHYSIOLOGICAL REGULATION OF PACEMAKER FREQUENCY

The normal rhythmic heartbeat reflects a cyclic change in the membrane potentials of the pacemaker cells in the SA node. Beginning at the end of a preceding repolarization, nonpropagated diastolic depolarization proceeds relatively slowly, accelerates through the threshold for excitation, and results in the rapid propagated depolarization of the pacemaker action potential. With repolarization the cycle begins again. The tracings sketched in figure 1.6 illustrate the possible changes in membrane electrical function that relate to the frequency of impulse origin (Hoffman and Cranefield, 1960).

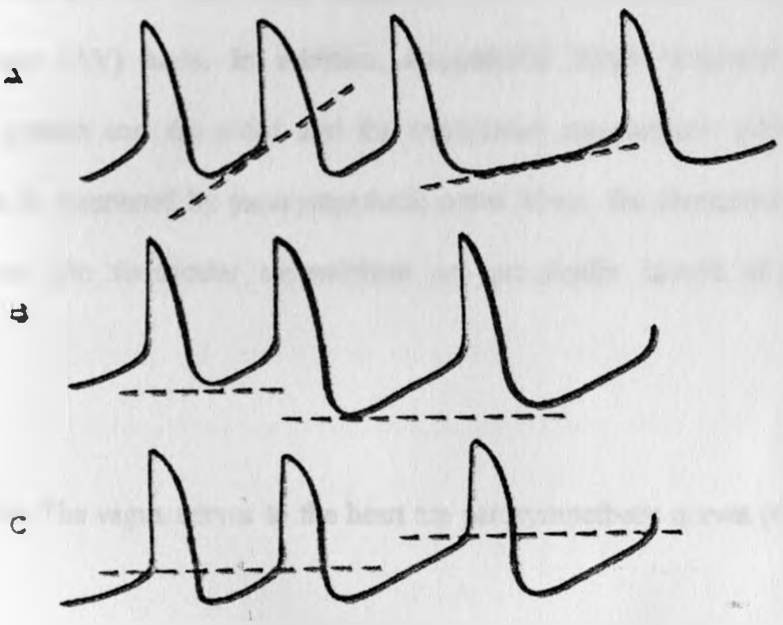
The slope of diastolic depolarization might be changed. Assuming that other factors remain constant, an increase in slope would cause a positive chronotropic response; if the slope were decreased, the consequence would be a negative chronotropic effect.

The magnitude of the maximal diastolic membrane potential will be a factor. For example, an increase in post-excitation membrane polarization would increase the cycle duration (i.e., reduce the heart rate) if other factors remained constant.

Finally, a change in the membrane threshold potential at which regenerative rapid depolarization occurs would produce a change in cycle duration if other factors are not changed.

Modification of the sinoatrial node frequency by efferent fibers of the autonomic nervous system is an important element in the physiological regulation of heart rate. The sinoatrial node is richly supplied by neural structures, including nerve fibers, nerve bundles, and ganglia (Truex, 1961).

Figure 1.6

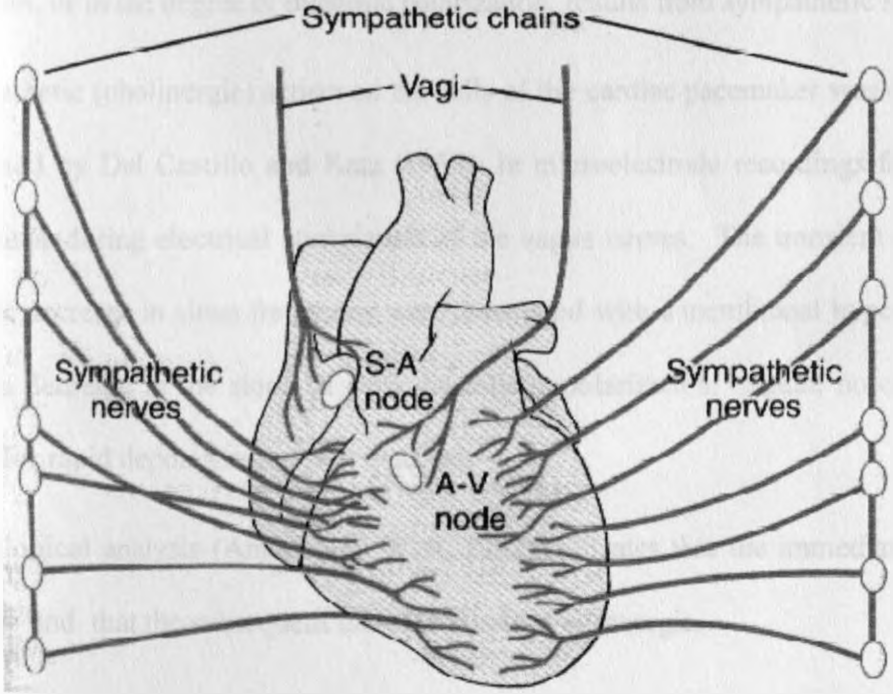


Ways in which changes in membrane potential can alter the spontaneous frequency of pacemaker fibers. Pacemaker action potentials drawn to show: changes in slope of diastolic depolarization (A); magnitude of maximum diastolic depolarization (B) and level of threshold potential (C), (Hoffman and Cranefield, 1960).

Generally, in the mammal, postganglionic sympathetic fibers from the stellate and other ganglia of the bilateral sympathetic chains supply the heart. Pre-and postganglionic parasympathetic fibers and parasympathetic ganglia are terminal structures of the right and left vagus nerves. The autonomic cholinergic and adrenergic fibers also supply the atrioventricular (AV) node. In addition, sympathetic fibers innervate the ventricular conduction system and the atrial and the ventricular myocardium. Although the atrial myocardium is innervated by parasympathetic nerve fibers, the conduction system of the ventricles and the ventricular myocardium are practically devoid of parasympathetic innervation.

Figure 1.7

Cardiac nerves.The vagus nerves to the heart are parasympathetic nerves (Guyton and Hall, 2000).



The excitation of the cardiac sympathetics normally results in a sinus tachycardia. Associated with the positive chronotropic effect of sympathetic stimulation is a slight positive dromotropic effect, that is seen as an increase in the rate of conduction through the AV node and Purkinje system (Hoffman and Cranefield, 1960).

The excitation of vagal fibers supplying the heart results in a negative chronotropic response. Depending on the intensity and duration of the stimulus, asystole may be induced for several seconds. The right vagus has been shown to affect the SA node more than does the left. However, left vagal stimulation affects AV nodal function more than does the stimulation of the right. This phenomenon has been illustrated clearly for the dog by Truex (1961). Thus, the ventricular frequency can be severely reduced by left vagal stimulation because of AV nodal conduction block, even though the atrial frequency is much less affected.

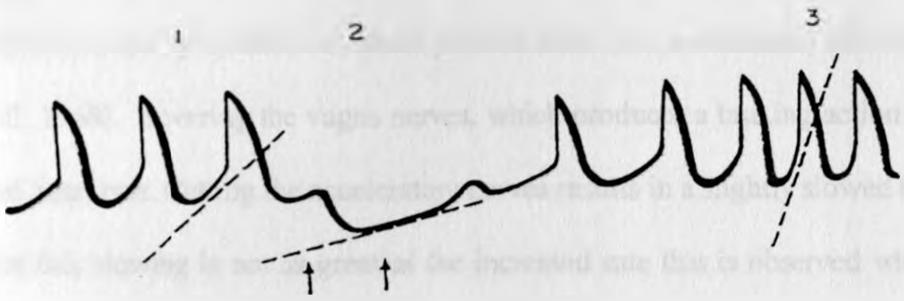
The sympathetic (adrenergic) action on the cells of the cardiac pacemaker effects an increase in the slope of diastolic depolarization (Hutter and Trautwein, 1956) and is associated with an increase in the frequency of spontaneous excitation. Little or no change in the threshold of excitation, or in the degree of diastolic polarization, results from sympathetic stimulation.

Parasympathetic (cholinergic) action on the cells of the cardiac pacemaker was convincingly demonstrated by Del Castillo and Katz (1955) in microelectrode recordings from the frog sinus venosus during electrical stimulation of the vagus nerves. The transient asystole and subsequent decrease in sinus frequency were associated with a membranal hyperpolarization and with a decrease in the slope of slow diastolic depolarization. Again, no change in the threshold for rapid depolarization was detected.

Pharmacological analysis (Amory and West, 1962) indicates that the immediate slowing is cholinergic and that the subsequent increase in rate is adrenergic.

Upon physiological analysis (Vincenzi and West, 1963), graded variations in stimulus intensity were used to show that the induced changes in pacemaker frequency result from the excitation of intramural nerve fibers. Specifically, the stimulated cholinergic fibers appear to be postganglionic, as are the sympathetic fibers. By using the method of transmural stimulation it was possible to demonstrate, in the mammalian SA node, the patterns of response to cholinergic and adrenergic stimulation (Amory and West, 1962).

Figure 1.8



The membrane effects of parasympathetic and sympathetic intranodal nerve stimulation, as recorded in rabbit sinoatrial node. Composite tracing synthesized from data of Amory and West (1962) and Vincenzi and West (1963). Transmural electrical stimulation of intranodal nerve endings between arrows. At 1, control slope of diastolic depolarization; at 2, cholinergic hyperpolarization and reduction of diastolic slope, and at 3, positive chronotropic response and increased diastolic slope from adrenergic stimulation.

1.3.2 REGULATORY INNERVATION OF THE HEART

Regulatory innervation is supplied to the vertebrate heart via the parasympathetic and sympathetic divisions of the autonomic nervous system. It is believed that both the parasympathetic and sympathetic systems provide tonic (i.e., continuous) activity to the heart (Marshall, 1968). Severing the vagus nerves, which produces a braking action, results in an increased heart rate. Cutting the acceleratory nerves results in a slightly slowed heart rate; the degree of this slowing is not as great as the increased rate that is observed when the vagus nerves are cut. If both the vagus and the sympathetic nerves to the heart are cut, the heart rate increases; thus the vagal effects predominate in the resting condition (Warner and Russell, 1969). It has been generally accepted that normal changes in the heart rate depend on reciprocal variations in the balance between the sympathetic and parasympathetic systems (Thames and Kontos, 1970). Glick and Braunwald (1965), however, suggested that reciprocity of the two systems is not necessary: the parasympathetic system, alone, can slow the heart in response to a physiological variable such as increased arterial pressure; the sympathetic system, alone, can accelerate the heart.

