UTERINE AND GASTROINTESTINAL TRACT RESPONSES TO METHANOLIC EXTRACT OF *CROTON PENDULIFLORUS* IN LABORATORY MAMMALS

BY

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ABSTRACT

Croton penduliflorus of the family Euphorbiaceae is an important medicinal plant in Southern Nigeria. It has been used extensively as a remedy for several stomach complaints. The plant is also known traditionally to be used as abortifacient agent. The present study was undertaken to evaluate some of these claims. The experiments which were both in-vitro and in-vivo were carried out on mice, rats and guinea pigs. The in-vivo studies included the effects of methanolic extract of *Croton penduliflorus* (MECP) on acute toxicity test in mice, basal and stimulated gastric acid secretion (GAS) in rats, and gastrointestinal motility in mice. The other investigation into the effect of MECP on guinea pig ileum (GPI) and uterine contractions in rats were in-vitro.

The plant's seeds were ground using laboratory pestle and mortar. Two extracts of petroleum ether and methanol were prepared from the ground sample using soxhlet apparatus. Column and thin layer chromatographic separation of the extract gave eight precipitates. Toxicity of the extract in mice was tested using conventional method. The effects of MECP, column fractions and histamine on gastric acid secretion were investigated in rats. In

another experiment in rats, the possibility of drug mechanism of action between MECP and histamine on GAS was assessed. Four groups of mice were used to study in-vivo intestinal transit of charcoal using MECP, normal saline, carbachol and atropine. These drugs were given intraperitoneally prior to oral administration of charcoal in tragacanth meal.

The toxicity test gave an intraperitoneal LD_{50} of 891 mg/kg body weight of mice. On the gastric acid secretion, the extract stimulated group of rats secreted significantly more acid than their mean basal acid secretory values at doses of 10^{-4} and 10^{-3} g/ml ($P<0.05$). However, there was no significant change in GAS with a dose of 10^{-5} g/ml extract compared with the mean basal acid secretion ($P>0.05$). There was also a significant reduction in histamine-induced GAS when extract was injected before histamine ($P<0.05$). The study on intestinal transit of charcoal in mice showed significant reduction in the intestinal transit of charcoal in extract treated mice ($P<0.05$) giving a peristaltic index of 1.31 ± 0.88 compared with control that gave 51.90 ± 10.32.

In the in-vitro studies, responses of guinea pig ileum (GPI) and isolated rat uterus strip (IRUS) preparations to extract were dose-

dependent. The contractile responses of ileal smooth muscle strips to extract were inhibited in a dose-related fashion by promethazine (a H_1-receptor antagonist) but not by atropine (a muscarinic receptor blocker). There was also contractile response of GPI strip to fraction; Cf_6 with melting point 210°C. In the IRUS study, the extract-induced increase in frequency of uterine contractions were not affected by oxytocin but were significantly reduced by indomethacin (an inhibitor of prostaglandin synthesis). However, none of the different fractions of the extract showed contractile effect on the IRUS preparation.

The results showed that the extract possesses contractile effect on both the GPI and IRUS preparations. The effect on rat uterus appears to be independent of oxytocin mediation, while that on ileal smooth muscle seems to be involved with H_1-receptor interaction. The gastric acid secretory effect is manifested at high doses which are relatively below the doses required for their contractile activity. The study in mice showed that the extract appears capable of reducing gastric emptying and thus preventing rapid evacuation of the stomach.

DEDICATION

The work is dedicated to Almighty God whose ceaseless grace and continuous mercy have consistently sustained me.

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CERTIFICATION

I certify that this work was carried out by Mr. F. S. OLUWOLE in the Department of Physiology, College of Medicine, University of Ibadan.

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ABBREVIATIONS

TBA	-	Traditional birth attendants
WHO	-	World Health Organisation
MECP	-	Methanol extract of Croton penduliflorus
PEECP	-	Petroleum Ether extract of Croton penduliflorus
GPI	-	Guinea pig ileum
IRUS	-	Isolated rat uterine strip
ENS	-	Enteric nervous system
MMC	-	Migrating motility complex
ATP	-	Adenosine triphosphate
H⁺-K⁺ATPase-		Hydrogen-Potassium-Adenosine triphosphatase
CNS	-	Central nervous system
DMNV	-	Dorsomotor nucleus of the vagus
NTS	-	Nucleus tractus solitarius
VMH	-	Ventromedial hypothalamus
LH	-	Lateral hypothalamus
MFB	-	Medial forebrain bundle
ECL	-	Enterochromaffin-like cell
G-cell	-	Gastrin-producing cell

$M_1/M_2/M_3$	-	Muscarinic receptor subtypes 1,2, & 3
N	-	Normality
I.U.	-	International Unit
g	-	gramme
GAS	-	Gastric acid secretion

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INTRODUCTION

INTRODUCTION

Prior to the advent of orthodox medicine, there was a well developed indigenous medical system in Africa. Under this system the Africans relied on traditional medicine for prophylactic and curative purposes (Seal, 1971). The treatment and prevention of diseases in Nigeria were for many years handled solely by traditional healers. These healers were the equivalent of our present day medical practitioners and medical specialists. The knowledge they accumulated over years of practice was handed down by oral tradition from generation to generation. Some of their methods and practices got mixed up in the process of handing down. However, some survived to the present time (Akubue, 1986). In Africa, this set of people that practice herbal medicine are called "the herbalists", the East Africans call them "bwana mganga". In Nigeria, they are called "dibia" in Igbo language, the Yorubas call them "babalowo" and the Hausas call them "mai maganì" (Kokwaro, 1976;Sofowora, 1982). An earlier theory was proposed that the knowledge of medicinal plants was gained by accident. This theory has been refuted by a number of traditional medicine practitioners, who claimed that information on

plants were communicated to their ancestors in various ways (Akpata and Lambo, 1979). Amongst these traditional healers were some who specialised in specific areas like bone setting (now called orthopaedics), those trained in comprehensive child delivery services who were generally referred to as traditional birth attendants(TBA) or traditional midwives, those specialised in eye diseases, psychiatric medicine and many others. Their practice involved the use of herbs, animals or mineral substances and/or the use of incantations involving some rituals (Akubue, 1986).

Traditional medicine as defined by the World Health Organisation in 1976, is the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance. The practice relies exclusively on practical experience and observations handed down from generation to generation, whether verbally or in writing. The traditional healer, as defined by W.H.O. (1976) is a person who is recognised by the community in which he lives as competent to provide health care by using herbs, animal and mineral substances. Employed in this act are certain methods based on social, cultural

and religious background, as well as on the knowledge, attributes and beliefs that are prevalent in the community. Such methods have regard for physical, mental and social well being and the causation of diseases and disability (Fella, 1986).

The itch for the discovery of prototype drugs from herbs is rapidly gaining ground as it is estimated that, only a fraction (approximately 10%) of the world's plants have been investigated in detail up to date (Stanberg and Bruhn, 1979). The last hundred years have resulted in major advances in the isolation and identification of some active principles in these plants (Stanberg and Bruhn, 1979). The study by the United Nation Commission for Trade and Development (UNCTAD) indicated that about 33 percent of the drugs produced in the developed countries are derived from plants (UNCTAD/GATT, 1974), and that if microbes are added, 60 percent of the medicinal products are of natural origin (Sofowora, 1981).

According to other sources almost 80 percent of the present day medicines are directly or indirectly derived from plants (Myers, 1982). These medicinal plants are therefore the sources of many important scientific drugs of the modern world. Quinine from

Cinchona bark, Reserpine from Rauwolfia root and leaf, Hyoscyamine from Hyocyamus and Datura leaves and roots, Coniine from Conium fruit, Morphine from Opium capsule, Sennosides from Cassia leaf, Colchicine from Colchicum corm and Vincristine and Vinblastine from Catharanthus root. These are a few examples of the modern scientific drugs that have been prepared from the medicinal plants (Ghani, 1986).

The use of herbs has therefore formed an important aspect of traditional medical practice in Nigeria. This has equally stimulated increasing interest in the search for medicinal plants which could be useful in the health care delivery of developing countries. This development has given birth to registered traditional clinics and associations. Many herbal preparations are also openly commercialised in both national and international trade fairs. This shows the level of government awareness and interest in traditional medicine. Presently in Nigeria, scientific work is going on in many research institutes like Ife Institute for Drug Research in Obafemi Awolowo University (formerly, University of Ife) and National Institute for Pharmaceutical Research and Development, Idu, Abuja. The use

of herbs in the treatment of certain diseases is now given prominence due partly to increasingly high cost of orthodox drugs (Assefa, 1987; Salganik et al, 1987; Claveau, 1988).

Among the medicinal plant preparation in current use in Nigeria is the ground form of the seeds of Croton penduliflorus of the family Euphorbiaceae. The medicinal applications of the species in the family varied and include cures for warts and tumours by the genus Euphorbia; E kemeruma, E characias, E polychroma (Hartwell, 1969), the seeds of Jatropha curcas and Ricinus communis are used as anticonceptives (Mameesh, 1963; Okwuasaba et al, 1990). Some species of the Euphorbiaceae family are known to possess purgative property. In most cases the bioactive principle which causes purgation in the seed is present in the oil. The oils of the seed of Jatropha curcas, J gossypiifolia and J multifida are known as powerful purgative. Croton penduliflorus, Croton tiglium and Ricinus communis which are also members of the Euphorbiaceae possess powerful purgative principles which are said to be present in both their seeds and oil (Trease and Evans, 1983; Purseglove, 1988). Interestingly alkaloids are present in Croton

pendulifiorus (Shetty et al, 1983), Croton tigliun, Ricinus communis and Jatropha curcas (Trease and Evans, 1983) which are all known to possess anti-constipatory property. The oil of these specie is formed from esters of the tetracyclic diterpenes; acetic, capric, lauric and palmitic acids for Croton tiglium (Trease and Evans, 1983), palmitic, stearic, oleic, linoleic, myristic and arachidonic acids for Jatropha curcas (Irvine, 1961), ricinoleic and oleic acids for Ricinus communis (Stewart and Bass, 1976), palmitic, stearic and arachidic acids in approximately equimolar concentrations for Croton pendulifiorus (Asuzu et al, 1988). Croton oil if taken internally, is a very rapid, drastic cathartic, and is given in certain cases of apoplexy and of obstinate constipation (Wallis, 1967). This form of the seed is claimed by a traditional herbalist, Mrs. Oyhu Aziga to relieve severe constipation when administered orally with pap (boiled semi-fluid drink from maize flour). The plant is also known traditionally to be used as abortifacient agent. However there is no information regarding this action in the literature. Also, apart from the report of Adesogan (1981) who reported that the plant is used extensively as a remedy from several stomach problems, and that of Asuzu et al,

(1988), who isolated and identified the purgative component in the seed oil as white crystals, no other references are available in literature to show its usefulness in gastrointestinal tract disorders. It is therefore of great interest to study the effects of the seed's extract and its column fractions on gastrointestinal and uterine smooth muscle preparations and its secretory effect on gastric acid. The study which was carried out on mice, rats and guinea pigs examined the effects of methanolic extract of Croton pendulliflorus (MECP) on acute toxicity in mice, basal and stimulated gastric acid secretion (GAS) in rats, gastro-intestinal motility in mice, and the effects of MECP on guinea pig ileum (GPI) and isolated rat uterine strip(IRUS) preparations.

LITERATURE REVIEWS

2.1 Background Information on Croton penduliflorus

The genus Croton belongs to the family Euphorbiaceae. The specie penduliflorus syn. longiracemosus is indigenous to the South-Western part of Nigeria where it is known as aworoso, amoroso and jesebe by the Yorubas (Keay, 1989; Gill, 1992). Its English name is purging croton (Gill, 1992). There are some Croton genera which are found in temperate regions of the South East Asia and in many African countries. Croton tiglium was introduced from India about 1819 (Wallis, 1967). Croton macrostachus is well distributed in Guinea, Ivory Coast and Cameroons (Irvine, 1961).Herbarium information from Botany department of the University of Ibadan, and Forestry Research Institute of Nigeria, Jeri[illegible] Ibadan, Nigeria have identified the following localities where Croton penduliflorus is grown: Ijebu-Ode - Benin Road 30 km west of Ijebu Ode; Ejigbo Agurodo roadside, Osun state; Idanre Forest Research, Ondo state; Iperu, Ogun state; Usonigbe Forest Research , Benin, Edo state; Ikorodu, Lagos state; Kasewe Forest , Sierra-Leone, Relic Forest of University of Ibadan, Ibadan.

2.1.1 Botanical Information

Croton penduliflorus is a forest tree with conspicuous pendulus terminal racemes, sometimes over 30 cm long and crowded with cream-coloured flowers (Keay, 1989). The tree is up to 25m high and 2m in girth. The bore is straight, bark greyish; slash thick, yellowish-brown, more or less granular. The leaves are 5-15 cm long by 3-9 cm broad; broadly elliptic, shortly acuminate, cuneate or rounded at the base, the margin with small regular teeth curving inwards and terminating in a very small gland; the two glands at the base usually conspicuously stalked, sometimes sparsely and minutely stellate hairy on both surfaces. It flowers between May and June, crowded on narrow terminal racemes up to 45 cm long; the females mostly on the lower two-thirds of the central stem and the males at the end. Female flowers with stout, hairy stalks less than 2mm long; ovary is globose, hairy with three prominent styles about 6 mm long, each branching from the base. Male flowers with cream coloured petals 5mm across, on slender stalks about 2mm long. It

fruits between May and August and are three-lobed, shortly stalked, about 10 mm broad and long (Keay, 1989).

2.1.2 Medicinal Uses

The plant is used extensively as a remedy for several stomach complaints (Adesogan, 1981). A leaf infusion is applied externally for fever (Irvine, 1961). The dried-stembark, rootbark and seeds of Croton penduliflorus were reported to possess multicomponents (Shetty *et al.* 1983). Decoction made from the root powder (Gill, 1992), stembark and seeds (Shetty et al. 1983) is reported to be effective as a purgative when taken orally. The oil extracted from the seeds is a severe hydrogogue purgative and is given when efficient action is required (Gill, 1992). The wood decoction in small doses is diuretic and powerful diaphoretic (Gill, 1992). The paste of the powdered seeds mixed with vaseline is applied over tumours and obstinate buboes while the oil is used as liniment for acute rheumatism, arthritis, neuralgia and diseases of the joints (Gill, 1992). The seeds are normally crushed, ground and made into powder form by a traditional herbalist, Mrs. Oyhu Aziga of Jos, Plateau-state who dispenses it for the relieve of constipation when taken orally with cereal pap.

2.1.3 Biological Uses

Chloroform, methanol and petroleum ether extracts of the dried rootbark and dried stembark were tested and have been reported to stimulate increased activity in frog's rectus abdominus muscle (Shetty et al, 1983). Asuzu *et al*(1989) reported that the gut-stimulating principles of Croton penduliflorus seed oil has the ability to stimulate the central nervous system, reduce the potency of opoiods(except codeine) and prolong the duration of their analgesic action. The chronic oral administration of the gut-stimulating crystals of Croton penduliflorus seed oil in both sexes of mice at 21mg/kg body weight showed anti-fertility effects (Asuzu *et al*, 1990).

2.1.4 Biochemical Analysis

Phytochemical tests had revealed the main constituents as alkaloids flavonoids, sterol or triterpenes and the absence of glycosides and tannins in both the leaf and seed of Croton penduliflorus (Shetty *et al*, 1983). The purgative principles, otherwise called gut-stimulating principles in the plant were isolated as white crystals from the seed oil using bioassay guided chromatographic separation process (Asuzu *et al*, 1988). These crystals are mixture of

palmitic, stearic and arachidic acids in approximately equimolar concentration (Asuzu *et al*, 1988).

2.2 Gastrointestinal Tract

2.2.1 Gastrointestinal Innervation

It is well known that the motor and secretory activities of the gastrointestinal tract is under several controlling factors; psychic, neurogenic (nerves) and humoral (chemical agents like hormones). There are both intrinsic and extrinsic components of the nervous control. The intrinsic nervous control is also known as the enteric nervous system (ENS) which is made up of elaborate nerve plexuses of interconnected ganglion cells found within the gut walls. The organisation and function of the ENS is such that, it relies on information generated from all segments of the gastrointestinal tract (Hersey and Sachs, 1995). The mycnteric plexus (Auerbach's plexus) lies between the circular and longitudinal layers of smooth muscle and is primarily associated with coordination of motility. The submucosal plexus (Meissner's plexus) has direct nerve fibre supply to the mucosal cells as well as to the loosely arranged smooth muscle contained within the submucosal layer (Hersey and Sachs, 1995).

Studies based on neuronal tracing using histochemical markers for specific neurotransmitters indicate that submucous ganglia receive a variety of synaptic inputs (Bornstein, Costa and Furness, 1986; Bornstein, Costa and Furness, 1988). These plexuses are involved in local reflexes along the tract. The sensory endings are responsive to a variety of stimuli which include stretch, change in pH, osmolarity and products of digestion. The important feature of the enteric nervous system as with other components of the autonomic nervous system, is that, the postganglionic nerve fibres are polymodal, secreting two or more neurotransmitters (Costa and Furness, 1989).

In many cases individual nerve fibres have been shown to contain both a conventional neurotransmitter, e.g. Acetylcholine or Norepinephrine, and one or more neuropeptides. Another feature of the postganglionic nerve fibres of the ENS is that transmitter release can occur along an extended length of the nerve axon. This is due to the existence of periodic swelling or varicosities that are assumed to be the sites for transmitter release (Wood, 1987). In human and other mammalian species, the mycntcric plexus of the ENS consists of two main populations of neurons that can be distinguished by their content of peptide transmitter. The neurons contain either vasoactive

intestinal peptide (VIP) and its homologue peptide histidine methionine (PHM in human) or substance P (SP) and its homologue substance K (SK) also known as neurokinin (Wattchow et al, 1988; Llewellyn-smith *et al*, 1988).

The autonomic nerves are classified as forming the extrinsic nervous system or precisely described as autonomic nervous system. This nervous control is divided into sympathetic and parasympathetic. The parasympathetic nerve supply to the gastrointestinal smooth muscle comes mainly from the vagus which has its origin in the dorsal motor nucleus. However, the pelvic nerves supply the distal portion of the colon. The parasympathetic fibres, whether vagus or pelvic in origin, do not end directly on the smooth muscle cells but end by making synapses with cells in the ENS (Carlson *et al*, 1922). The vagus fibres innervate the esophagus down to the level of the midtransverse colon. The vagus nerve does not innervate the parietal cells directly but synapse with ganglion cells of the ENS (Radke, Stach and Weiss, 1980). The effects of acetylcholine which is the neurotransmitter released from vagal efferents can therefore be blocked at two levels; at the junction between the vagi and the enteric ganglia (that is, the nicotinic action of acetylcholine)

or at the junction between the enteric neuron and the effector cell (the muscarinic action of acetylcholine). The sympathetic supply to the stomach and small intestine is by way of the splanchnic nerves. The caecum, appendix, ascending and transverse colons are supplied by nerves which arise from the superior mesenteric plexus. The lower part of the rectum is supplied by sympathetic fibres which arise from the upper and lower divisions of the hypogastric plexus, that is , lumbar colonic nerves and hypogastric nerves. The preganglionic sympathetic fibres end in three ganglia namely; the celiac, superior mesenteric and inferior mesenteric ganglia. The sympathetic fibres that reach the intestine are postganglionic (Carlson *et al*, 1922). Most of the sympathetic fibres are concerned with the vasomotor supply of the numerous blood vessels in the intestine but some go to the intestinal smooth muscle in the muscularis mucosae (Kuntz, 1953). The gastrointestinal tract is capable of carrying out its major functions after all the extrinsic nerves must have been severed. This automaticity may be attributed partly to the properties of the smooth muscle. Both the neurogenic and myogenic functions are regulated through the central nervous system reflexes by way of the autonomic nerves.

2.2.2 Gatrointestinal Motility

The type of movement of the intestinal stream depends upon whether the alimentary canal (gastrointestinal tract) contains recently eaten food or is in interdigestive state (Schultz, 1983). It is noteworthy also to mention that, normal digestion and absorption of foodstuffs by the gastrointestinal tract are dependent on the orderly and controlled transit of intraluminal contents. The ever-decreasing volume of contents is propelled towards the rectum. In the rectum, the faecal matter is temporarily stored until defecation, an act involving both voluntary and involuntary movements of the colon, the anal sphincters, and muscles of the pelvic floor, abdomen, and chest (Schultz, 1983).

