# FURTHER STUDIES ON THE PHARMACOLOGICAL AND TOXICOLOGICAL PROFILE OF CRUDE EXTRACTS OF *HELIOTROPIUM INDICUM* LINN

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by

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# DECLARATION

The experimental work described in this thesis was carried out at the Department of Pharmacology, KNUST. This work has not been submitted for any other degree. I hereby declare that this thesis has been composed by myself, that it has not been accepted in any previous application for a degree, that the work of which it is a record has been done by myself and that all sources of information have been specifically acknowledged by means of references

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Alex Boye

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Prof. A. K. Abaitey

# ABSTRACT

*Heliotropium indicum* Linn has various medicinal uses in the treatment of disease conditions such as abdominal pains, amenorrhoea, dysmenorrhoea, skin rashes, wounds, hypertension, ocular infections, convulsion, dizziness and also used as a component of ''abemuduro'' (a local herbal preparation used mostly by Asante women to manage post - labour inflammatory reactions). The purpose of this work was to evaluate the pharmacological activity of crude extracts of *Heliotropium indicum* on isolated smooth muscle preparations, the cardiovascular system of the anaesthetised cat, isolated rabbit and frog hearts and also the analgesic and some toxicological effects in *in vivo* and *in vitro* studies to explain some of its reported local uses.

*In vitro* studies of the effects of the crude extracts on smooth muscle preparations ( Isolated guinea – pig ileum, isolated rabbit jejunum, and isolated rat uterus) showed contractile activity which was possibly mediated by stimulating muscarinic and nicotinic receptors, since the contractions were readily inhibited by atropine and hexamethonium. Two prostaglandin synthetase inhibitors indomethacin and diclofenac sodium could not modify the effects of the crude extracts and oxytocin on the isolated pregnant and non – pregnant rat uterus preparations. The crude extracts of *Heliotropium indicum* showed significant ($P < 0.05$) stability to plasma cholinesterase comparable to those of methylcholine and carbamylcholine.

*In vivo* and *in vitro* studies of the effects of crude extracts on the cardiovascular system of the anaesthetized cat showed negative inotropic and chronotropic effects. The cardiovascular effects of the crude extracts could be mediated through $M_2$ – receptor stimulation or by direct action on the heart muscle.

Crude extracts of *Heliotropium indicum* (30 - 300 $mg/kg^{-1}$) insignificantly [$F_{3,8} = 2.633$, $P = 0.5391$, $F_{3,8} = 8.633$, $p = 0.3321$] inhibited both the first and second phases of formalin induced pain as compared to those of morphine and diclofenac sodium (1 – 10 $mg/kg^{-1}$)

[$F_{3,8}$ = 2.699, P = 0.3926 and $F_{3,8}$ = 1.442, p = 0.9923; $F_{3,8}$ = 1.678, P = 0.9234, and $F_{3,8}$ = 2.344, p = 0.4121] respectively.

Oral doses of 3 g/kg in mice and 5 g/kg in rats of the crude extracts were tolerated, however, in crude extract treated rats histopathological damages were observed in the liver, kidney, lungs, and heart.

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# DEDICATION

I dedicate this thesis to the memory of my late mother, Madame Georgina Abena Puopele Samba.

## TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF PLATES

# ABBREVIATIONS

- HIE – crude extract of *Heliotropium indicum*
- $HIE^{A}$ – Aqueous extract of *Heliotropium indicum*
- $HIE^{E}$ – Ethanolic extract of *Heliotropium indicum*
- MABP - Mean Arterial Blood Pressure
- ACh - Acetylcholine
- EDTA - Ethylenediamine tetra acetic acid
- PA - Pyrrolizidine Alkaloid
- ICR - Imprinting Controlled Region.
- CHD - Coronary Heart Disease
- RBC - Red Blood Cells
- WBC - White Blood Cells
- i.p. – Intraperitoneal
- p.o. – Oral administration of drug
- i.v. - intravenous
- S.R. – Sarcoplasmic Recticulum.
- mAChR – muscarinic Acetylcholine Receptors
- W N R – Within Normal Range

*Chapter 1*

# INTRODUCTION

## 1.1 GENERAL INTRODUCTION

Kloss (1939) had reported the use of herbs as the oldest medical science. Herbs have been reported to be the most widely used in traditional medicine (Orwa, 2002). Much of the medicinal use of plants seems to have been developed through observations of wild animals uneventful use of herbs as food and also by trial and error. As time went on tribes added the medicinal power of herbs in their area to their knowledge base. In the time past, a common practice in herbal medicine was based on the application of the doctrine of "signature" which was a belief in the principle of "like cures like". Under this doctrine, the selection of a particular plant part as a local remedy was based upon its odour, shape, or colour. For example, the dandelion stem, which contains "milk", was used to stimulate lactation; plants with red outlook were used to treat blood - related problems; and plants with yellow appearance were appropriate in the treatment of jaundice (Chandler, Freeman and Hooper, 1979). Tribes methodically collected information on herbs and developed well-defined herbal pharmacopoeias.

Indeed, well into the $20^{th}$ century much of the pharmacopoeias of scientific medicine were derived from the herbal lore of native people. Many drugs commonly used today are of plant origin. A report has it that about 25 % of prescription drugs dispensed in the USA contain at least one active ingredient derived from plant material, or made from plant extracts, while others are synthesized to mimic a natural plant compound. The desire to

exert more responsibility and control over one's body and life-style has led to resurgence in self - care practices. This self - treatment frequently reflects health-care practices influenced by folk remedies and the use of medicinal plants for maintaining health and treating common diseases. Depersonalisation frequently experienced in today's technologically oriented medical - care delivery system, increasing public dissatisfaction with the cost of prescription medications, combined with an interest in returning to natural or organic sources of remedy, perhaps should serve as an impetus for us to start looking for more traditionally affordable, and readily available, cheaper alternative sources of health care of which herbal medicine is an integral. It is in line with this that the World Health Organization (WHO) had acknowledged the need for inexpensive and effective treatment for common diseases especially in low-income countries (WHO, 2002). According to WHO, most traditional medicine practitioners live and work at the community level, which makes their treatment available and affordable to most of the people (WHO, 2002).

However, choices between the use of herbal medicine and conventional medicine vary across ethnic groups and social classes. At any given time, treatment may reflect exclusive use of herbal medicine, or the application of conventional medical practice alone or as an adjunct to herbal medicine. Recently, the WHO estimated that 80 % of people worldwide rely on herbal medicines for some aspect of their primary healthcare ( World Bank, 2002).

Nonetheless, the bane of herbal medicine is that it has usually been practiced without the appropriate scientific framework to ensure that cause and effect can be properly documented to institute a system of verification or assessment of the efficacy of treatment.

Thus, whereas, the local herbal medicine practitioner is quick to profess the efficacy of his remedy, he stops short of providing a sound scientific basis and explanation for his remedy and more often than not thrives on the long, continuous uneventful use of the remedy for treatment (Robbers and Tyler, 1999). A plant may contain vitamins essential for good health; nitrogenous organic compounds (alkaloids) that act on the vascular and central nervous systems; antibiotics that attack microorganisms; and essential oils, heterosides (glucides or sugars), acids, or minerals that interact chemically to affect certain body organs selectively (Schauenberger and Paris, 1977), but these scientific information on most medicinal plants in use are lacking. As part of the efforts to promote the use of herbal medicines either as an alternative or adjunct to conventional medical care or bridging the gap between conventional and herbal medicines, it is necessary for scientists to carry out preliminary scientific investigations into herbal medicines. These scientific investigations could help characterize the active compounds in herbal medicines and also help to predict the pharmacological activity of these active compounds in herbal medicines. It could also help to promote the rational use of herbal remedies within the cultural context or system in which they are used

## 1.2 THE PLANT HELIOTROPIUM INDICUM LINN

*Heliotropium indicum* Linn (Family Boraginaceae) is a medicinal plant. It is known commonly as “Cock’s comb”. *Heliotropium indicum* has the following vernacular names:

**Twi**: *Akomfem-triko; ansam-konakyi; akokotubatuba; akukopea; akomfetiko.*

**Fante***: Akoko-nyenedem; akokodam*

**Ga-Adangbe***: Kokodenebaa*

**Nzema***: Dodowhirede; apusupusuo*

**Ewe***: Koklotutsu; agama-ble; litsa-ble*

**Hausa***: Kalkashin; korama*.

### *1.2.1 Morphology of Heliotropium indicum Linn*

It is an annual herb, 30 - 90 cm tall, bears flowers throughout the year. The flowers are small, blue or white, in long curled terminal spikes; only few flowers open at a time, the remainder of the spikes covered with fruits and buds; spikes curled towards the tip; leaves broadly ovate, up to 11 cm long, 8 cm broad, and undulate - dentate.

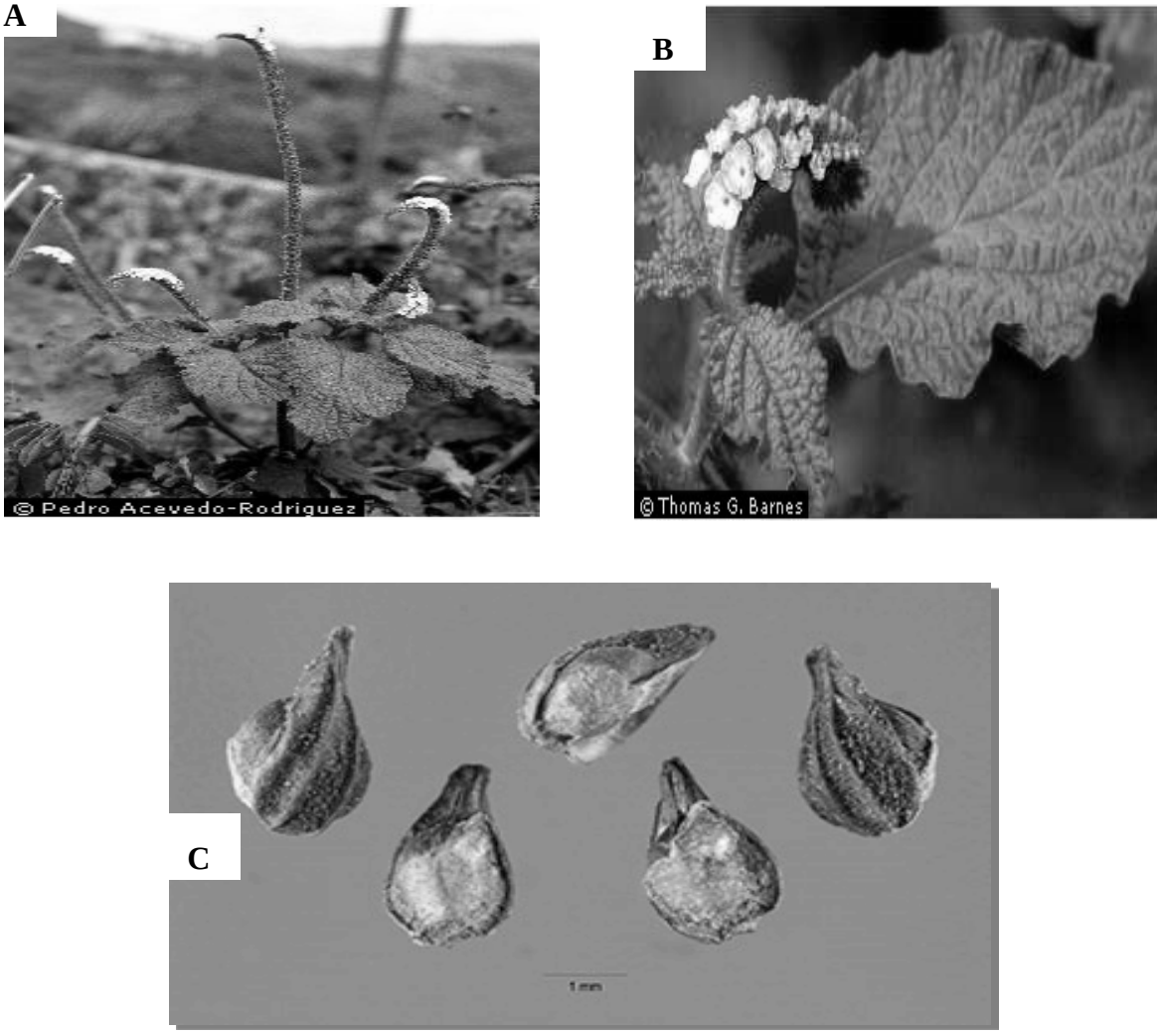


**Figure1.1 Shows the aerial parts (A), flowers and leaves (B), and the fruits (C) of *Heliotropium indicum* Linn.**

## *1.2.2 Ecological Distribution*

It is usually associated with the moist rich soils of the lowland tropics near rivers and lakes, on the roadsides, and also in waste places. It may also be found in moist sandy soils or in shallow swamps (Holm *et al*., 1977).

### *1.2.3 Propagation*

It reproduces by seeds which are produced from the long, curled, one-sided spikes (Holm *et al*., 1977). Datta *et a.l*., (2003) had reported propagation of *Heliotropium indicum* in callus cultures.

### *1.2.4 Chemical Composition*

The genus *Heliotropium* had been reported to yield alkaloids which closely resemble those isolated from plants in the genus *Senecio* (Zhao *et al*., 1989). Betanabhatla *et al*., (2007) had reported the presence of alkaloids in the chloroform extract of *H. indicum.* Ghana Herbal Pharmacopoiea, (2007) had reported presence of Pyrrolizidine alkaloids and their N-oxides: indicine, echinatine, lasiocarpine, indicine N - oxide, Laziocarpine N - oxide, hydrolizable tannins, and reducing sugars in *Heliotropium indicum*. Birecka *et al*., (1984) had also reported that *Heliotropium indicum* contains the pyrrolizidine alkaloids indicine, indicine – N - oxide, acetyl - indicine, indicinine, heleurine, heliotrine, supinine, supinidine and lindelofidine, all of which are thought to have hepatotoxic activity. Furthermore work had also isolated the alkaloids trachelanthamidine and retronecine and the pyrrolizidine precursor amines (in leaves and inflorescence) putrescine, spermidine and spermine. The seeds of *Heliotropium indicum* are found to contain 12 % oil and 1.8 % nitrogen ( Birecka *et al.,* 1984). The nitrogen-containing lipid fraction contained $C_{16}$ and $C_{18}$ acids esterified with 1 – cyano – 2 – hydroxymethylprop – 1 – en – 3 - ol (Birecka *et al.,* 1984).

*Heliotropium indicum* grown under greenhouse conditions showed the highest alkaloid content at the beginning of the flowering period. The young leaves, seedlings and inflorescences showed high alkaloid levels and with ageing, the level of alkaloids decreased 20 fold in the leaves. The highest alkaloid content was found in the roots and inflorescence and these also had the highest relative amounts of N - oxide, ranging from 60 – 90 % of the total alkaloid content. No significant age-dependent differences in N-oxides were found (Hartman, 1999).

Kugelman *et al*., (1976) isolated N-oxide of the alkaloid indicine from *Heliotropium indicum.* Many reports had indicated the presence of pyrrolizidine alkaloids in *Heliotropium indicum* (Sammy *et al*., 2005; Mattock, 1961; Jose, 2000, Ghana Herbal Pharmacopoiea, 2007; Kerharo and Bouquet, 1950). Pyrrolizidine alkaloids are considered of great pharmacological, biological, and chemotaxonomic interest (Reddy *et al*., 2002; Reina *et al*., 1998; Catalfamo *et al*., 1982).

Sixteen free fatty acids from the crude hexane extract of *Heliotropium indicum* Linn have been identified after conversion to their methyl esters with boron trifluoride-methanol followed by quantification by GC - FID and identification by GC - MS analysis (Theeraphan *et al*., 2008).

According to Theeraphan *et al*., (2008) the sixteen fatty acids accounted for 95 % of the chromatographable components, with 9, 12 - octadecadienoic acid, (39.7 %), 9 -

octadecenoic acid (32.4 %), hexadecanoic acid (14.2 %) and octadecanoic acid (5.1 %), as the major constituents.

A small amount of 6,10,14-trimethyl-2-pentadecanone and 3,7,11,15 – tetramethyl – 2 – hexadecen – 1 - ol as well as a homologous series of n - alkanes present at trace level and ranging from $C_{25}$ to $C_{31}$ were also found Theeraphan *et al*., (2008).

Rosmarinic acid and methyl rosmarinate had also been identified in *Heliotropium indicum* (Derianiyagala *et al*., 2002).

### *1.2.5 Folk Uses*

#### *1.2.5.1 Medicinal Uses In Ghana*

*Heliotropium indicum* Linn is used extensively among villagers and some urban dwellers in Ashanti, Ghana, in the treatment of various disease conditions. Some of the uses include the external application of the poultice of the leaves to sores (Irvine, 1930); cold infusion of the leaves used as an enema stops abdominal pains; this preparation also removes cataract in the eye; the juice from the leaves is squeezed into the eye to stop dizziness; decoction of the whole plant is used to treat convulsion in children; the roasted aerial parts of the plant together with certain ingredients are used as an enema for expulsion of clotted blood in a woman who has recently given birth; the poultice of the leaves mixed with honey is externally applied to the penis to restore virility; the juice from the mashed leaves is used as ear drops (Ghana Herbal Pharmacopoiea, 1992; Burkill,1985; Kerharo and Adams, 1974);

and the poultice is topically applied to swollen glands of the neck (Kerharo and Adams, 1974). Irvine, (1960) had reported the use of the concoction of the leaves with clay as an anti-abortive agent among Asante women.

### *1.2.5.2 Medicinal Uses In Other Countries*

*Heliotropium indicum* is also put to various uses in many other West African countries. For instance, in Senegal, the leaf powder is applied to skin rashes, suppurating eczema and impetigo in children (Kerharo and Adams, 1974). The plant has the reputation of being toxic to certain animals (Kerharo and Adams, 1974). In Ivory Coast, the dried leaf powder is snuffed as a decongestant in treating cold and sinusitis (Kerharo and Adams, 1974 cited by Adiaratou *et al*., 2005; Bouquet and Debray, 1974). A preparation of the plant is applied to erysipelas in Liberia and therefore, commonly known as "erysipelas plant" in Liberia (Dalziel, 1937). In Siby, a village in Mali it is used against vomiting, amenorrhoea, reduced baby weight, ocular infections and high blood pressure (Adiaratou *et al*., 2005).