1.3.2. (a) EFFERENT PARASYMPATHETIC INNERVATION

Parasympathetic preganglionic fibers destined for the heart arise in the vagal dorsal motor nuclei of the medulla (the cardioinhibitory center). They pass along the main vagal trunks and then the vagal cardiac branches to reach the cardiac plexus. Some fibers synapse within the ganglia in the plexus while others continue and synapse in the intrinsic cardiac ganglia that are distributed over the surfaces of the atria.

The parasympathetic postganglionic fibers supply abundant innervation to the pacemaker tissues of the sinoatrial (SA) node, the atrioventricular (AV) node, and the bundle of His; they also supply the atrial musculature and the blood vessels of the atria and the ventricles. It has been stated that, in most mammals, the ventricle does not receive parasympathetic innervation (Marshall, 1968).

However, some anatomical evidence (Napolitano *et al.*, 1965) and experiments showing that stimulation of the vagi produces direct negative inotropic effects on the ventricular myocardium (De Geest *et al.*, 1965; Levy *et al.*, 1966; Harman and Reeves, 1968; Dempsey and Cooper, 1969; Levy and Zieske, 1969) suggest that at least a few postganglionic parasympathetic fibers reach the ventricles of mammalian hearts. Furnival *et al.*, (1968) and Snow *et al.*, (1969), however, were unable to demonstrate a direct effect of vagal stimulation on the ventricular myocardium of the dog heart. In addition, Ekstrom (1970) found choline acetyltransferase activity in the ventricles, as well as the atria, of cat, rabbit, and rat hearts; although this alone is not sufficient to prove the presence of parasympathetic innervation in the ventricles, it does lend support to the electro-physiological and anatomical evidence.

1.3.2 (b) EFFERENT SYMPATHETIC INNERVATION

The sympathetic preganglionic fibers arise in the intermediolateral gray columns of the top four or five thoracic segments of the spinal cord. They proceed through the ventral roots and the white rami communicantes to the adjacent ganglia of the sympathetic trunks. Some fibers synapse within these ganglia; others ascend and synapse in the inferior, middle, and superior cervical ganglia. A few fibers may proceed through these ganglia and synapse near or within

the heart itself (Brown, 1967 b; Priola and Fulton, 1969; Wechsler *et al.*, 1969). Smith (1970), using electrical stimulation techniques and drugs on the dog heart, found evidence for intracardiac adrenergic neurons innervated by cholinergic fibers that reach the heart via the vagus.

1.3.2. (c) AFFERENT PARASYMPATHETIC AND SYMPATHETIC FIBERS

Afferent, as well as efferent, fibers run in the sympathetic and parasympathetic nerves. The afferent parasympathetic fibers arise from baroreceptors (mechanoreceptors) in the heart and great vessels and pass to the cardioinhibitory center. They are concerned with reflexes that depress cardiac activity. The baroreceptors have been described as unencapsulated (Miller and Kasahara, 1964) stretch receptors (Coleridge *et al.*, 1957) and while using silver and methylene blue staining, the terminations of the fibers were described as fine fibrillae. Dropman (1968), however, using electron microscopy, found lamellated mechanoreceptor structures resembling Pacinian corpuscles in the carotid sinus. The baroreceptors are thought to be stimulated by distension of the tissue in which they are located (Coleridge *et al.*, 1957; Sleight and Widdicombe, 1965). The sympathetic afferent fibers proceed in the sympathetic nerve branches to reach the upper thoracic spinal roots. These fibers are believed to carry pain signals (Brown, 1967a; Netter, 1969).

1.3.3 STIMULATION OF CARDIAC NERVES

1.3.3. (a) STIMULATION OF THE PARASYMPATHETIC (VAGUS) NERVES

Stimulation of the vagus nerves to the vertebrate heart produces a reduced heartbeat rate, diminished contractile strength of the atria, and reduced conduction velocity through the atrioventricular node. With increased intensity of stimulation there may be a dissociation of atrial and ventricular contractions (AV block) or complete arrest of the heartbeat (Netter, 1969; Marshall, 1968). The muscle fibers of the atria show a greater tendency to fibrillate in response to direct electrical stimulation during vagal stimulation than during its absence (Marshall, 1968). Stimulation of the vagi probably has some direct negative ionotropic effect on the ventricles. The ventricles are slowed indirectly as well by the parasympathetic activity on the sinoatrial and atrioventricular nodes and the atria (Milnor, 1968). By analyzing the heartbeat intervals of rabbit hearts before and during vagal stimulation at varying frequencies, Versprille (1970) concluded that an arithmetic increase in the rate of stimulation of the vagus causes a geometrical increase in its effect.

Acetylcholine (Ach) applied to the pacemaker tissues and the atria produces effects similar to those produced by stimulation of the vagus nerves.

It has been proposed that ACh produces its effects by causing an increase in permeability of the cardiac surface membrane to both inward and outward movements of potassium (Hutter and Trautwein, 1956).

To produce the pronounced breakdown of the membrane resistance, it must be assumed that the cardiac tissue is densely innervated to provide sufficient receptor sites on which the ACh may act (Hutter, 1957).

An increase in potassium permeability will tend to drive the membrane potential toward the equilibrium potential for potassium. Thus the membrane potential of cells that normally show membrane potentials less negative than the potassium equilibrium potential will be increased. This is the case in pacemaker cells that possess—due to the slow diastolic depolarization—a diastolic membrane potential 10-20 mV more positive than nonpacemaker cells. Nonpacemaker cells usually show only a small degree of hyperpolarization, or none, to the application of ACh, since the difference between the membrane potential and the equilibrium potential for potassium is small. Trautwein and Dudel (1958a), using excised dog atria, showed that the membranes of cells made artificially more negative than the potassium equilibrium potential by applied currents respond to ACh by depolarizing. Further, when the concentration of potassium in the bath was increased, the equilibrium potential for ACh became more positive; the relation was characteristic of a potassium electrode (Trautwein and Dudel, 1958a). Burgen and Terroux (1953) also showed that increasing the concentration of potassium in the bath diminished the inhibitory effects of ACh on the cat atrium by causing a decrease in the resting potential.

A ten-fold increase in the potassium, however, did not produce a 58 mV change in the resting potential, as would be expected for a potassium electrode, but only 38 mV. Still, on the basis of such experiments it appears that ACh effects a specific increase in potassium permeability (Marshall,1968).

Increased permeability to potassium can sufficiently account for the inhibitory effects produced by vagal stimulation and ACh. Increased outflow of potassium would tend to accelerate the repolarization phase of the action potential; this effect would shorten the duration of the action potential and, if severe enough, reduce its amplitude. Shortening the action potential would result in shortening its refractory period, because in cardiac muscle, the duration of the refractory period is proportional to the duration of the action potential (Marshall, 1968). The relatively free flux of potassium ions would tend to drive the membrane potential toward the potassium equilibrium potential. This can account for the increased membrane potential that is observed, especially in pacemaker tissues, if one assumes that the potassium equilibrium potential is more negative than the diastolic membrane potential. This increased membrane potential can also account for the suppression of the pacemaker potential, since a greater degree of depolarization will be required to reach threshold and trigger the action potential.

1.3.3. (b) STIMULATION OF THE SYMPATHETIC NERVES

Stimulation of the sympathetic nerves to the vertebrate heart produces an increased heartbeat rate, augmented force of heart contraction and increased conduction velocity through the atria, AV node, and ventricles. The Purkinje fibers tend to exhibit dominant pacemaker activity during sympathetic stimulation and this can result in ventricular extrasystoles or fibrillation (Marshall, 1968). Although the sympathetic nerves do not produce maintained tone in the ventricles, the force of ventricular contractions can be influenced by the frequency of impulses in these nerves (Milnor, 1968).

The application of noradrenaline (NA) or adrenaline (A) also increases the rate and force of the heartbeat.

Hutter and Trautwein (1956) suggested that the mechanism involved in sympathetic stimulation may be either an increase in the sodium influx or a decrease in potassium permeability—that is, the reversal of the parasympathetic mechanism.

Trautwein (1963) points out that sodium ions carry the depolarizing current, since depolarization does not occur if the sodium in the bath is halved, and that this sodium current is probably increased by the application of adrenaline or by sympathetic stimulation. Increased permeability to sodium would account for the increased slope of the pacemaker potentials and the increased amplitude of the action potentials. Trautwein (1963) suggested, however, that increased sodium permeability cannot be the only effect produced by adrenaline: increased permeability to sodium can explain neither why depolarization of the membrane potential during diastole may not occur when there is an increased slope of the pacemaker potential, nor why the membrane potential may increase in the presence of adrenaline (Hutter and Trautwein, 1956).

Antoni and Delius (1965) observed at fast sweep speeds, two phases of the upstroke of action potentials recorded from isolated frog myocardial trabeculae. Raising the potassium in the bath produced a selective reduction of the first phase, while replacing 70% of the sodium in the bath with sucrose caused a reduction of both the first and second phases. A high extracellular concentration of magnesium (5-15 mM) increased the resting potential, decreased the overshoot of the action potential, increased the amplitude of the first phase, and abolished the second phase. When adrenaline was added to the bath in potassium-and

magnesium-rich or in sodium-free conditions, the second phase of the upstroke was selectively augmented.

On the basis of their experiments, Antoni and Delius (1965), concluded that the first phase of the upstroke is produced by a sodium current, while the second phase—the beginning of the plateau— is produced partly by sodium but also by other ions.

Reuter (1966) obtained current-voltage curves from Purkinje fibers of sheep hearts that were placed in sodium-free solutions with varying concentrations of calcium [0, 1.8 (normal), 7.2 mM]. Square pulses in calcium-free solution produced electrotonic responses showing simple capacitative distortion. In normal or high calcium solutions the depolarizing square pulses resulted in a response of two phases; the first phase was slow while the second phase had a threshold and consisted of an accelerated rise from threshold toward a steady potential. In the presence of calcium, adrenaline augmented the amplitude of the second phase; it had no effect on the electrotonic responses in calcium-free solution. Reuter suggested, then, that an inward calcium current contributes to the depolarization and that adrenaline increases the calcium movement.