2.2.3 Propulsion as a concept

The intestine executes a variety of movements within its lumen in order to both mix and move intestinal content along its length. Geometrically the intestine is basically a fluid-containing tube. As a fluid-propelling unit, however, it is an extremely complex unit (Williams, 1983). The concept of propulsion has its origin in that physical science of mechanics (Halliday and Resnick, 1966).

Mechanics is concerned with the study of motion of objects and traditionally views motion as having two different aspects - kinematic and dynamic (Halliday and Resnick, 1966; Fox and McDonald, 1973).

The subspecialty of mechanics called kinematics describes the motion of an object relative to a reference frame and is not concerned with the forces that act on the object to alter its motion. Velocity and acceleration are two examples of kinematic quantities. A number of studies have considered the motion of intestinal content from a basic kinematic viewpoint. Studies of intestinal transport (Cramer, 1959; Summers *et al*, 1970; Bueno *et al*, 1975) are in effect kinematic in nature. An example of such a study is when radioactive or radiopaque particles are placed in the intestinal lumen and the time required for various amount of these particles to move a given distance is determined (Derblom *et al*, 1966). Procedures of this general type permit kinematic quantities such as average velocity to be obtained but provide no information regarding the dynamic aspects of the transport process.

The subspecialty of mechanics called dynamics, on the other hand, deals with motion relative to forces associated with it and to the properties like mass, volume and density of the moving object.

One of the fundamental problems of dynamics is to determine how an object will move when we know the forces that act on it. Inherent in this problem is the idea that a system imparts motion to an object by the application of a force referred to as a propulsive force. The propulsive force ability of any system can thus be regarded as that system's ability to produce and apply propulsive forces. The first study to provide information regarding the intestinal system's ability to do propulsive work on fluid loads was conducted by Trendelenburg in 1917. He demonstrated that in vitro segments of guinea pig ileum have intrinsic ability to propel luminal fluid up a pressure gradient when intraluminal pressure was initially set at a value of 1-2 cm of water. This qualitative observation was confirmed by Kosterlitz, Pirc and Robinson (1956) when they employed Trendelenburg's basic method to begin a semi-qualitative evaluation of some of the intrinsic capabilities of the guinea pig ileum to propel luminal fluid. Other scientists who had employed this method on other animals to study the effects of various agents on peristaltic reflex included Bulbring *et al* (1958), Fontaine *et al* (1973) and Van Nueten *et al* (1973).

2.2.4 Pattern of Gastrointestinal Motility

Bayliss and Starling (1899, 1901) pioneered the basic understanding of intestinal motility. They proposed in their classical studies "the law of the intestine" which stated that, the response of the small intestine to local stimuli consists of a contraction of the smooth muscle above and relaxation below the stimulated area. Shortly thereafter, Cannon (1902) using radiographic techniques to follow the progression of bismuth through the small intestine of fed cats described different patterns of movements of intraluminal contents. These he said, were caused by definite patterns of contractions of the intestinal muscle. These two basic movements are segmentation and peristalsis.

Segmentation was thought to be due to stationary ring of contractions that would divide contents but would cause no net movement in either the oral or aboral direction (Cannon, 1902). Segmentation is now known to be myogenic in nature and involves the alternate contraction and relaxation of the circular smooth muscle layer of the intestine. This is mainly dependent on myenteric plexus (Guyton 1976). Peristalsis was believed to be due to ring of

contractions that occurred in an aboral sequence, thus propelling intraluminal contents in that direction (Cannon, 1902). Peristalsis is neurogenic, that is, it is carried out through a local reflex mediated through intrinsic nerve plexuses within the intestinal wall. These contraction waves are in two phases involving longitudinal muscle contraction and circular muscle relaxation and vice versa. It was later in 1912, that Cannon proposed that, the reflex responsible for the law of the intestine be designated the myenteric reflex, since this reflex will disappear if the intestine is paralysed by applying such drugs as nicotine or cocaine to the serosal surface. The explanation was that, these drugs penetrate the longitudinal muscle layer and paralyse the underlying nervous mechanism. Vasoactive intestinal peptide (VIP) and its homologues have the unique ability to cause relaxation in circular smooth muscle throughout the digestive tract (Biancani *et al*, 1985; Grider *et al*, 1985). Evidence also accrued in the fact that, vasoactive intestinal peptide is released during electrical or reflex activation of myenteric neurons and its release is proportional to the intensity of stimulation and accompanied by a corresponding increase in relaxation (Grider and Makhlouf, 1987). Earlier studies on rat and guinea pig intestine indicated that VIP

motor neurons regulated the descending relaxation component of the peristaltic reflex, whereas substance P (SP) motor neurons regulated in part the ascending contraction component of the reflex (Grider and Makhlouf, 1986). The evidence for SP and substance K (SK) as contractile transmitters in myenteric motor neurons is based on substance P and SK causing direct and cholinergically mediated contraction (Souquet *et al*, 1987), and their release during electrical or reflex activation of myenteric neurons (Grider and Makhlouf, 1988).

The renewed interest in intestinal motility resulted in the clear recognition of a distinct pattern of contractions called Migrating motility complex (MMC). Reinke *et al* (1967), and Carlson *et al* (1970) described MMC as a cycle of contractile activity from any site on the small bowel of dogs that had been fasted for twelve hours or more. These electrical events of the MMC indicate a pattern of motility. In each cycle, there is a quiescent period of around 50 minutes without contractions, followed by a period of 10-20 minutes of seemingly random contractions; these built up to a 10-20 minute period of large amplitude contractions at the maximum frequency seen in that portion of the bowel. The contractions end up abruptly and the cycle

starts again. Carlson *et al* (1972), Code and Marlett (1975) further described this cyclical activity and named it the interdigestive myoelectric complex. They divided each complex into four phases. Phase 1 equalled the phase of no activity. Phase 2 was the period of seemingly random activity. Phase 3 equalled the period of intense activity, while phase 4 was the transition period between phase 3 and 1. Migrating motility complex is associated with bursts of depolarisation from the esophagus to the ileocecal valve at frequencies of one every 1½ - 2 hr. The depolarisations are coincident with wave of contractions. This results in a propulsive movement which empties the upper gastrointestinal tract to the caecum (Frank, 1981). The term "interdigestive" can no longer be applied universally because the pattern is seen in some animals feeding ad lib (Ruckebusch and Fioramonti, 1975). It was found that feeding failed to interrupt the MMC in the transplanted isolated jejunal loops (Sarr and Kelly, 1981). During fasting, the volume of liquid passing through a jejunal segment per unit time was greatest during phase 3 and least in phase 1. Also, after feeding, the movement of liquid was similar to that found during phase 2 (Sarr

and Kelly, 1980). This pattern of motility is known to effectively 'clean out' the stomach and small intestine.

Some of the Factors affecting Migrating Motility Complex

1. Motilin, is an intestinal hormone with its producing cells mostly found in the duodenal mucosa. It is taken as a candidate hormone to initiate migrating motor complexes in the stomach as its concentrations rise with the onset of the gastric migraing motor complex in the fasting state. It was also noted that, exogenous motilin raised the plasma immunoreactive motilin level and thus induced phase 3 activity of the MMC in the stomach and duodenum of dog (Lee et al, 1978) and man (Vantrappen et al, 1979).
2. Somatostatin was found to inhibit motilin induced MMC in the dog (Ormsbee et al, 1978).
3. Pancreatic polypeptide: Bueno *et al* (1982) suggested that pancreatic polypeptide may be involved in the inhibition of the MMC in the dog.
4. Lipid meals inhibited the MMC for longer periods than carbohydrates or proteins (Rees et al, 1982).

5. Human factor: Observations over 24 hours with pressure sensing capsules indicated considerable human variation in the length of phases of the MMC within and between subjects (Thompson et al, 1980).

6. Sleep: Sleep significantly reduced the duration of phase 2 of the MMC (Thompson et al, 1980). Point of origin of MMC during sleep is mostly from the duodenum (Finch et al, 1982).

7. Peptide YY (PYY) is a 36 amino acid polypeptide isolated from the intestinal mucosa (Tatemoto, 1982). The peptide inhibited interdigestive migrating motor complexes in the innervated canine stomach but did not affect contractions of denervated gastric pouches (Sukuki *et al*, 1983).

2.3 THE STOMACH

2.3.1 Anatomy

The stomach is divided into four portions (Fig. 2.1). From the diaphragmatic end, these are the cardia, fundus, corpus (body), and antrum. The cardiac area is located distal to the entry of the esophagus, and is a few millimetres in length. It contains entirely mucous cells but no parietal cells or chief cells. The fundus is the dome-shaped portion of the stomach that extends above the cardia.

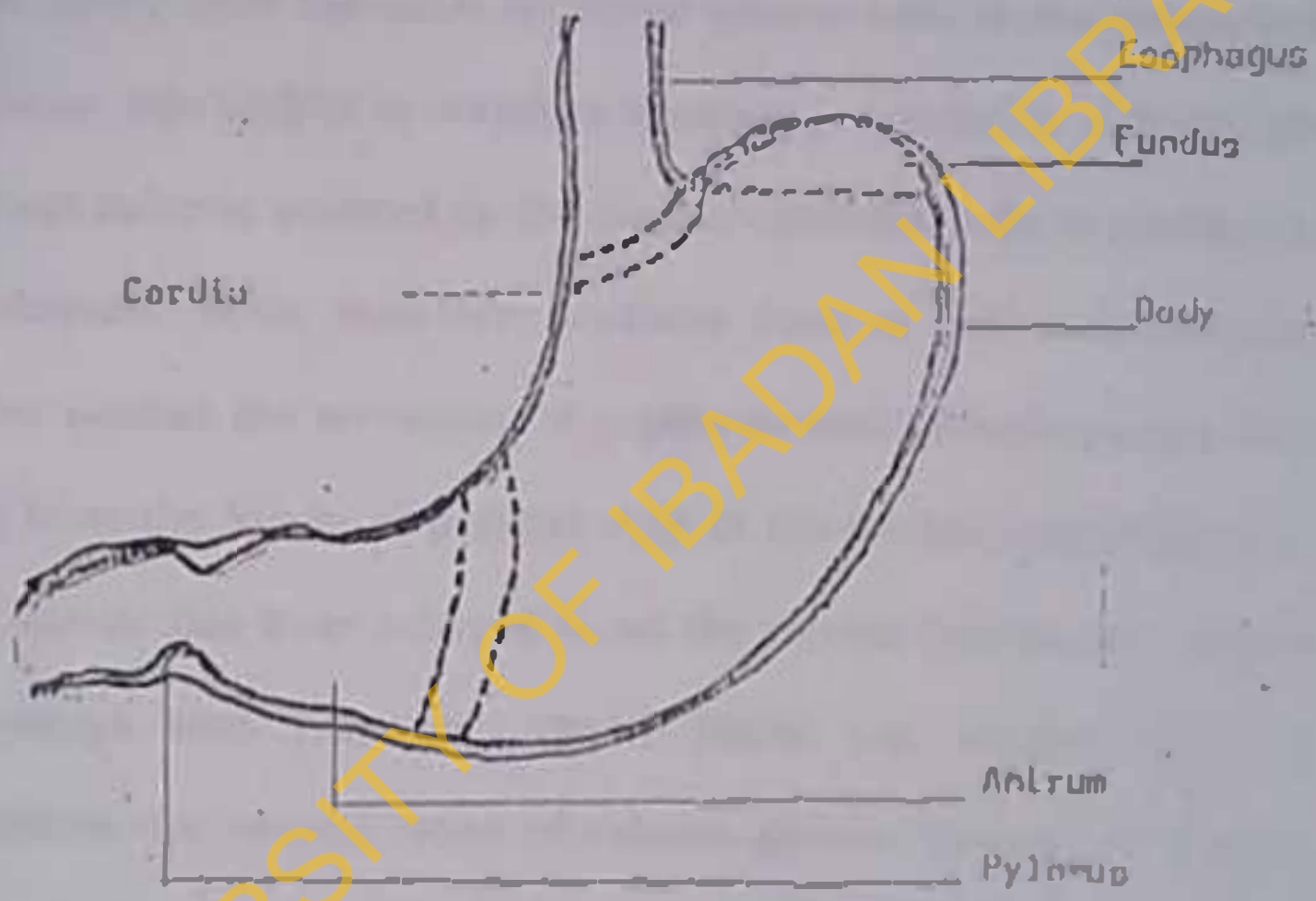

Fig. 2.1: Shows the various divisions of the stomach Redrawn from "Basic Histology. 291p. Longe Medical Publications. 2nd Ed. 1978

The body of the stomach is the largest portion representing about 80% of the entire stomach. The gastric antrum, or antral gland area occupies about 20% of the stomach area (Ito, 1987). The gastric mucosa lining is made up of columnar epithelial cells. This epithelium secretes mucous. The mucous membrane of the stomach is separated from the outer muscular gastric wall by the muscularis mucosae. The surface is therefore constantly covered by a thick layer of tough mucous secreted by the surface epithelial cells in addition to bicarbonate. Both secretory products form a rather continuous barrier against the movement of pepsin as well as hydrochloric acid (HCl) from the lumen of parietal cells to the surface epithelial cells. This barrier has been referred to as the mucus-bicarbonate barrier (Flemstrom and Turnberg, 1984). Below the surface layer of epithelium are various types of tubular glands. Parietal or oxyntic cells are found in the fundus and body of the stomach. These gastric glands are tubulo-vesicular in nature, as they open on a common chamber called gastric pit (fovcolus) that opens in turn on to the surface of the mucosa (Ganong, 1989). A gland is divided into three areas; the isthmus, the neck and the base. The first two areas are well distributed with parietal cells while the base contains mainly of

chief cells. Other cells found in the gland are those of mucous and endocrine.

2.3.2 Gastric Secretion

The stomach serves as a temporary reservoir for food after the ingestion of meals. However, it has a number of important physiological functions among which are, to secrete hydrochloric acid at a concentration of 160meq/1, the proteolytic enzyme pepsins, the intrinsic factor necessary to bind with Vitamin B_{12}, also known as extrinsic factor, so as to facilitate the absorption of vitamin B_{12} in the terminal ileum, the polypeptide hormone, gastrin which stimulates the parietal cell to secrete the highly acidic juice, bicarbonate, serotonin, somatostatin as well as a variety of mucoid substances including blood group substances. The other components of gastric secretion are water and electrolytes which are transported by the cells lining the gastric glands.

2.3.3 Production of gastric acid by the stomach

In an attempt to explain the secretion of acid, Bernard (1858) had suggested that the stomach secreted a precursor substance that was converted to acid in the stomach lumen. However, Golgi (1893) was the first scientist to postulate that the gastric parietal cell was

the source of this acid. This was based on his obscrvation that the intracellular canaliculus of the parietal cell expanded during active secretion. The actual presence of acid in the canaliculus was established by Dibona *et al* (1979) and Berglindh *et al*(1980). The canaliculi are extensions of a system of fine canals with projecting microvilli. The microvilli of the secretory canaculi are long and numerous and face the lumen of the stomach. They serve to increase the luminal cell surface area by approximately four to fivefold. H^+/K^+-ATPase (the enzyme involved in the final step of H^+ secretion) is located within the cell membrane of the intracellular canaliculi. Immediately beneath the microvilli are multiple tubulovesicles. When gastric acid secretion is stimulated, the microvillar surface increases rapidly, and is accompanied with a reduction in the number of vesicles. Current evidence indicates that tubulovesicles fuse with the cell membrane and thereby expand the intracellular microvillar membrane (Schofield *et al*, 1979).

2.3.4 Mechanism of gastric acid secretion

The accepted concept that gastric acid secretion is not a continuous process but a rather tightly regulated process controlled by a variety of mechanisms was deduced by Beaumont (1833). This

assertion originated from the survival of an 18 year old boy Alexis St. Martin from an accidental discharge from a shot gun. He was however left with a permanent fistula. Davenport (1939) was the first scientist to give a clue to this observation in his carbonic anhydrase theory of gastric acid secretion. In subsequent study concerning the mechanism of acid formation by the stomach, it was generally accepted that only water could supply sufficient protons to account for the observed rate of gastric secretion. Subsequent studies were then focused on the possible mechanisms by which the parietal cell could separate H^+ and OH^- (Davies, 1951).

(a) **Carbonic anhydrase theory**

Davenport (1939) demonstrated that the parietal cell of the gastric mucosa contains a high concentration of the enzyme, carbonic anhydrase. The reactions leading to the elaboration of hydrochloric acid by these cells were analogous to the well established chloride shift in the red blood cells explained below:

Chloride-shift of red blood cells:

The red blood cell membrane is a specialised membrane which is extremely permeable to anions and specifically permits the rapid exchange of Cl^- and HCO_3^-. The cations pass through it very slowly.

When blood is in tissue capillaries, bicarbonate ions formed inside the red cells as a result of hydration of carbon-dioxide and the release of hydrogen ions in the presence of carbonic anhydrase diffuse into the plasma. Since these ions cannot be accompanied by cations, the red cells are left with a positive electric charge which tends to draw anions into the cells. Chloride ions being the most abundant anions migrate inward in exchange for bicarbonate (chloride-shift). These reactions in the parietal cells are suggested to be a reversal of the red blood cells chloride shift mechanism. Unlike the entry of chloride ions into red blood cells, chloride ions leave the parietal cells for gastric lumen. Davenport then proposed two assumptions based on his discovery. These were stated thus carbonic anhydrase played a necessary role in the hydration of carbon-dioxide to bicarbonate ions, thereby permitting the exchange with an agent which partially blocked carbonic anhydrase activity, also, that the formation of bicarbonate and the reversed chloride shift should be directly proportional to the fraction of uninhibited enzyme.

Few years later, Davenport courageously retracted the second assumption in his write up tagged "In Memoriam" where he suggested thereafter that, as in the case of erythrocytes, there is an

enormous carbonic anhydrase in parietal cells such that, every fractional inhibition of enzyme leaves enough enzyme still uninhibited, thus any conceivable amount of catalysis which could be required by the secretion of the cells could still be carried out. The first assumption was equally disputed. Neither sulphanilamide nor thiophane-2-sulphonamide, each a potent inhibitor of carbonic anhydrase inhibited gastric acid secretion in mammals when the drugs were present in gastric mucosa in concentrations sufficient to inhibit more than 99% of the enzyme. The most plausible explanation given was that, increase in the rate of reaction by catalysis (Carbonic anhydrase) is not necessary as the uncatalysed rate of hydration of carbon-dioxide at the temperature of mammalian body is fast enough to provide all the carbonic acid required by the acid secreting mechanism.

Alternatively, other explanations are needed for the source of hydrogen ions secretion.