Many countries use *Heliotropium indicum* in various forms for the treatment of gonorrhoea, for instance, in Gambia the leaf infusion; in Ivory Coast the leaf-sap with citron - juice; and in Nigeria the leaf infusion are used for the treatment of gonorrhea (Kerharo and Bouquet, 1950). In Gabon, a leaf preparation of the plant is used in the treatment of inflammation of gums and genitals (Walker, 1953; Walker and Sillans, 1961). The plant is a common ingredient of the Yoruba Agbo infusion for fever in children

(Dalziel, 1937). In Sierra Leone, a leaf decoction is used to wash new born babies (Bouquet and Debray, 1974).

*Heliotropium indicum* has many uses in other countries outside West Africa. Among these countries are Sudan where poultice of the leaves is used to treat headaches and Indonesia where the leaf decoction is used for thrush (Burkill, 1935). In Zaire, a leaf infusion is used as remedy for fever (Taton, 1970).

### *1.2.5.3. Other Medicinal Uses*

Other medicinal uses of *Heliotropium indicum* comprises the use of juice of the leaves as an antiseptic and anti - inflammatory agent when applied to wounds, sores, boils, gum boils and pimples on the face. Boiled with castor oil, it is applied to sores from scorpion bites and also locally used in treating nophthalmia, when the cornea is inflamed or excoriated (Kham, 2004; Soerjani *et al*., 1987; Radanachaless *et al*., 1994). *Heliotropium indicum* leaves are used for the treatment of ophthalmic disorders, erysipelas and pharyngodynia (Chadah, 1981). Braga, (1976) had reported the use of *Heliotropium indicum* in folk medicine in the treatment of skin rashes and as a powerful expectorant.

### *1.2.5.4.* ***Non-Medicinal Uses***

In Gambia the whole plant is buried for the fleshy tissues to decompose away leaving the fibre which is used to make false hair for women.

### *1.2.6 Previous Work Done on Heliotropium indicum*

#### *1.2.6.1 Wound Healing Activity*

Many reports had indicated wound healing activity of *Heliotropium indicum* (Reddy *et al*., 2002; Diwan *et al*., 1982; Udupa *et al*., 1989). They showed that topical application of 10 % w/v of *Heliotropium indicum* increased the percentage of wound contraction and completed wound healing by $14^{th}$ day indicating rapid epithelisation and collagenisation, whiles the control used healed a similar wound in 23 days. Muthu *et al*., (2006) had also reported the use of *Heliotropium indicum* for the treatment of wounds and skin infections. Also, Suresh *et al*., (2002) had shown the wound-healing activity of *Heliotropium indicum* in rats.

#### *1.2.6.2 Anti-Inflammatory Activity*

Betanabhatla *et al*., (2007) reported that chloroform extract of *Heliotropium indicum* dose - dependently inhibited the carrageenan induced rat paw oedema and also showed anti-nociceptive activity in rats. Srinivas *et al*., (2000) and Abubakar et al., (2007) had reported the anti - inflammatory activity of *Heliotropium indicum*. The presence of phytocompounds in the crude extract of *Heliotropium indicum* had been reported as a possible cause of its anti - inflammatory and anti - nociceptive activities (Betanabhatla *et al*., 2007).

### *1.2.6.3 Anti-Tumour Activity*

Kugelman *et al*., (1976) isolated N - oxide of the alkaloid indicine from *Heliotropium indicum* and observed significant anti-tumor activity of the compound in W - 256 carcinosarcoma, L - 1210 leukaemia, P - 1534 leukaemia and melanoma B - 16 tumor systems. On the basis of these results, the compound was selected for human clinical trials. Miser *et al*., (1991) isolated indicine – N - oxide from *Heliotropium indicum* and used it to treat 46 children with malignant solid tumors (17 with osteosarcoma 12 with neuroblastoma 13 with a brain tumor, and 4 with other miscellaneous tumors).

### *1.2.6.4 Anti-Microbial Activity*

A preliminary biological testing showed that the crude hexane extract of *Heliotropium indicum* had antituberculosis activity against *Mycobacterium tuberculosis* (H37Ra) in alamar blue assay system with MIC of $1x10^{-4}$ mg/mL Theeraphan *et al*., (2008).

The antimicrobial activity of *Heliotropium indicum* extract against various microbes has been shown (Rao *et al*., 2001, Abdulazeez *et al*., 2008*;* Rao *et al.,* 2006, Nethaji and Manokaran, 2009; Deshmukh *et al*., 2009).

### *1.2.6.5 Anti-Fungal Activity*

Srikumar *et al*., (2009) had reported that *Heliotropium indicum* has anti - fungal activity against *Aspergillus flavus.*

### *1.2.6.6 Gastroprotective Activity*

Abdulazeez *et al.,* (2008) had shown that aqueous extract of dry leaves of *Heliotropium indicum* dose - dependently reduced indomethacin induced gastric mucosa ulceration in rats when administered orally. In the investigation it was shown that gastric mucosa ulceration induced by 80 mg/kg/bodyweight of indomethacin was reduced 31.0 %, 81.0 %, and 87.5 % by 100, 200, and 400 mg/kg of the extract respectively. It is observed that the gastric ulceration due to indomethacin is via the inhibition of prostaglandin biosynthesis (Abdulazeez *et al.,* 2008) but the mechanism of action of the gastroprotective activity of *Heliotropium indicum* is unclear, it is possible that its gastroprotective activity could be mediated via the mobilization of endogenous prostaglandins and also in part due to the presence of tannins and saponins (Abdulazeez *et al*., 2008).

### *1.2.6.7 Toxicity*

Pyrrolizidine alkaloids had been implicated in liver damage, a form of hepatic - veno occlusive disease, (Casarett and Doull, 1996). Human deaths had been reported in several countries for the accidental consumption of some species of *Heliotropium* (Casarett and Doull, 1997), including South Africa, Jamaica and Barbados. In Afghanistan, there was an epidemic of hepatic - veno occlusive disease from consumption of a wheat crop contaminated with seeds of a species of *Heliotropium* ( Tandon *et al*., 1978). The clinical

signs associated with the liver damage resemble those of cirrhosis and some hepatic tumors and may be mistaken for those conditions (McDermott and Ridker, 1990). The clinical condition had been described as a form of Budd - Chiari Syndrome with portal hypertension and obliteration of small hepatic veins (Ridker *et al*., 1985). Damage to hepatocytes has been proposed to be due to the formation of pyrrole metabolites from Pyrrolizidine alkaloids by liver microsomal oxidation, with cross linking of DNA strands by the pyrrole metabolites (Carballo *et al*., 1992). Pyrrolizidine alkaloids produce necrosis or inhibition of mitosis depending on the dose but independent of the route of administration (Bull *et al*., 1968). Heliotrine, a pyrrolizidine alkaloid derived from *Heliotropium indicum* is reported to be associated with a major outbreak of disease in broiler chickens in Australia with the disease characterized by depression, ascites and hepatic degeneration (Pass *et al*., 1979). Subsequent experimental work showed that ingestion of *Heliotropium europaeum* the only source of heliotrine and lasiocarpine in Australia produced identical lesions seen in the natural disease (Pass *et al*., 1979). Further work showed that a diet containing 0.5 % heliotrope seeds and a total alkaloid content of 0.009 % caused reduced rate of growth and minimal liver lesions in young adult chickens (Caple, Heath, and Pass unpublished data cited by Pass *et al*., 1979).

## 1.3 REVIEW OF PHYTO - CONSTITUENTS OF *HELIOTROPIUM INDICUM*

### *1.3.1 Alkaloids*

Typical alkaloids are derived from plant sources, they are basic, they contain one or more nitrogen atoms usually in a heterocyclic ring and they usually have a marked physiological action on man or other animals (Trease and Evans, 1989). There are many types of alkaloids but two alkaloids (pyrrolizidine alkaloids and indicine – N - oxide) have been reported in *Heliotropium indicum* (Zhao *et al.,* 1989; Ghana Herbal Pharmacopoiea, 2007; Birecka *et al*., 1984).

### *1.3.2 Pyrrolizidine Alkaloids*

Although these alkaloids have at present no great medicinal significance, yet they are important in that they constitute the poisonous hepatotoxic constituents of plants in the genera senecio and boraginaceae, well known for their toxic effects on livestock (Trease and Evans, 1989). Some of the alkaloids also show carcinogenic and mutagenic properties raising safety concerns, for example pyrrolizidine alkaloids have been reported in some herbal products such as comfrey from the plant genus Boraginaceae (Trease and Evans, 1989). Casarett and Doull, (1996) and Culvenor *et al*., (1968) had also implicated pyrrolizidine alkaloids in liver damage.

### *1.3.3 Indicine-N-oxide*

Indicine – N - oxide isolated from *Heliotropium indicum* has been shown to have activity against some human leukemias (Letendre *et al*., 1981; Kugelman *et al.,* 1976; Philipson *et al*., 1978)). However this agent has been reported to have adverse effects on humans including hepatotoxicity secondary to veno - occlusive disease (McLean, 1970). Indicine – N - oxide when given intravenously to monkeys and dogs caused transient changes in hepatic function, however no histologic evidence of hepatic damage was observed in those animals (Castles *et al*., 1976; Rakieten *et al*., 1974).

### *1.3.4 Tannins*

Tannins are phenolic compounds that form complexes with proteins, divalent metals, cellulose, hemicelluloses, pectin, and other carbohydrates (Mahanato *et al*., 1982). Consumption of food containing high amount of tannins is dangerous to health, being a phenolic secondary plant metabolite with one or more hydroxyl substitutes bonded to an aromatic ring, it produces anthrocyanides a toxic product on acid degradation (Gatachew, *et al*., 2000; Waterman and Cole, 1994). Perez Maldonado, *et al.,* (1996) had observed that complexes formed by tannins with proteins are not digestible. In a similar work Mole and Waterman, (1987) showed that addition of tannic acid to standard trypsin solution led to the formation of insoluble complexes which are irreversible, therefore herbal products with high amount of tannins raises safety concerns.

### *1.3.5 Saponins*

Saponins have been reported to decrease plasma cholesterol and also increase bile acid production (Oakenful and Sidhu, 1990; Mahanato**,** *et al***.,** 1982). Saponins have also been reported to reduce rate of heart beat and also as an abortifacient in pregnant rats (Mahanato *et al*., 1982). The abortifacient effect is reported to be non - species specific (Mahanato, *et al*., 1982). Price, *et al*., (1987) had reported the formation of irreversible complexes between saponins and animal cell membranes, rendering the membranes impermeable. Saponins have also been implicated in haemolysis (Onning, *et al*., 1996; Casarett and Doull, 1996). In an earlier work Lalitha, *et al*., (1990) had shown that oral administration of saponins 300 mg/kg to rats caused histopathological changes in the liver and kidney of rats.

### *1.3.6 Cyanogenic Glycosides*

Cyanogenic glycosides are present in a number of food plants. Hydrogen cyanide (HCN) is normally released from cyanogenic glycosides when fresh plant material is macerated as in chewing which allows enzymes and cyanogenic glycosides to come into contact releasing hydrogen cyanide. Cyanide is one of the most potent and rapidly acting poisons known. Cyanide inhibits the oxidative process of cells. However, the human system is able to rapidly degrade cyanide within the concentration range of 50 - 60 parts per million (ppm) for an hour without any serious consequences. Generally, plants containing more than 20

mg HCN/100 g of fresh plant material are considered potentially dangerous but several factors determine whether poisoning will actually take place (Kingsbury, 1964). Montgomery, (1980) had reported the inhibition of metalloenzymes such as cytochrome oxidases by cyanogenic glycosides leading to oxygen deprivation and subsequent cessation of cellular respiration.

### *1.3.7 Steroids*

Plant steroids are key hormones throughout the plant kingdom. They regulate many aspects of growth and development; mutants deficient in plant steroids are often extremely stunted and infertile. Plant steroids are similar in many respects to animal steroids, but appear to function very differently at the cellular level. Animal cells respond to steroids using internal receptor molecules within the cell nucleus, whereas in plants the receptors are anchored to the outer surface of the cell membranes. Plant steroids are present in very small amounts in many fruits, vegetables, nuts, seeds, cereals, legumes, vegetable oils, and other plant sources. Plant steroids from dietary sources are reported to reduce cholesterol levels in the body, therefore have the ability to reduce risk of coronary heart disease (CHD).

## 1.4 JUSTIFICATION OF THE STUDY

The WHO estimates that four billion people (80 % of the world population) presently use herbal medicines for some aspect of their primary health care. Herbal medicine is a major

component in all indigenous people's traditional medicine and a common element in Ayurvedic, homeopathy, naturopathy, and even traditional oriental and native American-Indian medicine. WHO had reported that of 119 plants - derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlate directly with their traditional uses as plant medicines by native culture. WHO (2003) reported that countries in Africa, Asia, and Latin America use traditional medicine to help meet some of their primary health care needs. In Africa, up to 80 % of the population uses traditional medicine for primary health care (WHO, 2003).

Also a survey in 1983 confirmed that developing states are more interested than ever in making use of traditional indigenous resources in managing their primary health care programmes (Abbiw, 1990).

Therefore, it was not for nothing, when in 1978, the WHO set forth criteria for the study and documentation of medicinal plants of importance in the developing countries of the world. The WHO recognized the necessity of using indigenous treatment in providing healthcare. It has become important, therefore, to have accurate information about the actual use of herbal medicines in a given region, accompanied by any available scientific data on their pharmacological efficacy. As a result, the WHO has clarified the definition of medicinal plants and vegetable drugs and has established a procedure for the "botanical and ethnopharmacognostic investigation of a plant used in herbal medicine" (Penso, 1980). According to WHO, a medicinal plant is defined as "any plant which in one or more of its organs contains substance that can be used for therapeutic purpose or which are precursors

for chemo-pharmaceutical semi synthesis". The term "vegetable drug" refers to the part of the medicinal plant used for therapeutic purposes (Penso, 1980).

As healthcare gets increasingly expensive and ineffective in many African Countries, a growing number of people are turning to traditional herbal medicine. The WHO estimates seven out of ten people in Africa rely on traditional herbal medicine. In many countries, however, these herbal medicines are not certified and their use raises safety concerns. In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60 % of children with high fever resulting from malaria is the use of herbal medicine at home (WHO, 2003). Traditional as well as modern medicines are integral components of the health care delivery system in Ghana. In Ghana, a country of scarce medical resources and transitioning cultural traditions and beliefs, approach to health care is based on a personal understanding of one's health, life and being (Hevi, 1989; Van Eeden, 1993). Ghana is administratively divided into ten regions, each with a regional hospital, yet access to health care defined as living within 3 - 5 miles of a healthcare facility is lacking (Tabi, 1994). Approximately 70 % of the population of Ghana lives in rural areas where access to health care is a problem. In Ghana, the ratio of medical doctor to population is 1:20,000 whiles the ratio of traditional healer to the population is 1:200 (Patterson, 2001; Tabi and Frimpong, 2003). Often for the rural and urban poor, traditional healers provide the only affordable and accessible form of healthcare (Cocks and Moller, 2002; Sodi, 1996; Tabi, 1994). Research indicates that at least 60 % of Ghanaians use herbal medicine, either because it is cheaper, more convenient or simply believed to be more effective. With the renewed interest in the use of herbal

medicine as against conventional medicine especially among the rural and urban poor in developing countries like Ghana, it is important to promote the rational use of herbal medicine to serve as an alternative or an adjunct or better still bridge the gap between herbal and conventional medicinal use and to provide reliable scientific data concerning the pharmacological and therapeutic activities of herbal medicines.

The bane of herbal medicine had been their non-standardization as compared to conventional medicine, whereas the latter contain specific active ingredients of constant composition and expected biochemical/pharmacological effect with guarded safety, the former are used in the crude form as infusions or decoctions and the only evidence of efficacy is the prolonged and apparently uneventful use Robbers and (Tyler, 1999). Though it is well accepted that medicinal plants have components with various pharmacological activities, conscious effort should be made to identify these pharmacological activities; standardize the herbal preparations and to come out with consistent herbal products, which could take the place of conventional drugs in the treatment of disease conditions.

It is in the pursuit of the above, that this thesis aims at investigating the preliminary *in vitro* and *in vivo* pharmacological activity of *Heliotropium indicum* extract on the following :

- Smooth muscle preparations
- Cardiovascular system.

Also, analgesic and toxicological effects of *Heliotropium indicum* will be investigated *in vivo*.

*Chapter 2*

# MATERIALS AND METHODS

## 2.1 EXPERIMENTAL ANIMALS

Cats of either sex (1 – 3 kg), guinea – pigs of either sex ( 210 – 280 g), pregnant and non – pregnant female rats ( 200 - 260 g), ICR mice of either sex ( 20 – 35 g) and rabbits of either sex ( 2.2 – 3 kg) obtained from the animal house of the Department of Pharmacology, KNUST, were used for the experiments. All animals were kept under normal day - night cycle, and also under normal temperature and humidity conditions. They were allowed access to food and water *ad libitum*, however this was varied to suit the specific requirements of a particular experiment. Frogs (30 – 40 g) used were obtained from ponds on the KNUST Campus.

## 2.2 COLLECTION, IDENTIFICATION AND AUTHENTICATION OF *HELIOTROPIUM INDICUM*

The aerial parts of *Heliotropium indicum* were collected from Buokrom in the Ashanti Region in September, 2007 and authenticated by the curator (Mr. Adator K. Brown) of the

Department of Theoretical and Applied Biology, College of Sciences, KNUST, where a voucher specimen (KNUST/ BSC / F621) was deposited.

## 2.3 PREPARATION OF *HELIOTROPIUM INDICUM* EXTRACT (HIE)

### *2.3.1. Preparation of Ethanolic Extract of Heliotropium indicum ($HIE^E$)*

Sun - dried aerial parts of the plant were comminuted to coarse powder. A quantity of 500 g of the coarse powder was cold macerated with 4 litres of 70 % alcohol in a glass-stopped flask for three days. The macerate was filtered to obtain a dark - brown filtrate. A rotary evaporator was used to retrieve the alcohol leaving a dark - brown liquid which was oven - dried to finally yield a semi - solid gummy extract which was named ($HIE^E$) and it had a pH of 8.0. The percentage yield was 13.5 %.