In addition to Antoni and Delius (1965), Carmeliet and Vereecke (1969), using cow Purkinje fibers, showed that conduction that had been blocked by a high extracellular concentration of potassium could be restored by the addition of adrenaline. Carmeliet and Vereecke (1969) observed that the action potentials that took place in the presence of both increased potassium and adrenaline proceeded in two steps. The first step-a sodium spike was lowered in amplitude when the sodium concentration of the bath was decreased and was specifically blocked by tetrodotoxin. The second step, the plateau, was neither affected by artificial changes of the membrane potential between -85 and -55 mV nor by reduction of the

extracellular sodium: it was specifically blocked by manganese ions and beta-receptor blocking agents and depended on a critical extracellular concentration of calcium ions. The amplitude of the plateau increased 17 mV for a ten-fold change in the calcium concentration. On the basis of these experiments Carmeliet and Vereecke (1969), like Reuter (1966) suggested that in the presence of adrenaline there is an increase of calcium inward current during the plateau of the action potential.

The theoretical value of membrane potential change for a tenfold change in extracellular calcium is 28 mV. Carmeliet and Vereecke (1969) therefore proposed that the membrane is permeable to other ions as well as to calcium. Sodium conductance probably contributes to the second depolarization since tetrodotoxin slows its rising phase (Carmeliet and Vereeck, 1969). Although early radioisotope experiments using ^{42}K did not succeed in showing a diminution of ^{42}K permeability during sympathetic stimulation (Hutter, 1957)), Hauswirth *et al.* (1968), by performing voltage clamp experiments, found that in the presence of noradrenaline there is a decline of potassium conductance following an action potential

Such a decline results in enhanced pacemaker activity. Furthermore, in preliminary experiments, Carmeliet and Vereecke (1969) found that replacement of chloride by acetylglycinate caused a marked increase in the duration of the electrical response in the presence of adrenaline, although the amplitude was increased by only a few millivolts. A decline in chloride conductance would be expected to favor depolarization.

Thus, although the complete ionic fluxes that occur during sympathetic stimulation or application of adrenaline must be further elucidated, it appears that the membrane conductance changes involve several species of ions.

The receptors of effector cells on which adrenergic transmitters act were named, for working convenience, on the basis of their relative responsiveness to administered agonists and blocking agents (explained by Ahlquist, 1967). Although Govier *et al.* (1966) reported evidence for the presence of alpha receptors in rabbit atria, it is generally accepted that the heart possesses only receptors classified as beta (Furchgott, 1967). The beta receptors of the heart are associated with an increase in the frequency of action potentials and increased force of contraction developed with each action potential (Axelsson, 1971). The beta-receptor blocking agents slow the heart and block the positive chronotropic action of applied catecholamines (Ahlquist, 1968).

The character of the actual receptor and the mechanism by which it functions to mediate responses of the effector cell are not known. It has been proposed that the effects associated with beta-receptors are produced by an increased level of intracellular cyclic AMP and that the receptor itself is a part of the adenyl cyclase system (Robinson *et al.*, 1967). Indeed La Raia and Sonnenblick (1971) showed for the rabbit heart parallel development of the amount of tension, adenyl cyclase, and cyclic AMP in the presence of NA and parallel reductions of these parameters in the presence of carbamylcholine. The atria responded significantly to NA whereas the ventricles responded only slightly. The cholinergically mediated responses were blocked by atropine. La Raia and Sonnenblick (1971) indicate that it must yet be established whether the cyclic AMP is directly associated with the excitation-contraction mechanism or only with changes in the intermediary metabolism that accompany it. Another suggestion is that the beta-receptors that mediate enhanced contraction of cardiac cells constitute the cellular actomyosin (Honig and Stam, 1967).

In view of this extensive review of the heart motor state. it is worth noting that in an intact animal, tonic vagal discharge predominates and is referred to as vagal tone which is normally abolished by atropine that reversibly blocks the M_2 receptors. Agonists that slows down the heart rate and the heart force does so by increasing the K^+ conductance and slowing down opening of ca^{++} channels. Most of the evidence based antihypertensives works on this principle. It is therefore prudent to investigate the effects of *Mondia Whytei* aqueous root extract on isolated rabbit heart inorder to delineate its proper use as an antihypertensive and confirm the folklore claims regarding its use in the management of hypertension.

1.4.0 RATIONALE OF THE STUDY

From time immemorial, *Mondia Whytei* roots have been chewed fresh and dry in the management of various ailments.

Upon ingestion, the extract comes into contact with the lining of gastrointestinal tract, therefore it would be prudent to investigate the effects it might cause on the activity of the smooth muscle of the gastrointestinal tract upon swallowing.

It is possible that once absorbed into the circulatory system, the extract would have an effect on vascular smooth muscle of circulatory system and even on cardiac muscle. If the extract does affect the heart muscle, then this means that it would have profound effects in all the systems of the body.

It is this probable sequence of events that necessiated investigating the effects of *Mondia Whytei* extract on the isolated rabbit jejunum and heart preparations.

1.5.0 HYPOTHESIS

Mondia Whytei aqueous root extracts may alter the motor activity of gastrointestinal smooth muscle and cardiac muscle.

1.6.0 AIMS

1. To investigate the activity of *Mondia Whytei* aqueous root extract on the isolated rabbit heart and jejunum preparations.

1.7.0 OBJECTIVES

1. To test the effects of *Mondia Whytei* aqueous root extract on cardiac muscle using an isolated rabbit heart preparation.
2. To test the effects of *Mondia Whytei* aqueous root extract on the smooth muscles of an isolated rabbit jejunum preparation.

2.0.0 MATERIALS AND METHODS

2.1.0 PREPARATION OF *MONDIA WHYTEI* AQUEOUS ROOT EXTRACT

Mondia Whytei roots were harvested and cleaned off soil using water. The skeels (the outer part of the root) were peeled off while the roots were still fresh, cut into small pieces and then kept under shade in order to dry. The drying time was three weeks and the dry skeels were then powdered using a grinder. The *Mondia Whytei* roots that were harvested yielded one kilogramme of powder.

Water extraction was done by mixing 100 g of the powder with 500 ml of water and boiling for one hour. The mixture was then decanted and filtered using sintered glass. Subsequently, the filtrate was solidified using a mixture of dry carbon dioxide and acetone. Freeze-drying was carried out for a duration of fourty eight hours, after which the freeze-dried material was kept in an air tight container and stored in a desiccator.

2.2.0 ANIMALS

Fifteen male and female California white rabbits were used with exclusion of the pregnant ones. They were obtained from International Livestock Research Institute (ILRI) Kenya and housed in animal cages within the animal house . The room temperature was maintained at $25^{o}C \pm 2$. The animals were allowed water *ad libitum* and they were handled humanely in accordance with the institutional animal care and committee guidelines.

2.3.0 TEST SYSTEMS

2.3.1 Effects of *Mondia Whytei* aqueous root extract on isolated rabbit heart in Langerdoff preparation.

This experimental set-up was meant to investigate the actions of the extract on cardiac muscle (without the modifying influence of blood pressure control system) and also on coronary circulation.

A rabbit was sacrificed by a blow at the back of the head. The chest wall and rib cage were quickly opened. The whole heart was removed ensuring that at least 1cm of the aorta was left intact. Immediately after the heart was isolated from the animal, it was placed into a dish containing cold oxygenated Ringer locke solution and gently squeezed to remove blood which would otherwise clot inside the heart.

The heart was then freed from extraneous tissue and immediately attached through the aorta to a cannula at the base of the Langerdoff apparatus. The rates of perfusion and oxygenation were adjusted until the heart was beating satisfactorily. At this point, the apex of the heart was hooked and attached by a thread through a pulley system to a Starling heart lever. The normal heart activity was recorded over a two minute period on a Havard recorder prior to administration of the test extract.

Experimental Parameters

Organ Bath Volume - 5 ml

Bath Solution - Ringer locke Solution

Bath Aeration - 95% O_2, 5% CO_2

Bath Temperature - 37^{0}C

Recording - Isometric

Equilibration Time - 5 minutes

Contact Time - 1 minute

Reagents under investigation

1. Normal Saline - 9 % w/v
2. *Mondia Whytei* - 12 mg/ml

Experimental Protocol

1. Record the normal intrinsic heart activity over a control period of two minutes.
2. Add 0.2 ml normal saline to the organ bath, and record its effects on the heart rate and force for one minute. Repeat this step 5 times in order to generate 5 sets of tracings.
3. Repeat step 2 but add 0.1 ml *Mondia Whytei* aqueous root extract to the organ bath instead of the normal saline. Generate 5 sets of tracings using 0.1 ml *Mondia Whytei* aqueous root extract.
4. Repeat step 3, using 0.2 ml *Mondia Whytei* aqueous root extract.
5. Repeat step 3, using 0.3 ml *Mondia Whytei* aqueous root extract.
6. Repeat step 3, using 0.4 ml *Mondia Whytei* aqueous root extract.

the following precautions were taken before proceeding to the next step

i. Five minutes were allowed for the heart to recover from the effects of the reagent administered.

ii. The baseline activity of the heart was recorded over a period of one minute.

2.3.2 Effects of *Mondia Whytei* aqueous root extract on isolated rabbit jejunum.

The rabbit jejunum exhibits normal regular pendular movements and therefore it was used to study the effects of the extract on the motility and the tone of the intestines.

A piece of jejunum (approximately 5-10 cm below the stomach) was cut from a rabbit, shortly after sacrificing it by a blow at the back of the head. The rabbit was starved for twenty four hours prior sacrifice. A 3 cm long piece of the jejunum was set up in an organ bath containing Tyrode solution.