(b) **Oxidation and Reduction theory**

In 1957, Davenport put forward another view in which, he proposed that, the secretory process is a cyclic oxidation and reduction process (Fig. 2.2). According to Davenport (1961), he

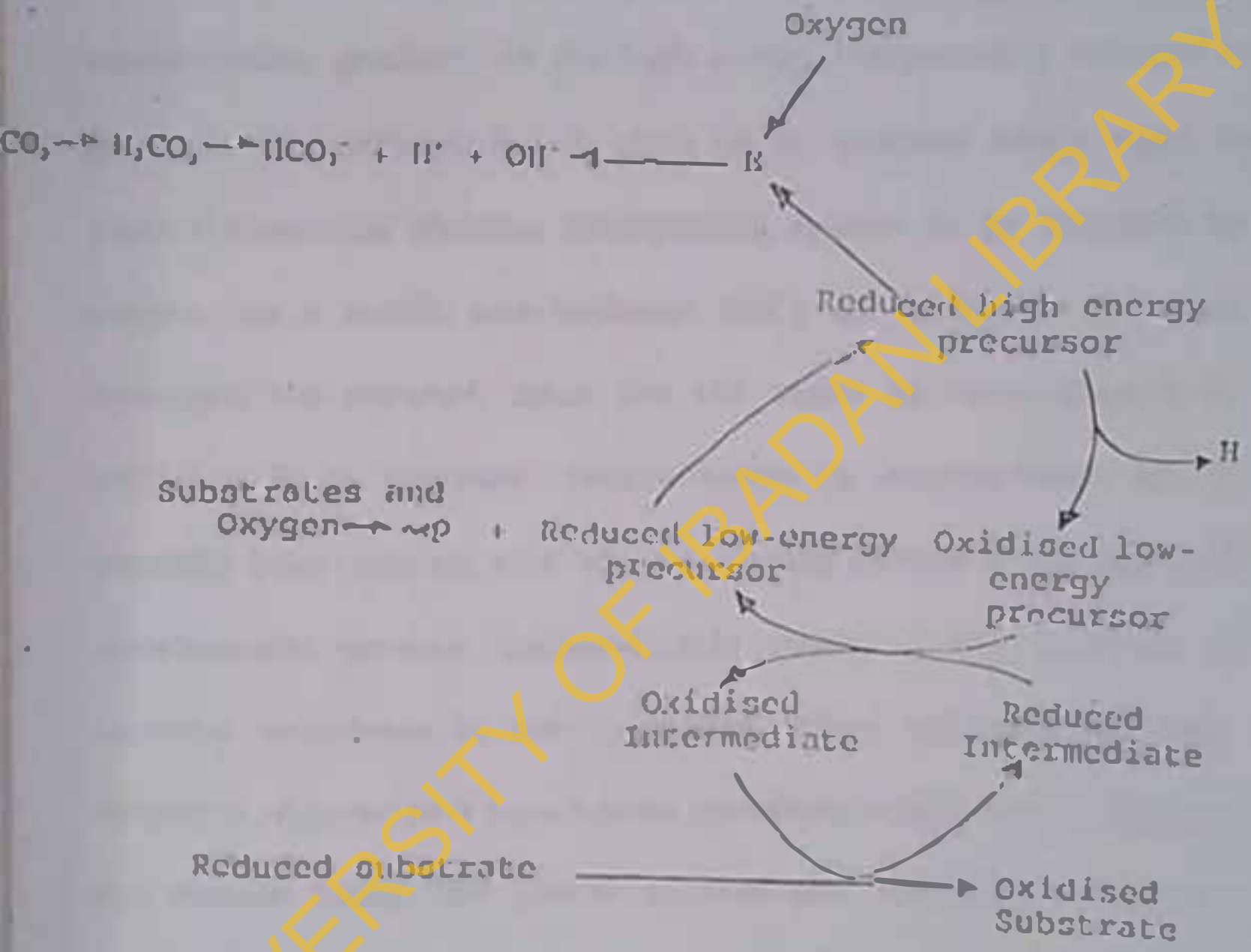

Fig. 2.2: Shows a scheme of the metabolic basis of gastric acid secretion (Davenport, 1957).

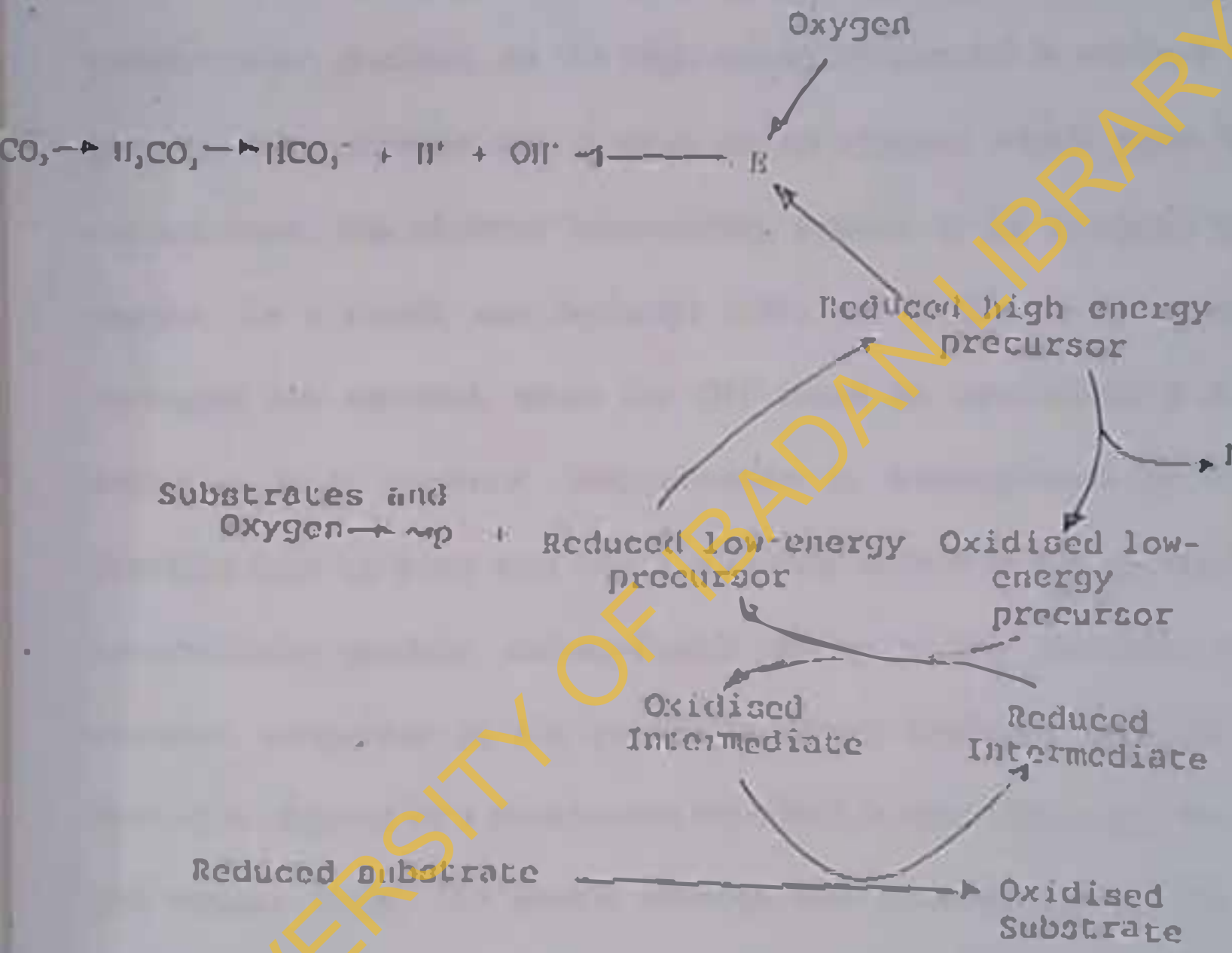

Fig. 2.2: Shows a scheme of the metabolic basis of gastric acid secretion (Davenport, 1957).

reported that, the crucial reactions in hydrogen ions secretion is an oxidation in which a high energy intermediate compound is oxidised to a low-energy product and a hydrogen ion. The energy liberated is used to transfer the hydrogen ion into the gastric juice against the concentration gradient. As the high energy compound is oxidised to generate the hydrogen ion, it gives up an electron which must be carried down the electron transporting system to be accepted by oxygen. As a result, one hydroxyl (OH^-) ion is formed for every hydrogen ion secreted, since the OH^- must be neutralised if H^+ secretion is to continue. Neutralisation is accomplished by H^+ provided from carbonic acid which is rapidly formed in the cell from metabolically produce carbon-dioxide owing to the presence of carbonic anhydrase in the cytoplasm. Every hydroxyl ion (OH^-) formed is replaced by a bicarbonate ion which is then discharged into the venous blood. The gastric mucosa also possesses a chloride pump. Chloride ions move from the cell into the gastric juice not only against a concentration gradient but, also against the electrical gradient of 60 mv (negative), that is, the mucosa with respect to the serosa surface of the stomach. Bell *et al* (1982) were in agreement with the metabolic basis of gastric acid secretion going by their

assertion that, the concentration of hydrogen ions in the parietal cells' secretion is a million times greater than that of the plasma. Therefore, there is an energy required to drive the secretion of hydrogen ions against this concentration gradient called aerobic-oxidation.

(c) **H^+ - K^+- ATPase theory**

H^+- activated ATPase was identified by Ganser and Forte (1973) and Sachs *et al* (1976) as being unique to gastric membranes. It was also found to be activated by K^+ but not Na^+ as it was not inhibited by ouabain, a selective inhibitor of sodium pump. The enzyme was described as catalysing an electroneutral exchange of H^+ for K^+ and hence so designated H^+-K^+-ATPase as the gastric proton pump. The identification of the pump was however solved by the discovery of omeprazole as a proven highly valuable inhibitor for investigating the mechanism of gastric acid secretion (Fellenius et al, 1981).

Using vesicle preparation from isolated parietal cell microsomes, H^+-K^+-ATPase, an enzyme isolated by Sachs and Berglindh (1981) was found to be responsible for the take up of hydrogen ion and the release of potassium ion inside the vesicle in the presence of ATP (Fig. 2.3).

Key:

Cnl = Canaliculus

Ca = Carbonic anhydrase

S = Symport

A = Antiport

P = Pump

C = Conductance

Fig 2.1: Shows a model of secretant parietal cell and the various pathways.
(Adapted from Sachs and Berglindh, 1981).

The model proposed for the secretory parietal cell shows that, there are two levels of ionic exchanges in the parietal cell. These are at the basolateral surface of the oxyntic cell and on the canalicular membrane.

On the basolateral surface, two exchange mechanisms are involved, a bicarbonate-chloride exchanger, which is carrier mediated, and a sodium-potassium pump (active). In addition to this, is another portal for the entry of sodium chloride. Located on the canalicular membrane are the ATP-driven pump, H^+-K^+-ATPase and a port for the exit of potassium chloride. The primary source of the secreted H^+ is the ionisation of water. The carbonic anhydrase located near the H^+-K^+-ATPase provides a mechanism for converting the remaining hydroxyl ions (OH^-) to bicarbonate ions (HCO_3^-) (Vander *et al*, 1985). The bicarbonate exchanges for chloride ions. The chloride ions now pass through conductive pathways across the luminal surface of the cell. The enzyme, H^+-K^+-ATPase is responsible for the active secretion of H^+ into gastric juice in exchange for K^+. High intracellular K^+ and low Na^+ concentrations are maintained by the Na^+-K^+-ATPase at the basolateral membrane, while K^+ also moves through conductive pathways into the lumen thereby producing K^+

for the H^+-K^+- ATPase across the canalicular membrane. The overall process generates H^+Cl^- secretion that is followed by passive water flow (Pandol, 1990).

2.3.5 Regulation of Gastric Acid Secretion:

Regulatory mechanism of gastric acid secretion can be broadly grouped into central, that originating from the central nervous system, and peripheral, that due to intrinsic mechanisms of the stomach itself. These terms will replace the former description; "phases". Cephalic phase will be equivalent to central nervous system (CNS) originating control, while both gastric and intestinal phases are grouped as peripheral control (Hersey and Sachs, 1995). It is generally recognised that sight , smell, taste or thought of food can stimulate acid secretion. however, it is less well appreciated that the strongest central stimulus is hypoglycaemia (Simili *et al*, 1927).

2.3.6 Central Regulation

Dorsomotor nucleus of the vagus (DMNV) integrates sensory inputs arising primarily from these central structures: hypothalamus and nucleus tractus solitarius (NTS) for visceral input (Kadekaro *et al*, 1980). These assertions are made on the premise that DMNV supplies stimulatory efferent fibres to the stomach through the vagus

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nerve (Fox and Powley, 1985; Gwyn *et al*, 1985), destruction of the DMNV eliminates central stimulation of acid secretion (Kerr and Preshaw, 1969), whereas electrical stimulation of the DMNV results in a strong secretoty response (Shiraishi, 1980; Wyrwicka and Garcia, 1979).

The ventromedial hypothalamus (VMH) appears to exert a tonic inhibitory influence on acid secretion as its destruction enhances secretion (Ridley and Brooks, 1965), while electrical stimulation of VMH suppress acid secretion (Ishikawa *et al*, 1983). The influence of VMH on gastric acid secretion is indirect, as it was found that stimulatory signals from both lateral hypothalamus (LH) and median forebrain bundle (MFB) were inhibited by VMH (Kadckaro *et al*, 1980). Both LH and MFB are shown to be responsible for glucoprivial or hypoglycaemic stimulation of acid secretion (TerHorst *et al*, 1984; Colin Jones and Himsworth, 1970).

The nucleus tractus solitarius (NTS) receives major afferent inputs of the taste fibres and viscerals from synapses in the inferior ganglion of the vagus (Hoffman and Schnitzlein, 1961). Also, NTS responded directly to glucose deprivation with a strong stimulation of acid secretion mediated through DMNV (Kndekaro *et al*, 1980).

2.3.7 Peripheral regulation

The regulation of gastric acid secretion is achieved in the periphery by interplay between three major gastric endocrine cells; the enterochromaffin-like (ECL) cell producing histamine, the gastrin or G cell producing gastrin, and the somatostatin or D cell producing somatostatin (SS) (Sachs *et al*, 1997). Regulation of these cells is via stimulatory or inhibitory paracrine, endocrine, and neural pathways (Sachs *et al*, 1997).

Histamine

Popielski (1919) was the first scientist to discover that histamine is a potent stimulus for gastric acid secretion. It was difficult to accept this property of histamine for several years due to the fact that there was lack of inhibition of acid secretion by conventional antihistamine drugs and also that histamine was undetectable in blood plasma during physiological stimulation of gastric acid (Code, 1965; Johnson, 1971). It was Black *et al* (1972) who showed that histamine is a secretagogue when they successfully developed selective antagonist drugs which blocked gastric histamine receptor, the H_2 receptor. Further light was thrown on the acceptance

of histamine as secretagogue by Larsson *et al* (1979) when they developed the paracrine regulation concept, which suggested that histamine could be released within the gastric mucosa and act locally without reaching sufficient concentrations to be detected in blood. Subsequently there was conflict on the source of gastric histamine for acid secretion. It was formerly thought that a specialised type of mast cell was responsible for releasing the histamine associated with acid secretion (Soll *et al*, 1988). However, in the rat, a specialised endocrine cell of the stomach enterochromaffin-like cell (ECL) was obviously known as the candidate for histamine release for the stimulation of acid secretion, since it is the major histamine containing cell; but this cell type is less abundant in species like human and dog, where mast cells are also a prominent source of histamine (Hakanson et al, 1986).

More recent studies have favoured the release of gastric histamine required for acid secretion from the ECL cell (Prinz et al, 1993). These ECL cells were found to comprise one - third to one-half of the endocrine cells in the oxyntic (acid secreting) mucosa of most vertebrate (Simonsson et al, 1988). Hakanson *et al* (1986) had shown by immunochemistry that the ECL cells store histamine, while

Kubota *et al* (1984) also showed that these cells contain histidine decarboxylase, the enzyme required for histamine synthesis.

Gastrin

Gastrin is formed primarily in the G-Cells of the gastric antrum and duodenum, with small amounts located in the pituitary and some vagal nerve fibres (Dockray and Gregory, 1989). The fetal and neonatal pancreas produce gastrin, and this was thought to be the source of neonatal hypergastrinemia (Euler *et al*, 1977); Lichtenberger, 1984). A heptadecapeptide, G-17 gastrin is the predominant form found in the circulation, although both shorter and longer forms have been reported to also exist (Dockray and Gregory, 1989). Gastrin shares an identical pentapeptide amide with CCK, but there is however, a differential effect of these peptides on acid secretion (Soll *et al*, 1985; Dockray and Gregory, 1989).

The G-cells of the gastric antrum are of the "open" type with their apical surfaces reaching the glandular lumen (Polak, 1989). It is therefore postulated that chemical effectors bind to the apical membrane to exert their influence (Giraud *et al*. 1987). Specific chemical components of food have been proposed to modulate the release of gastrin. Among the effectors are the pH of the antral

contents, which at values less than 3 completely suppress gastrin release (Elwin, 1974; Walsch and Grossman, 1975), the presence of amines and specific amino acids (Lichtenberger *et al*, 1982), peptone, a partially digested protein mix (Elwin, 1974). Also, significant distension of the antral portion of the stomach results in enhanced release of gastrin (Schiller *et al*, 1980; Hirschowitz, 1989). Neutrally released gastrin either that locally initiated by distension or through the vagus is mediated by gastrin - releasing peptide - GRP (Schubert *et al*, 1985). GRP is localised to enteric nerve fibres both in the antrum and in the fundus (Dockray *et al*, 1979; Buffa *et al*, 1982), and its release from enteric nerves of the antrum appears to occur at least in part, through nonmuscarinic cholinergic (nicotinic) pathways (Schubert and Makhlouf, 1982; Schubert *et al*, 1985).

Somatostatin

Somatostatin was first characterised as a tetradecapeptide (SOM-14) from Ovine hypothalamus that inhibited secretion of growth hormone, also a peptide with similar properties was then identified in stomach (Brazeau *et al*, 1973; Arimura *et al*, 1975). The peptide inhibited both basal gastric secretion and hormone induced secretion when administered by both intravenous and intragastric

routes (Konturek *et al*, 1976; Johansson *et al*, 1978). A 28-residue NH_2-terminally extended form of somatostatin (SOM-28) was isolated from porcine duodenum (Pradayrol *et al*, 1980). Both SOM-14 and SOM-28 forms of mammalian somatostotin were derived from a common precursor of 116 residue, and were found to inhibit acid secretion and gastrin release (Arimura *et al*, 1975). SOM-14 is over five times more potent than SOM28 in inhibiting gastric acid secretion (Seal *et al*, 1982). Somatostatin exerts a tonic inhibition on both the basal parietal cell and G-cell secretions (Debas and Carvajal, 1994).

In addition, somatostatin was found to depress pancreatic endocrine and exocrine function in man (Albertin *et al*, 1973; Hanssen *et al*, 1977), has a stimulatory effect on gastric mucus production (Johansson and Aly, 1982), decreased the level of intrinsic factor (Bloom *et al*, 1974; Barros *et al*, 1975; Schrumpf *et al*, 1978) and reduced gastric motility and splanchnic blood flow (Keller *et al*, 1978; Sonnenberg *et al*, 1981).

Gastrin and CCK interaction on D-cells

CCK (CCK - 8,) stimulates somatostatin secretion by both the CCK -A and CCK -B/gastrin receptors, while gastrin acts via the CCK

-B/gastrin receptor alone (Zavros and Shulkes, 1997). These receptors are present on D-cells of the antrum and fundus (Song *et al*, 1996). It is thus suggested that CCK is a negative regulator of gastrin as either the blockade of type A or B receptor will result in an increase in circulatory gastrin (Zavros and Schulkes, 1997).

Acetylcholine

The efferent fibres of the vagus nerve do not innervate the parietal cells directly but synapse with ganglion cells of the enteric nervous system (Radke *et al*, 1980). The postganglionic nerve fibres of the enteric nervous system (ENS) and other components of the ANS are polymodal, releasing two or more neurotransmitters, that is, they contain both a conventional neurotransmitter e.g. acetylcholine and one or more neuropeptides (Costa and Furness, 1989). Regional release of acetylcholine (Ach) activates the parietal cells directly by binding to an M_3 subtype muscarinic receptor (Wilkes *et al*, 1991). Also, vagal stimulation of the parietal cells occur through the same cholinergic receptor. Similarly, Ach stimulates the fundic enterochromaffin-like (ECL) cell to release histamine, which in turn stimulates the parietal cell by binding to an H_2 histamine receptor (Black *et al*, 1972; Debas and Carvajal, 1994). In the antrum, Ach

stimulates the G cells to secrete gastrin into circulation where it travels to the fundus and stimulates the ECL cell to release histamine (Prinz *et al*, 1993). Ach or vagal stimulation inhibits the release of somatostatin from D cells (Chiba *et al*, 1981). Ach thus augment the direct stimulation of the G cell by releasing the tonic inhibition of gastrin release (Debas and Carvajal, 1994).

2.4 THE UTERUS

The uterus is a muscular, pear-shaped organ with a dilated portion, the body, whose upper part is the fundus of the uterus and a lower cylindrical part called cervix or uterine neck (Junqueira *et al*, 1978). The cervix bulges into the lumen of the vagina. The wall is formed by three coats; the outer is either the serosa (connective tissue and mesothelium) or adventitia (connective tissue only), the middle muscular coat called myometrium and the inner mucosal lining called endometrium. The uterus is held in place by the bony pelvis, strong muscles and fibres between the anal canal and the urogenital area, so also the broad ligaments of the uterus (Strand, 1978). There are three openings to the uterus, two lateral ones at the entrance of the oviducts (Fallopian tubes) and external Os, which is a small opening in the cervix that project into the vagina. The cervix or

coilum is the lower cylindrical part of the uterus with peculiarly fewer smooth muscle fibres but large quantity of connective tissue (Junquicra *et al*, 1978).

Generally speaking, the endometrium undergoes a functional sequence of cyclical activities resulting in the cstrus cycle in lower experimental animals and menstrual cycle in human.