### *2.3.2. Preparation of Aqueous Extract of Heliotropium indicum ($HIE^A$)*

A 600 g quantity of the coarse powder was mixed with 5 litres of water and warmed for 15 minutes. The infusion was filtered to obtain a dark - brown filtrate, concentrated by evaporation over a hot water bath and later in a hot air-oven at $60^oC$ until a constant weight was obtained. It was cooled in a desicator to yield dark - brown solid extract which was named ($HIE^A$).

## 2.4 PROCEDURES FOR PHYTOCHEMICAL SCREENING

The ethanolic and aqueous extracts of *Heliotroprium indicum* were screened for possible active principles using standard phytochemical methods as described by Wall *et al;* 1952; Trease and Evans, 1983; and Sofowora, 1993.

### *2.4.1 Test for Alkaloids*

To 0.5 g of $HIE^{A}$, 5 ml of 1 % aqueous hydrochloric acid (HCl) was added and stirred. The solution was warmed and filtered. A 1 ml sample each of the filtrate was transferred into two test tubes. To one of the test tubes was added a few drops of Mayer's reagent, to the other test tube was added a few drops of Dragendorff's reagent and observed.

### *2.4.2 Test for Tannins*

A 0.5 g of $HIE^{A}$ was boiled with 10 ml of distilled water for 5 minutes, filtered whiles hot and cooled subsequently. The filtrate was diluted to 20 ml with distilled water. A 1 ml of the above solution was further diluted with distilled water to 5 ml, after which a few drops of 0.1 % ferric chloride solution was added and observations made.

### *2.4.3 Test for Saponins*

A 0.5 g of $HIE^{A}$ was boiled with 10 ml of distilled water for 10 minutes and filtered whiles hot, and allowed to cool. A 2.5 ml of the filtrate was diluted with 10 ml of distilled water and shaken thoroughly. Two drops of castor oil were added to the solution and shaken vigorously for two minutes and observations were made.

### *2.4.4 Test for Cyanogenic Glycosides*

Cyanogenic glycosides were detected using the technique of the picrate - impregnated paper as described by Harbone (1984) with modification. The assay was performed in triplicate. A 1 g of $HIE^{A}$ was moistened with a few drops of dilute sulphuric acid in a test tube. The test tube was stoppered with a cork containing a strip of picrate - impregnated paper hanging down from the stopper and the set up incubated at ambient temperature for 2 h.

#### *2.4.4.1 Preparation Of The Picrate Paper*

Strips of filter paper (5.0 cm x 1.5 cm) were soaked in an aqueous solution of 0.05 M picric acid earlier neutralized with sodium bicarbonate and filtered. The impregnated paper was allowed to dry completely at ambient temperature.

### *2.4.5 Test for Steroids*

A mass of 0.5 g of $HIE^A$ was dissolved in 2 ml chloroform in a test tube. Concentrated sulphiric acid was carefully added down the inner sides of test tube which formed a lower layer. A reddish – brown colour appeared at the interface which indicates the presence of steroids.

## 2.5 EFFECT OF $HIE^A$ ON SMOOTH MUSCLE PREPARATIONS

### *2.5.1 Isolation and Mounting of Guinea-Pig Ileum*

A guinea - pig was fasted for 12 h before it was used, this ensured a clean gut which could be used immediately, without the need for washing and also to avoid the possibility of damaging the preparation in the process of washing. The guinea - pig was sacrificed later and the ileum isolated according to the method described by Okpako and Taiwo (1984). The abdomen was cut open, the first 10 cm of the intestine near the ileo - caecal junction was discarded, and the remaining piece of ileum placed in a warm aerated Tyrode physiological solution (Appendix 5.1). When the tissue had relaxed completely, 2 - 3 cm portion was mounted in the 20 ml inner bath in the organ bath of the Harvard Isolated Tissue / Organ Bath as described below. A thread was attached to each end of the isolated ileum by inserting a needle from the inside of the gut (isolated ileum) outwards making sure the lumen of the gut was not closed off. One thread was tied to the aerator tube in the inner bath and the other to a lever system which has a frontal writing point. The bath temperature was maintained at $32^oC$. The tissue was aerated with ordinary air using

Corning – Eel, 850 air compressor (Evans electroselenium Ltd, England). A load of 0.5 g which generated a tension of 5 mN was applied to the isolated guinea – pig ileum to minimize spontaneous activity if any, so that contractions due to added drugs could clearly be observed.

#### *2.5.1.1 Experimental Design*

The effects of acetylcholine ($1 \times 10^{-3} – 2.6 \times 10^{-1}$ mg/ml), histamine ($1 \times 10^{-3} – 2.6 \times 10^{-1}$ mg/ml), nicotine ($1 \times 10^{-3} – 2.6 \times 10^{-1}$ mg/ml) and $HIE^A$ ($2 \times 10^{-2} – 5.12$ mg/ml) were tested on the isolated guinea – pig ileum. The contractions of the longitudinal muscles of the isolated guinea - pig ileum were recorded by means of a pendular lever system with the frontal writing point moving on a white kymograph paper wound around a cylinder of 30 cm diameter revolving at a rate of 4 mm per minute. A 3 minute time cycle was used (0 second: kymograph was started; 30 seconds: drug was administered; 60 seconds: kymograph was stopped and tissue washed; 180 seconds: kymograph was started again).

#### *2.5.1.2 Determination of Site of Action of $HIE^A$*

Complete dose - response tracings of acetylcholine ($8 \times 10^{-5} – 1.3 \times 10^{-3}$ mg/ml) were obtained using the mounted isolated guinea - pig ileum and the sub - maximal response produced by a dose of acetylcholine ($6.4 \times 10^{-4}$ mg/ml) selected. Equipotent responses produced by nicotine ($4 \times 10^{-4}$ mg/ml), histamine ($3.2 \times 10^{-4}$ mg/ml), and $HIE^A$ (2.7 mg/ml) were obtained. The effects of hexamethonium (0.005 mg/ml), atropine ($1 \times 10^{-4}$ mg/ml) and

mepyramine (2 x $10^{-2}$ mg/ml) on the contractile activity of $HIE^A$ were compared with those of their respective agonists.

In another set of experiments to investigate the type of inhibitions, complete dose – response tracings were obtained for $HIE^A$ (1.3 x $10^{-1}$ – 34.1 mg/ml) in the absence and presence of atropine (5 x $10^{-9}$ mg/ml, 2 x $10^{-8}$ mg/ml, 1 x $10^{-7}$ mg/ml) and hexamethonium (0.005 mg/ml, 0.05 mg/ml).

#### *2.5.1.3 Test for Stability of $HIE^A$ to Plasma Cholinesterase*

Complete dose - response tracings of acetylcholine were obtained and a sub - maximal response (70 % of the maximum response) was chosen as a reference. Equipotent responses were obtained for methylcholine, carbamylcholine and $HIE^A$. Ten test tubes were labelled I – X and treated as shown in Table 2.1.

Tenfold sub - maximal dose of acetylcholine and equipotent doses of methylcholine, carbamylcholine and $HIE^A$ were added to test tubes I - IV respectively and made up with distilled water up to 10 ml to serve as controls. To the tenfold dose of acetylcholine (test tube V), methylcholine (test tube VI), carbamylcholine (test tube VII) and the $HIE^A$ (test tube VIII) was added 3 drops of blood (source of cholinesterase) and then made up to 10 ml with distilled water.

To test tube IX (control), 3 drops of blood and 5 ml distilled water were added, boiled for 5 minutes and cooled before the addition of the 10 - fold dose of acetylcholine. It was then

made up with distilled water to 10 ml. To confirm that cholinesterase was responsible for the expected inhibitions, 3 drops of blood and 1 ml of physostigmine ($5 \times 10^{-2}$ mg/ml) was added to test tube X before the addition of the 10 - fold dose of acetylcholine. It was then made up with distilled water to 10 mls. The test tubes were incubated for 15 minutes in water bath at 37 $^{o}$C, which is the optimum temperature for cholinesterase activity. After the incubation period, 1 ml of each drug equivalent to the selected dose in the respective test – tubes were administered onto the mounted isolated guinea – pig ileum preparation in succession and the dose - response tracings obtained were analyzed.

**Table 2.1 Treatment of test tubes I-X in the determination of stability of Acetylcholine, Methylcholine, Carbamylcholine and HIE to cholinesterase activity.**

| **Test tube** | **I** | **II** | **III** | **IV** | **V** | **VI** | **VII** | **VIII** | **IX** | **X** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Acetylcholine** ($1x10^{-6}$ mg/ml) | 0.2 ml | | | | 0.2 ml | | | | 0.2 ml | 0.2 ml |
| **Methylcholine** ($1x10^{-6}$ mg/ml) | | 0.5 ml | | | | 0.5 ml | | | | |
| **Carbamylcholine** ($3x10^{-6}$ mg/ml) | | | 0.5 ml | | | | 0.5 ml | | | |
| **HIE** (8.5 mg/ml) | | | | 0.5 ml | | | | 0.5 ml | | |
| **Blood** | | | | | 3 drops | 3 drops | 3 drops | 3 drops | 3 drops* | 3 drops |
| **Physostigmine** ($5x10^{-2}$ mg/ml) | | | | | | | | | | 1 ml |
| **Distilled water** | 10 ml | 10 ml | 10 ml | 10 ml | 10 ml | 10 ml | 10 ml | 10 ml | 10 ml | 10 ml |

*** The blood was added to distilled water, boiled and cooled before adding 10x volume of acetylcholine.**

### *2.5.2 Isolation and Mounting of Isolated Rabbit Jejunum*

A rabbit was sacrificed and the jejunum isolated according Okpako and Taiwo (1984). After opening the abdomen, the jejunum was carefully removed and placed in warm Ringer Locke physiological solution (Appendix 5.2) at 37$^{o}$C to facilitate washing of the intestine. The isolated tissue was aerated with ordinary air using Corning - Eel, 850 air compressor (Evans Electroselenium Ltd, Halstead, and Essex, England).

#### *2.5.2.1 Experimental Design*

Reference drugs (acetylcholine, noradrenaline) and the HIE$^{A}$ were administered using 1 ml syringe. Effects of HIE$^{A}$ (3 x $10^{-1}$ – 34 mg/ml) and acetylcholine (1 x $10^{-4}$ – 3.2 x $10^{-2}$ mg/ml) were investigated. The effect of atropine was investigated on the responses of the tissue to acetylcholine and HIE$^{A}$. The inner bath capacity was 20ml. The tension on the tissue was 10 mN. The bath temperature was maintained at 37$^{o}$C.

The contractions of the longitudinal muscles of the isolated rabbit jejunum were recorded with a pendular lever system with a frontal writing point moving on a white kymograph paper wound around a cylinder of 30 cm diameter revolving at a rate of 8 mm per minute.

The following time cycle was used:

0 second: the kymograph was started,

30 seconds: the dose of drug was added.

60 seconds: the kymograph was stopped and drug washed off the preparation.

180 seconds: the kymograph was started again.

### *2.5.3 Isolation and Mounting of Isolated Rat Uterus*

A rat was sacrificed and the abdomen opened. The two horns of the uterus were isolated and dissected. The dissected uterine horns were transferred to a dish containing De Jalon's physiological solution. The two horns were separated and freed from fat and connective tissues and each was cut open longitudinally so that the uterine horn was now a sheet of muscle instead of a narrow tube.

Each horn was further divided longitudinally to obtain four pieces. A strip of the horn about 2 - 3 cm was cut out. A thread was attached to one end of the isolated strip of uterus and was tied to the aerator tube in the organ bath containing De Jalon's physiological solution. Another thread was attached to the other end of the isolated uterus and fixed to a lever system fitted with a frontal writing point. The tissue was aerated with ordinary air using Corning - Eel, 850 air compressor (Evans Electroselenium Ltd, Halstead Essex England). The load on the tissue was 0.5 g (a tension of 5 mN). The temperature of the organ bath was maintained at $32^{o}$C. The isolated strip of uterus was allowed to stay in the De Jalon's physiological solution for one hour before use.

### *2.5.3.1 Experimental Design*

Effects of oxytocin (1 $x10^{-3}$ – 6.67 x $10^{-2}$ mg/ml), acetylcholine (1 $x10^{-3}$ – 3.2 x $10^{-2}$ mg/ml), and $HIE^{A}$ (1.3 x $10^{-1}$ – 17.1 mg/ml) were investigated on both the mounted isolated pregnant and non – pregnant rat uterus. Dose - response tracings were obtained for each drug.

The site of action of $HIE^{A}$ (6 mg/ml, 4.26 mg/ml, and 8.5 mg/ml) on the isolated rat uterus was investigated using salbutamol (4.2 x $10^{-4}$ mg/ml), atropine (4.2 x $10^{-4}$ mg/ml), indomethacin (4.8 mg/ml), and diclofenac sodium (1.7 x $10^{-1}$ mg/ml) as against their respective specific agonists. Drugs were washed off the preparation using the overflow method. The contractions of the longitudinal muscle of the isolated rat uterus were recorded with a lever system fitted with a frontal writing point moving on a white kymograph paper wound around a cylinder of 30 cm diameter revolving at a rate of 4 mm per minute.

The time cycle below was used:

0 second the kymograph was started

30 seconds: the dose of drug was added.

60 seconds: the kymograph was stopped and drug washed off the preparation.

180 seconds: the kymograph was started again.

## 2.6 EFFECT OF HIE$^{A}$ ON THE CARDIOVASCULAR SYSTEM

### *2.6.1 Isolation and Mounting of Isolated Frog Heart*

A frog was stunned by hitting its head sharply against the edge of a bench. The dorsal region of the frog's head was removed and the spinal cord destroyed by pushing a large pin down the vertebral canal. The frog's chest was opened and the heart carefully freed from connective tissue whilst ensuring that the arteries remain intact. The heart was continuously kept moistened by a cotton wool soaked in frog - Ringer solution (Appendix 5.3). The preparation was set up at normal room temperature. A small clip used to hold the tip of the ventricle was tied to a lever system fitted with a frontal writing point moving on a white kymograph paper wound around a cylinder of 30 cm diameter revolving at a rate of 4 mm per minute.

#### *2.6.1.1 Experimental Design*

Acetylcholine, histamine, adrenaline ($1 \times 10^{-3} - 3.2 \times 10^{-2}$) and HIE$^{A}$ ($1 \times 10^{-1}$ 12.8 mg/ml) were administered directly onto the frog heart using 1 ml syringe.

Dose - response tracings were obtained for each drug and analyzed statistically. The normal rhythmical force of contraction of the frog's heart was recorded as the control tracings.

The time cycle below was used:

0 second the kymograph was started

30 seconds: the dose of drug was added to the preparation

60 seconds: the kymograph was stopped and drug washed off the preparation

180 seconds: the kymograph was started again.

### *2.6.2 Isolation and Mounting of Perfused Rabbit Heart*

A rabbit was sacrificed and the heart with at least 1 cm of aorta attached removed as quickly as possible and placed in a dish of Ringer Locke physiological solution (Appendix 5.4) at room temperature. The rabbit was injected with heparin (1000 IU) through the marginal ear vein before being killed as a precaution to prevent clot formation in the heart. The preparation was squeezed several times when first placed in Ringer Locke solution so as to remove as much blood as possible from the heart. The aorta was located and dissected free from all connective tissue. All other vessels connected to the heart were trimmed away. The aorta was cut just below the point where it divides and the heart was transferred to the perfusion apparatus where the aorta was tied firmly on a glass cannula with a thread. Care was taken to ensure that no air bubble got into the aorta and also bubbles formed in the glass cannula were removed by a thin long polythene pipette. The perfusion fluid from a reservoir was oxygenated at a constant pressure and maintained at 37 $^{o}$C. A clip was attached to the tip of the ventricle of the perfused rabbit heart and connected to a spring lever by thread and the effects of the HIE$^{A}$ on the amplitude of contractions of the heart were recorded via a universal Oscillograph (model 50 - 8622, Harvard Apparatus Limited, Kent, England). The rate of flow was measured by placing a graduated cylinder beneath the lower chamber of the preparation. This was possible because the pressure of the perfusion

fluid closed the aortic valve, forcing fluid to pass through the coronary vessels, escaping from the inferior vena cava to form drops which dripped off into the graduated cylinder.

### *2.6.2.1 Experimental Design*

Drugs (acetylcholine, $HIE^A$, and atropine) were added to the preparation by injection through a rubber cup into the perfusion fluid. Gas collected below the rubber cup was removed from time to time by a syringe. Further doses of drugs were not given until the preparation had recovered fully from the effect of a previously administered drug.

### *2.6.3 The Anaesthetized Cat Experiment*

Each cat was anaesthetized using chloralose (1 % w/v) at a dose of 80 mg/kg administered intraperitoneally. The arterial blood pressure was recorded through a carotid artery cannula (polythene tubing, 1.02 - 1.27 mm) via a pressure transducer (model P23Gb, Statham) and the phasic arterial pressure was continuously monitored via a universal Oscillograph (model 50 - 822, Harvard Apparatus Ltd, Kent, England). Drugs were injected through a cannula inserted into the femoral vein on one of the hind legs. The venous catheter was continuously flashed with 0.5 ml of 0.9 % normal saline after each dose was administered. This was to ensure that drugs collected in the catheter were all pushed into the femoral vein. The whole preparation was allowed to equilibrate for 30 minutes before doses of drugs were added. Effects of $HIE^A$ (0.3 – 90 mg) and acetylcholine ($3.2 \times 10^{-4}$ - $1.2 \times 10^{-}$

$^{3}$mg) were tested on the preparation. After each drug treatment, cardiovascular variables were allowed to stabilize for an additional 15 minutes before any further administration of drugs were made.

## 2.7 ANALGESIA

### *2.7.1 Formalin-Induced Nociception*

The formalin - induced nociception was carried out as described by (Dubuisson and Denis, 1977; Malmberg and Yaksh, 1992) with some modification.