Experimental Parameters

Organ Bath Volume	-	40 ml
Bath Solution	-	Tyrode Solution
Aeration	-	95% O_2, 5% CO_2
Bath Temperature	-	37°C
Recording	-	Isotonic
Equilibration Time	-	20 minutes
Contact Time	-	1 minute

Drugs

1. Acetylcholine	-	10 ug/ ml
2. Adrenaline	-	100 ug/ml
3. *Mondia Whytei*	-	12 mg /ml

erimental Protocol

dd 0.2 ml acetylcholine into the organ bath and record its effects on the jejunum reparation.

dd 0.2 ml of adrenaline into the organ bath and record its effects.

dd 0.3 ml *Mondia Whytei* and record its effects.

epeat step 3 and generate 5 sets of tracings.

llowing precautions were taken before proceeding to the next step

i. The preparation was washed out twice by filling the organ bath with Tyrode solution and then draining it after each step and time was allowed for the piece of jejunum to recover its baseline activity (approximately ten minutes).

ii. Baseline activity was recorded over a period of two minutes.

libration time refer to the time it took from mounting an organ into the bath until the ct was added whereas contact time referred to the time it took to start recording from the the extract was added into the organ bath.

3.0 RESULTS

3.1.0 FINAL ORGAN BATH CONCENTRATION

120 mg of freeze dried *Mondia Whytei* was accurately weighed and dissolved in 10 ml of Ringer Locke solution. The same was done with Tyrode solution. Therefore the resultant stock solution had a concentration of 12 mg/ml freeze dried *Mondia Whytei* root powder.

Therefore 0.1 ml of the stock solution contained 1.2 mg *Mondia Whytei* root powder.

Considering the dead volume of Langerdoff equipment equal to 5.0 ml

Therefore 1.2 mg dissolved in 5.0 ml. The final organ bath concentration therefore was 0.24 mg/ml (from 0.1 ml extract stock solution)

i.e

$$\frac{1.2 \text{ mg}}{5.0 \text{ ml}} = 0.24 \text{ mg/ml}$$

Similarly, the final organ bath concentration for the other concentrations are shown in table 1.

Table 1

Volume of stock solution	Amount of *Mondia Whytei*	Final organ bath concentration
0.1 ml *Mondia Whytei*	1.2 mg	0.24 mg/ml
0.2 ml *Mondia Whytei*	2.4 mg	0.48 mg/ml
0.3 ml *Mondia Whytei*	3.6 mg	0.72 mg/ ml
0.4 ml *Mondia Whytei*	4.8 mg	0.96 mg/ml

3.2.0 ISOLATED RABBIT HEART RESPONSE

The data presentation was based on the size and frequency of the twitches before and after adding the *Mondia Whytei* aqueous root extract.

The recorded intrinsic heart activity formed the basis of the control experiment.

The speed of the recorder was set at 2.5 mm per second. Therefore the length run by recording paper over a control period of 20 seconds was 5 cm. [i.e. 2.5 mm/sec x 20 seconds = 50 mm]

The percentage change in the size and the number of twitches after adding *Mondia Whytei* known concentration was calculated.

Normal saline was used as a control in this experiment. For each known concentration of *Mondia Whytei,* five sets of experiment were done and the average percentage change in size and number of twitches established.

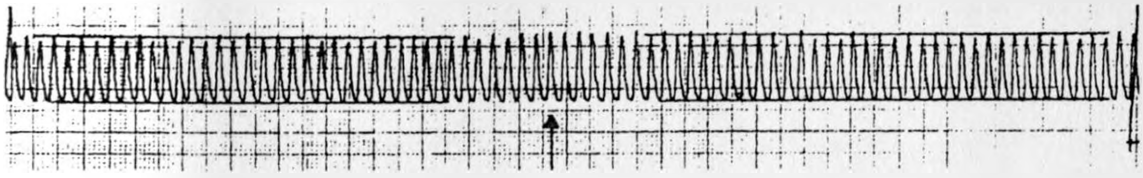
Various concentrations (multiples of two) of *Mondia Whytei* were tested and the results tabulated. These results were used to plot a graph of both change in the heart force and heart rate versus the concentration.

The size (height) of the twitch represented the heart force whereas the frequency of the twitches represented the heart rate.

3.2.1 PRESENTATION OF PERCENTAGE CHANGE IN FREQUENCY AND SIZE (HEIGHT) OF TWITCHES FOR EACH CONCENTRATION

A. 0.2 ml Normal Saline

Figure 3.1a



The arrow (↑) indicates the point at which 0.2 ml of normal saline was added into the organ bath. The frequency and size of the twitches before adding 0.2 ml normal saline was 28 twitches per 5 cm and 0.8 cm in height respectively. After adding 0.2 ml normal saline the frequency and size of the twitches recorded was 28 twitches per 5 cm and 0.8 cm in height respectively.

Assuming that one twitch was equivalent to one heart beat, then 28 twitches per 20 seconds was equivalent to 84 beats per minute. The percentage change in heart rate is therefore:

$$\frac{(84 - 84)}{84} \times 100\ \% = 0\ \% \text{ (zero)}$$

Similarly the percentage change in heart force is:

$$\frac{(0.8 - 0.8)}{0.8}\ \text{cm} \times 100\ \% = 0\ \%\ \text{change}$$

Figure 3.1b

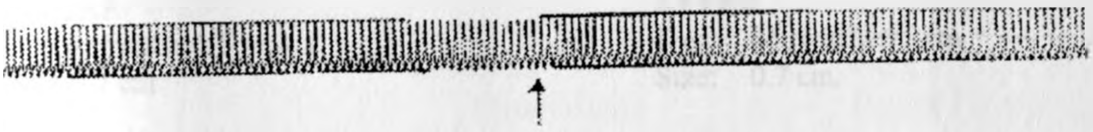


Figure 3.1c

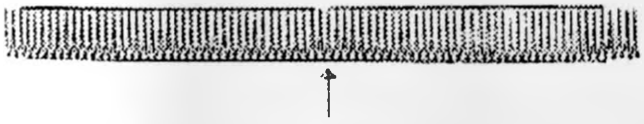


Figure 3.1d

Figure 3.1e

Working out similarly for the other results the following values were determined as tabulated below.

Figure 3.1b

BEFORE
Freq: 46 twitches/5 cm
Size: 0.7 cm

AFTER
Freq: 46 twitches/5 cm
Size: 0.7 cm.

Figure 3.1c

BEFORE
Freq: 28 twitches/3 cm
Size: 0.7 cm

AFTER
Freq: 28 twitches/3 cm
Size: 0.7 cm.

Figure 3.1d

BEFORE
Freq: 33 twitches/5 cm
Size : 0.8 cm

AFTER
Freq: 33 twitches/5 cm
Size : 0.8 cm.

Figure 3.1e

BEFORE
Freq: 48 twitches/5 cm
Size : 0.7 cm

AFTER
Freq: 48 twitches/5 cm
Size: 0.7 cm

Table 2:

Figure Number	Percentage change in Heart Rate	Percentage change in Heart Force
3.1a	0	0
3.1b	0	0
3.1c	0	0
3.1d	0	0
3.1e	0	0
Mean	0	0
Standard deviation	$\pm$0	$\pm$0

0.2 ml normal saline added into the organ bath resulted in no change of frequency and size of the heart twitches.

The arrow [↑] indicates the point at which 0.1 ml (1.2 mg) *Mondia Whytei* was added into the organ bath.

B. 0.1 ml *Mondia Whytei* (1.2 mg)

Figure 3.2a

Figure 3.2b

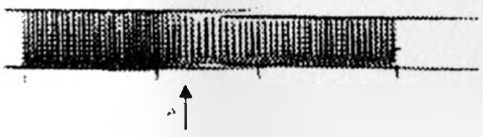


Figure 3.2c

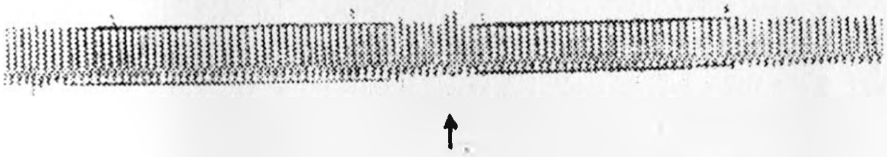


Figure 3.2d

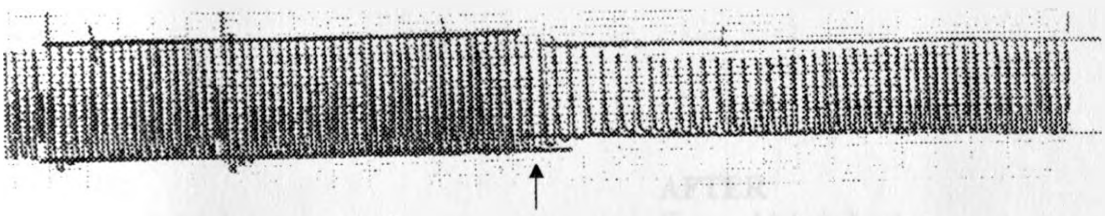
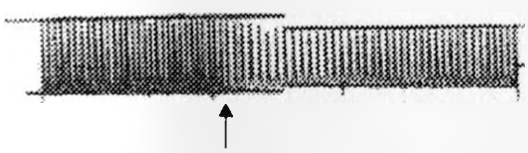


Figure 3.2e



Similarly the following values were obtained for various concentration of the *Mondia Whytei.*

Figure 3.2a

BEFORE
Freq: 23 twitches/5 cm
Size: 0.7 cm

AFTER
Freq: 22 twitches /5 cm
Size: 0.6 cm.

Figure 3.2b

BEFORE
Freq: 17 twitches/cm
Size: 0.9 cm

AFTER
Freq: 13 twitches/cm
Size: 0.7 cm

Figure 3.2c

BEFORE
Freq: 50 twitches/5 cm
Size : 0.8 cm

AFTER
Freq: 48 twitches/5 cm
Size : 0.6 cm

Figure 3.2d

BEFORE
Freq: 40 twitches/5 cm
Size : 1.6 cm

AFTER
Freq: 30 twitches/5 cm
Size : 1.3 cm.