These changes arc due to the hormonal control to which the uterus is subjected. The rat uterus is more sensitive to drugs at estrus. In human, rhythmic contractions are depressed during early pregnancy, but they increase in force towards the end of pregnancy. The spontaneous contractions are however feeble in most pregnant uterus. The contractions are of low amplitude at ovulation and very frequent. Some two to three days thereafter, these are replaced by contractions with irregular amplitudes, and these become larger, less frequent but regular towards the end of menstrual cycle (Wilson *et al*, 1975).

2.4.1 UTERUS AND THE DRUGS

Posterior pituitary extract oxytocin, and ergot alkaloids most especially ergometrine are the most powerful uterine stimulants (Wilson *et al*, 1975). Posterior pituitary extract effect on the uterus is

dependent upon sex hormones. Estrogens are known to enhance the action of oxytocin while progesterone depresses it. However, the oxytocic action of ergot alkaloids is independent of hormonal influences (Wilson *et al*, 1975).

It is unlikely that oxytocin released from the posterior pituitary gland initiates parturition since blood oxytocin level increases only after the uterus starts to show regular rhythmic contraction and anti-oxytocin serum fails to delay the onset of labor (Huguchi et al, 1994). There is increased expression of oxytocin, so also the number of oxytocin receptors in the myometrium increases just before the onset of parturition, which results in very high sensitivity of the myometrium to oxytocin at parturition (Huguchi *et al*, 1994).

Nitric oxide, an endogenous smooth muscle relaxant is reported to exist in the uterus. Thus, nitric oxide synthetase activity is elevated during pregnancy and rapidly decreased in parturition (Huguchi *et al*, 1994).

Opioids inhibits stimulated oxytocin (OXY) secretion; mu-opioids, like morphine, and kappa-opioids inhibits the firing of oxytocin neurones but kappa-opioids act also on OXY terminals in the posterior pituitary (Russell, 1994).

Both PGE_2 and $PGF_{2\alpha}$ when given by various routes are effective inducers of labour and are therefore very useful abortifacients. The history of prostaglandins dated back to early 1930's when two American gynaecologists, Kurzrok and Lieb (1930) observed that strips of human uterus relax or contract when exposed to human semen.

Vane (1971) discovered that aspirin can inhibit prostaglandin synthesis, so also are other compounds of diverse structures but known to possess similar activities as aspirin and indomethacin were found to cause inhibition of uterine contractions both in-vivo and in-vitro (Vane and Williams, 1973; Dubin *et al*, 1979). These inhibitors act on the cyclooxygenases, the precursors of prostacylin and thromboxane as well as $PGF_{2\alpha}$ and PGE_2.

It has been demonstrated that, the uterine tissues of rats and rabbits contain high-affinity gamma aminobutyric acid (GABA) receptor binding sites in a high density. It was therefore suspected that, GABA may have a role to play in the uterine functions (Erdo, 1984).

2.4.2 FUNCTIONS OF UTERUS

1. Endocrine:

The uterus was first noticed to have endocrine function by Loeb (1923). According to him, he performed hysterectomy on guinea pig and discovered that, this operation prevented regression of the corpus lutea and thus resulted in prolonged estrous cycles of between 60-120 days. In his report, he then postulated the existence of an uterine luteolytic hormone. Years later, other workers, Asdell and Hammond (1933) using rabbit, Bradbury (1937) using rats, Wiltbank and Casida (1956) using cows, and Spies *et al* (1958) working on horse and pig confirmed that the uterus controls the life-span of the functional corpus luteum in many animal species. This luteolytic substance is now known to be $PGF_{2\alpha}$ produced in the endometrium and acts directly on the myometrium to stimulate contractility (oxytocic effect) and in some animals species to cause luteal regression (luteolytic-effect) (Thorburn and Challis, 1979). A lutcolytic substance will cause the corpus luteum to regress histologically and plasma progesterone levels to fall.

The menstrual cycle, that is, the cyclic build up and sloughing of the endometrium, a notable function of the uterus is controlled by hormonal secretions of the ovary.

2. Transportation:

The uterus favours sperm transport preparatory to fertilisation. The external os of the cervix serves this purpose by allowing sperm to transverse the uterus and penetrate into the oviducts to fertilise the ovum. The external os of the cervix also serves as an opening through which the debris of menstrual flow must pass down to reach the vagina and the exterior.

3. Protective:

The cervical mucous glands undergo little secretory variations and changes during the menstrual cycle, however, they proliferate extensively during pregnancy to secrete a more viscous and abundant mucous which normally form the protective mucus plug of pregnancy guiding the external Os (Junqueira et al, 1978).

The external aspects of the cervix is greatly modified with stratified squamous epithelial cells to prevent physical desquamation (Junqueira et al, 1978).

4. Adequate and conducive environment for foetus:

Myometrium is unique in that, a major part of its time, it is found to be in a relatively quiescent state to facilitate implantation and development of the foetus, and will only develop brief episodes of the violent activity associated with parturition and delivery of the foetus and placenta at term (Thorburn and Challis, 1979).

It provides the conditions necessary for the maintenance of the fertilised ovum like increased glycogen contents of endometrial cells, increased secretory products and tortuous endometrial blood vessels. It also responds to implantation by developing the maternal part of the placenta necessary for the nourishment of the embryo (Strand, 1978).

5. Prostaglandins production:

The secretory endometrium of the uterus produces large quantities of prostaglandins which are thought to be responsible for vascular necrosis and bleeding observed during menstrual cycle (Ganong, 1989).

6. Non-coagulability of menstrual flow:

The necrotic endometrial material of the uterus contains a fibrinolysin which prevents the menstrual fluid from clotting (Guyton, 1976).

7. Provides uterine immunity:

The uterus is said to be leucorrhea during menstruation, that is, it discharges tremendous numbers of leucocytes along with the necrotic material and blood. This is believed to be responsible for its resistance to infection even though the endometrial surfaces are denuded (Guyton, 1976).

MATERIALS AND METHODS

3.0 MATERIALS AND METHODS

3.1. SEEDS OF CROTON PENDULIFLORUS

3.1.1 Plant material

The seeds of *Croton penduliflorus* were purchased from a traditional herbalist in Plateau state, Mrs Oyhu Aziga employed by University of Jos, to teach traditional healing methods to medical students in the Department of Pharmacology. Additional quantity of the seeds was purchased from a local herbs' seller at Bodija market, Ibadan.

3.1.2. Authentication

The seeds were identified and authenticated by Prof. A. Egunyomi and Mr. A.E. Ayodele, both of the Department of Botany and Microbiology, University of Ibadan. These seeds were verified and confirmed as herbarium specimen FHI 91302 by Messrs T.K. Odewo and A. Magbagbeola of the Federal College of Forestry, Herbarium section, Jericho, Ibadan.

3.1.3 Preparation of extracts and column fractions

(A) Methanolic extract of Croton penduliflorus (MECP)

Large quantity of *Croton penduliflorus* seeds were weighed, crushed and ground thoroughly into powdered form using laboratory mortar and pestle. Weighted portions of this sample were separately transferred into the extracting chamber and the top surface was covered with cotton wool in the Soxhlet apparatus (Fig 3.1). Methanol was poured into the round bottom flask fitted to an upper extracting chamber. Exhaustive soxhlet extraction of each sample portion was then carried out at a constant temperature of 65°C maintained by a regulated water bath. The escaping vapour carried with it extractives which later condensed back into the solvent due to a cooling device, the condenser fitted to the set up. The extract was concentrated in vacuo using a rotary evaporator assembly.

The concentrates from the sample portions were air dried to constant weight. The weights of the extracts were recorded and the percentage yield calculated before their storage. A total starting sample of 1,699.7g of ground seeds gave a mean yield of 10.68±3.23g of extract (where n = 4).

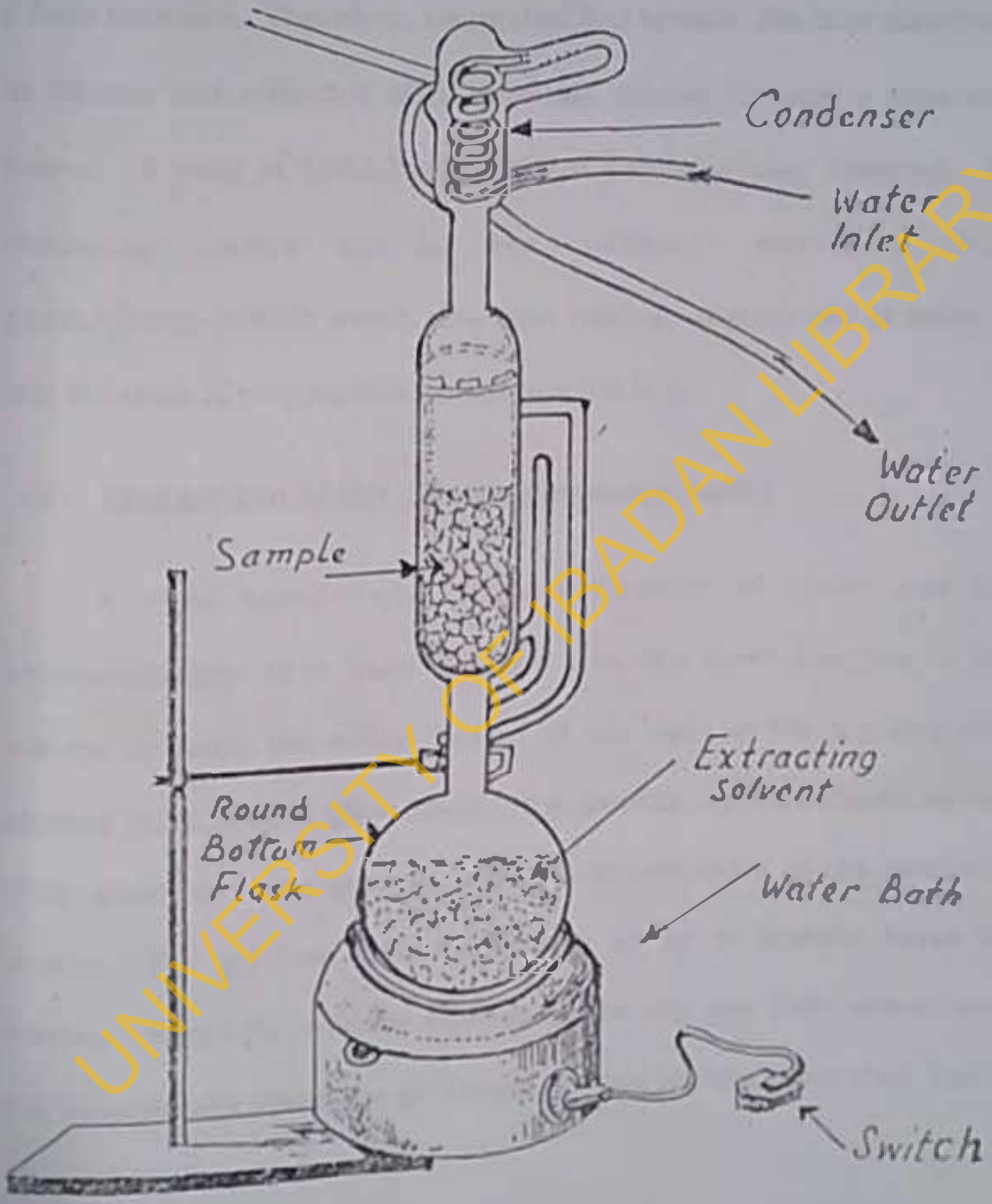


Fig. 3.1: Diagram showing the set up of Soxhlet apparat

(B) **Fractionation**

The initial methanol extract of *Croton penduliflorus* (MECP) gave a fatty material. Therefore, using distilled hexane, fraction dissolvable in hexane was collected after thorough mixing through a separating funnel. A yield of 142.00 millilitres of brown oil was obtained. The remaining fraction was fat free methanol extract of *Croton penduliflorus* (MECP) which was then used as appropriate to make up test solution of varying concentrations (Plate I).

(C) i. **Preparation of the column chromatography**

A 50ml burette with internal diameter of 15mm was the chromatography tube used. Before use, the lower junction of the burette between the wider portion of the tube to the tap end was blocked by a plug of glass wool. The burette tap was closed as the thick slurry of silica gel was filled up to one-halve of the column's length. The gel was added gradually so as to prevent break in column. When the column was ready, the tap was then opened and the supernatant above the gel flowed out but leaving some clear liquid above the column.

Plate 1: Shows from left, methanolic extract of Croton penta*androus* and the seeds.

(ii) Loading the column

The dark brown solvent-free methanol extract of *Croton penduliflorus* seeds was air dried by spreading it out in powdery form. This fine powdery material was introduced into the column which had been carefully drained by suction pump. The sample was layered on the surface of the silica gel without disturbing the surface. To isolate the compounds, varying solvent systems of chloroform - methanol of increasing polarity; from ratio 9:1 up to 5:5 were delivered to the top of the column and used to elute the extract already pre-absorbed in silica gel. Successive serial collections were done using a 50ml conical flask. Under no circumstance was the surface of the column allowed to run dry during this procedure.

Analytical TLC was performed on the fractions using commercially precoated gel plates of silica GF_{254}, 0.25mm thick (Merck, sharp and Dohme). The developing solvents were chosen as appropriate for the different fractions. Based on the TLC appearance and R.f of the fractions, similar fractions were bulked and evaporated

Twenty three (23) fractions were produced with TLC analysis of which only eight (8) fractions showed solid precipitates after evaporation.

3.2 Determination of melting point of the column fractions

Melting point was determined by using an electrothermal Gallenkamp melting point apparatus. One melting point capillary tube s ealed at one end was filled with a little of each fraction whose melting point was to be determined. The packed tube was then introduced into the equipment in a vertical position. The dry sample was electrically heated to the point of melting. The thermometer reading was read at the start of melting of the sample to the point of complete melting. This therefore gave a range for the melting point.

The results showing the melting point determination, colour, solubility in water and the Rfs' carried out are shown in Table 3.1. The column fractions were mostly brown solid plus two other solid precipitates that were white in colour (Cf6 and Cf7). The melting point determination revealed fraction Cf4 having the maximum melting point of 212°C and Cf1 with the minimum melting point.

Table 3.1 Some properties of the column fractions of extract (MECP)

Column Fractions	Weight of fraction (mg)	Properties of Column Fractions				
		Colour	Solubility in water	Melting point (±1°C)	RF Chloroform: Methanol	
					9:1	7:1
Cf_1	29.0	Brown	+	155	0.30	0.40
Cf_2	48.0	Brown	+	166	0.24	0.38
Cf_3	119.0	Brown	+	180	0.18	0.36
Cf_4	70.0	Brown	+	212	0.16	0.34
Cf_5	141.0	Brown	+	204	0.18	0.34
Cf_6	171.0	White	+	210	0.18	0.32
Cf_7	118.0	White	+	180	0.16	0.32
Cf_8	134.0	Brown	+	170	0.16	0.32

+ = Soluble; Cf = Column fraction

RF = Resolving factor = Ratio of the sample rise on the TLC plate to that of the solvent front.

3.3 Experimental animals

Adult albino rats of both sexes weighing between 200 and 260g and mice of weight range 20-25g bred in the Animal House of the Faculty of Medical Sciences, University of Jos, and Preclinical Animal House of the College of Medicine, University of Ibadan were used. These animals were fed twice a day on routine standard livestock pellets from Olaogun Co, Ltd, Ibadan and were given water ad libitum before the studies commenced. Guinea pigs (300-350g) locally purchased from Bodija market, Ibadan were also used for the studies. These animals had standard pellets but were supplemented with soft green vegetable in their diet. They also had free access to water.

3.4 (i) Physiological salt solutions (PSS)

The physiological solutions used were of the following composition:

Tyrode's solution (mM/l): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.4, $NaHCO_3$ 11.0 and glucose 5.6.

Kreb's solution (g/l): NaCl 6.90, KCl 0.35, $CaCl_2$, 0.28, $NaHCO_3$ 2.10, KH_2PO_4 0.16, $MgSO_4.7H_2O$ 0.29 and glucose 2.00.

(ii) **Reference drugs and chemicals**

The following drugs and chemicals were used: oxytocin (Sandoz), atropine sulphate (Sigma), acetylcholine chloride (BDH), stilbestrol (UCH), promethazine (Sandoz), histamine dihydrochloride, ethyl carbamate (Urethane), sodium chloride (May and Baker), Phenolphthalein, carbamylcholine chloride (Carbachol), activated charcoal (MSD), tragacanth (May and Baker), methanol, hexane and petroleum ether.

Mice were used for acute toxicity test and gastrointestinal transit of charcoal in tragacanth (in vivo).

Adult male albino Wistar strain rats were used to study gastric acid secretion, while the adult non-pregnant female albino rats were used in the study of isolated uterine strip contraction.

Guinea pigs were sacrificed and used in the isolated guinea pig ileum study.

Acute toxicity study in mice

Albino mice divided into six groups were used in this study. Each group was made up of six mice. They were kept in separate cages and were fed with mouse pellets and water ad-libitum prior to the day of the experiment. They were fasted for 24 hours prior to the

study. Different dosages of MECP 2.5,5.0,10.0,20.0 and 40.0 (mg per 10g body weight) were administered intraperitoneally into separate groups of mice.

The control group received equivolume (2.5ml) of distilled water. The doses of MECP given were selected after a preliminary experiment which showed no death with the least dose and 100% death with the highest dose. A lethal dose was regarded as one which resulted in the death of the animal within 24hr. The number of deaths were recorded for each group after 24hr following the method of Aguwa (1986). The percentage mortality was calculated and a graph of percentage mortality against log-dose was plotted from which the LD_{50} was extrapolated.

3.6 In vivo measurement of intestinal transit in mice

Mice starved for 24 hours were separated into four groups of eight per group.

The animals were allowed free access to water. One group was given 0.55 mg/kg body weight of MECP intraperitoneally. Another group received 1 mg/kg body weight of carbamylcholine chloride and the third group received 10 mg/kg body weight of atropine. The fourth

group received 0.55 ml/kg body weight of normal saline. The last group served as control. All the drugs were given intraperitoneally. The dosages of standard drugs administered were in accordance with those reported by Aguwa (1986).

Ten minutes thereafter, 0.5ml of 5% v/v activated charcoal in tragacanth mucilage was orally administered to all animals in the groups. The charcoal meal was to provide an opaque intestinal medium for easy measurement of distance covered by meal. In another twenty minutes after the meal, the mice were killed by a sharp blow to their heads. The abdomens were surgically opened, and the intestines carefully brought out and severed at the level of the lower esophageal sphincters and measured. The length moved by the charcoal meal from the stomach towards the caecum was measured and recorded in millimetres. This was expressed as percentage of the total length of the small intestine.

3.7 Gastric acid secretory study in rats

3.7.1 Surgical procedures:

Each animal was prepared for gastric acid secretion study following several steps. The rats were fasted overnight with free access to water so as to provide a clean stomach at the time of the experiment. They were then anaesthetised with an intraperitoneal injection of urethane solution (25% w/v) at 0.6ml per 100g body weight.

This dose produces a constant and satisfactory anaesthesia for up to 10hrs. Each rat was tied face up on a rat dissecting board. The neck region was opened slightly with an incision followed by blunt dissection so that a blood vessel and the vagus nerves were not cut. The trachea was exposed, cannulated with a size-4 polythene tracheal cannula and exteriorized to ensure free normal breathing throughout the period of the experiment. The abdomen was opened through the linea alba with a midline incision. A soft long esophageal cannula was passed through the mouth, inserted into the esophagus and the tip positioned in the ruminal portion of the stomach. A short thread was then used to make a tight ligature around the upper portion of the esophagus to prevent leakage of perfusate. The external end of the

flexible esophageal cannula was passed through Watson-Marlow H.R. flow-inducer with the free end placed inside a reservoir of normal saline in a round bottom flask. The stomach was brought out from its bed and delivered through the abdominal wound. A semitransected incision was made at the junction between the pylorus and the duodenum. Through this incision a polythene tube of about 20 cm in length was introduced into the stomach and firmly secured by a ligature around the pylorus. The stomach was returned and the abdominal wound closed by interrupted sutures (Plate II).

Perfusion technique

The continuous stomach perfusion technique described by Ghosh and Schild (1958) was modified for the experiments.