ICR mice were randomly divided into four groups of three mice per group. The groups were treated as follows: group one $HIE^{A}$ (30, 100, and 300 mg/kg body weight *p.o*), group two $HIE^{E}$ (30, 100, and 300 mg/kg body weight *p.o*), group three morphine (1, 3, 10 mg/kg body weight *i.p*) and group four diclofenac (1, 3, and 10 mg/kg bodyweight *i.p*). Groups three and four served as controls. Each animal was placed in one of twenty Perspex test chambers of linear dimensions (15x15x15 cm).

Morphine and diclofenac sodium were administered intra - peritoneally 30 minutes before the induction of nociceptive behaviours in the animals by a subcutaneous injection of 0.1 ml of 5 % formalin solution into the plantar tissues of the right hind paw of animals. The extracts were administered 1 hour before the induction of nociception. After the induction of nociceptive behaviours animals were quickly returned individually into the testing chamber. A mirror placed at $45^{o}$ beneath the testing chambers made possible an unobstructive viewing of the animals in the testing chamber. The nociceptive behaviors of

the animals were captured (60 min) by using a camcorder (Evario$^{TM}$ model GZ - MG1300, JVC, Tokyo, Japan) positioned in front of the mirror. Nociceptive responses were scored (60 min) for each animal immediately after formalin injection with the help of a public software JWatcher$^{TM}$ Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia ).The average nociceptive score for each time block was calculated by multiplying the frequency and time spent on licking and biting. The data were expressed as the mean ± sem scores between 0 - 10 and 10 - 60 minutes after the induction of nociceptive behaviours.

## 2.8 TOXICITY STUDIES

### *2.8.1 Experimental Design*

Experimental animals were randomly divided into five groups of four animals per group. Each group of animals was kept in an aluminium cage. Each animal in a group was marked to permit individual identification. The first four groups of animals were assigned to four doses of HIE$^{A}$ (1, 2, 4 and 5 g/kg *p.o*). The fifth group was used as the control group.

### *2.8.2 Acute Toxicity Test*

Five groups of animals were used, with four animals in each group. The animals were fasted for 24 hours prior to the administration of the extract. The first four groups of

animals were administered with the following increasing doses of $HIE^A$ (1, 2, 4, and 5 g/kg *p.o*).

The experiment above was repeated using mice dosed with $HIE^A$ (1, 1.5, 2, and 3 g/kg *p.o*).

After administration of $HIE^A$ the animals in each group were observed for seven hours for changes in movement, salivation, respiratory pattern, and frequency and consistency of stool. Also, the mortality of animals in each experimental group within twenty four hours was observed.

### *2.8.3 Sub - Acute Toxicity Test*

Four groups with four Spraque - Dawley rats in each group were used. Animals in the first three groups were respectively dosed daily for fourteen days with $HIE^A$ (2, 4 and 5 g/kg *p.o*). The fourth group served as control. Animals in each group were weighed on the first day before $HIE^A$ administration, on the seventh day and at the end of the fourteenth day.

Blood samples were collected from all four animals in each group through a cardiac puncture into ethylenediamine tetra - acetic acid (EDTA) treated tubes (5 ml) for haematological studies using sysmex haematology system (Model: KX - 21N, Kobe, Japan). The haematological system was used to measure RBC count, WBC count, haemoglobin concentrations and platelet levels. The results were analysed statistically. The animals in each group were sacrificed on the $14^{th}$ day.

### *2.8.4 Histopathology*

The following organs (lung, liver, kidney, and heart) were isolated and preserved in 10 % formalin. Portions of the organs were embedded in paraffin and cut according to standard micro techniques and stained with hematoxylin and eosin. Photomicrographs were made and examined for histopathological changes at the Histopathology Department of Komfo Anokye Teaching Hospital, Kumasi.

The absolute wet weights of the isolated organs (lung, heart, liver, and kidney) were compared to the body weight of the whole animals from which they were isolated and the results analyzed statistically. Also, the changes in body weights between the first, seventh and fourteenth days were analysed.

## 2.9 STATISTICAL ANALYSIS

The $ED_{50}$ ( The dose responsible for producing 50 % of the desired maximum effect) and all responses of drugs were analysed by using an iterative computer least squares method, Graphpad Version 5.00 ( Graphpad Software, San Diego, CA, USA) with the following non – linear regression ( Four – parameter logistic equation).

$$\mathbf{Y = a + (a - b) / 1 + 10^{(Log\ ED_{50} - X) \times Hill\ Slope)}}$$

Where **X** is the logarithm of concentration, **Y** is the response, which starts at **a** and goes to **b** with a sigmoid shape. The levels of significance were determined by analysis of variance (ANOVA) and Student – Neuman – Keuls post hoc test using graphpad prism Version

5.00. Values were expressed as mean ± sem $P < 0.05$ (significant), $P < 0.01$ (very significant), $P < 0.001$ (highly significant) and $P > 0.05$ (non – significant). $HIE^{A}$ was used in almost all the experiments in this thesis because all the reported local uses involve aqueous preparations however in experiments where $HIE^{E}$ was used it has clearly been stated.

# *Chapter 3*

# RESULTS

## 3.1 PHYTOCHEMICAL SCREENING

Phytochemical screening of the aqueous and ethanolic extracts of *Heliotropium indicum* showed the presence of alkaloids, cyanogenic glycosides, tannins, saponins, and steroids (Table 3.1).

**Table 3.1 Phytocompounds present in the aqueous and ethanolic extracts of *Heliotropium indicum*.**

| Active principles | Aqueous extract | Ethanolic extract |
|---|---|---|
| 1. Alkaloids | + | +++ |
| 2. Cyanogenic glycosides | + | ++ |
| 3. Saponins | ++ | ++ |
| 4. Tannins | + | +++ |
| 5. Steroids | ++ | ++ |

**Key: +, ++, and +++ describe the strength and frequency of color change.**

## 3.2 EFFECT OF HIE ON SMOOTH MUSCLE PREPARATIONS

### *3.2.1 Isolated Guinea - Pig Ileum*

$HIE^A$ produced dose - dependent contractions of the isolated guinea - pig ileum just as acetylcholine, histamine, and nicotine (Figure 3.1 and appendix 5.5). The $EC_{50}$ values for $HIE^A$, acetylcholine, histamine and nicotine are shown in Table 3.2.

#### *3.2.1.1 The Effect Of* **Hexamethonium, Atropine** *And* **Mepyramine** *On The Contractile Effect Of $HIE^A$ On The Isolated Guinea - Pig Ileum Preparation*

Hexamethonium inhibited the effect of nicotine by 86.2 % ($P < 0.001$) and that of $HIE^A$ by 38.9 % ($P < 0.001$) (Fig 3.2 and appendix 5.6). Atropine inhibited the effect of acetylcholine by 72.6 % ($P < 0.001$) and that of $HIE^A$ by 34.8 % ($P < 0.001$) (Fig 3.3 and appendix 5.7). Though mepyramine inhibited effect of histamine, it had no inhibitory effect on the contractile responses of the isolated guinea – pig ileum to $HIE^A$.

In a related experiment to investigate the type of inhibitions produced by atropine and hexamethonium on the contractile responses of the isolated guinea – pig ileum to $HIE^A$, the log dose - response curves of $HIE^A$ were shifted to the right, however the right shifts were not parallel (Figs 3.4, appendix 5.8 and fig 3.5, appendix 5.9 respectively). The $EC_{50}$ values of $HIE^A$ in the presence of atropine were higher than $HIE^A$ in the absence of atropine (Table 3.3). Similarly, $EC_{50}$ values of $HIE^A$ in the presence of hexamethonium increased as compared to those of the control (Tables 3.4).

Surprisingly, on a similar isolated guinea – pig ileum preparation pretreated with mepyramine the contractile responses of the guinea – pig ileum to $HIE^A$ was potentiated resulting in a shift of the log dose – response curves of $HIE^A$ to the left (Fig 3.6 and appendix 5.10). The $EC_{50}$ values of $HIE^A$ in the presence of mepyramine decreased compared to the control (Table 3.5).

#### *3.2.1.2 Investigation Of The Stability Of $HIE^A$,* Acetylcholine, Methylcholine *And* Carbamylcholine *To Plasma* Cholinesterase.

$HIE^A$ was more stable to cholinesterase activity than acetylcholine but less stable than carbamylcholine and methylcholine. The stability of $HIE^A$ to plasma cholinesterase activity was therefore significant ($P < 0.05$) (Fig 3.7 and appendix 5.11).

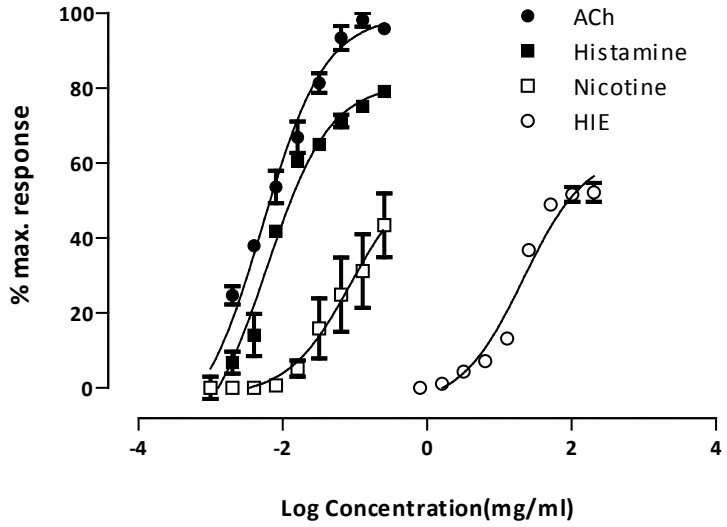


**Figure 3.1 Log dose - response curves of acetylcholine (1 x $10^{-3}$ - 2.6 x $10^{-1}$ mg/ml), histamine (1 x $10^{-3}$ - 2.6x$10^{-1}$ mg/ml), nicotine (1 x $10^{-3}$ - 2.6x$10^{-1}$ mg/ml) and HIE$^{A}$ (2 x $10^{-2}$ - 5.12 mg/ml) on the isolated guinea - pig ileum preparation. Each point represents the mean ± sem (n = 5)**

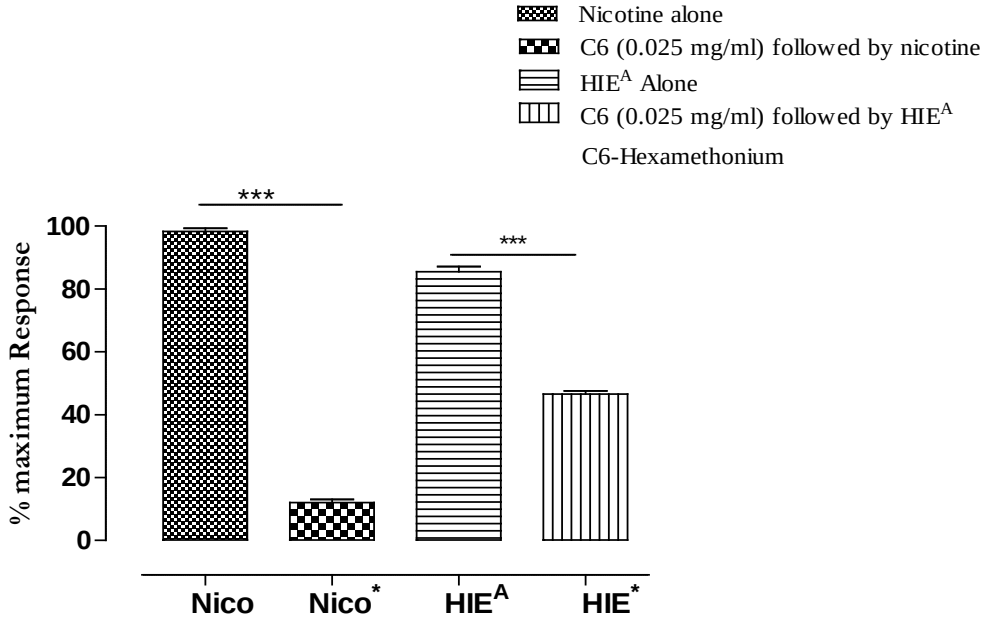


**Figure 3.2 Effect of Nicotine (4x$10^{-4}$ mg/ml) and HIE$^{A}$ (2.7 mg/ml) on the isolated guinea - pig ileum in the presence of Hexamethonium (0.025 mg/ml). Each column represents the mean ± sem (n = 3). The inhibitions of responses of nicotine and HIE$^{A}$ by hexamethonium were highly significant (98.28 ± 0.9954,12.07 ± 0.9954, P < 0.001 and 85.52 ± 1.593,46.55 ± 0.9954, P < 0.001 respectively).**

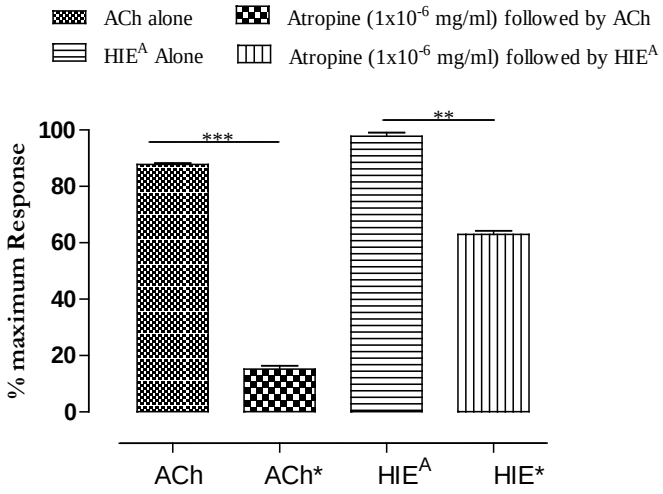


**Figure 3.3 Effect of Acetylcholine (6.4 x $10^{-4}$ mg/ml) and $HIE^A$ (0.1 mg/ml) on the isolated guinea - pig ileum preparation in the presence of Atropine (1 x $10^{-6}$ mg/ml). Each column represents the mean ± sem (n = 3).The inhibition of responses of acetylcholine by atropine was highly significant (P < 0.001) and inhibition of responses of $HIE^A$ was very significant (P < 0.01).**

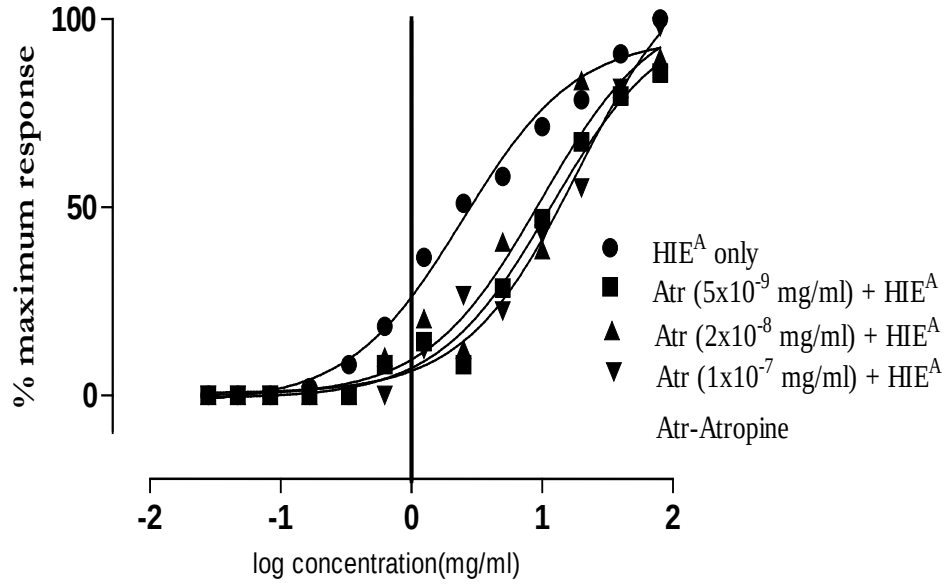


**Figure 3.4 Log dose - response curves of $HIE^A$ (0.02 - 34.1mg/ml) showing the effect of $HIE^A$ on the isolated guinea - pig ileum in the presence of three separate concentrations of atropine (5 x $10^{-9}$ mg/ml, 2 x $10^{-8}$ mg/ml, and 1 x $10^{-7}$mg/ml). Each point is the mean ± sem (n = 3).**

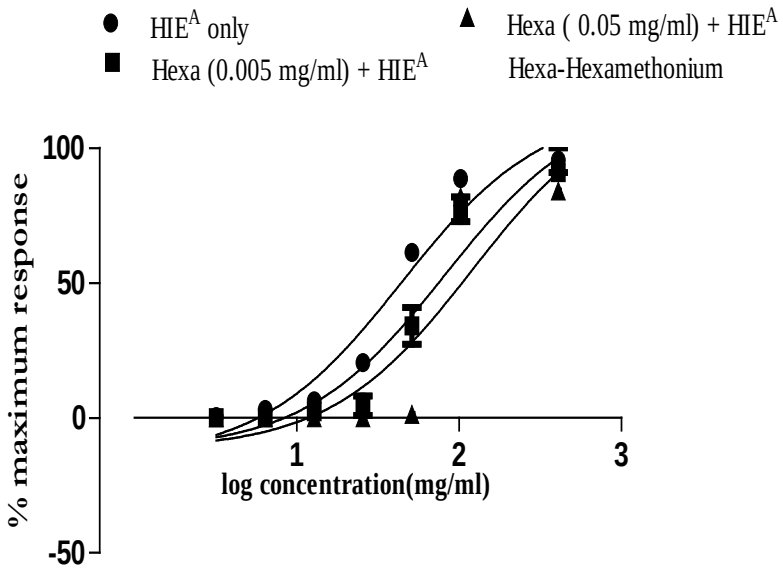


**Figure 3.5 Effect of HIE$^A$ (0.3 - 34.1mg/ml) on the guinea - pig isolated ileum in the presence of two separate concentrations of Hexamethonium (0.005 mg/ml, and 0.05 mg/ml). Each point is the mean ± sem (n = 3).**