Figure 3.2e

BEFORE
Freq: 13 twitches/cm
Size : 1.0 cm

AFTER
Freq: 11 twitches/cm
Size: 0.8 cm

Table 3

Figure Number	Percentage Change in Heart Rate	Percentage Change in Heart Force
3.2a	-4.3	-14.3
3.2b	-23.5	-22.2
3.2c	-4.0	-25.0
3.2d	-25.0	-18.7
3.2e	-15.4	-20.8
Mean	-16.5	-20.0
Standard deviation	$\pm$10.08	$\pm$ 3.94

Positive (+ve) sign indicates a percentage increase whereas negative (–ve) sign indicates percentage decrease in either the frequency (Heart Rate) or height of twitch (Heart Force) on adding 0.1 ml (1.2 mg) of the *Mondia Whytei* extract into the organ bath.

The heart rate and the heart force decreased by 16.5 ($\pm$ 10.08) and 20.0 ($\pm$ 3.94) percent respectively.

C. 0.2 ml *Mondia Whytei* (2.4 mg)

Figure 3.3a

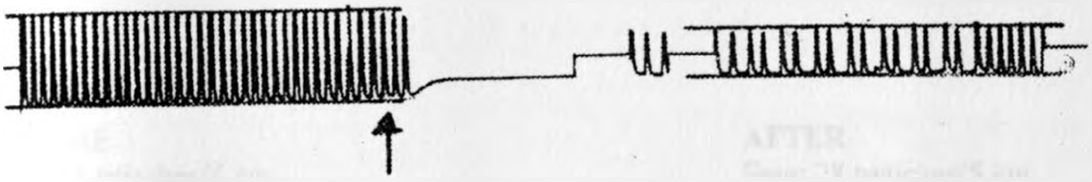


Figure 3.3b

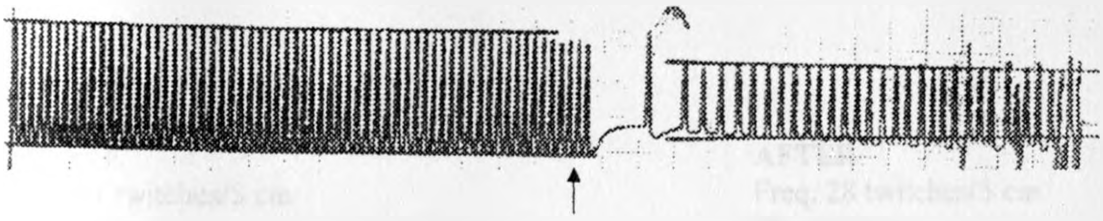


Figure 3.3c

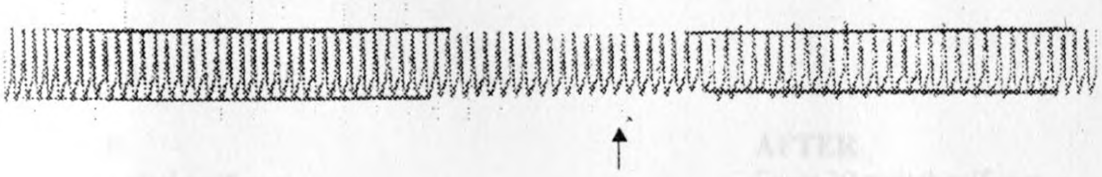


Figure 3.3d

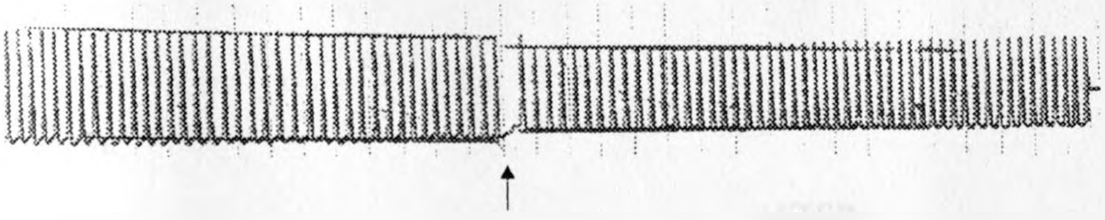


Figure 3.3e

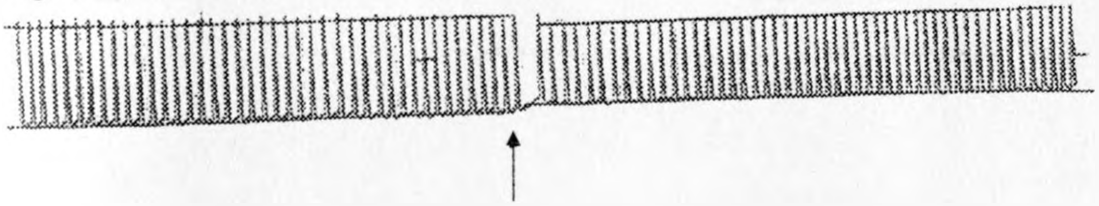


Figure 3.3a

BEFORE
Freq: 21 twitches /3 cm
Size : 1.3 cm

AFTER
Freq: 14 twitches/3 cm
Size : 0.7cm

Figure 3.3b

BEFORE
Freq: 41 twitches/5 cm
Size : 1.7 cm

AFTER
Freq: 28 twitches/5 cm
Size : 1.0 cm.

Figure 3.3c

BEFORE
Freq: 31 twitches/5 cm
Size : 1.0 cm

AFTER
Freq: 28 twitches/5 cm
Size 0.8 cm.

Figure 3.3d

BEFORE
Freq: 30 twitches/5 cm
Size :1.5 cm

AFTER
Freq: 30 twitches/5 cm
Size :1.1 cm.

Figure 3.3e

BEFORE
Freq : 32 twitches/5 cm
Size : 1.3 cm

AFTER
Freq: 31 twitches/5 cm
Size: 1.0 cm

Table 4

Figure Number	Percentage Change in Heart Rate	Percentage change in Heart Force
3.3a	-33.3	-46.2
3.3b	-31.7	-41.2
3.3c	-9.7	-20.0
3.3d	0.0	-26.7
3.3e	-3.1	-23.1
Mean	-15.6	-31.4
Standard deviation	±15.86	±11.58

The heart rate and the heart force decreased by 15.6 (± 15.86) and 31.4 (± 11.58) percent respectively on adding 0.2 ml (2.4 mg) *Mondia Whytei* aqueous root extract into the organ bath.