Adult male albino rats of Wistar strain weighing between 200 and 260g were used for the study. The stomach lumen of each rat was perfused with normal saline (0.15M) at an adjusted rate of 1±0.1ml/minute volume using Watson-Marlow HR flow-inducer. The gastric effluent was collected at ten minute intervals and assayed for titrable acid against 0.01N sodium hydroxide using phenolphthalein as an indicator. An assemblage of the set up is shown in Plate II.

3.7.3 Precautions

The body temperature was maintained with an electric bulb directed on the animal (Plate II).

The initial titres recorded for the effluent samples were high and hence discarded from the results. Mechanical trauma to the animal during operation might have been responsible for this observation. Therefore, the mean of four consecutive readings obtained after a steady state of acid secretion was recorded as basal secretion.

3.7.4 Areas of modification of Ghosh and Schild method.

(i) Perfusion fluid in this work was normal saline (0.15M NaCl) instead of $^{N}/_{4000}$ sodium hydroxide reported by Ghosh and Schild (1958). This modification provided an in-vitro quantitative analysis of acid secretion by employing the titration method. However, in Ghosh and Schild method, the level of gastric acidity was measured in-vivo by continuous recording of pH change of effluent over a pH glass electrode in a fitted U-shaped glass container connected to a direct reading pH meter.

(ii) A 40 Watts electric bulb was used to raise and maintain the body temperature of the animal while in Ghosh and Schild (1958) procedure, the body temperature was artificially stabilised at 30°C by

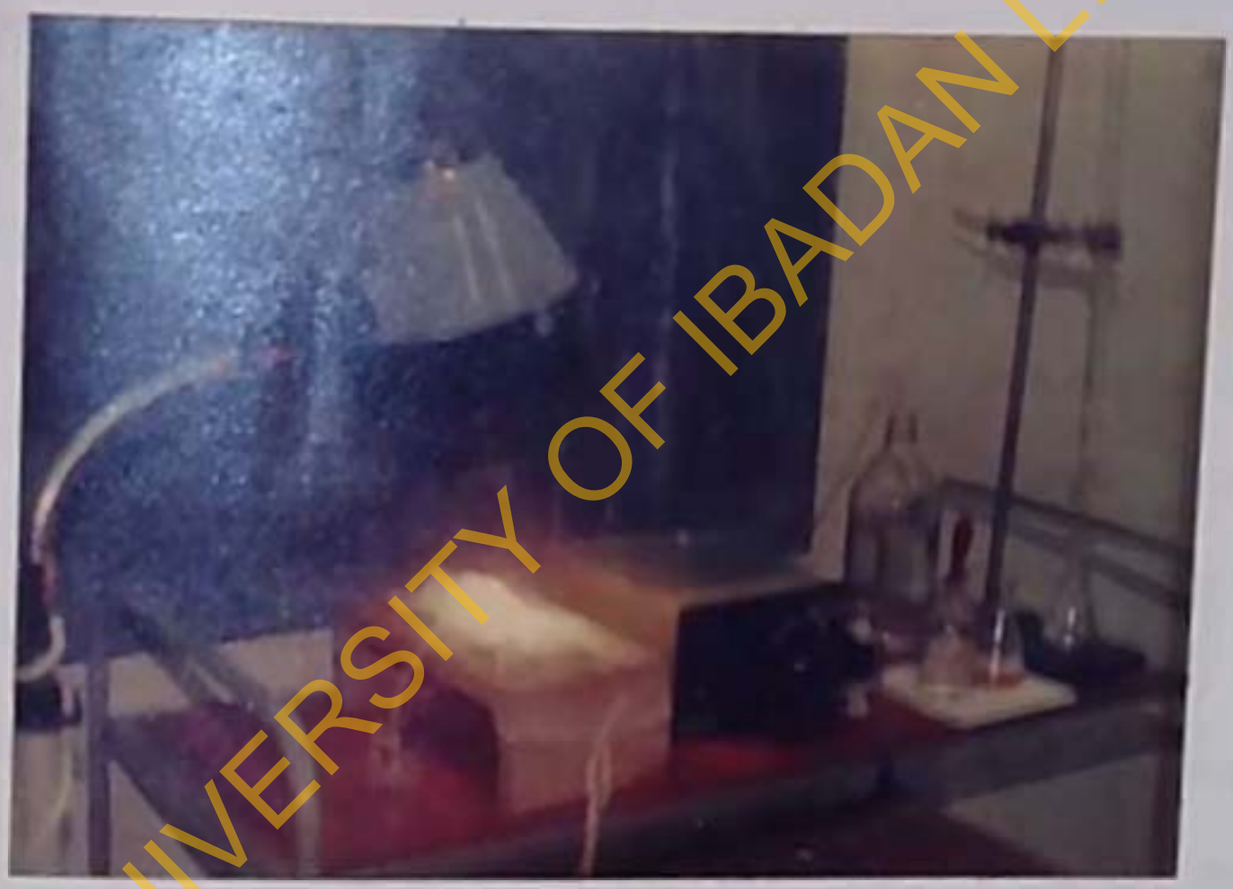


Plate II: An assemblage used for gastic acid secretion study.

means of a rectal contact thermometer which monitored the heating of the operating table through an electronic relay.

3.7.5 Effect of drugs

To investigate the effects of drugs on gastric acid secretion, the left femoral vein was cannulated with a size-1 polythene cannula. At the end of the basal collection, intravenous injection of MECP was given at varying doses (10^{-5} to 10^{-3}g/ml) to a set of eight adult male albino rats. Also to assess the gastric secretory responses to other drugs, second set of eight rats received a dose of 0.5mg/kg body weight (Bolarinwa and Amure, 1976) histamine dihydrochloride, while another set of eight rats, were given a single injection of 0.2×10^{-3} g/ml MECP before a dose of 0.5mg/kg body weight histamine dihydrochloride. In all cases the drugs were injected in a volume of 0.1 to 0.2ml followed by a washing injection of 0.1ml normal saline.

In another study, a group of thirty two male albino rats were used to investigate the effects of eight column fractions of MECP on gastric acid secretion. Four rats per group were similarly treated as above with each column fraction of MECP.

3.7.6 Choice of Indicator

All chemical indicators used in the laboratory have different pH range during which there is a change in colour. The choice of an indicator therefore depends on the range of pH of the net solution from a mixture of two or more. For example, when an acid solution of pH 4 is titrated against a base of pH 13, it would be useless in this case to choose an indicator whose pH range lies between 3 and 4. There would be no colour change, so the best indicator to use is the one with pH range between 8 and 10. In this study, the acid in the effluent is a weak one, and is being titrated against a relatively weak base, hence the choice of the indicator is phenolphthalein. The pH range is between 8 and 10. This indicator is prepared by dissolving 1g of powered phenolphthalein in 500ml of 50% ethanol.

3.7.7 Volumetric analysis calculation

The underlying principle involved in the measurement of acid strength is the matching of a constant volume of effluent (acid) against a variable volume of the base.

At the end point the two volumes are presumed equivalent. This is indicated through the colour change provided by phenolphthalein.

The hydrogen ions concentration can thus be derived from the principle of mole of a substance.

That is;

Mole of acid = Mole of base

(at end-point)

10ml of aN HCl= b ml of 0.01N NaOH

where aN and bml are both unknown.

These are normality of acid and millilitres of base respectively.

$$\text{Mole of HCl} = \frac{10}{1000} \times aN$$

$$\text{Mole of NaOH} = \frac{b}{1000} \times 0.01$$

$$\text{i.e. } \frac{10}{1000} \times aN = \frac{b}{1000} \times 0.01 \quad \text{----------(i)}$$

$$aN = \frac{b \times 0.01 \times 1000}{10 \times 1000}$$

$$aN = \frac{b \times 0.01}{10} \quad \text{----------(ii)}$$

$$\text{Mole of HCl (at Equivalence)} = \frac{10 \times aN}{1000}$$

$$1 \text{ Equivalence} = \frac{aN}{100}$$

Substituting for aN in the above equation:

$$1 \text{ Equivalence} = \frac{1}{} \times \frac{[b \times 0.01]}{}$$

$$= b \times 10^{-2} \times 10^{-3}$$
$$= b \times 10^{-5}$$

To magnify the above unit of equivalence;

$$1 \text{ Eq.} = 10^{3}\text{m Eq}$$

$$1 \text{ Eq.} = 10^{6}\mu \text{ Eq.}$$

Therefore;

$$1\ \mu\text{Eq} = b \times 10^{5} \times 10^{6} = b \times 10$$

All the titre values obtained in this work are multiplied by factor 10 as explained above to give micro-equivalence of effluent acid. This titration method measures total titrable acid in the effluent.

3.8 Isolated guinea pig ileum study

Guinea pigs of either sex were used for the study. Each animal was killed by a sharp blow to the head and bled by cutting through the neck region. The abdomen was opened by a midline incision to locate the region of terminal ileum with the caecum. Carefully, a fairly long strip of the ileum was removed but excluding a portion of about 10cm distal to the ileo-caecal junction. Suitable lengths of 2-3cm ileum were cut and their lumen flushed out of intestinal contents using Tyrode's solution. The ileum strips were bubbled with air and

maintained at 37°C in fresh tyrode solution in a petridish. Pieces of the ileal strips were suspended in 20ml capacity organ baths which is made up of an outer bath which regulate the temperature of the nutrient fluid and an inner bath in which the tissue is mounted. They were well aerated with air coming from a fairly long, small bore glass tubings while the bath temperature was maintained at 37°C with a BECKMAN model water circulator. The free ends of the ileal strips were connected to force transducers (FT 0.03, Grass Instruments). The strips were left suspended in the physiological solution for 60 minutes to allow for equilibration. During this time, the bath's solution was repeatedly changed.

3.8.1 Polygraph recording arrangement

An eight channel Grass polygraph (model 7D, Quincy, MA, USA) was used. The recorder was set at STANDBY position for one hour to allow for electrical stabilisation and was calibrated using the voltage balance control knob. This provided full scale pen deflection covering equal units of upwards and downwards length on the polygraphic sheet. Contractions were isometrically recorded as displacement force under an initial resting tension of 1g weight and with an adjusted sensitivity.

3.8.2 Effects of MECP and standard agonists on GPI preparation

The action of various concentrations of histamine (10^{-9} to 10^{-5}g), acetylcholine (10^{-9} to 10^{-5}g) and the extract (10^{-7} to $3x10^{-4}$g) were tested on several strips from different animals.

The responses of MECP in the presence of each of these two standard antagonists; promethazine and atropine were investigated to ascertain possible mechanism of action.

In another study, different column fractions of MECP were also used on the tissue strip preparation.

In all cases, a dose of each drug was allowed to remain in contact with the tissue for a minimum time for the response to peak. The drug was then washed and the tissue allowed to rest for 3 minutes before another dose was applied.

3.9 Isolated rat uterine strip study

Adult female non-pregnant albino rats were used for the study. These animals were pre-treated with stilbestrol (0.1mg/kg body weight) 24hr before the experiment. Each animal was killed by a blow to the head and then exsanguinated. To remove the uterus, the abdomen was opened and the two horns of the uterus dissected out.

On removing the adhering fats, connective tissues, and small vascular supply, the uterus was dissected and removed by the use of a pair of scissors and a pair of forceps, and transferred into a petri-dish containing krebs solution. The uterus was cut transversely into four sections. Each section was further cut longitudinally to form strips of length 10 - 12 mm and width 1 - 2 mm. Each strip was mounted in a 20ml tissue bath containing krebs solution aerated with 95% O_2: 5% CO_2 with the temperature maintained at 37°C by a water circulator. The upper free end of the tissue was fastened to a force - displacement transducer (FT 0.03) which was connected to a Grass polygraph, model 7D (Grass Instruments, Quincy MA). The strip was allowed to equilibrate for 60 minutes before the start of experiment. During the equilibration period the preparation was washed every 15 minutes.

3.9.1 Polygraph recording arrangement

All necessary polygraphic arrangements and adjustments were made as in the isolated guinea pig ileum (GPI) study. At the start of the experiment, normal spontaneous contractile acuvity of the uterus was obtained and recorded on Grass 8-channel polygraph for each preparation. As the responsiveness of individual tissue differed

considerably, the rats were brought into estrus 24 hrs prior to the work by injecting subcutaneously, a dose of 0.1 mg/kg B.W. stilbestrol in olive oil.

3.9.2 Effects of MECP and standard oxytocic agent (oxytocin) on IRUS preparation

The effects of MECP (10^{-2}g to 10^{-5}g) and oxytocin (10^{-1} to 10^{-3} I.U) on the change in frequency of contraction and force of contraction of the isolated rat uterine strip (IRUS) was studied and compared.

In another study, responses of isolated uterine muscle to the column fractions of MECP was assessed.

RESULTS

4.0 RESULTS

4.1 Acute toxicity test in mice

The results obtained from the toxicity test of the extract (MECP) on mice are presented in a sigmoid curve of percentage mortality against log dose (Fig 4.1). The LD_{50} for MECP was obtained by extrapolation from the graph of percentage mortality against log dose and was calculated to be 891 mg/kg body weight administered intraperitoneally.

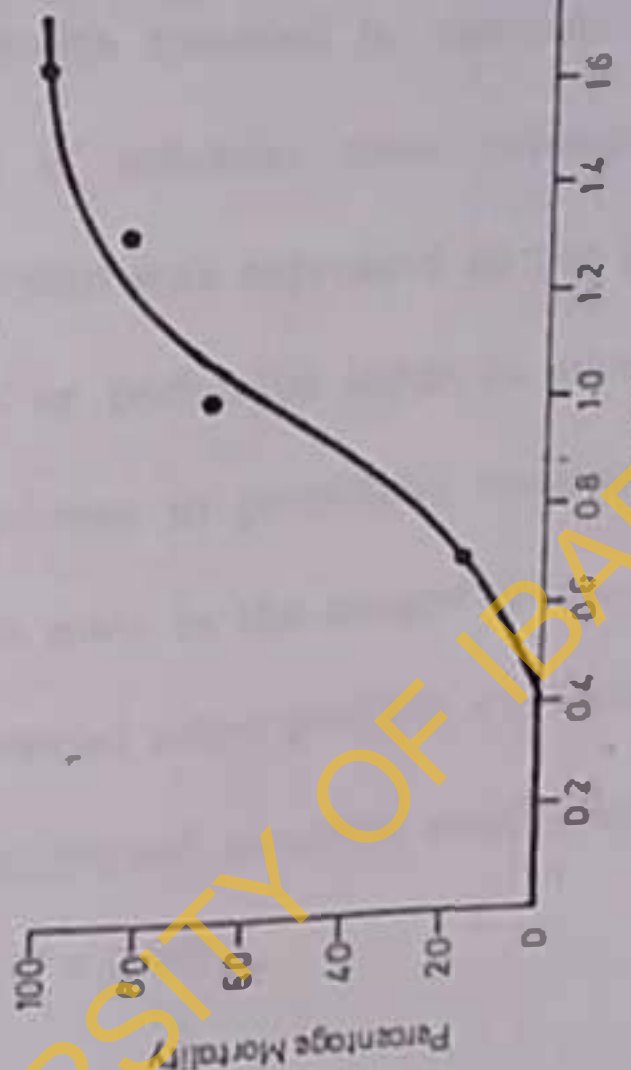


Fig.41 Shows sigmoid curve of percentage mortality versus log dose

4.2 In-vivo measurement of intestinal transit in mice.

The results of this study are shown in Table 4.1. The percentage of the total length travelled by charcoal meal in proportion to the entire length of intestine from pyloroduodenal junction to the ileocaecal junction was expressed as the in-vivo intestinal transit of charcoal meal or peristaltic index in mice. The extract produced a significant decrease in propulsive movement of the intestine when compared with mice in the control group which received 0.55ml/kg body weight normal saline ($P<0.05$). Carbachol at 1mg/kg body weight significantly increased charcoal meal transit when compared with normal saline ($P < 0.05$).

Table 4.1: The effects of methanolic extract of Croton pendulifloras (MECP) on intestinal transit in mice.

Group	Treatment (dose)	Percent of total length travelled by charcoal meal (%) n=8 (Peristaltic index)	P-Value
A	MECP (0.55 mg/kg)	1.31 ± 0.88	<0.05
B	Carbachol (1 mg/kg)	85.60 ± 6.95	<0.05
C	Atropine (10 mg/kg)	31.96 ± 4.79	
D	Normal Saline (0.55 ml/kg)	51.90 ± 10.32	

Values in the 3rd column are mean of eight determinations (n=8) ± SE.

Percent of total length travelled by charcoal meal is also known as Peristaltic index (John and Akingbade, 1996)

4.3 Gastric acid secretory studies in rats

The results of gastric acid secretory studies are shown in Figures 4.2, 4.3 and 4.4.

The mean basal acid secretion for the rats before the intravenous injection of extract (MECP) was 3.35 ± 0.04 μeq/10min. Significant increase in gastric acid secretion was produced in all animals after intravenous administration of high doses of extract (10^{-4}, 0.1 - 0.4×10^{-3} g/ml) ($P<0.05$). There was however no significant change between the basal and the secretory response to 10^{-5} g/ml of the extract ($P>0.05$) Fig 4.2.

When extract was given at a dose of 0.2×10^{-3} g/ml prior to histamine (0.05mg/100g body weight), it produced reduction in acid secretory effect of histamine from 8.28 ± 0.02 to 7.75 ± 0.01 μeq/10min (Fig 4.3). The difference was statistically significant ($P<0.05$).

In another study, the column fractions Cf_1, Cf_3, Cf_5, Cf_6, Cf_7 and Cf_8 of the extract showed no significant change in gastric acid secretion between presumulation values and values obtained after their intravenous injection ($P>0.05$) (Fig 4.4). Fractions Cf_3 (m. pt 156°C) and Cf_4 (m. pt 212°C) however significantly increased basal

acid secretion from 2.70 ± 0.08 to 3.43 ± 0.30 and 2.27 ± 0.19 to 3.63 ± 0.25 respectively.

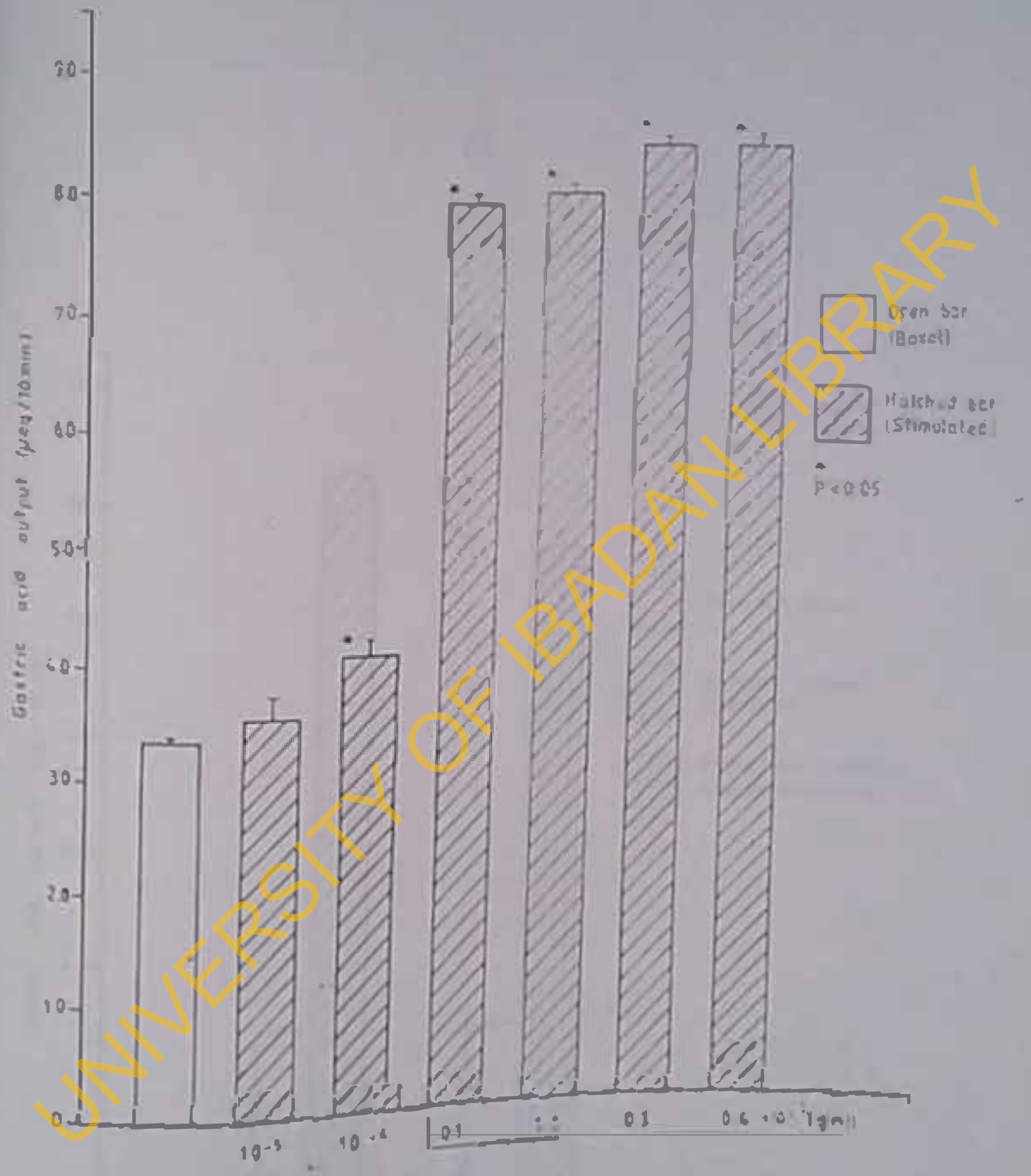


FIG 4.2: Effect of different doses of extract (10^{-5} to 10^{-3}g/ml) on gastric acid section

statistical significance is indicated by * P<0.05 when compared to basal secretion.