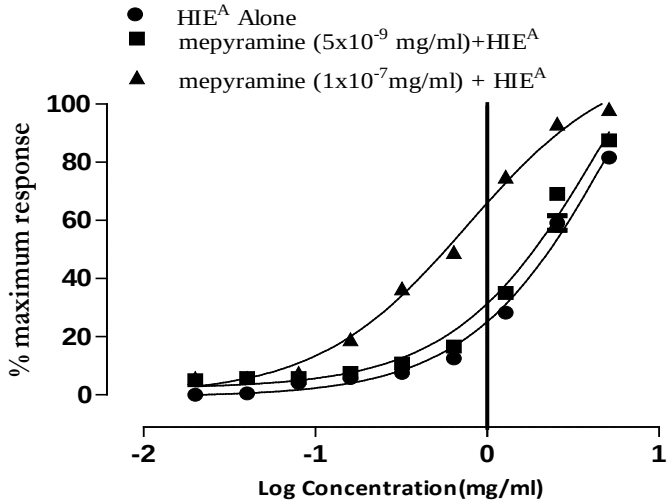


**Figure 3.6 Effect of HIE$^A$ (0.02 - 5.12 mg/ml) on the isolated guinea-pig ileum in the presence of two separate concentrations of mepyramine (5 x $10^{-9}$ mg/ml, 1 x $10^{-7}$ mg/ml). Each point represents the mean ± sem (n=4).**

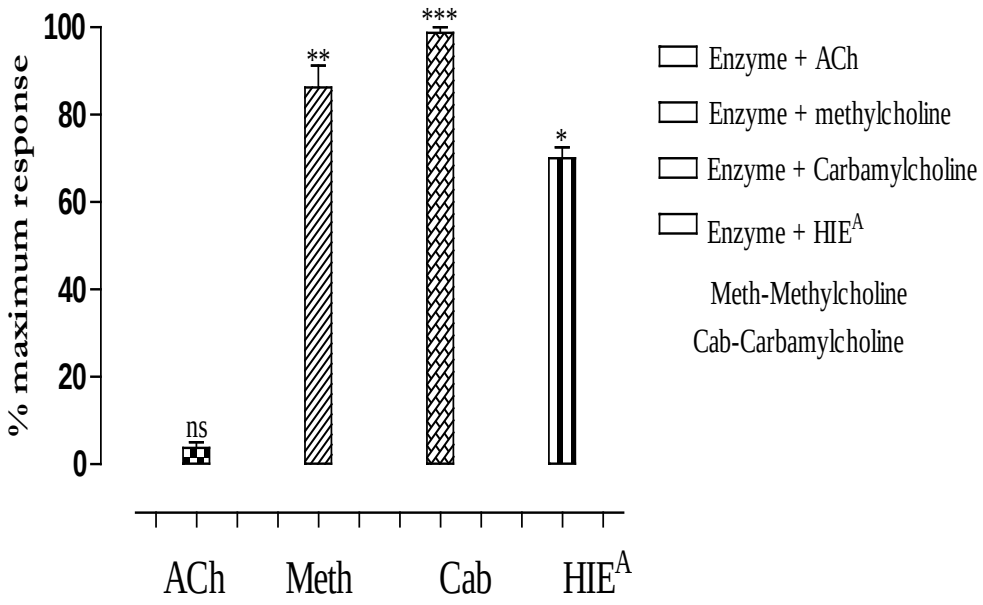


**Figure 3.7 The contractile effects of acetylcholine (1 x $10^{-6}$ mg/ml), methylcholine (1 x $10^{-6}$ mg /ml), carbamylcholine (3 x $10^{-6}$ mg/ml) and $HIE^A$ (8.5 mg/ml) on the guinea-pig isolated ileum in the presence of cholinesterase (Enzyme). Each column is the mean ± sem (n = 3). The degree of significance in activity in the presence of cholinesterase is shown as P < 0.001 (highly significant), P < 0.01 (very significant), P < 0.05 (significant) and ns (not significant)**

### *3.2.2 Isolated Rabbit Jejunum*

$HIE^A$ produced biphasic effect on the isolated rabbit jejunum and the effects were dose - dependent (Fig 3.8 and appendix 5.12).

#### *3.2.2.1.**Effects Of Atropine And Hexamethonium On The Contractile Responses Of The Isolated Rabbit Jejunum To $HIE^A$***

Atropine inhibited the effect of acetylcholine by 56.7 % ($P < 0.001$) and that of $HIE^A$ by 13.7 % ($P < 0.01$) however, hexamethonium that inhibited effect of nicotine by 50 % ($P < 0.01$) could not inhibit the effect of $HIE^A$ (ns $P > 0.05$) (Fig 3.9 and appendix 5.13). Table 3.6 shows the $EC_{50}$ and minimum and maximum concentrations of $HIE^A$ on the rabbit isolated jejunum.

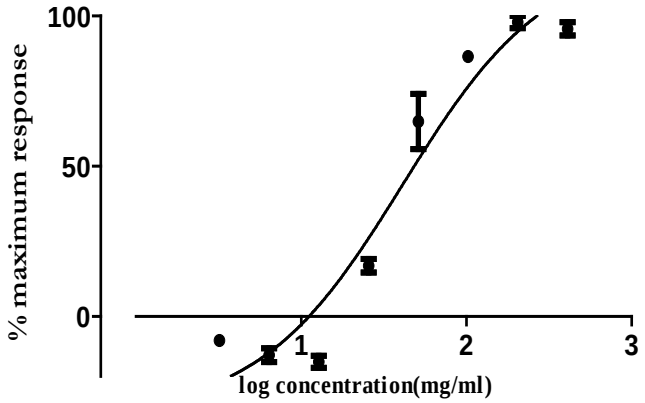


**Figure 3.8 Log dose - response curve of HIE$^{A}$ (0.3 - 34.1mg/ml) on the rabbit isolated jejunum. Each point is the mean ± sem (n = 4).**

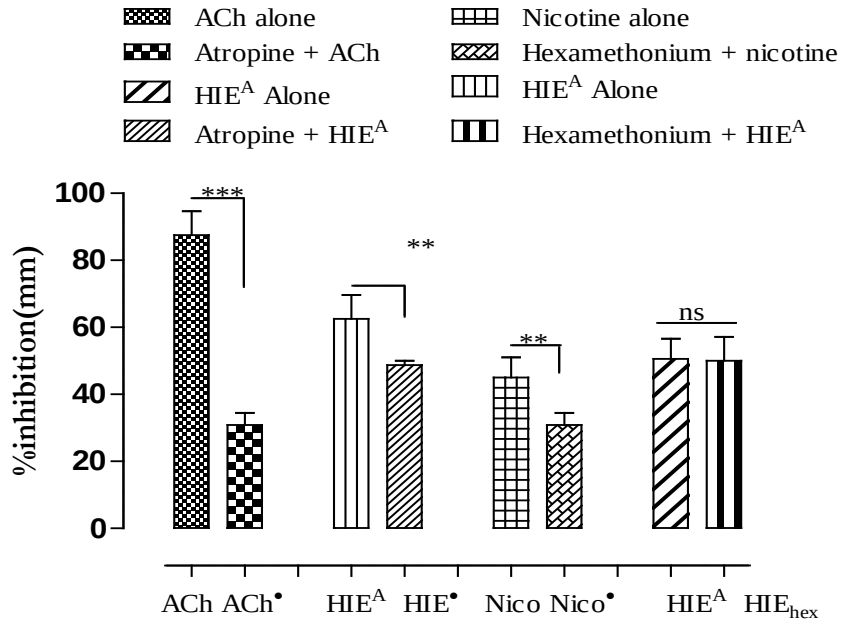


**Figure 3.9 Effect of acetylcholine (1.6 x $10^{-6}$ mg/ml), nicotine (1.25 x $10^{-2}$ mg/ml) and HIE$^{A}$ (3.2 mg/ml) on the rabbit isolated jejunum in the presence of atropine (8.3x$10^{-8}$ mg /ml) and hexamethonium (0.01 mg/ml). ACh (Inhibitions of responses of acetylcholine by atropine, P < 0.001), HIE$^{\bullet}$ (Inhibition of responses of HIE$^{A}$ by atropine, P < 0.01), Nico$^{\bullet}$ (Inhibition of responses of nicotine by hexamethonium, P < 0.01) and HIE$_{hex}$ (Inhibition of responses of HIE$^{A}$ by hexamethonium, ns P > 0.05). Each column is the mean ± sem (n = 4).**

### *3.2.3 Isolated Rat Uterus*

$HIE^A$ produced dose - dependent myometrial contractions on both the isolated non - pregnant and pregnant rat uterus preparations similar to those produced by acetylcholine and oxytocin (Fig 3.10 and appendix 5.14). $HIE^A$ was the least potent from the $EC_{50}$ values (Table 3.7).

#### *3.2.3.1 Effect Of* **Adrenaline** *On The Contraction Of The Isolated Rat Uterus Produced By* $HIE^A$ *And* **Acetylcholine**

Adrenaline physiologically inhibited contractile responses of the isolated rat uterus to acetylcholine by 75.0 % ($P < 0.001$) and that of $HIE^A$ by 73.9 % ($P < 0.001$) (Fig 3.11 and appendix 5.15).

#### **3.2.3.2** *Effect Of* **Atropine** *On The Contractions Of The Isolated Rat Uterus Produced By* $HIE^A$ *And* **Acetylcholine**

Atropine inhibited the myometrial contractions produced by acetylcholine by 40.0 % (** $P < 0.01$). The same dose of atropine inhibited $HIE^A$ by 30.0 % (* $P < 0.01$) (Figs 3.12 and appendix 5.16).

### *3.2.3.3 Effect Of* Salbutamol *($B_2$-Adrenoceptor Agonist) On The Contractile Responses Of The Isolated Rat Uterus To $HIE^A$ And* Oxytocin

Salbutamol inhibited the effect of oxytocin by 50.0 % ($P < 0.001$). The same dose of salbutamol inhibited the effect of $HIE^A$ by 40.1 % ($P < 0.001$) (Fig 3.13 and appendix 5.17).

### *3.2.3.4 Effects Of Two Prostaglandin Synthetase Inhibitors (Indomethacin And Diclofenac Sodium) On Contractile Responses Of The Isolated Rat Uterus To $HIE^A$ And Oxytocin*

Indomethacin could not inhibit the myometrial contractions produced by oxytocin (ns $P > 0.05$) and $HIE^A$ (ns $P > 0.05$) on the isolated rat uterus (Fig 3.14 and appendix 5.18). However, diclofenac sodium which could not modify the myometrial contractions of oxytocin (ns $p > 0.05$) inhibited myometrial contractions produced by $HIE^A$ by 27.4 % ($P < 0.05$) (Fig 3.15 and appendix 5.19).

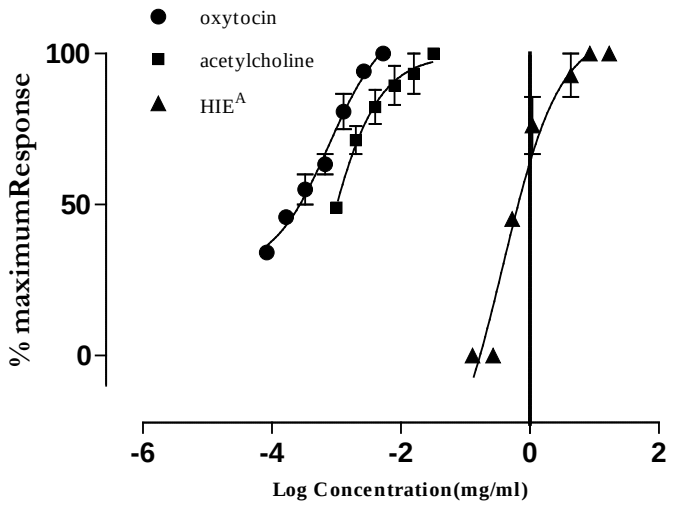


**Figure 3.10 Log dose - response curves of oxytocin (8.3 x $10^{-5}$ - 5.3 x $10^{-3}$ IU /ml), acetylcholine (1 x $10^{-3}$ - 3.2 x $10^{-2}$ mg/ml) and $HIE^A$ (0.13 - 17.1 mg/ml) on the isolated non pregnant rat uterus. Each point is the mean ± sem (n = 5).**

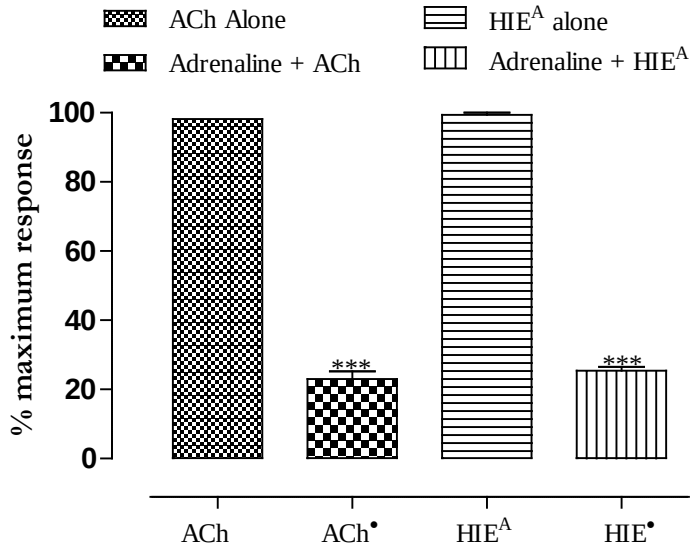


**Figure 3.11 Effect of Adrenaline (1 x $10^{-3}$ mg/ml) on the myometrial contractions of the isolated uterus to Ach (4x$10^{-3}$ mg/ml) and $HIE^A$ (8.5 mg/ml). Each column is the mean ± sem (n = 4). Inhibitions of responses are shown by P < 0.001(highly significant). ACh (acetylcholine), ACh• (Effect of acetylcholine in the presence of adrenaline), HIE• (Effect of HIEA in the presence of adrenaline).**

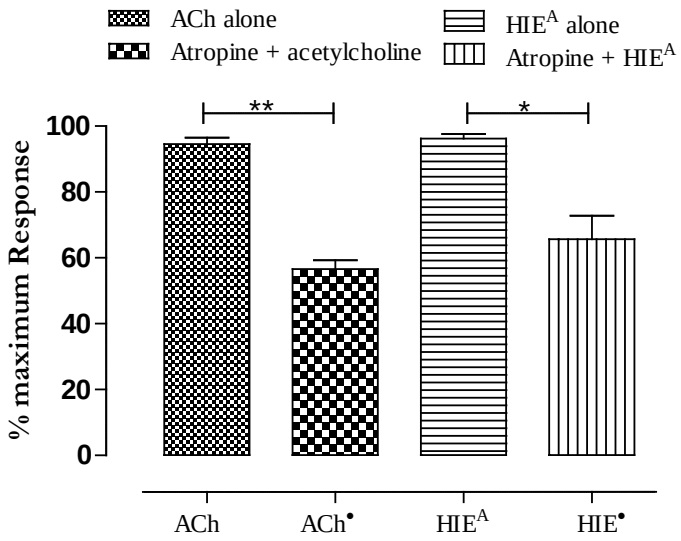


**Figure 3.12 Effect of ACh ($3.3 \times 10^{-2}$ mg/ml) and $HIE^A$ (6.0 mg/ml) in the presence of atropine ($4.2 \times 10^{-4}$ mg/ml) on the isolated non-pregnant rat uterus. The inhibition of responses is shown by $P < 0.01$ (Very significant) and $P < 0.05$ (significant). Each column is the mean ± sem (n = 6). ACh· (Effect of acetylcholine in the presence of atropine), $HIE^•$ (Effect of $HIE^A$ in the presence of atropine)**

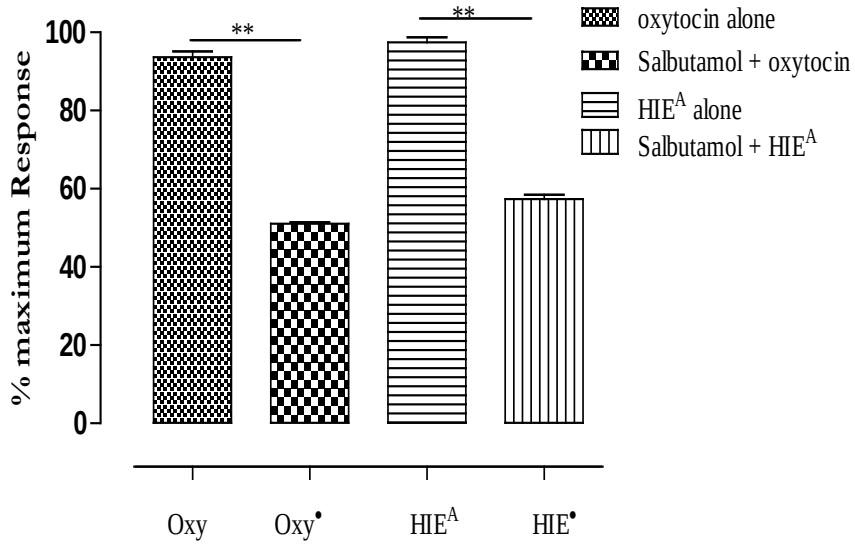


**Figure 3.13 Effects of oxytocin ($1.67 \times 10^{-2}$ IU/ml) and $HIE^A$ (8.5 mg/ml) in the presence of salbutamol ($4.2 \times 10^{-4}$ mg/ml) on the non - pregnant rat uterus. Inhibitions of responses are shown by $P < 0.01$ (very significant). Oxy· (Effect of oxytocin in the presence of salbutamol) and $HIE^•$ (Effect of $HIE^A$ in the presence of salbutamol). Each column is the mean ± sem (n = 5).**