D. 0.3 ml *Mondia Whytei* (3.6 mg)

Figure 3.4a

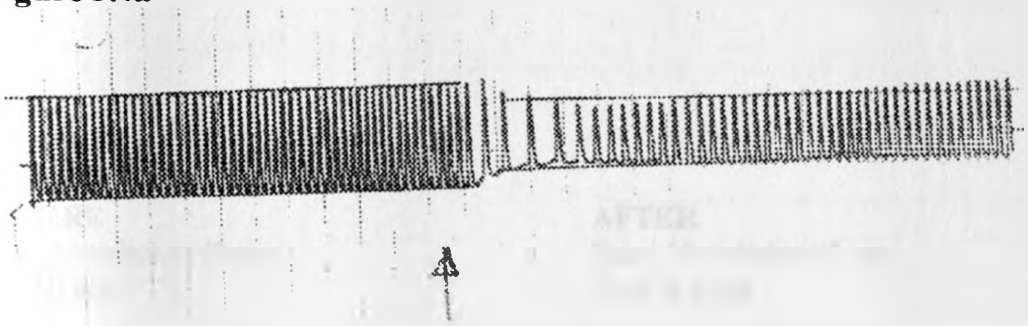


Figure 3.4b

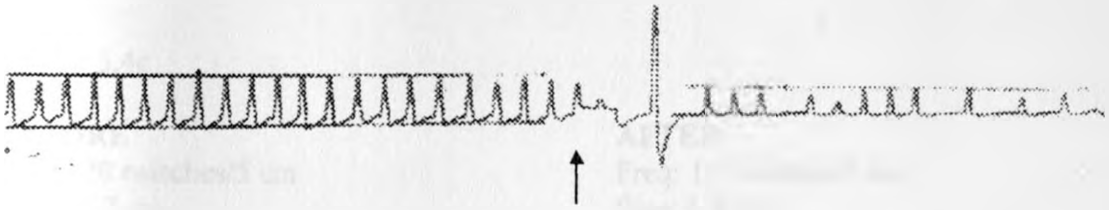


Figure 3.4c

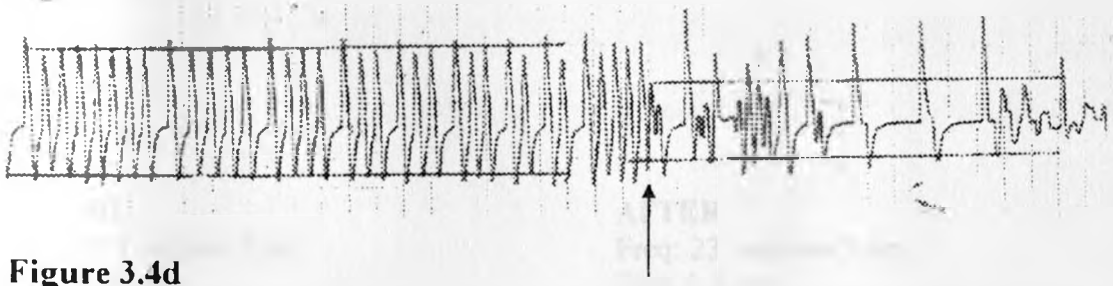


Figure 3.4d

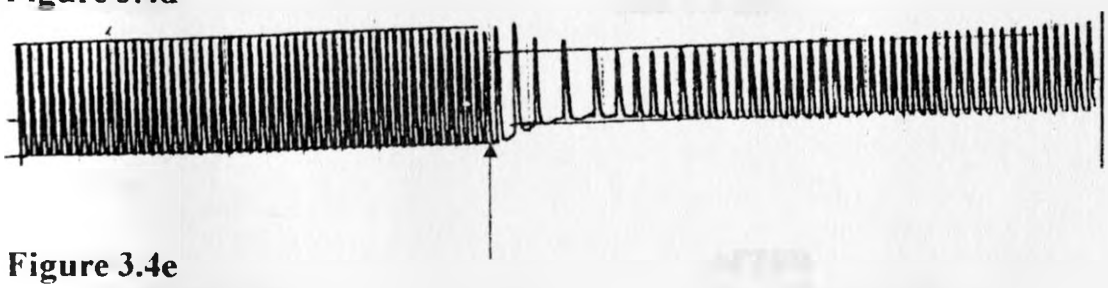


Figure 3.4e

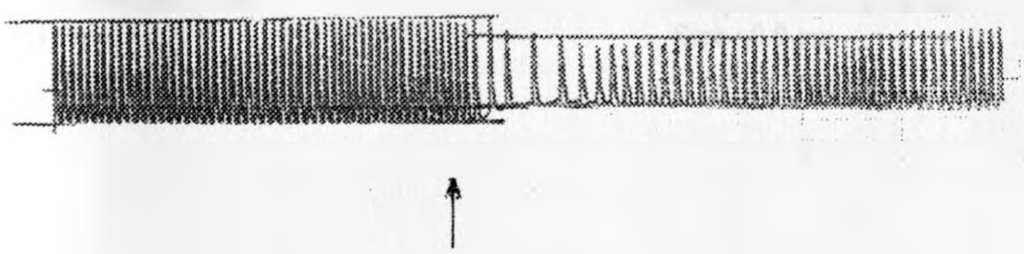


Figure 3.4a

BEFORE
Freq: 45 twitches/5 cm
Size : 1.4 cm

AFTER
Freq: 28 twitches/5 cm
Size: 0.9 cm

Figure 3.4b

BEFORE
Freq: 14 twitches /5 cm
Size: 0.6cm

AFTER
Freq: 10 twitches /5 cm
Size: 0.3 cm

Figure 3.4c

BEFORE
Freq: 20 twitches/5 cm
Size : 1.7 cm

AFTER
Freq: 12 twitches/5 cm
Size: 1.0 cm

Figure 3.4d

BEFORE
Freq: 40 Twitches/5 cm
Size: 1.5 cm

AFTER
Freq: 23 twitches/5 cm
Size: 1.0 cm

Figure 3.4e

BEFORE
Freq: 45 twitches/3 cm
Size :1.4 cm

AFTER
Freq: 28 twitches/3 cm
Size : 0.9 cm

Table 5

Figure Number	Percentage change in Heart Rate	Percentage Change in Heart Force
3.4a	-37.8	-35.7
3.4b	-28.6	-50.0
3.4c	-40.0	-41.2
3.4d	-42.5	-33.3
3.4e	-37.8	-35.7
Mean	-37.3	-39.2
Standard deviation	$\pm$ 5.21	$\pm$ 6.71

The heart rate and the heart force decreased by 37.3 ($\pm$ 5.21) and 39.2 ($\pm$ 6.71) percent respectively on adding 0.3 ml (3.6 mg) *Mondia Whytei* aqueous root extract into the organ bath.

E. 0.4 ml *Mondia Whytei* (4.8 mg)

Figure 3.5a

Figure 3.5b

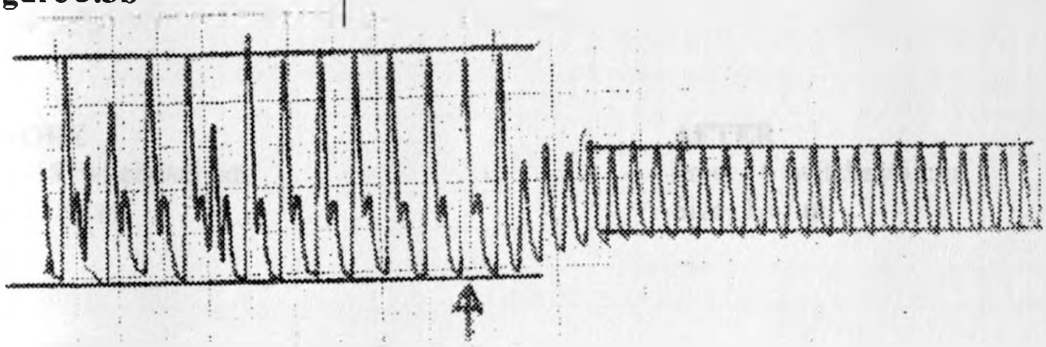


Figure 3.5c

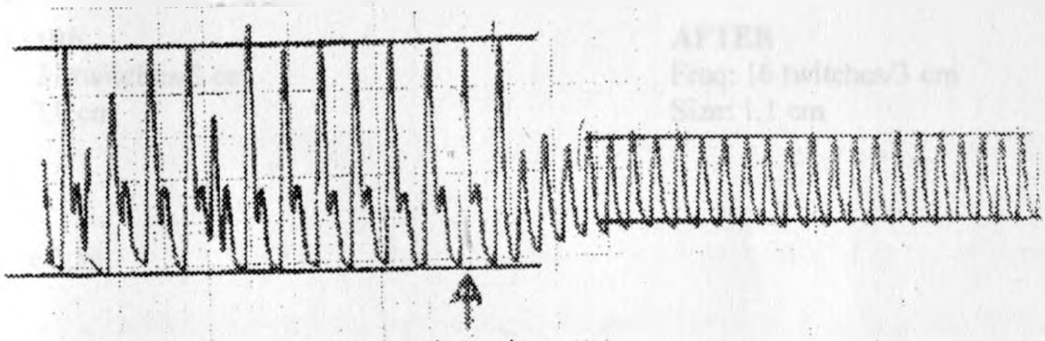


Figure 3.5d

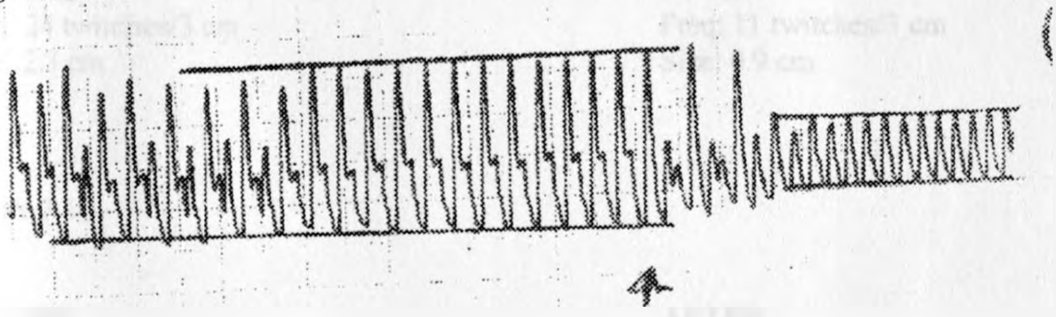


Figure 3.5e

Figure 3.5a

BEFORE
Freq: 13 twitches/5 cm
Size: 0.4 cm

AFTER
Freq: 4 twitches/5 cm
Size :0.3 cm

Figure 3.5b

BEFORE
Freq: 18 twitches/5 cm
Size : 3.0 cm

AFTER
Freq: 16 twitches/5 cm
Size: 1.1 cm

Figure 3.5c

BEFORE
Freq: 21 twitches/3 cm
Size: 3.0 cm

AFTER
Freq: 16 twitches/3 cm
Size: 1.1 cm

Figure 3.5d

BEFORE
Freq: 24 twitches/3 cm
Size: 2.3 cm

AFTER
Freq: 11 twitches/3 cm
Size: 0.9 cm

Figure 3.5e

BEFORE
Freq: 20 twitches/5 cm
Size: 0.4 cm

AFTER
Freq: 4 twitches/5 cm
Size: 0.1 cm

Table 6

Figure Number	Percentage Change in Heart Rate	Percentage change in Heart Force
3.5a	-69.2	-25.0
3.5b	-11.1	-63.3
3.5c	-23.8	-63.3
3.5d	-54.2	-60.9
3.5e	-80.0	-75.0
Mean	-47.7	-57.5
Standard deviation	±12.44	±18.98

The heart rate and the heart force decreased by 47.7 (± 12.44) and 57.5 (± 18.98) percent respectively on adding 0.4 ml (4.8 mg) *Mondia Whytei* aqueous root extract into the organ bath.

a. **Graphical Presentation**

The negative (-ve) sign represented a drop in either the heart rate or the heart force. In plotting the graph, the -ve sign was omitted and the term percentage drop was used instead of the percentage change in the heart rate and heart force.

Table 7

Percentage drop in the heart rate against concentration of *Mondia Whytei*

Concentration of *Mondia Whytei* (mg/ml)	Percentage drop in heart rate (%)
0.00	0.0
0.24	10.2
0.48	15.6
0.72	37.3
0.96	47.7