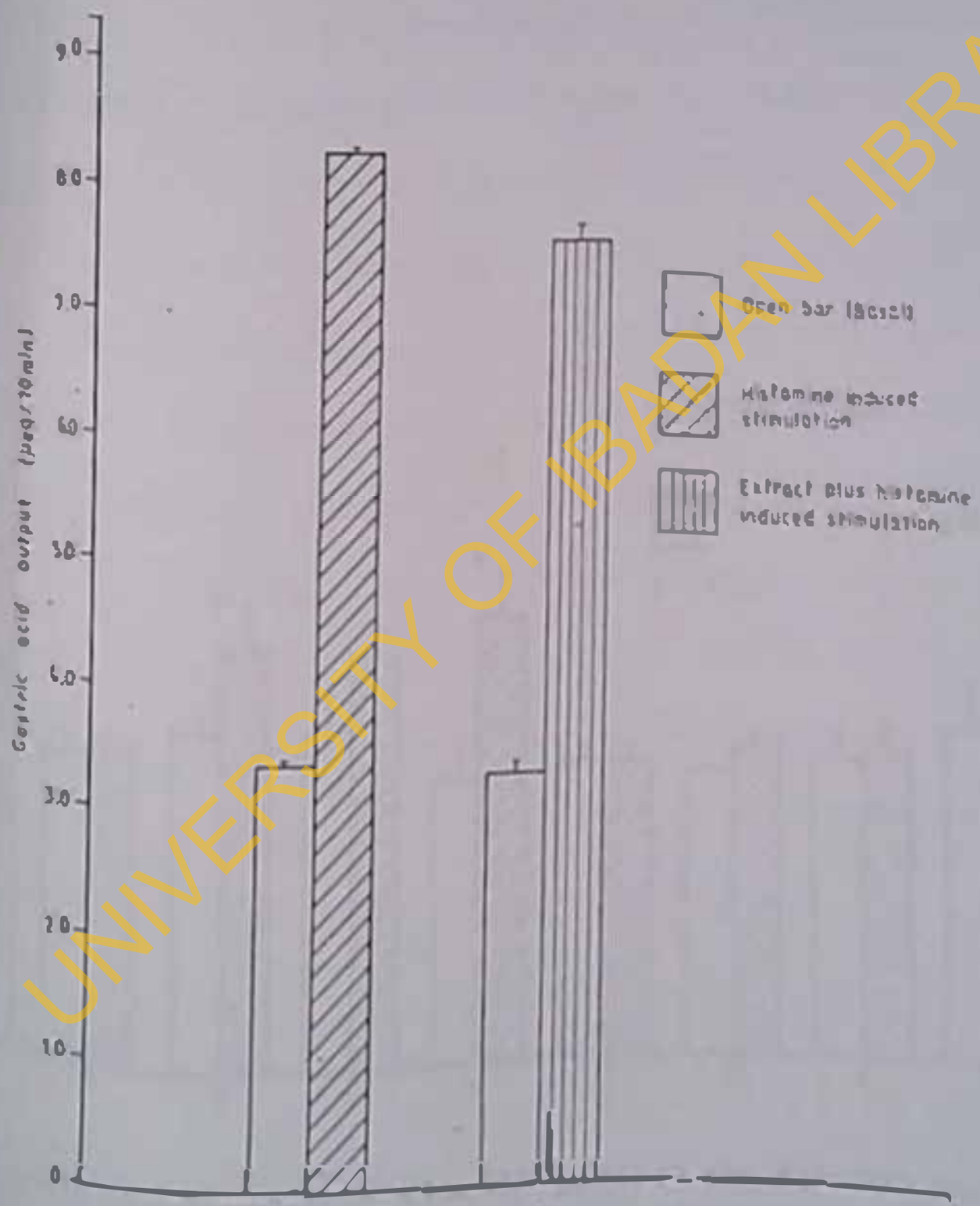


FIG. 4.3: Gastric acid secretory responses to histamine (0.05mg/100g BW) alone, extract (10^{-2}-10^{-1}g/ml) plus histamine 0.05mg/100 B W)

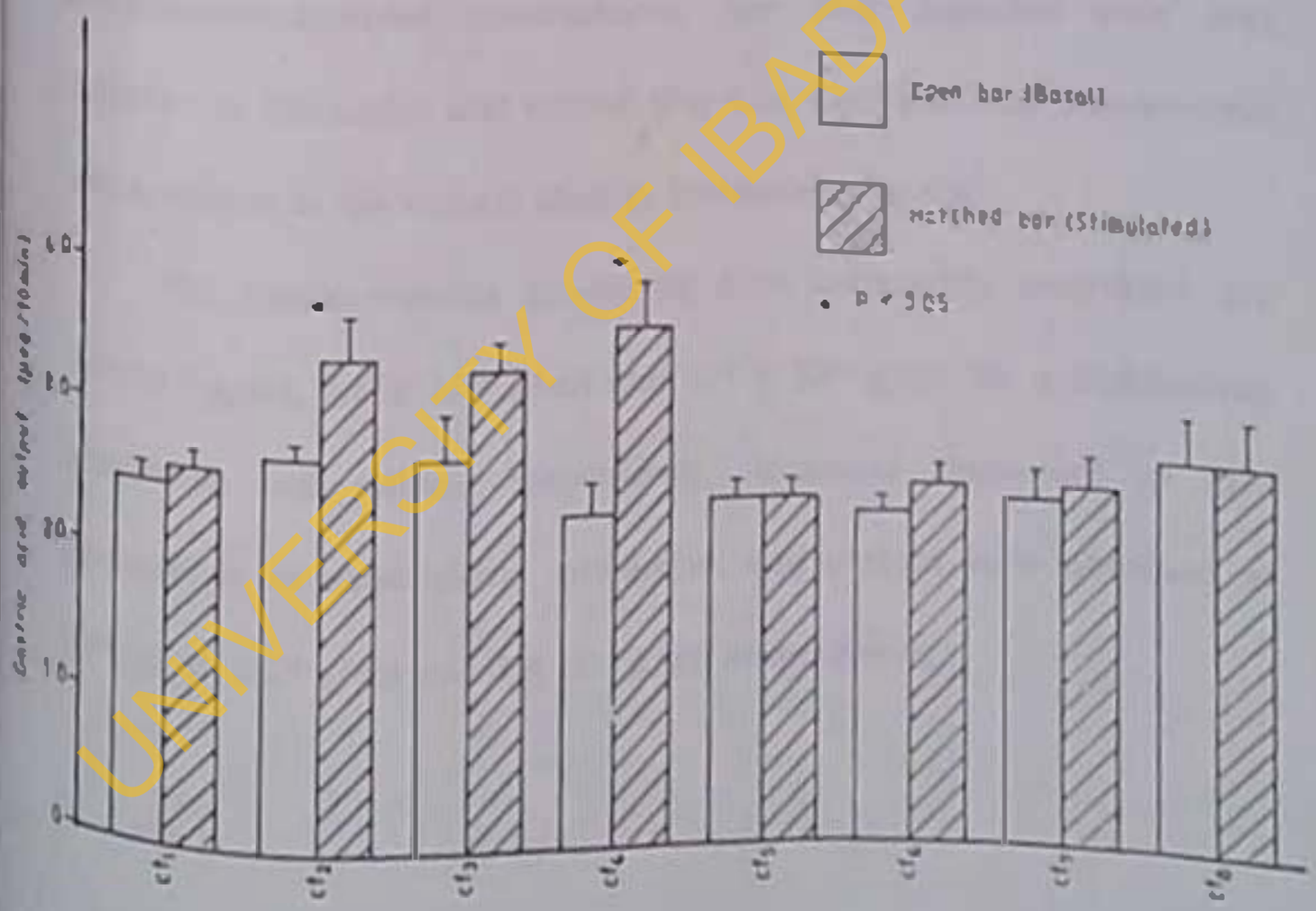


FIG. 4.4: Effect of the some dose (10^{-3}g/ml) of the different column fractions of extract on gastric acid secretion

Statistical significance is indicated at • $P < 0.05$ when compared to basal secretion.

4.4(a) Effects of drugs on isolated guinea pig ileum preparation

Acetylcholine (10^{-9} - 10^{-5} g/ml) and histamine (10^{-8} - 10^{-5} g/ml) were chosen as standard agonists used against which the responses to the extract (10^{-6} - 3×10^{-4} g/ml) on guinea pig ileum preparation could be compared. The drugs acetylcholine, histamine and extract caused dose-dependent contractions of the ileal muscles in all the experiments. Typical polygraphic traces showing these responses are obtained in Fig 4.5. The dose-response curves for the extract, histamine and acetylcholine are represented in Fig 4.6. Compared to acetylcholine-induced contractions, the ileal muscles were less sensitive to histamine and extract (Fig 4.6). Similarly, the tissues were less sensitive to the extract than to histamine (Fig 4.6).

The concentrations producing 50% contractile responses are 1.7×10^{-8} g/ml, 5.6×10^{-8} g/ml and 1.4×10^{-5} g/ml for acetylcholine, histamine and extract respectively. Maximal responses of GPI preparation to acetylcholine, histamine and extract were obtained at 10^{-6} g/ml, 10^{-5} g/ml and 3×10^{-4} g/ml respectively.

Fig 4.5 Polygraphic recordings of the contractions of guinea pig ileum strip superfused with aerated tyrode solution at temperature 37°C and speed at 5mm/min. Points a1, b1 & c1 all represent 10^{-3} of MECP stock. Points a2 b2 & c2 represent 10^{-7} 10^{-6} and 10^{-5} of Histamine dihydrochloride. Points a3 b3 & c3 represent 10^{-7} 10^{-6} and 10^{-5} of Acetylcholine.

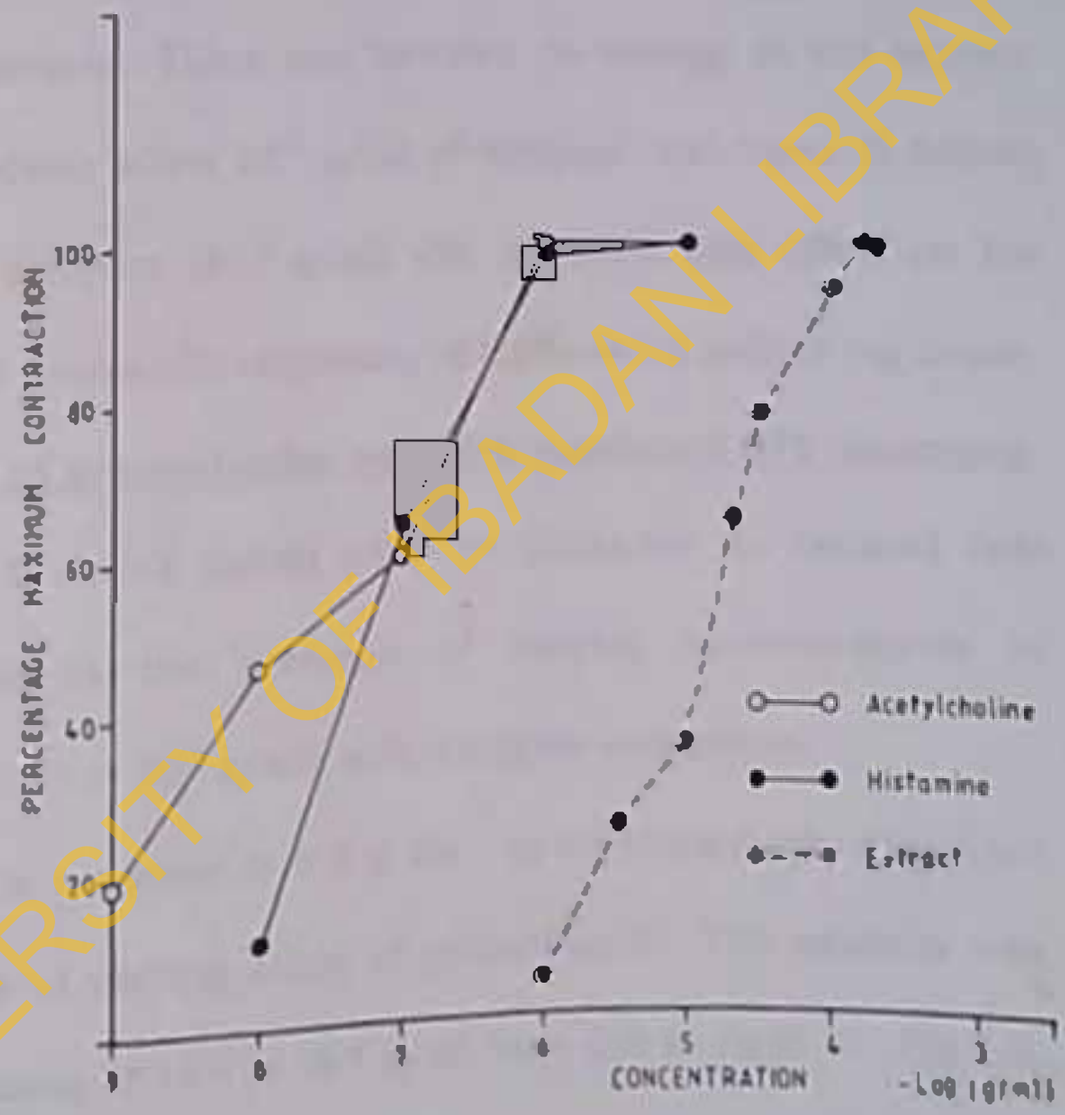


FIG 46 Concentration - response curve to acetylcholine, histamine and extract (MECP) on guinea pig ileum preparations.

Each point represents Mean ± SEM of six experiments

(b) **Effect of atropine on extract-induced GPI responses**

Typical effects of atropine on both acetylcholine - and extract induced contractions are shown in Fig 4.7. The contractile responses with acetylcholine (Ach) were reduced dose-dependently with 10^{-8} and 10^{-7} g/ml of atropine. There was however no change in the extract-induced contraction when 10^{-7} g/ml of atropine was used. It follows therefore that atropine (10^{-7} g/ml) did not have any effect on the extract-induced contractile responses of the isolated guinea pig ileum.

(c) **Effect of promethazine on extract-induced GPI responses**

Figure 4.8 shows typical effect of histamine on isolated ileal smooth muscle in the presence of varying concentrations of promethazine (10^{-8} to 10^{-6} g/ml), a H_1-receptor antagonist.

Contractile response to 0.4×10^{-3} g/ml extract was examined in the presence of varying doses of promethazine. The addition was sequential at doses of 10^{-8} to 10^{-5} g/ml from left to right in Fig 4.9. Promethazine inhibited the contractile response in a dose-related fashion (Fig 4.9).

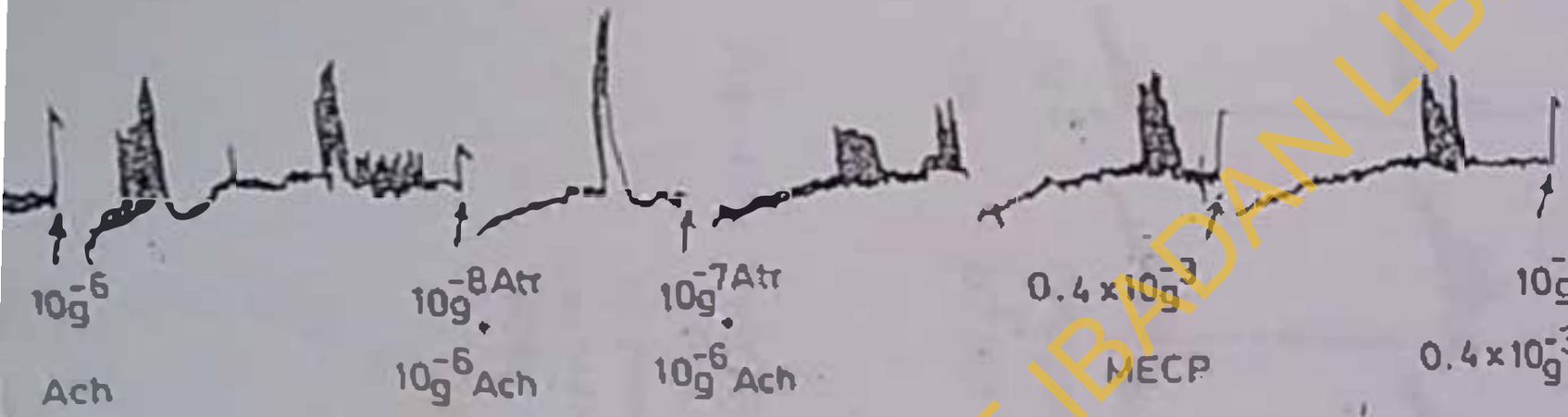


Fig. 4.7: Polygraph traces showing typical effect of different concentrations of atropine on Ach - and extract-induced responses on guinea pig ileum preparations.

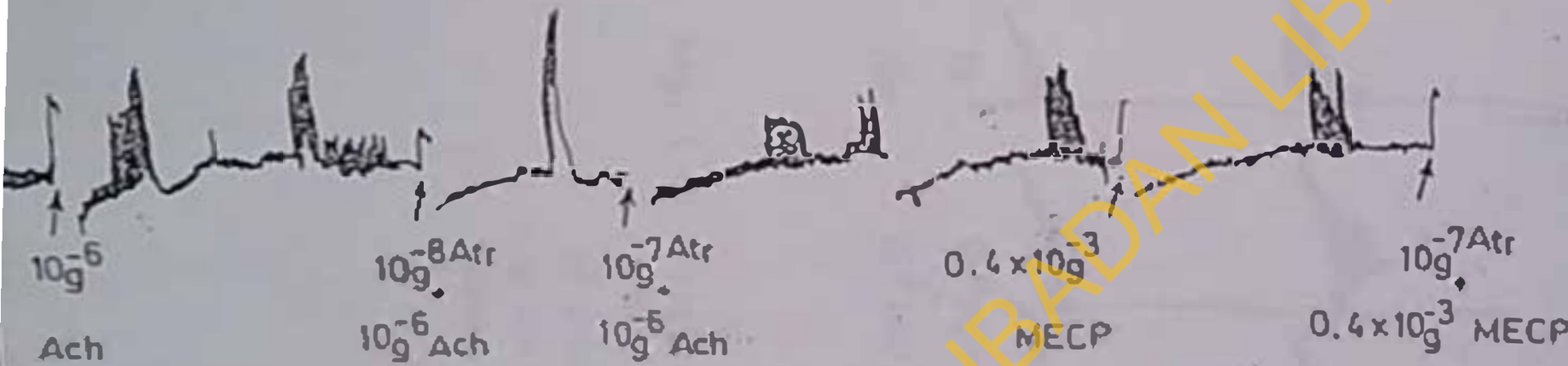


Fig. 4.7: Polygraph traces showing typical effect of different concentrations of atropine on Ach - and extract-induced responses on guinea pig ileum preparations.