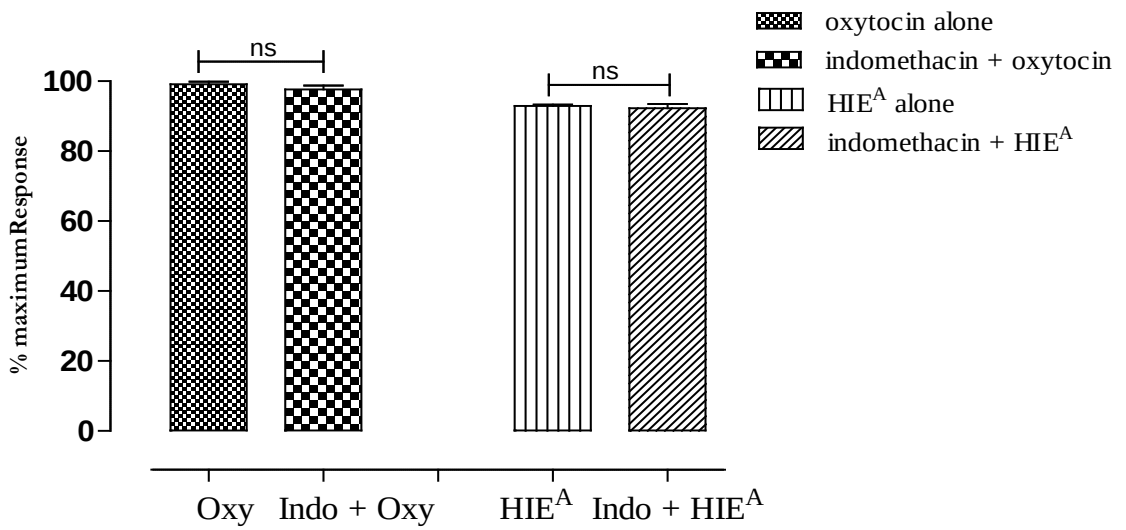


**Figure 3.14 Effect of indomethacin (4.8 mg/ml) on the myometrial contractions produced by oxytocin (3 x $10^{-2}$IU/ml) and HIE$^A$ (6.0 mg/ml) on the isolated non pregnant rat uterus. Each column represents the mean ± sem(n = 4).**

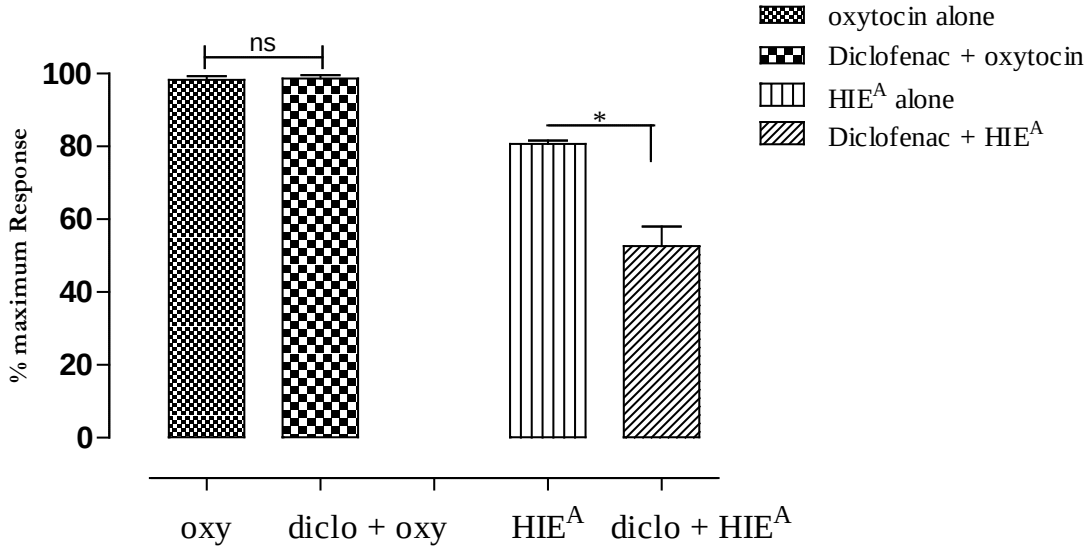


**Figure 3.15 Effect of diclofenac sodium (1.7 x $10^{-1}$ mg/ml) on the myometrial contractions produced by oxytocin (3 x $10^{-2}$ IU/ml) and HIE$^A$ (6.0 mg/ml) on the isolated non pregnant rat uterus. Each column is the mean ± sem (n = 4)**

## 3.3 EFFECT OF HIE ON THE CARDIOVASCULAR SYSTEM

### *3.3.1 Isolated Frog Heart*

$HIE^A$ and $HIE^E$ produced dose - dependent depressor effects on the force of contraction of the isolated frog heart. Effects of $HIE^A$, acetylcholine, histamine, and adrenaline on the force of contraction of the isolated frog heart are shown in (Fig 3.16 and appendix 5.21).

$HIE^A$ was the least potent in terms of depressor effect from the $EC_{50}$ values (Table 3.8). Figs 3.17, appendix 5.22 and fig 3.18, appendix 5.23 show the effects of $HIE^A$ on the force of contraction and the rate of heart beat of the isolated frog heart respectively. Tables 3.9 and 3.10 show the $EC_{50}$ values of $HIE^A$ on the force of contraction and the rate of heart beat of the isolated frog heart respectively.

Mepyramine which inhibited histamine by 50.1 % did not have any inhibitory effect on the depressor effect of $HIE^A$. $HIE^A$ had no inhibitory effect on the pressor effect of adrenaline on the isolated frog heart. Atropine inhibited depressor effect of acetylcholine by 19.0 % ($P < 0.05$) (Fig 3.19a and appendix 5.24) and that of $HIE^A$ by 11.5 % ($P < 0.05$) (Fig 3.19b appendix 5.25).

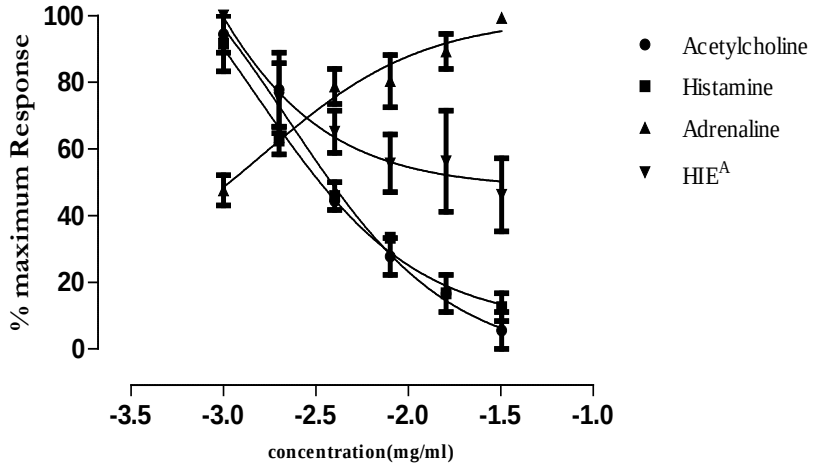


**Figure 3.16 Log dose - response curves of ACh, Hist, and Adrenaline ($1 \times 10^{-3}$ - $3.2 \times 10^{-2}$ mg/ml) and $HIE^A$ (0.1 – 12.8 mg/ml) showing their effects on the cardiovascular system of the isolated frog heart. Each point is the mean ± sem (n = 4).**

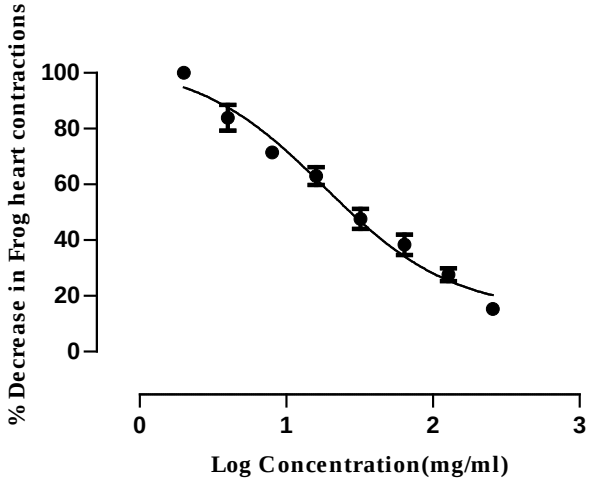


**Figure 3.17 Log dose - response curve of $HIE^A$ (0.1 - 12.8 mg/ml) showing the effect of $HIE^A$ on the force of contraction of the isolated frog heart preparation. Each point represents the mean ± sem (n = 4).**

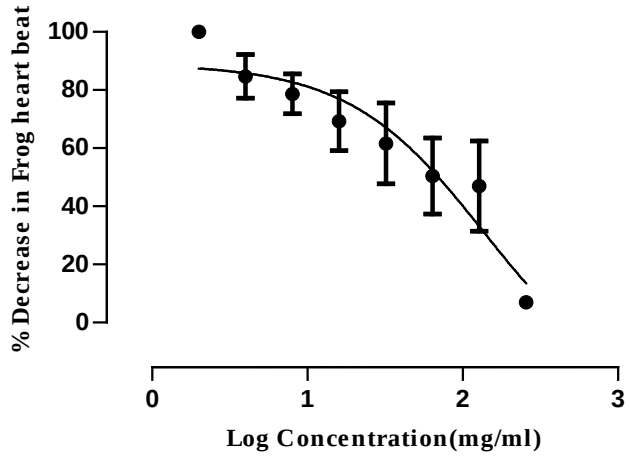


**Figure 3.18 Log dose - response curve showing the effect of HIE$^{A}$ (0.1 - 12.8 mg/ml) on the rate of beat of the isolated frog heart preparation. Each point represents the mean ± sem (n = 4).**

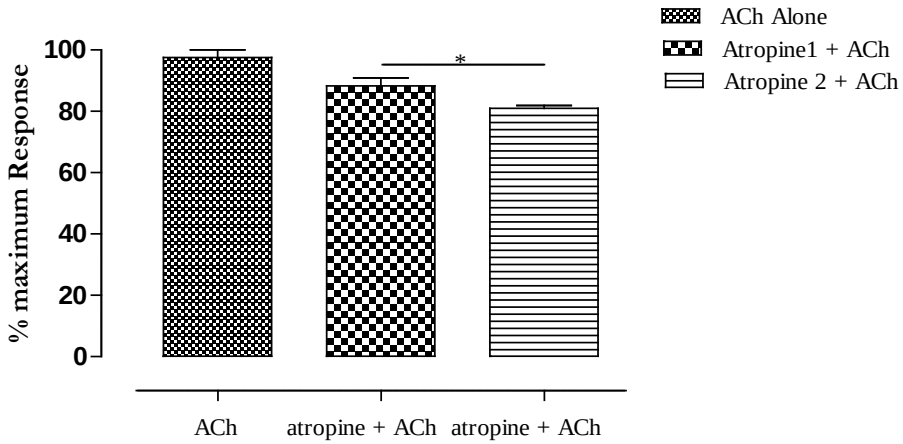


**Figure 3.19a Effect of acetylcholine (4 x $10^{-6}$ mg/ml) in the presence of two separate concentrations of atropine (5 x $10^{-6}$ mg/ml and 8 x $10^{-6}$ mg/ml) on the frog isolated heart. Each column is the mean ± sem (n = 4). Inhibition of responses produced by acetylcholine by atropine was significant (P < 0.05).**

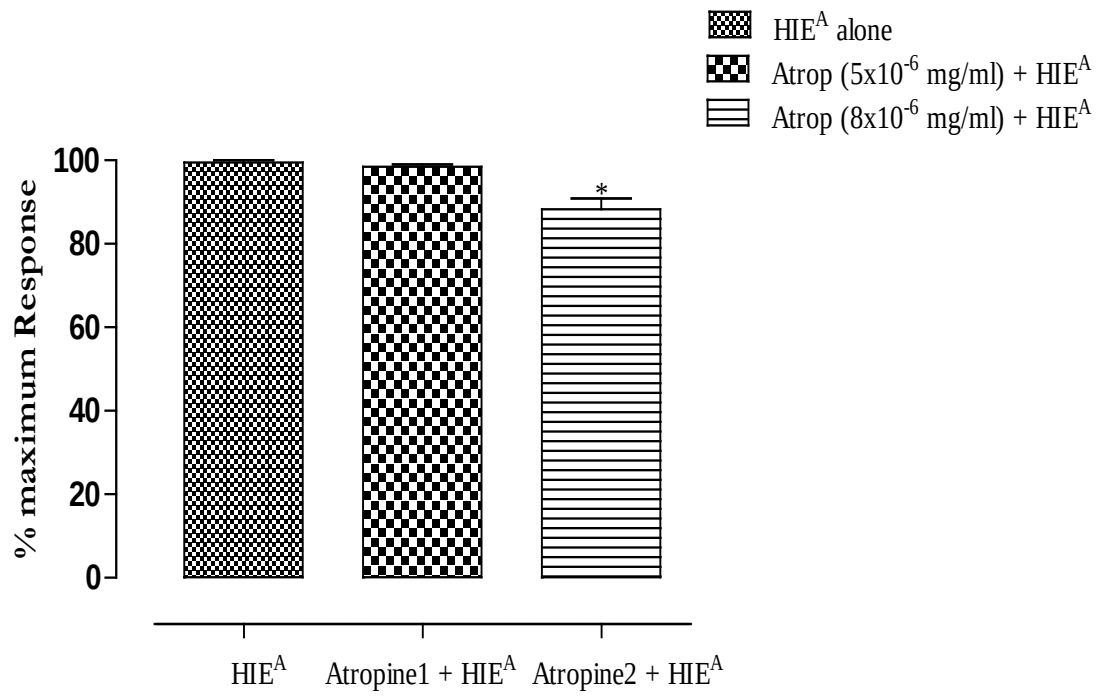


**Figure 3.19b Effect of $HIE^A$ (4.2 mg/ml) in the presence of two separate concentrations of atropine (5 x $10^{-6}$ mg/ml and $8x10^{-6}$ mg/ml) on the frog isolated heart. Each column is the mean ± sem (n = 4). Inhibition of $HIE^A$ responses by atropine was significant with the high dose ($P < 0.05$).**

### *3.3.2 The Perfused Rabbit Heart Experiment*

$HIE^A$ produced dose - dependent depressor effects on the force of contraction of the perfused rabbit heart and also reduced the coronary perfusion rate of the perfused rabbit heart (Fig 3.20 and appendix 25).

### *3.3.3 Effect of $HIE^A$ on the Blood Pressure of an Anaesthetized Cat*

$HIE^A$ produced a dose - dependent reduction in the systemic blood pressure of the anaesthetized cat (Figs 3.21 and appendix 5.27).

The depressor effects of acetylcholine and $HIE^A$ on the anaesthetized cat were inhibited by atropine 65.6 % ($P < 0.001$) and 0.6 % ($P < 0.05$) respectively (Fig 3.22 and appendix 5.28). Table 3.11 shows the $EC_{50}$ values of $HIE^A$ on the anaesthetized cat.

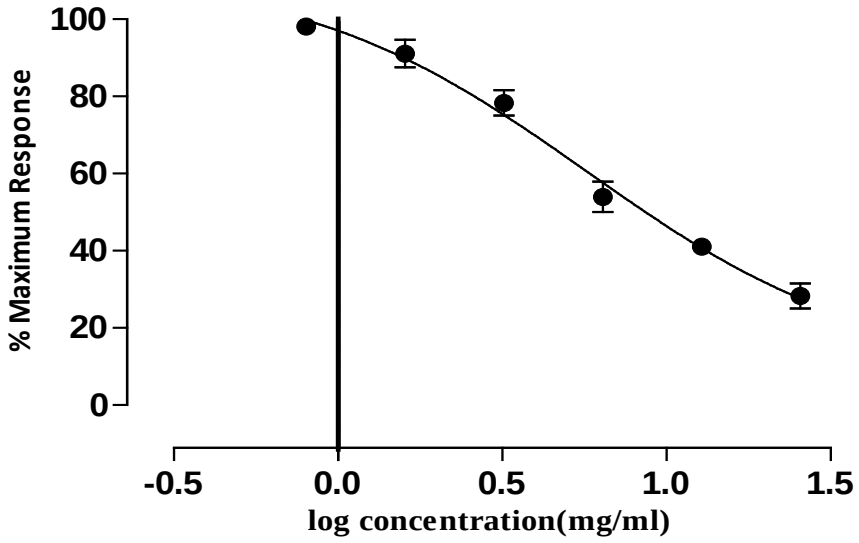


Figure 3.160 Effect of HIE$^{A}$ (0.8 - 25.6 mg/ml) on the rate of coronary perfusion of the perfused rabbit heart. Each point is the mean ± sem (n = 4).