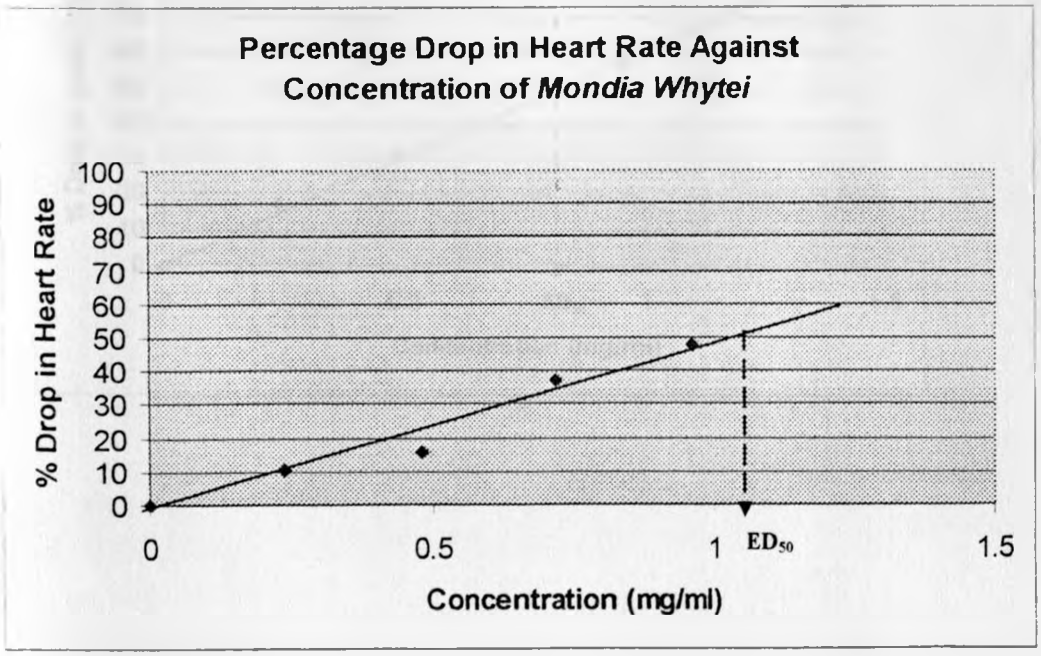
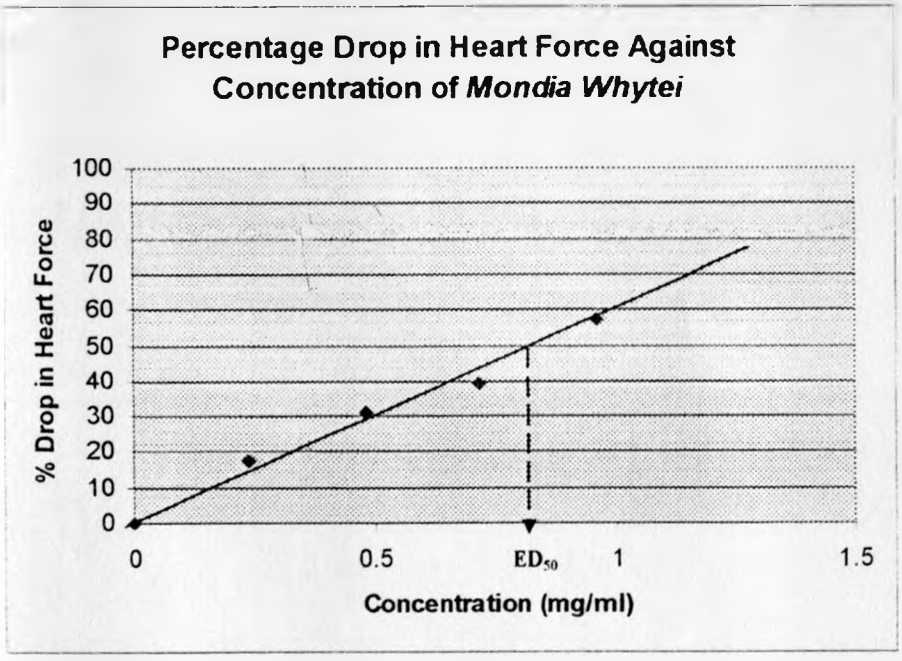


Table 8

Percentage drop in the heart force against concentration of *Mondia Whytei*

Concentration of *Mondia Whytei* (mg/ml)	Percentage drop in heart force (%)
0.00	0.0
0.24	17.5
0.48	31.4
0.72	39.2
0.96	57.5



The amount of *Mondia Whytei* that effectively slowed down the heart rate and reduced the heart force by half the original rate and force respectively (ED_{50}) was determined.

i.e The ED_{50} for the percentage drop in heart force is equal to 0.8 mg/ml. 0.1 ml of *Mondia Whytei* stock solution gives 0.24 mg/ml final bath concentration. Thus, the amount of *Mondia Whytei* stock solution that would give 0.8 mg / ml in the final organ bath is equal to;

$$\frac{0.8 \text{ mg/ ml}}{0.24 \text{ mg/ml}} \times 0.1\text{ml} = \underline{0.3 \text{ ml}}$$

But 1.0 ml stock solution had 12.0 mg freeze-dried *Mondia Whytei*, therefore 0.3 ml is equivalent to 3.6 mg.

Likewise the ED_{50} for the percentage drop in the heart rate equals 5.25 mg of freeze dried *Mondia Whytei*

3.3.0 ISOLATED RABBIT JEJUNUM RESPONSE

a. Effects of *Mondia Whytei* aqueous root extract on the contractility of the isolated rabbit jejunum

Figure 3.6a

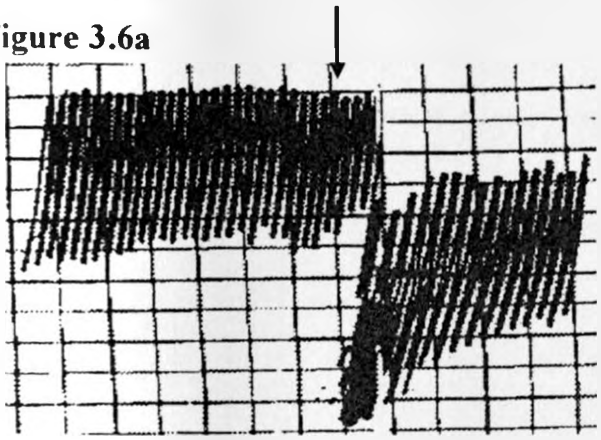


Figure3.6b

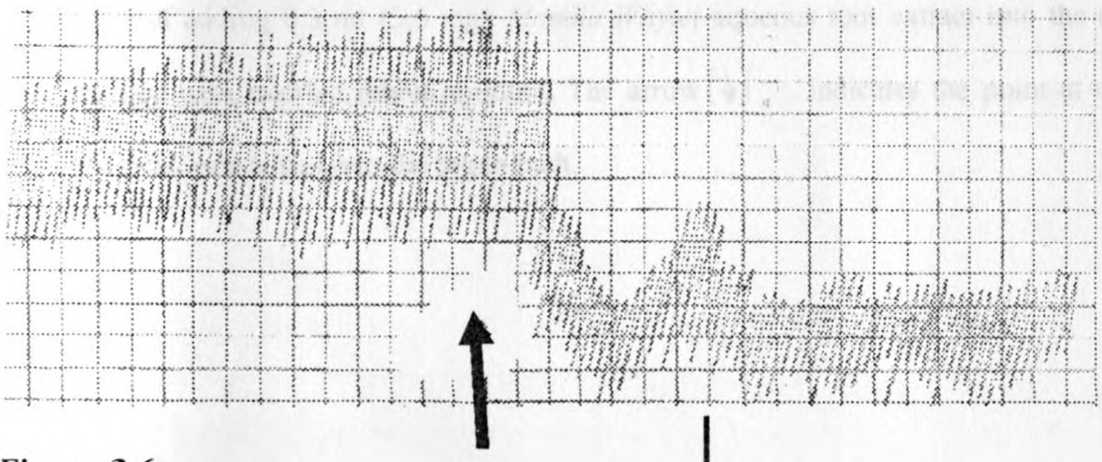


Figure 3.6c

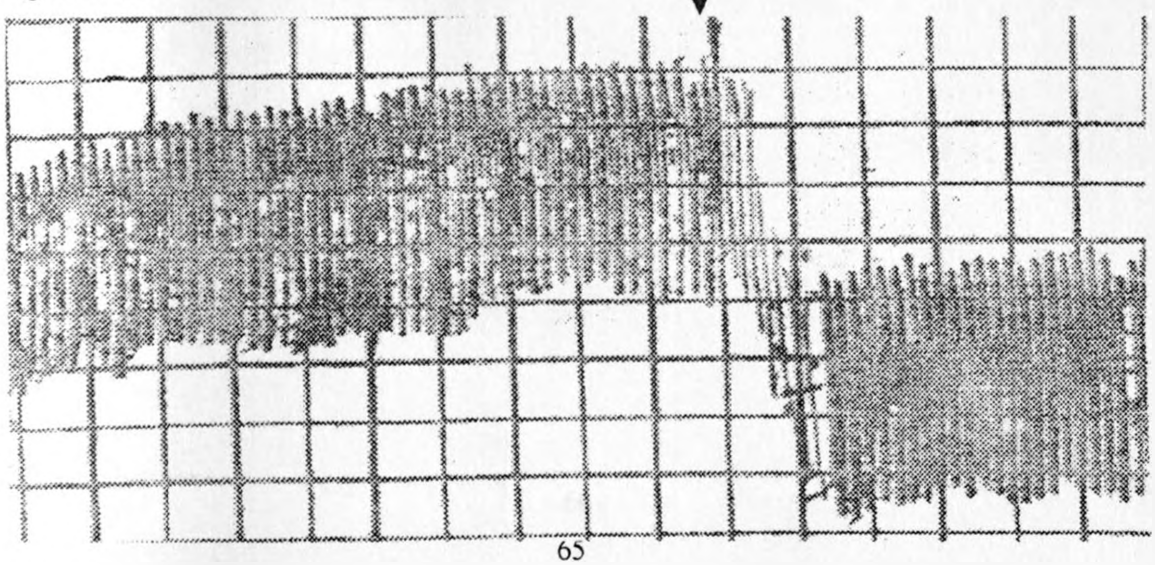


Figure 3.6a, 3.6b and 3.6c

The effects of adding 0.3 ml (3.6 mg) *Mondia Whytei* aqueous root extract into the organ bath mounted with isolated rabbit jejunum. The arrow (↓) indicates the point at which *Mondia Whytei* was added into the organ bath.