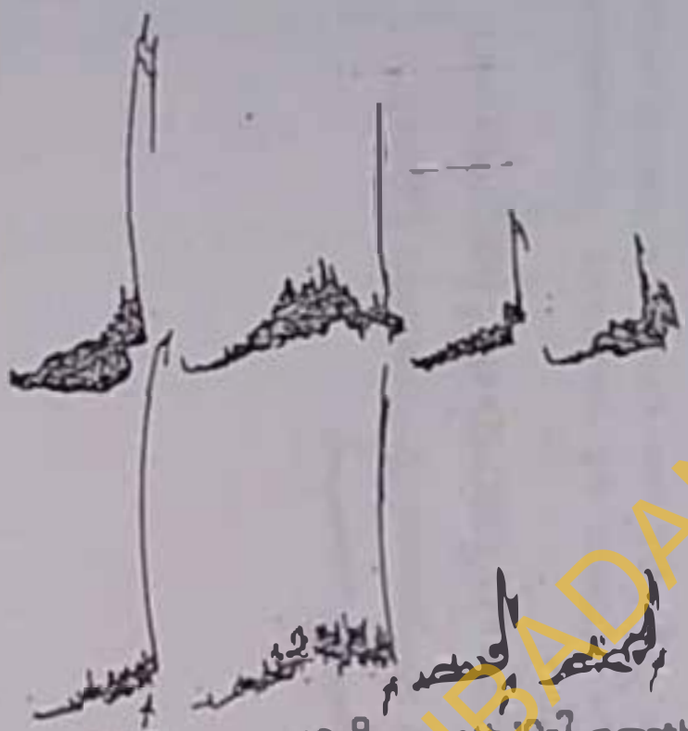


Fig. 4.8: Polygraph traces showing the effect of varying concentrations of promethazine (10^{-8} to 10^{-6} g/ml) on histamine-induced responses of guinea pig ileum preparations.

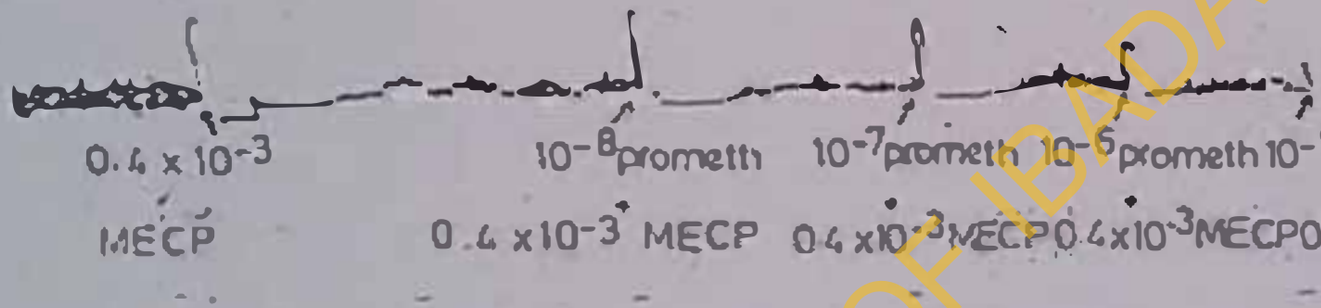
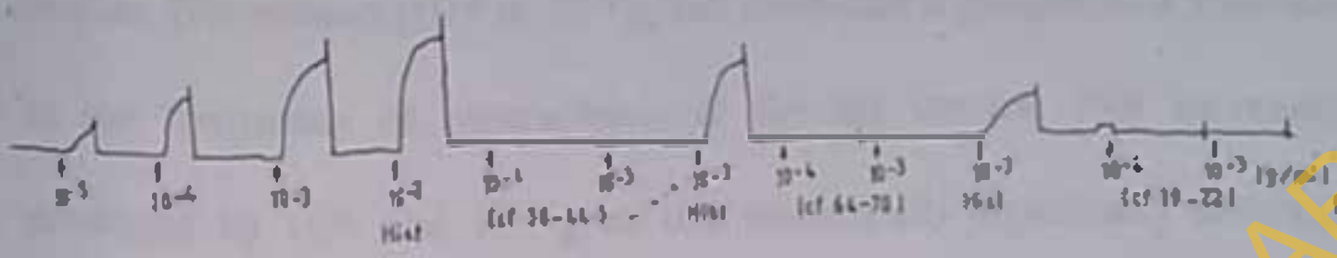


Fig. 4.9: Polygraph traces showing the effect of varying concentrations of promethazine (10^{-8} to 10^{-5} g/ml) on extract (MECP)-induced responses of guinea pig ileum preparations.

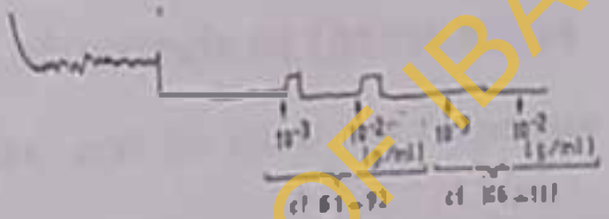
(d) **Effect of different column fractions on guinea pig ileum responses**

The investigation on the effects of different column fractions of extract on guinea pig ileum are shown on Fig 4.10 (a) and (b). Cf_6 81-92 with melting point 210°C was the only fraction that produced contractile responses of ileal smooth muscles at concentrations 10^{-3} and 10^{-2} g/ml.



* Hist = Histamine

a



b

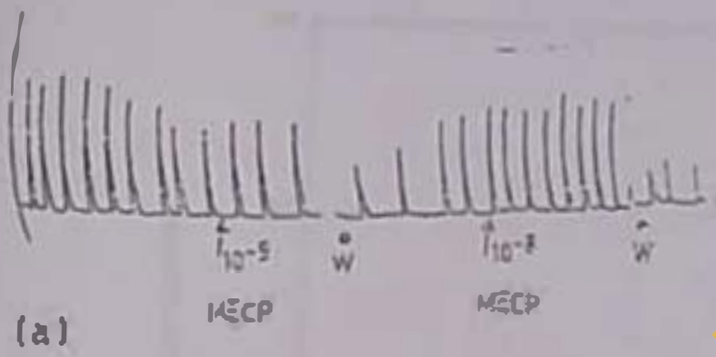
Fig. 4.10 (a) & (b): Show dose-response curves for histamine (10^{-5} - 10^{-2} g/ml) and the subsequent screening of different fractions (cf_2 38-44, cf_4 61-70, cf_1 19-22, cf_6 81-92 and cf_8 106-117) of extract on guinea pig ileum preparations.

4.5(a) Effect of extract on the rat uterus

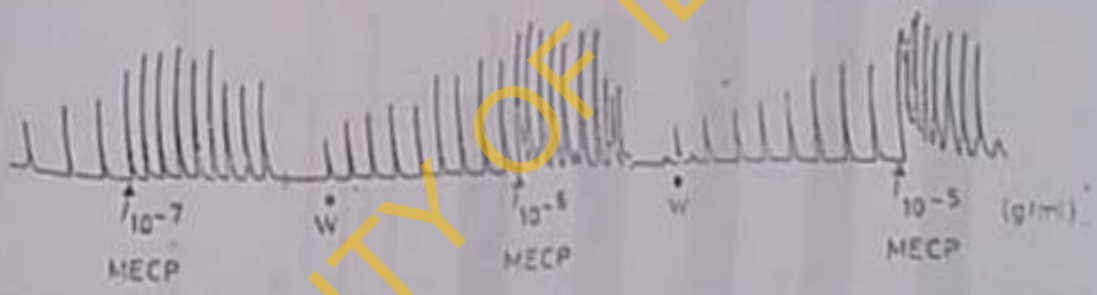
Fig 4.11(a) and (b) show typical effects of the extract on the rat uterus. The extract (10^{-9} to 10^{-6} g/ml) produced a progressive increase in the frequency of contractions of the rat uterus. The increases produced by 10^{-6} and 10^{-5} g/ml are statistically significant different from those of basal spontaneous contractions ($P<0.05$). The graphical representation of these data is shown in Fig 4.12. On Fig 4.11 (b), a dose of 10^{-5} g/ml extract produced contractile response, which was almost sustaining.

(b) Effect of oxytocin on the rat uterus

Fig 4.13(a) and (b) show the responses of isolated rat uterus strip preparation (IRUS) to various concentrations of oxytocin. Oxytocin, at concentration of 10^{-5} to 10^{2} i.u /ml, increased the frequency of uterine contractions in a dose-dependent fashion. At 10^{2} i.u/ml, a sustained contraction was observed (Fig 4.13b). At dose 10^{-4} to 10^{-3} i.u/ml, oxytocin significantly increase frequency of uterine contractions when compared to basal spontaneous contraction ($P<0.05$) (Fig 4.14).



(a)



(b)

Fig. 4.11 (a) & (b) : Typical responses of the spontaneously contracting isolated rat uterus to extract (MECP).

Figure Histogram showing the mean frequency of uterine contractions per second against different concentrations of the extract (MECP). Each bar represents mean ± SEM. n=6

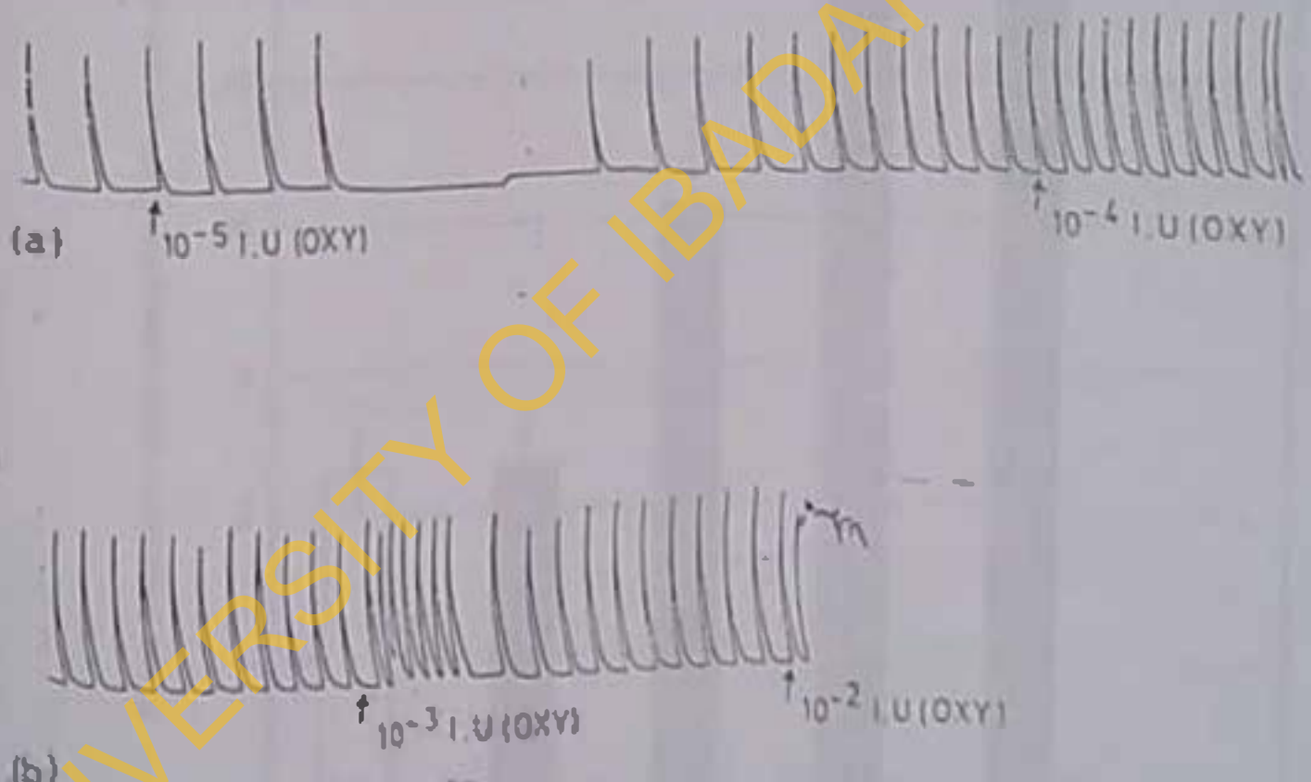


Fig. 4.13 (a) & (b): Typical responses of the spontaneously contracting isolated rat uterus to oxytocin (OXY).

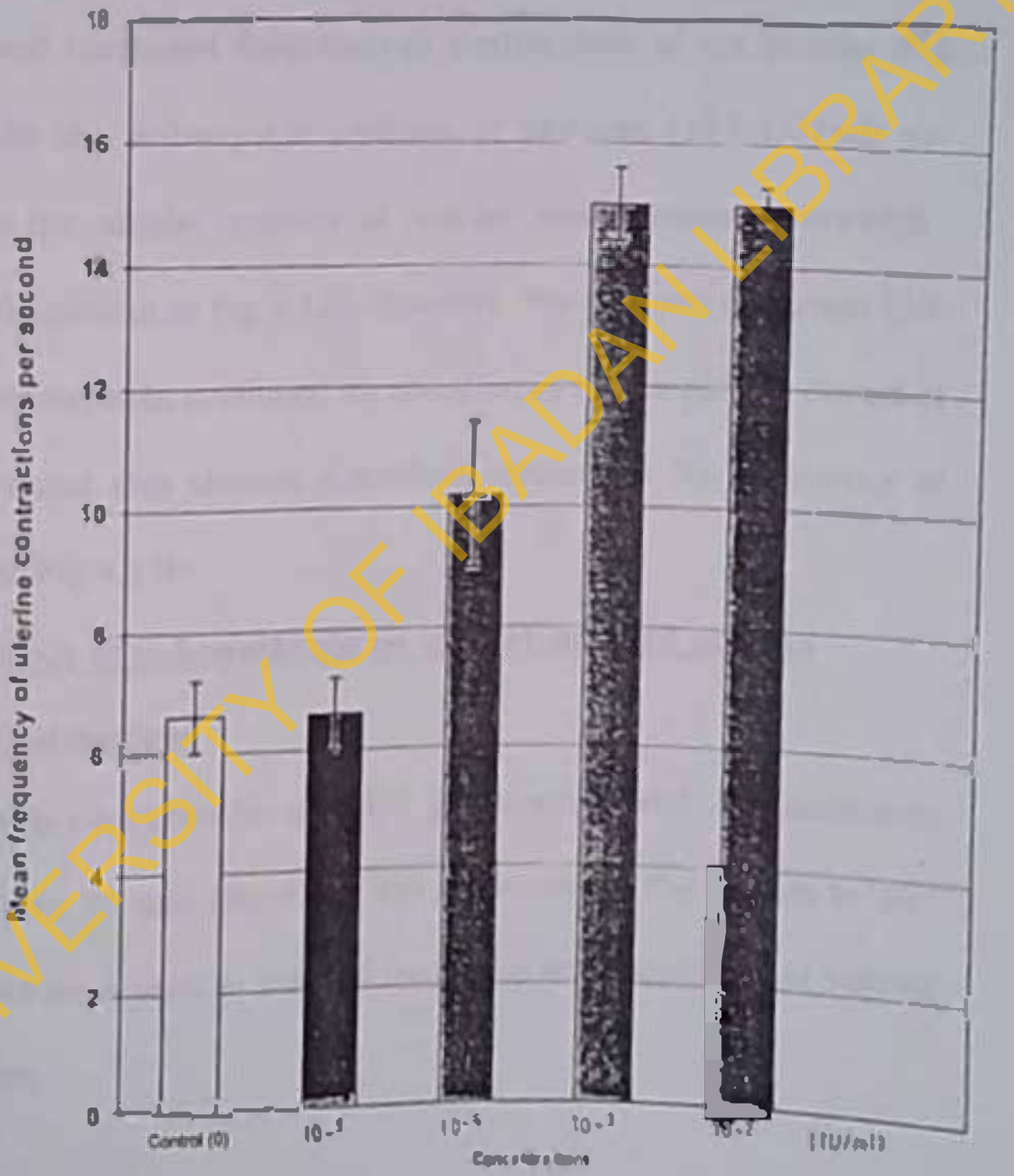


Fig 4.14. Histogram showing the mean frequency of uterine contractions per second against different concentrations of oxytocin. Each bar represents mean ± SEM. n = 6.

(c) **Effect of extract on oxytocin-induced contraction on rat uterus**

Figures 4.15(a) and (b) illustrate the effect of extract on oxytocin-induced uterine contractions. Prior addition of extract (10^{-6} g/ml) caused increased frequency of contractions of rat uterus (Fig 4.15b) while the subsequent addition of oxytocin (10^{-3} I.U/ml) re-established the similar pattern of uterine contractions shown with 10^{-3} I.U/ml oxytocin in Fig 4.13b. However, the addition of extract (10^{-6} g/ml) after oxytocin produced an elevation in the amplitude (force) of contraction and also showed a marked increase in the frequency of contraction (Fig 4.15b).

(d) **Effect of indomethacin on extract-induced uterine contractions**

After pre-incubation of IRUS preparation with indomethacin (10^{-4} g/ml) for 30 min (Fig 4.16), the responses of the uterus to 10^{-7} g/ml extract were seen as lowered frequency and amplitude of uterine contractions.

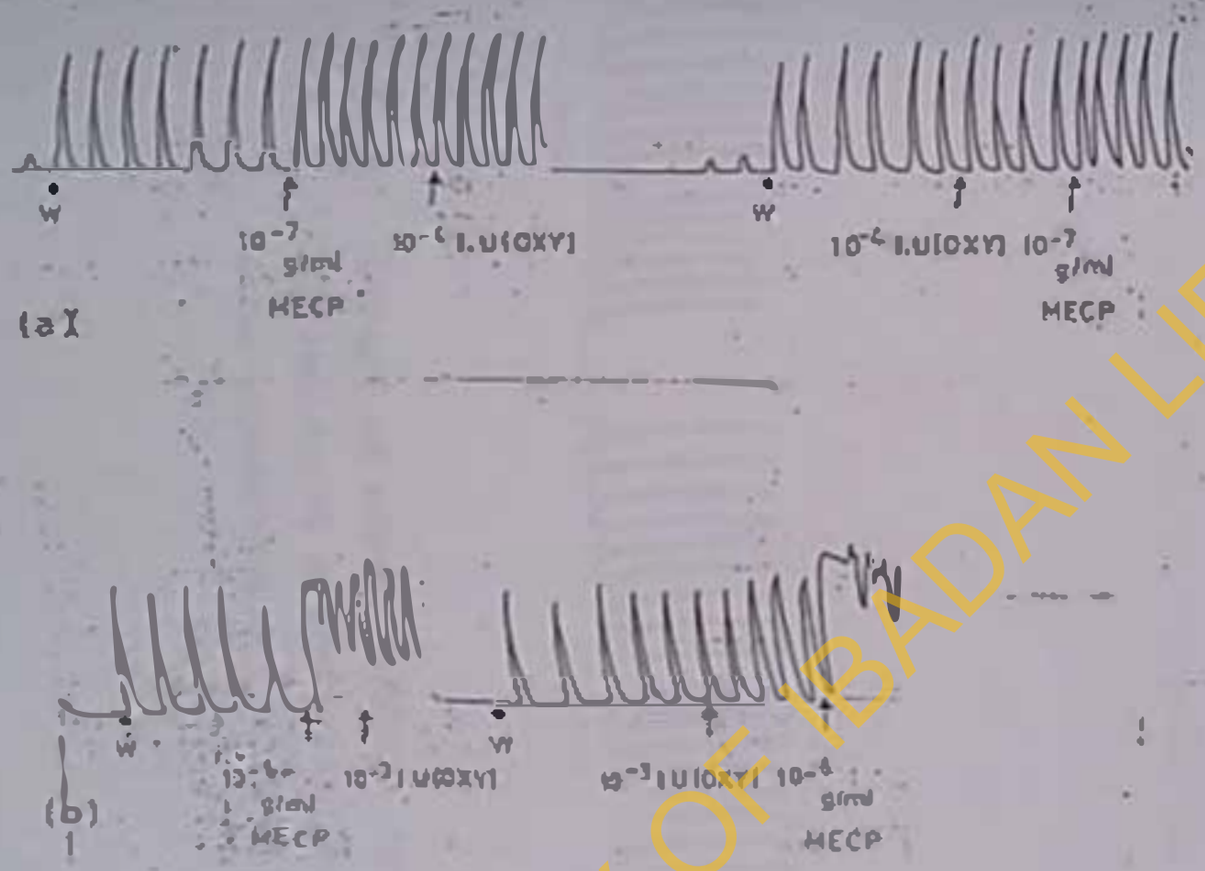


Fig. 4.15: (a) & (b): Effect of extract (MECP) on oxytocin-induced contraction in rat uterus.