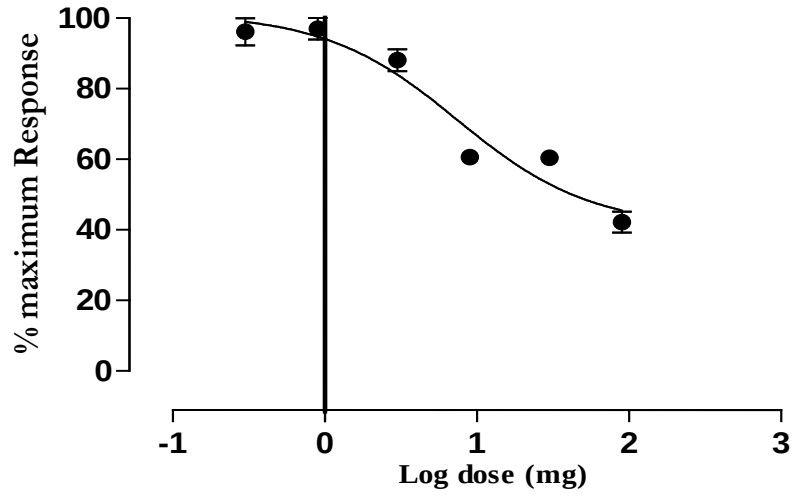


**Figure 3.21 Log dose - response curve of HIE$^{A}$ (0.3 - 90 mg) on the blood pressure of the anaesthetized cat. Each point is the mean ± sem (n = 4).**

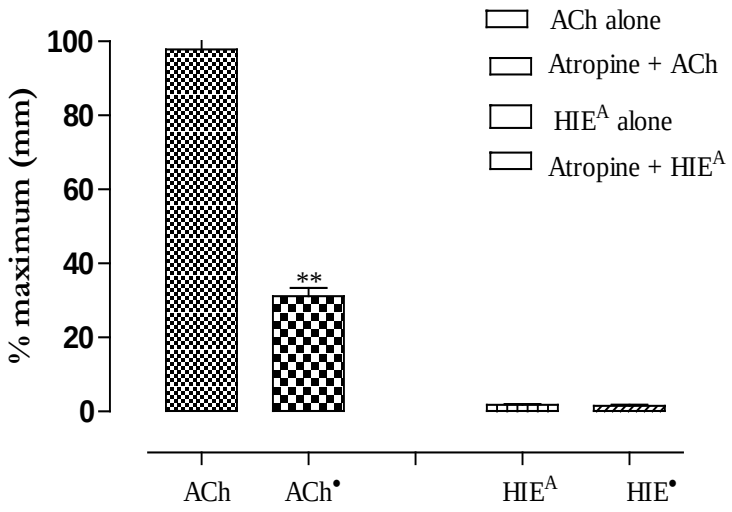


**Figure 3.22 Effect of intravenous administration of ACh ($4.4 \times 10^{-5}$ mg) and $HIE^A$ (9.0 mg) on the systemic blood pressure of the anaesthetised cat in the presence of atropine ($1.2 \times 10^{-6}$ mg). $ACh^\bullet$ (Effect of acetylcholine in the presence of atropine) and $HIE^\bullet$ (Effect of $HIE^A$ in the presence of atropine). Inhibition of responses of ACh by atropine was very significant ($P < 0.01$) and the inhibition of responses of HIE by atropine was not significant (ns $P > 0.05$). Each column represents the mean ± sem ($n = 4$).**

**Table 3.2. $EC_{50}$, values of ACh, Histamine Nicotine and $HIE^A$ on the isolated guinea - pig ileum preparation.**

| | *ACh* | *Histamine* | *Nicotine* | *$HIE^A$* |
|---|---|---|---|---|
| **$EC_{50}$** | 0.005 | 0.006 | 0.09 | 21.5 |

**Table 3.3 $EC_{50}$ values of $HIE^A$ showing its effects in the presence of three separate concentrations of atropine on the isolated guinea- pig ileum.**

| | *$HIE^A$ alone* | *$HIE^A$ + Atr ($5x10^{-9}$ mg/ml)* | *$HIE^A$ + Atr ($2x10^{-8}$ mg/ml)* | *$HIE^A$ + Atr ($1x10^{-7}$ mg/ml)* |
|---|---|---|---|---|
| $EC_{50}$ | 2.5 | 11.8 | 12.9 | 19.0 |

**Table 3.4 $EC_{50}$ values of $HIE^A$ in the presence of two separate concentrations of hexamethonium on the isplated guinea - pig ileum.**

| | *$HIE^A$ alone* | *Hex (0.005 mg/ml) + $HIE^A$* | *Hex (0.05 mg/ml) + $HIE^A$* |
|---|---|---|---|
| **$EC_{50}$** | 44.3 | 82.1 | 120.4 |

**Table 3.5 $EC_{50}$ values of $HIE^A$ in the presence of two separate concentrations of mepyramine on the isolated guinea - pig ileum.**

| | *$HIE^A$ alone* | *Mepyramine ($5x10^{-9}$ mg/ml) + $HIE^A$* | *Mepyramine ($1x10^{-7}$ mg/ml) + $HIE^A$* |
|---|---|---|---|
| **$EC_{50}$** | 6.3 | 5.0 | 0.8 |

**Table 3.6 $EC_{50}$ value of $HIE^A$ on the isolated rabbit jejunum preparation.**

| | |
|---|---|
| *$EC_{50}$* | ***41.2*** |

**Table 3.7 $EC_{50}$ values of $HIE^A$, ACh, and Oxytocin on the isolated frog heart preparation.**

| | *HIE* | *Ach* | *Oxytocin* |
|---|---|---|---|
| **$EC_{50}$** | 0.4 | 0.0005 | 0.0008 |

**Table 3.8 $EC_{50}$ values of ACh, Histamine, $HIE^A$ and Adrenaline on the isolated frog heart preparation.**

| | *ACh* | *Histamine* | *$HIE^A$* | *Adrenaline* |
|---|---|---|---|---|
| **$EC_{50}$** | **0.004** | **0.003** | **0.02** | **0.001** |

**Table 3.9 $EC_{50}$ value of $HIE^A$ on the force of contraction of the isolated frog heart preparation.**

| | |
|---|---|
| ***$EC_{50}$*** | *18.3* |

**Table 3.10 $EC_{50}$ value of $HIE^A$ on the rate of beat of the isolated frog heart preparation.**

| | |
|---|---|
| ***$EC_{50}$*** | *19.7* |

**Table 3.11 $EC_{50}$ value of HIE on the anaesthetised cat.**

| | |
|---|---|
| ***$EC_{50}$*** | *7.5* |

## 3.4 ANALGESIA

### *3.4.1 Formalin-Induced Nociception Test*

Formalin induced a characteristic nociceptive response exhibited as biting or licking injected paw. The response to pain was biphasic as previously reported (Dubuisson and Dennis, 1977; Wheeler - Aceto *et.al.*, 1990), comprising an initial intense response to pain beginning immediately after formalin injection and rapidly waning within 10 minutes (first phase). The first phase was then followed by a slowly rising but longer lasting response from 10 - 60 minutes after formalin injection with maximum effect at approximately 20 - 30 minutes after formalin injection (second phase) (Wang *et.al.,* 1999; Hayashida *et.al.*,2003).

Administration of $HIE^E$ and $HIE^A$ (30 - 300 mg/kg$^{-1}$*p.o*) an hour before formalin injection inhibited, but insignificantly both first and second phases of formalin induced paw licking and biting [$F_{3, 8}$ = 2.633, P = 0.5391 and $F_{3, 8}$ = 8.633, p = 0.3321] respectively, one - way ANOVA (treatment x time) followed by Neuman - Keul's multiple comparison test] (Figs 3.23 and 3.24). Similarly, morphine (1 - 10 mg/kg$^{-1}$ *i.p*) an opioid analgesic and diclofenac sodium (1 - 10 mg/kg$^{-1}$ *i.p*) an NSAID both dose - dependently inhibited both the first and second phases of pain elicited as paw licking and biting induced by formalin insignificantly [$F_{3, 8}$ = 2.699, P = 0.3926, and $F_{3, 8}$ = 1.442, p = 0.9923] respectively one - way ANOVA followed by Neuman - Keul's multiple comparison test] (Figs 3.25 and 3.26). Also, analysis of the AUCs for morphine, diclofenac sodium, $HIE^E$ and $HIE^A$ produced dose - dependent inhibitions though these inhibitions were not significant as compared to the control.

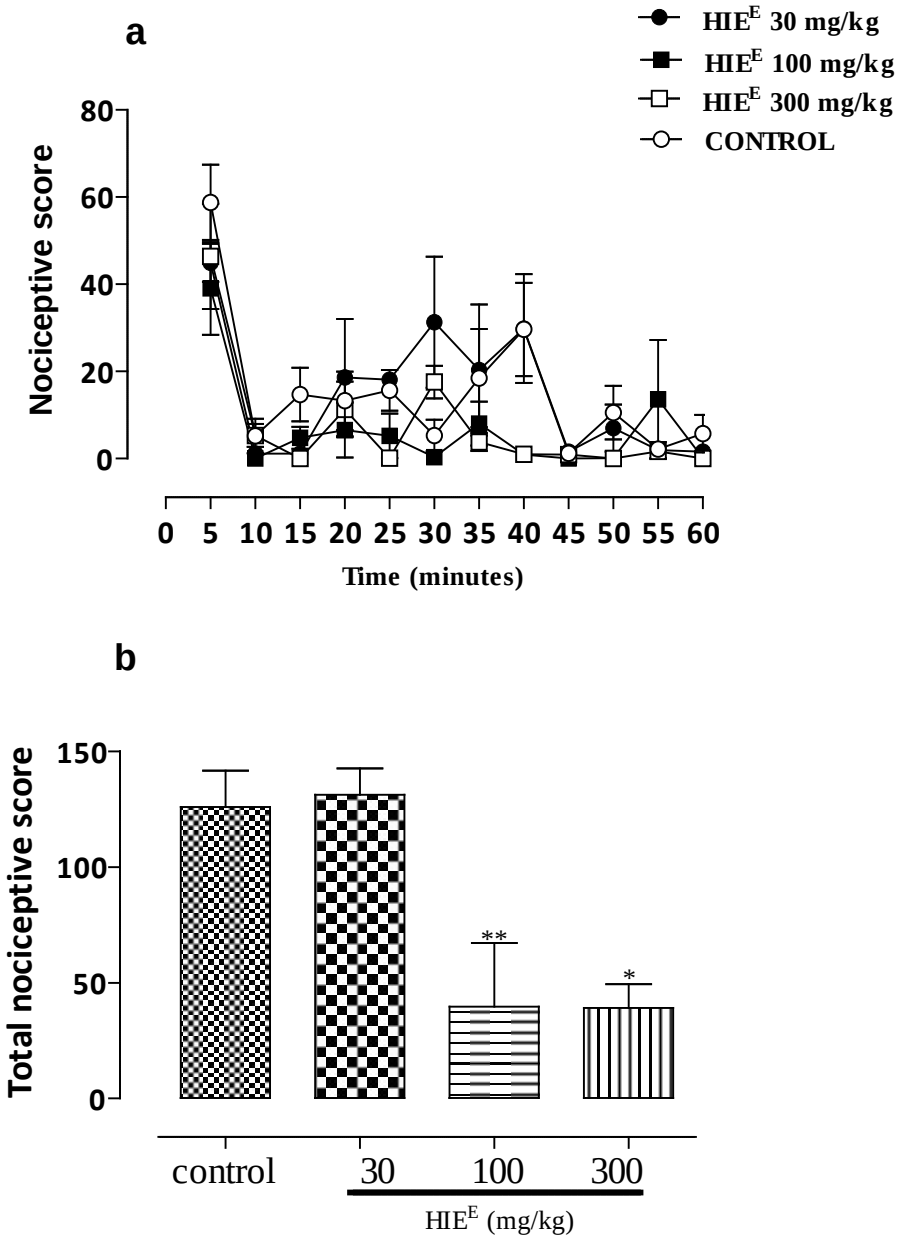


**Figure 3.23 Effect of HIE$^{E}$ (30 - 300 mg/kg$^{-1}$ p.o.) on the time course (a) and the total nociceptive score calculated from AUCs (Area under curve) (b) over both phases of formalin - induced pain in rats. Each point/column is the mean ± sem (n = 3). The significant differences from the mean of the control are shown by P ≤ 0.05, P ≤ 0.01 (one - way ANOVA followed by Neuman - Keul's multiple comparison test).**

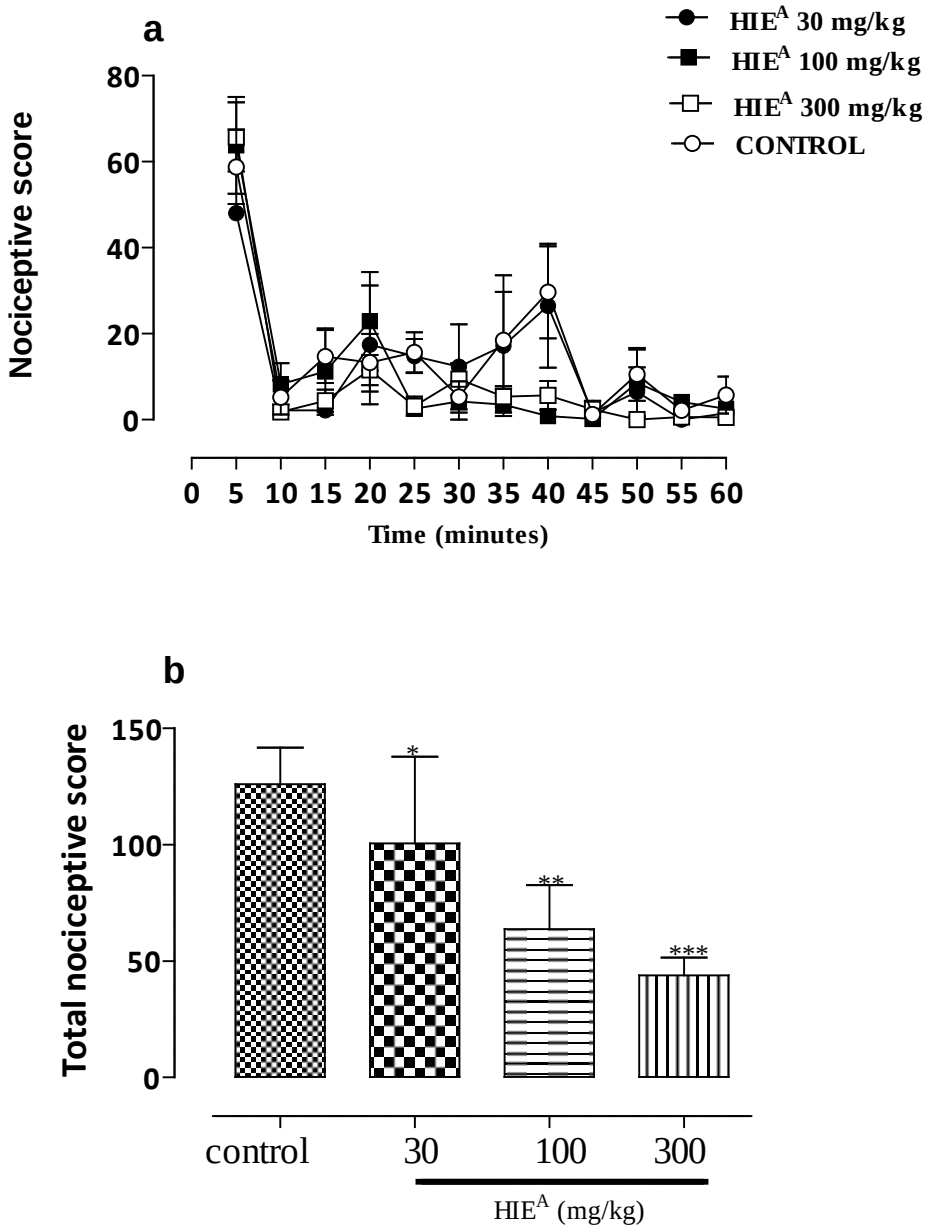


**Figure 3.24 Effect of HIE$^{A}$ (30 - 300 mg/kg$^{-1}$ p.o.) on the time course (a) and the total nociceptive score calculated from AUCs (Area under curve) (b) over both phases of formalin - induced pain in rats. Each point/column is the mean ± sem (n = 3). The significant differences from the mean of the control are shown by P ≤ 0.05, P ≤ 0.01, P ≤0.001 (one - way ANOVA followed by Neuman - Keul's multiple comparison test).**

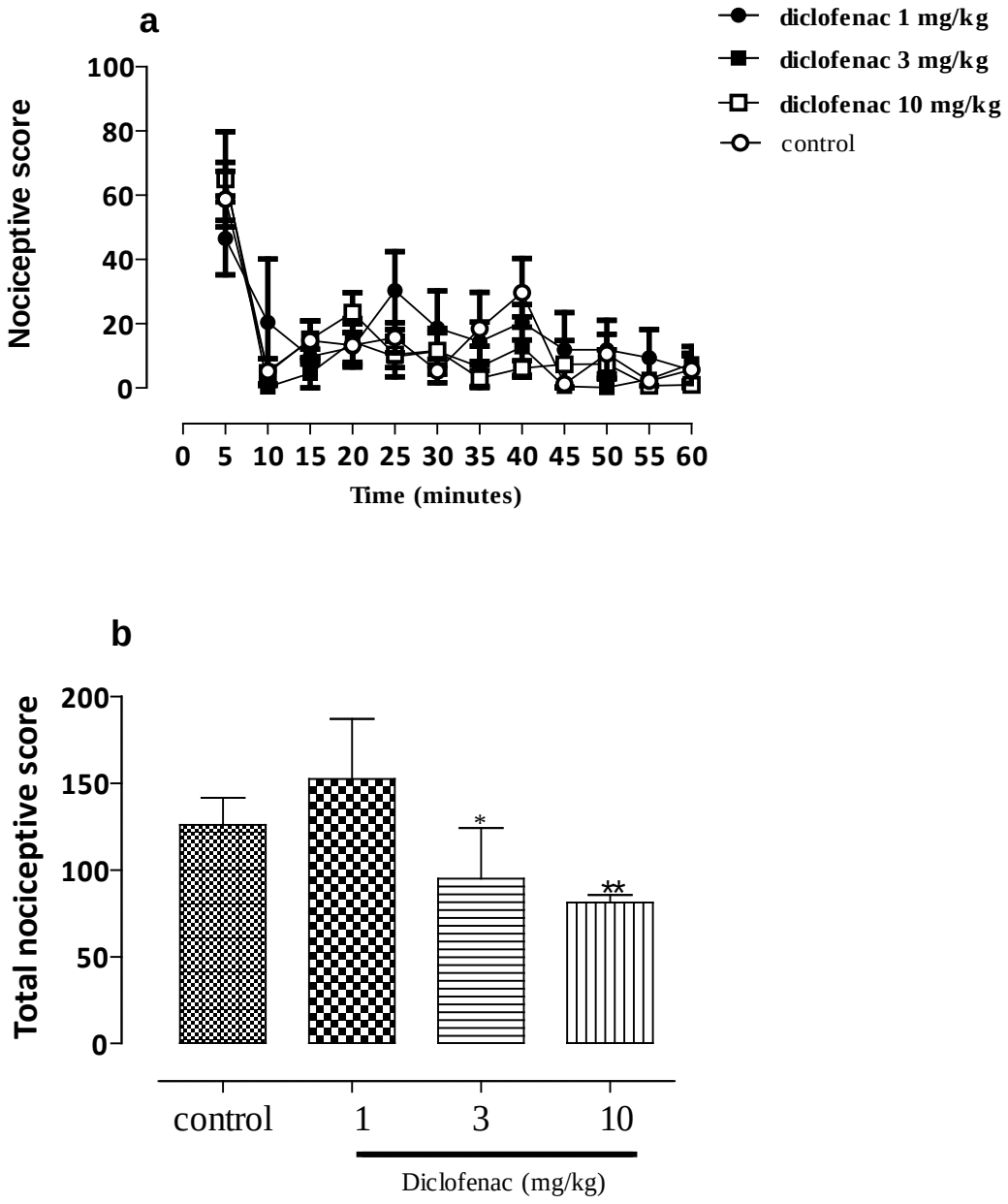


**Figure 3.25 Effect of diclofenac sodium (1 - 10 mg/kg$^{-1}$ i.p.) on the time course (a) and the total nociceptive score calculated from AUCs (Area under curve) (b) over both phases of formalin - induced pain in rats. Each point/column is the mean ± sem (n = 3). The significant differences from the mean of the control are shown by P ≤ 0.05, P ≤0.01 (one - way ANOVA followed by Neuman - Keul's multiple comparison test).**