Figure 3.6d

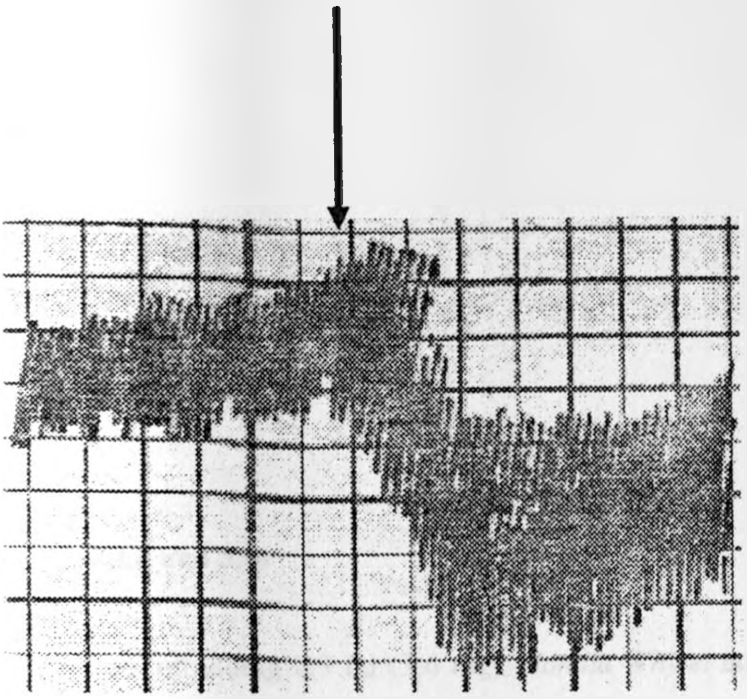


Figure 3.6e

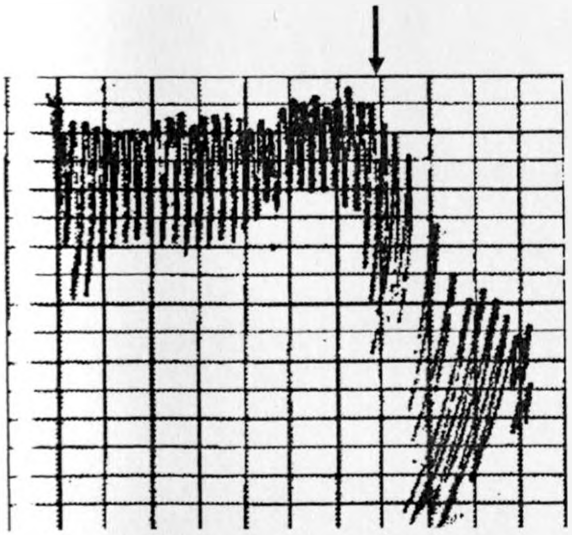
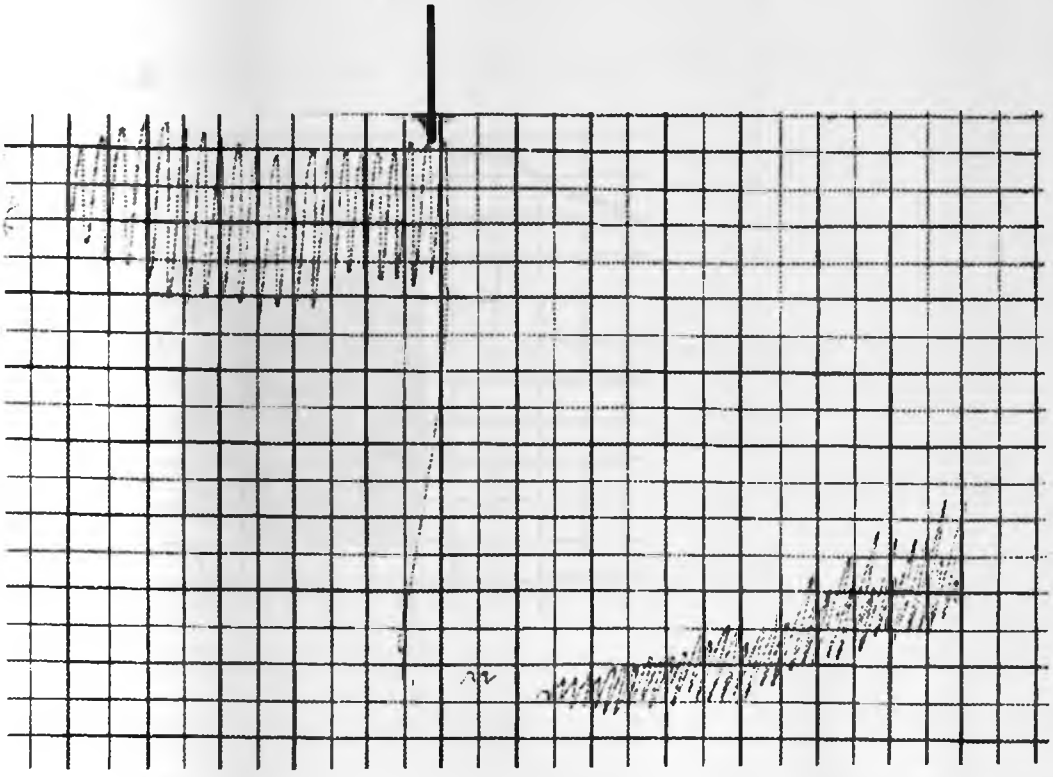


Figure 3.6d and 3.6e

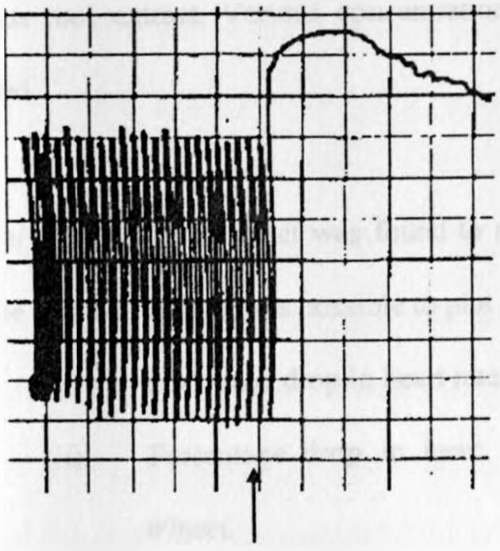
The effects of adding 0.3 ml (3.6 mg) *Mondia Whytei* aqueous root extract into the organ bath mounted with isolated rabbit jejunum. The arrow (↓) indicates the point at which *Mondia Whytei* was added into the organ bath.

Figure 3.7



The arrow (↓) indicates the point at which 2.0 micrograms acetylcholine was added into the organ bath. Acetylcholine caused contraction of isolated rabbit jejunum.

Figure 3.8



The arrow [↑] indicates the point at which 20.0 micrograms adrenaline was added into the organ bath. Adrenaline

4.0. DISCUSSION

The contractility of the rabbit heart was based on the size (height in centimeters) and frequency (number of twitches per unit time) of the twitches before and after adding *Mondia Whytei* aqueous root extract. Various concentrations of *Mondia Whytei* were tested and results tabulated.

Mondia Whytei aqueous root extract was found to reduce both the heart rate and the heart force. From the data obtained, it was possible to plot the graph of;

(i) percentage drop in heart rate versus concentration of *Mondia Whytei*.

(ii) Percentage drop in heart force versus concentration of *Mondia Whytei*.

It was assumed that there was no change in the heart rate and the heart force before adding *Mondia Whytei* since a stable myocardial activity of the heart was maintained. Therefore the graph was plotted as "a line of best fit" starting at zero. This was preferable because the data obtained formed a scatter diagram.

At higher concentration (above 4.8 mg) *Mondia Whytei* aqueous root extract caused a ceasation of the contractility of the isolated heart (Figure 3.5a) but at low concentration it had both negative chronotropic and ionotropic effects i.e the extract reduced both the heart rate and the heart force respectively.

Using the best line of fit, it was possible to determine the effective dose that slowed down the heart rate to half the original rate (ED_{50}). The ED_{50} for the heart force was also determined (Table 7 and 8). The ED_{50} for both the heart force and the heart rate were found to be 3.6 mg and 5.25 mg of freeze dried *Mondia Whytei* root powder.

Figure 3.1a, d and e were recorded from the same isolated heart preparation whereas figure 3.1. b and c were from a different isolated heart preparation. Similarly, figure 3.2 b, c, d and e were recorded from the same isolated heart preparation different from figure 3.2a.

Recording of figure 3.4 a, d and e was done using the same isolated heart preparation whereas figure 3.4 b and c were recorded from a different set-up.

Figure 3.5 a and e are unique from figure 3.5 b, c and d in that they were recorded from a different isolated heart preparation set-up and that the contractility of the heart seemed to cease in a and e.

On the isolated rabbit jejunum, *Mondia Whytei* aqueous root extract caused contraction but the organ seemed to recover its intrinsic activity over a period of approximately ten minutes. There are five possible sites through which an extract can act on a jejunum, these are adrenergic alpha and beta receptors, cholinergic muscarinic receptors, cholinergic nicotinic receptors, drug acting directly on smooth muscles and on the surface of the nerve as a local anaesthetic.

Muscarinic, histaminergic and serotonergic receptors are present on smooth muscles of the jejunum and when stimulated by their agonists they lead to contraction of jejunum. *Mondia Whytei* aqueous root extract could have acted via either of the routes mentioned above or through a mechanism that is yet to be established.

It was also observed that jejunum recovered from the effects (contraction) of *Mondia Whytei* even without washing it. This meant probably that, there could be some specific degrading enzymes which rendered *Mondia Whytei* inactive.

5.0 CONCLUSION

Freeze-dried extract of *Mondia Whytei* root was used in this project. The freeze-drying of *Mondia Whytei* powdered roots made it possible to quantify the amount used in the experiments. It also enabled its storage for a longer time with minimum degradation.

On isolated rabbit jejunum, the extract caused contraction. This contraction was not sustained as the jejunum recovered its intrinsic activity over a period of ten minutes.

On the heart muscle, the extract slowed down the heart rate and the heart force. At high concentrations it totally abolished the myogenic activity of the heart. It was possible to calculate the effective dose that lowered the heart rate and the heart force by half the normal original activity (ED_{50}) which was 3.6 mg of *Mondia Whyteil* root powder.

Decreasing the heart rate and the heart force in a closed circulatory system lowers the blood pressure. Thus, if these two effects were to be reproduced in an intact animal, *Mondia Whytei* could therefore manage hypertension. Also by exhibiting parasympathomimetic activities in the jejunum therefore means that the plant can actually be used to manage dyspepsia since it is a prokinetic agent. This tallies well with the folklore claims.

Therefore, further scientific work should be done in order to isolate pure compounds and elucidate mechanisms through which *Mondia Whytei* aqueous root extract mediates the effects presented here. Likewise, *Mondia Whytei* have extensively been used as an antidepressant agent. In this regard extensive work ought to be done in order to compare it with the existing conventional evidence based antidepressants and delineate its proper use in

this regard. Also its activity at various levels of the nervous system i.e central nervous system, spinal cord level and neuromuscular junction ought to be documented. Toxicological profile ought to be carried out.

During the course of this project, several limitations were encountered namely: financing the whole course as a self sponsored student. Lack of freeze drier machine in the department, financial burden in accessing literature review materials in the internet and cost of typing, printing and binding the booklets from a ccommercial computer bureau to mention but a few and help to appreciate some of the difficulties i have encountered.

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