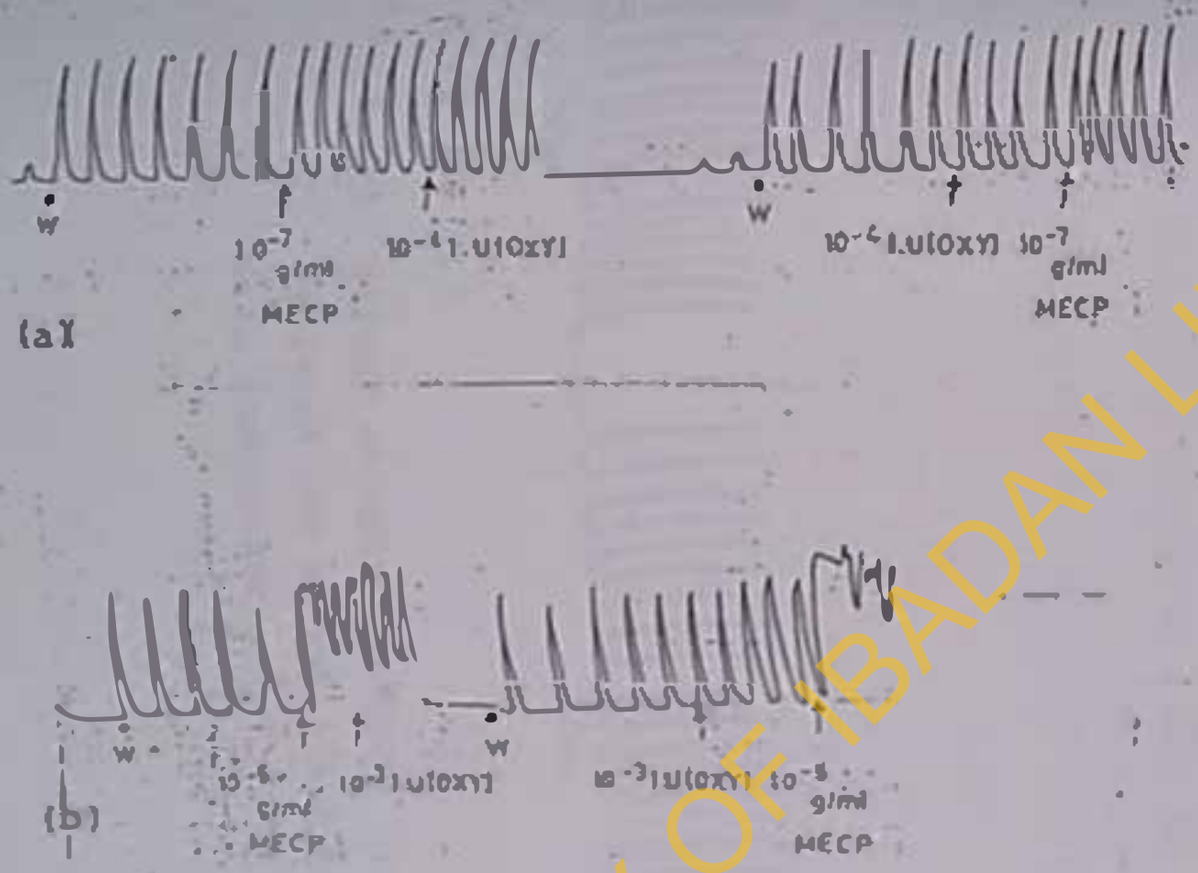
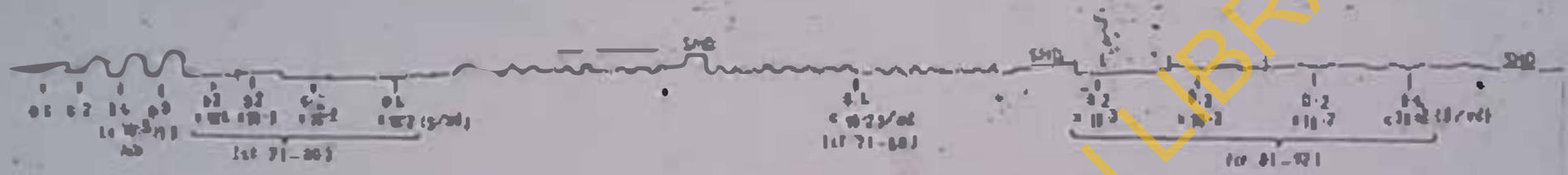


Fig. 4.15: (a) & (b): Effect of extract (MECP) on oxytocin-induced contraction in rat uterus.

Fig. 4.16: Effect of extract (MECP) (10^{-7} g/ml) on isolated rat uterus after pre-incubation with indomethacin (10^{-4} g/ml) for 30 mins.

(e) **Effect of column fractions of extract on rat uterus**

In the experiment designed to study the effects of column fractions of MECP on rat uterus, all the fractions (Cf_1 to Cf_8) indicated on Table 3.1 were tested in the study. Typical traces for the observed effects are shown in Fig 4.17. Dose-response relationship was initially established with a standard drug acetylcholine ($0.1 - 0.8 \times 10^{-5}$ M). Intermittent addition of the fractions after the submaximal dose of Ach (0.4×10^{-5}M) showed that the tissues were unresponsive to all the fractions.



* SMD = Submaximal dose of Acetylcholine (0.4×10^{-5}M Ach)

Fig. 4.17: Effect of different column fractions of MECP on isolated rat uterus strip preparations.

DISCUSSION

DISCUSSION

The mixture of activated charcoal in tragacanth is found as a useful opaque medium in the in – vivo intestinal transit study in mice because it is well tolerated in extremely high does up to 100g in man (Orisakwe and Akintonwa, 1991).

The in – vivo study in mice showed that the extract significantly reduced intestinal transit of charcoal when compared to normal saline treated mice acting as the control ($P<0.05$). This effect of the extract is similar in action to that of opiate derivatives. The opiate compounds are reported to diminish intestinal transit in – vivo in mice and also contract longitudinal muscles of the guines pig in – vitro (Orrego and spero, 1989). This action suggest that the extract reduces gastric emptying.

In the study showing the effect of graded does of extract on gastric acid secretion, the extract produced significant increase in gastric output ($P<0.05$). The results are presented in fig 4.2. However, a low dose of 10.5g/ml extract did not show any significant difference with the basal acid secretion ($P>0.05$). Compounds that increase gastric acid secretion are described as secretagogues. The results probably show that the extract produced secretagogue - like activity at fairly high doses. The study on the intravenous injection of the extract before histamine on gastric acid secretion showed significant

reduction in histamine – induced gastric acid secretion ($P<0.05$). This observation indicates a possible interaction of extract on histaminergic receptors of the stomach (H_2 receptors) which are responsible for gastric acid secretion. The fact that fractions Cf_2 and Cf_4 with melting points of 166°C and 212°C respectively caused significant increase in gastric acid secretion when compared to basal acid values (Fig 4.4) may be indicative of the presence of gastric acid secretory principles present in these fractions. Since parietal cell is known to be the unit cell responsible for gastric acid secretion, these Principles may be stimulating more parietal cells to become active at increasing dose (Bolarinwa and Amure, 1976). The hypothesis seems possible as the secretagogue effect of the extract was evident at high doses. This can then partly explain and account for the extract - induced gastric acid secretion. Although there are two other receptors in the stomach namely muscarinic and gastrinergic which mediate gastric acid secretion (Sachs, 1986), increase in gastric acid secretion associated with the extract was found to be histamine mediated (Fig 4.3). This mechanism of action represents the final pathway for gastric acid secretion (Berghindh, 1984).

On the isolated guinea pig ileum preparation, the extract caused dose dependent contractions which resembled those induced by acetylcholine and histamine (Fig 4.5).

In terms of potency, the extract was not as sensitive as histamine and acetylcholine on the ileal smooth muscle strips (Fig 4.6). Atropine (10^{-7}g/ml) which abolished acetylcholine (10^{-6}g/ml) responses failed to have effect on extract (0.4 x 10^{-3}g/ml) induced contraction (Fig 4.7). This suggests that cholinergic receptor interaction may not be involved in the explanation of the contractile action of extract on GPI preparation. It is however worthy of note that acetylcholine effects its action by interacting with muscarinic receptors to cause an influx of extracellular calcium which then triggers the release of intracellular calcium. The calcium ions combines with the contractile protein calmodulin to initiate contraction (Edman and schild, 1962). In another set of experiment, the contractions produced by the extract on GPI strips were blocked in a dose - dependent fashion by promethazine, a member of the selective H_1 - receptor blocker (Fig 4.9). Promethazine antagonism of this contractile effect of the extract on GPI strips suggest that histaminergic mechanism may be involved in the extract mode of

action. Although some plants are now known to contain appreciable amount of histamine (Parry and Wenyika, 1994), the phytochemical findings on the plant have not indicated the presence of this compound (Shetty *et al*, 1983). Following the results obtained using different fractions of extract on GPI preparations, the contractile activity of the seed's extract may probably be present in Cf_6 (M.pt 210°C). This assertion is made based on the fact that Cf_6 was the only fraction that produced contractile responses (Fig 4.10b).

In the study of the extract effect on IRUS preparation, different doses (10^{-9} to 10^{-5}g/ml) of the seed methanolic extract of *Croton penduliflorus* produced progressive increase in the frequency of uterine contractions (Fig 4.11). Similarly, the same pattern of dose – dependent increase in the frequency of uterine contractions were recorded for oxytocin (Fig 4.13). The extract thus displays oxytocic property by causing a dose – dependent increase in frequency of uterine contractions. Oxytocin was used as standard drug in this study. The drug effects its action by interacting with oxytocic receptors of the uterus. This interaction varies with number of oxytocic receptors and also depends on the hormonal status of the animal (Parry and Wenyika, 1994). The observation that there was no

change in the amplitude and frequency of uterine contractions when oxytocin (10^{-3} I.U/ml) was added after MECP (10^{-6}g/ml) would suggest that the contractile effect of the extract on the uterus cannot be explained in terms of direct interactions with oxytocin receptors. In yet another experiment, appreciable and significant reduction in the frequency and amplitude of uterine contractions were exhibited when rat uterus was incubated in indomethacin for 30 min prior to addition of 10^{-7} g/ml MECP (Fig 4.16). This effect of indomethacin on the uterine activity may partly explain the mechanism of action of the extract on the rat uterus. The extract is probably promoting the synthesis of prostnglandins. This hypothesis is based on the experimental findings that indomethacin which is an inhibitor of prostnglandin synthesis caused significant reduction in frequency of uterine contractions evoked by the extract. It should be recalled that prostaglandins are powerful myometrial stimulants and have been reported to mediate the activity of most agents that stimulate the uterus (Solloff, 1979). The contractile effect of the extract on the uterus appear to support the previously reported findings of Asuzu et al (1990) on mice that chronic oral administration of the purgative principles of *Croton penduliflorus* seed oil showed anti-fertility effect.

This inference is made on the understanding that, a drug which enhances the contractile activity of the uterus will make it very unsteady and unresponsive for implantation.

From the experimental findings in this work that the extract contracted isolated ileal smooth muscle strips and that of the rat's uterus will therefore support both its use in relieving constipation in Plateau - State and the traditional use as abortifacient agent respectively. However, infertility cases in women who often take to herbal preparations may be associated with the ignorant use of this category of drugs which render the uterus unstable and unresponsive to fertilised ovum.

CONCLUSION

CONCLUSION

Going by the activities of the extract on different tissue preparations, the extract probably contain several active natural compounds.

Among the probable active principles are those that showed gastric acid secretory effect via H_2 - receptor at high doses, reduced in-vivo contractile movement of the mice intestinal smooth muscle. These effects favour decrease gastric emptying, efficient food digestion and prevent speedy evacuation of the stomach. Another compound produced dose - dependent contractile responses in GPI preparations. This is believed to be mediated via H_1 - receptor. The active principle for this effect may likely be present in fraction CF_6 with melting point 210°C that caused contraction of ileal smooth muscle (Fig 4.10b). The extract also contain a compound with oxytocic property. It may thus be contraindicated for women with fertility problems or to pregnant women that may likely need to use herbal preporation for gastro intestinal disorders like constipation

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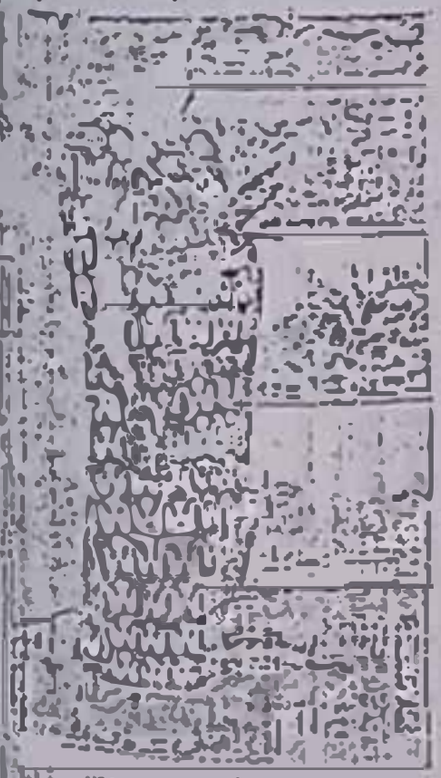
APPENDIX 1

Healing Nature's Way

By Zainab Dabup



APPENDIX 2

Relationship between different dosages of extract and mortality in mice

Drug Concentration (mg/ 10g Body weight)	Log-dose	Proportion Killed	Percentage Mortality
Control	0.00	0/6	0
2.5	0.40	0/6	0
5.0	0.70	1/6	17
10.0	1.00	4/6	67
20.0	1.30	5/6	83
40.0	1.60	6/6	100

Number of mice per group (n) = 6

Effect of different doses of extract, histamine and histamine plus extract on Gastric acid secretion

ANIMALS	EXPERIMENT I							EXPERIMENT II		EXPERIMENT III	
	Basal	After i/v of extract (MECP)						Basal	After i/v of histamine (0.05mg /100 g B.W)	Basal	After i/v of histamine (0.05mg/100 g B.W) plus 0.2 x 10^{-3} g/ml extract
		10^{-2}	10^{-1}	10^{-3} (g/ml)							
		0.2	0.2	0.1	0.2	0.3	0.4				
I	0.33	0.34	0.41	0.84	0.79	0.89	0.90	0.35	0.78	0.35	0.73
II	0.32	0.38	0.39	0.73	0.76	0.87	0.89	0.33	0.78	0.31	0.78
III	0.34	0.35	0.45	0.90	0.78	0.85	0.88	0.33	0.83	0.34	0.80
IV	0.35	0.35	0.40	0.85	0.80	0.85	0.85	0.34	0.88	0.33	0.78
V	0.33	0.37	0.43	0.75	0.90	0.83	0.83	0.34	0.80	0.30	0.78
VI	0.34	0.36	0.38	0.75	0.80	0.80	0.75	0.34	0.90	0.33	0.78
Mean ± SEM (Titre)	0.335 ± 0.004	0.358± 0.146	0.410± 0.011	0.803± 0.028	0.805± 0.019	0.848± 0.013	0.850± 0.023	0.338± 0.003	0.828± 0.021	0.327 ± 0.008	0.775 ± 0.010
Mean gastric acid secretion (meq/l/hrin)	3.35 ± 0.04	3.58 ± 0.15	*4.10± 0.01	*8.03± 0.03	*8.05± 0.02	*8.48± 0.01	*8.50± 0.02	3.38± 0.00	*8.28 ± 0.023	*2.7±0.01	*7.75 ± 0.01

Each value represents Mean ± SEM of Six(6) observations

- $P<0.05$ compared with the value for the best secretion

Effect of different column fractions of *Croton pendulifloris* on gastric acid Secretion.

COLUMN FRACTIONS		EXPERIMENTS								MEAN ± SEM (Gastric Acid Secretion) µeq/10miins		
		B1	S1	B2	S2	B3	S3	B4	S4	B	S	
19-22	Cf_1	2.68	2.65	2.63	2.70	2.40	2.50	2.30	2.35	2.50 ± 0.09	2.55 ± 0.08	NS
38-44	Cf_2	2.85	4.20	2.50	2.90	2.78	3.00	2.65	3.60	2.70 ± 0.08	3.43 ± 0.30	S
45-58	Cf_3	2.31	2.80	2.60	3.10	2.30	3.40	3.60	3.90	2.70 ± 0.31	3.30 ± 0.23	NS
63-70	Cf_4	2.23	4.10	1.75	4.00	2.60	3.10	2.50	3.30	2.27 ± 0.19	3.63 ± 0.25	S
71-80	Cf_5	2.63	2.50	2.10	2.20	2.40	2.35	2.60	2.70	2.44 ± 0.12	2.44 ± 0.11	NS
81-92	Cf_6	2.53	2.50	2.35	2.50	2.45	2.60	2.00	2.00	2.33 ± 0.12	2.45 ± 0.09	NS
93-105	Cf_7	2.33	2.60	2.40	2.40	2.90	2.95	2.00	2.00	2.41 ± 0.19	2.49 ± 0.20	NS
106-117	Cf_8	2.35	2.43	2.50	2.50	3.60	3.60	3.60	2.20	2.66 ± 0.32	2.68 ± 0.31	NS

Alphabets "B" and "S" represent basal and stimulated gastric acid secretion respectively. All values are in µeq/10 minutes n =4 observations

• $P<0.05$ compared with basal value indicated.

APPENDIX 5

Effect of different doses of acetylcholine on isolated guinea pig ileum preparation

Drug Concentration (g/ml)	Height of contraction (cm) Mean ±SEM	Percentage Maximum contraction	n
1×10^{-9}	0.50 ± 0.17	19.61	6
1×10^{-8}	1.18 ± 0.06	46.27	6
1×10^{-7}	1.54 ± 0.10	60.39	6
1×10^{-6}	2.55 ± 0.03	100.00	6
1×10^{-5}	2.55 ± 0.03	100.00	6

n = Number of observations

APPENDIX 6

Effect of different doses of histamine on isolated guinea pig ileum preparation

Drug Concentration (g/ml)	Height of contraction (cm) Mean ±SEM	Percentage Maximum contraction	n
1×10^{-8}	0.54 ± 0.10	11.16	6
1×10^{-7}	3.10 ± 0.45	64.05	6
1×10^{-6}	4.78 ± 0.73	98.76	6
1×10^{-5}	4.84 ± 0.75	100.00	6

n = Number of observations

APPENDIX 7

Effect of different doses of ~~extract~~(MECP) on isolated guinea pig ileum preparation

Drug Concentration (g/ml)	Height of contraction (cm) Mean ±SEM	Percentage Maximum contraction	n
1×10^{-6}	0.33 ± 0.01	4.58	6
5×10^{-6}	1.73 ± 0.15	24.03	6
1×10^{-5}	2.55 ± 0.25	35.42	6
2×10^{-5}	3.79 ± 0.26	52.64	6
3×10^{-5}	4.61 ± 0.48	64.03	6
4×10^{-5}	5.60 ± 0.51	77.78	6
5×10^{-5}	5.62 ± 0.51	78.06	6
1×10^{-4}	6.80 ± 0.06	94.44	6
2×10^{-4}	7.18 ± 0.05	99.72	6
3×10^{-4}	7.20 ± 0.04	100.00	6

n = Number of observations

Effect of different doses of extract (MECP) on contractile responses of IRUS preparation

Drug Concentration (g/ml)	Mean Frequency of uterine contractions per second ± SEM	n
Control(0)	28.8 ± 5.4	6
10^{-9}	30.0 ± 4.2	6
10^{-8}	34.2 ± 4.8	6
10^{-7}	36.0 ± 4.8	6
10^{-6}	*48.0 ± 2.4	6
10^{-5}	*48.0 ± 0.0	6

Key:

n = Number of observations

Control(0) = Basal spontaneous contractions

* P<0.05 significantly different from control.

Effect of different doses of oxytocin on contractile responses of IRUS preparation

Drug Concentration (g/ml)	Mean Frequency of uterine contractions per second ± SEM	n
Control(0)	6.60 ± 0.60	6
10^{-5}	6.60 ± 0.60	6
10^{-4}	*10.20 ± 1.20	6
10^{-3}	*15.00 ± 0.60	6
10^{-2}	*15.00 ± 0.00	6

n = Number of observations

Control(0) = Basal spontaneous contractions

* $P<0.05$ significantly different from control.