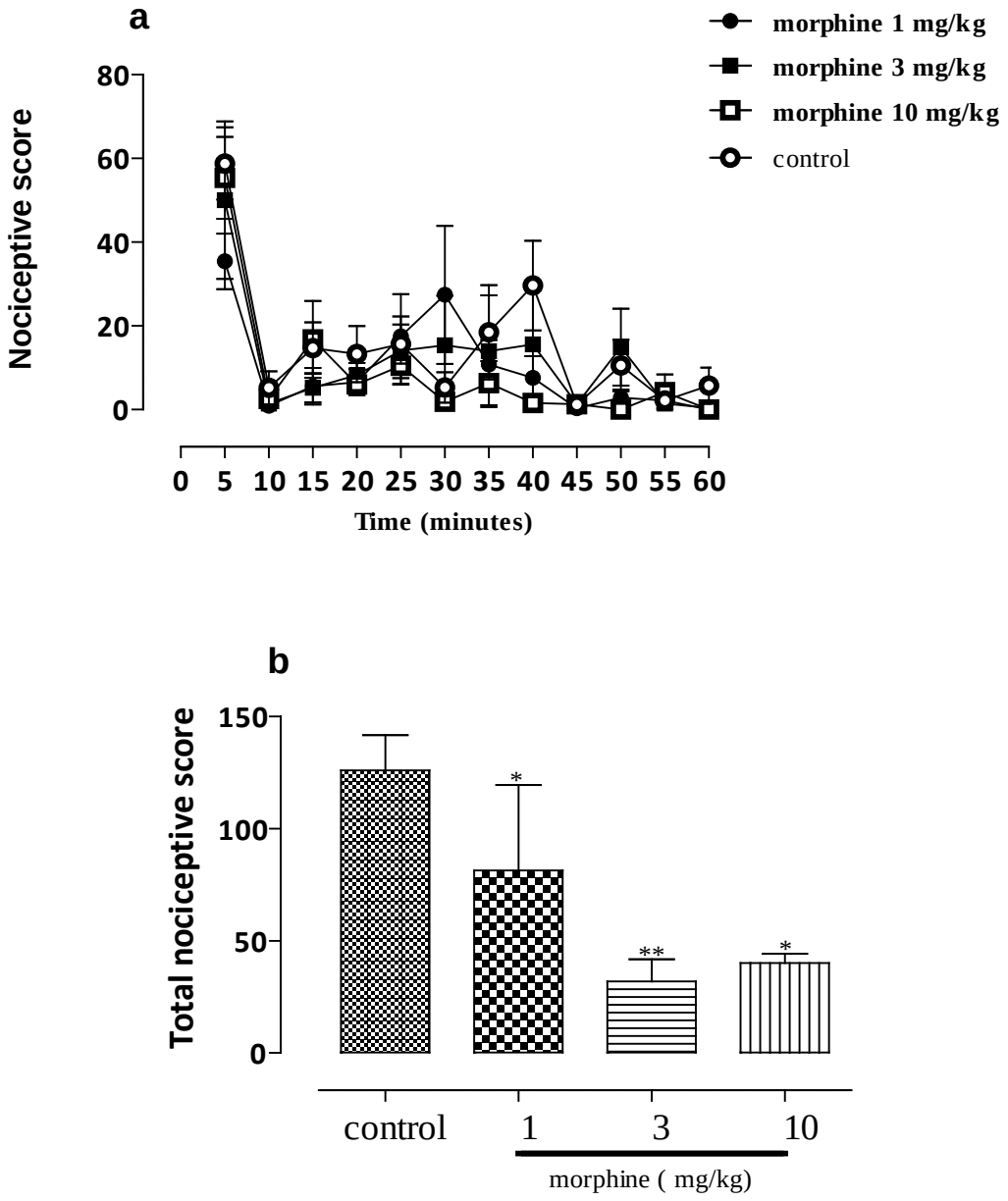


**Figure 3.26 Effect of morphine (1 - 10 mg/kg$^{-1}$ i.p.) on the time course (a) and the total nociceptive score calculated from AUCs (Area under curve) (b) over both phases of formalin - induced pain in rats. Each point/column is the mean ± sem (n = 3). The significant differences from the mean of the control are shown by P ≤ 0.05, P ≤0.01 (one - way ANOVA followed by Neuman - Keul's multiple comparison test).**

## 3.5 TOXICITY STUDY

### *3.5.1 Acute Toxicity Study*

Administration of $HIE^A$ (1, 2 and 3 g/kg *p.o*) to ICR mice and (1, 2, 4 and 5 g/kg *p.o*) to rats species did not kill any of the experimental animals over a period of twenty four hours. However among the rats, the groups that received 4 g/kg and 5 g/kg of $HIE^A$ showed little activity in terms of movement after twenty four hours. All mice in the $HIE^A$ treated groups initially displayed low activity particularly in terms of movement, however this observation waned as the animals returned to normal activity after two hours.

### *3.5.2 Sub-Acute Toxicity Study*

No experimental animal died over the fourteen day period. However, rats in the $HIE^A$ treated group that received doses (4 and 5 $g/kg^{-1}$ *p.o*) showed prostration and were most of the time found gathered together at the corners of the aluminium cages in which they were kept.

The behavior and appearance of the $HIE^A$ treated animals were comparable to those of the control group in the last seven days of the fourteen day period.

Change in body weight of $HIE^A$ treated rats between the first, seventh and the fourteenth day was insignificant (Fig 3.27). There was no significant change (ns $P > 0.05$) in the wet weights of isolated hearts of $HIE^A$ treated rats compared to control rats. However, the wet weights of the isolated liver, lungs and kidney of $HIE^A$ treated rats showed significant

increase ($P < 0.05$) as compared to those of the control rats, particularly those $HIE^A$ treated rats that received 4 g/kg and 5 g/kg doses (Fig 3.28). Haematological investigations of the blood parameters of the control and $HIE^A$ treated rats in terms of their haemoglobin concentration, RBC count, WBC count, and platelet concentration did not show consistent pattern of change (Figs 3.29 – 3.30).

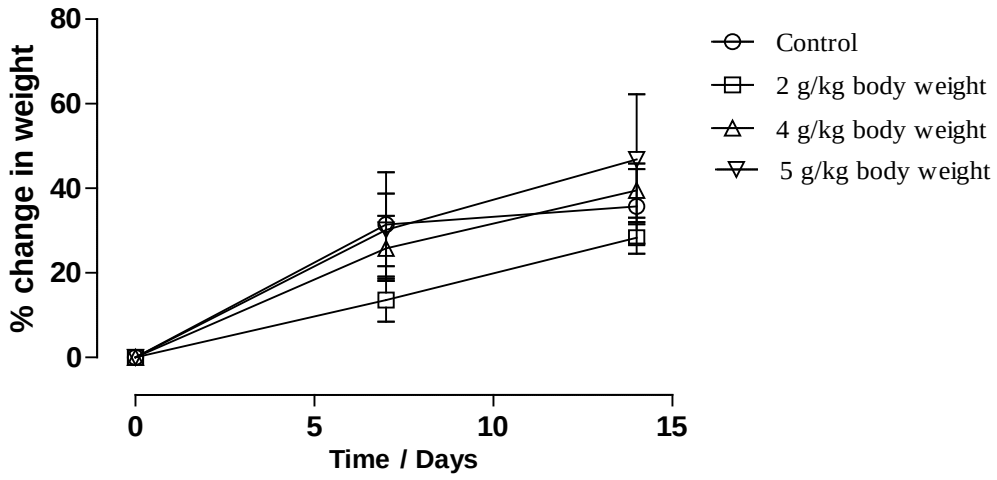


**Figure 3.27 Effect of daily oral administration of $HIE^A$ (2 - 5 g/kg) on the body weight of Spraque - Dawley rats for the period of 14 days. Each point is the mean ± sem (n = 4).**

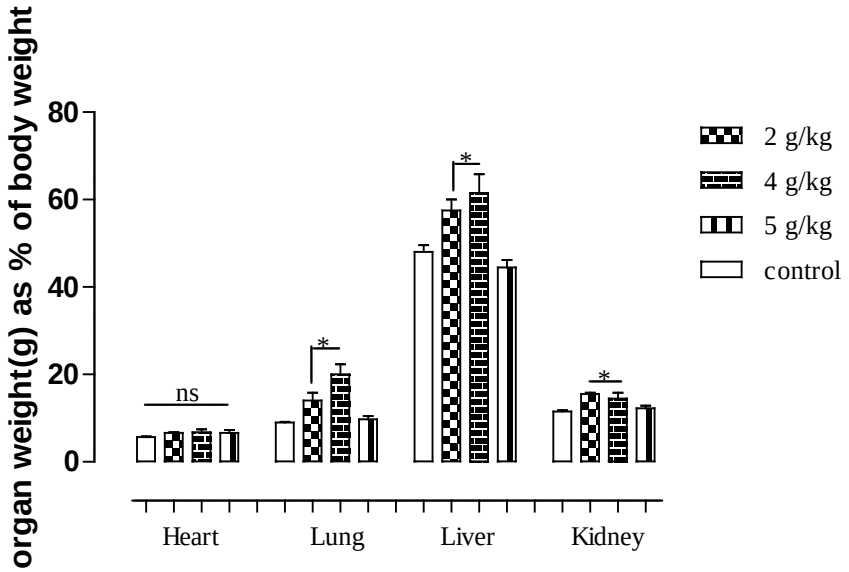


**Figure 3.28 Effect of daily oral administration of HIE[A] (2 - 5 g/kg) on the wet organ weights of rats after 14 days. Each column represents the mean ± sem (n = 4). Significant changes in the mean of organ weights of treated animals compared to the mean of controls are shown as ns (P > 0.05), insignificant (heart), (P < 0.05) significant (lung), (P < 0.05) significant (liver) and (P < 0.05) significant (kidney).**

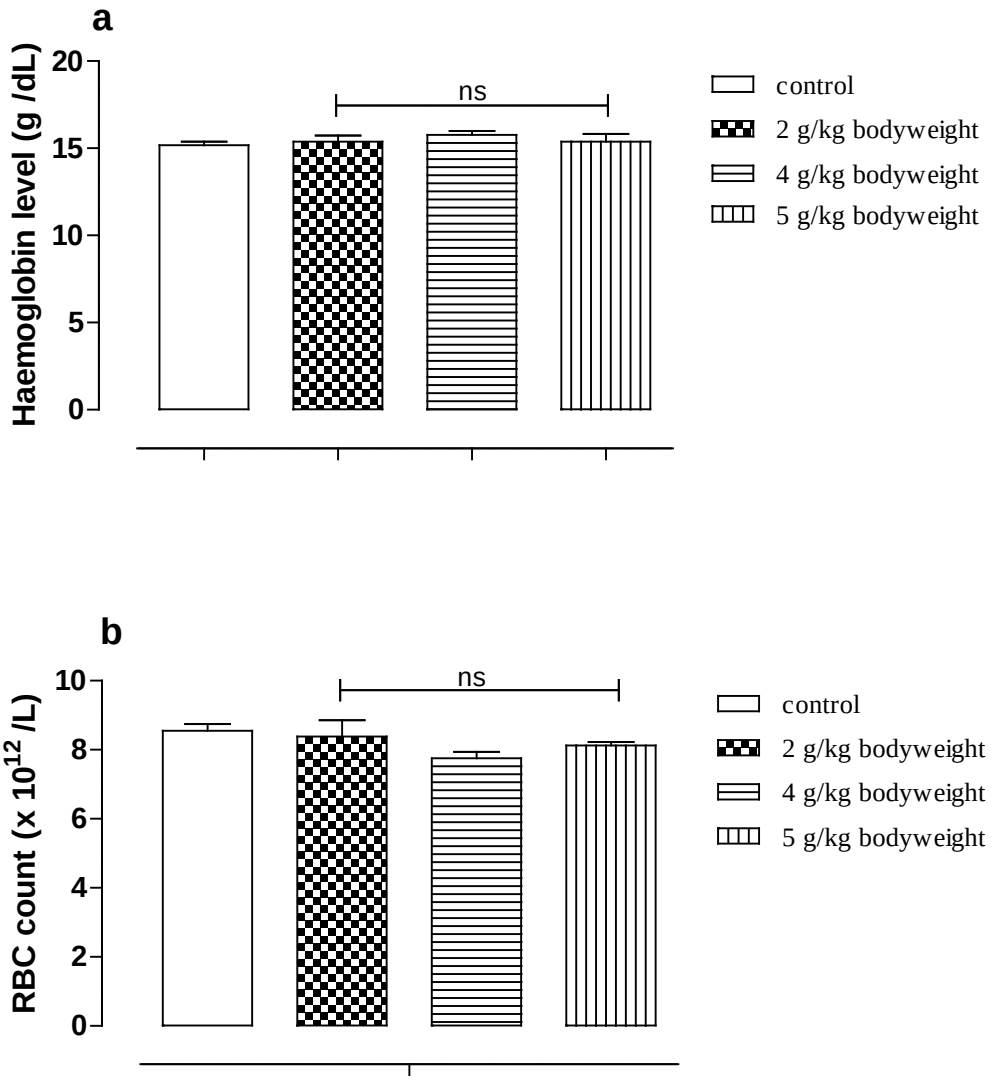


**Figure 3.29 Effect of daily oral administration of HIE$^{A}$ (2 - 5g/kg) for 14 days on haemoglobin concentration (a) and red blood cell count (b) of rats. Each column is the mean ± sem (n = 4).The mean change in haemoglobin concentration/ red blood cell count of the treated rats from those of the control rats is shown as P > 0.05 (ns).**

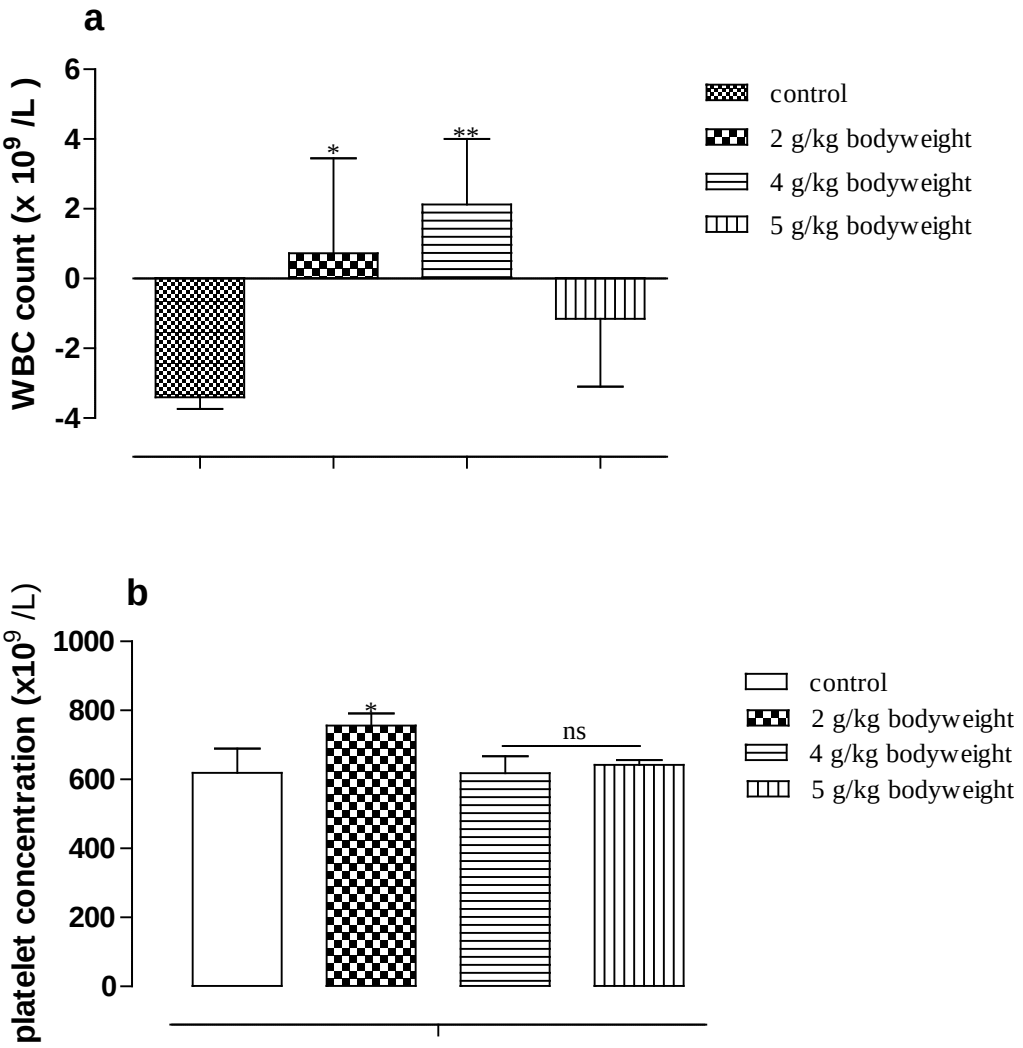


**Figure 3.30 Effect of daily oral administration of HIE$^{A}$ (2 - 5 g/kg) for 14 days on the white blood cell count (a) and platelet concentration (b) of rats. Each column represents the mean ± sem (n = 4). The mean change in white blood cell count/ platelet concentration of treated rats from those control rats is (P < 0.05, significant) and (P < 0.01, very significant).**

### *3.5.3 Histopathology*

Histopathological examination of the isolated organs (liver, lungs, kidney, and heart) of $HIE^{A}$ treated rats showed marked histopathological changes compared to those of the control rats. For example, the liver of $HIE^{A}$ treated rats showed histopathological damages including vascular congestion with mild architectural distortions which were not comparable to those of the control rats (Plates 3.1). Similar damages were observed in the lungs (Plates 3.2), kidney (Plates 3.3) and heart (Plate 3.4) of $HIE^{A}$ treated rats.

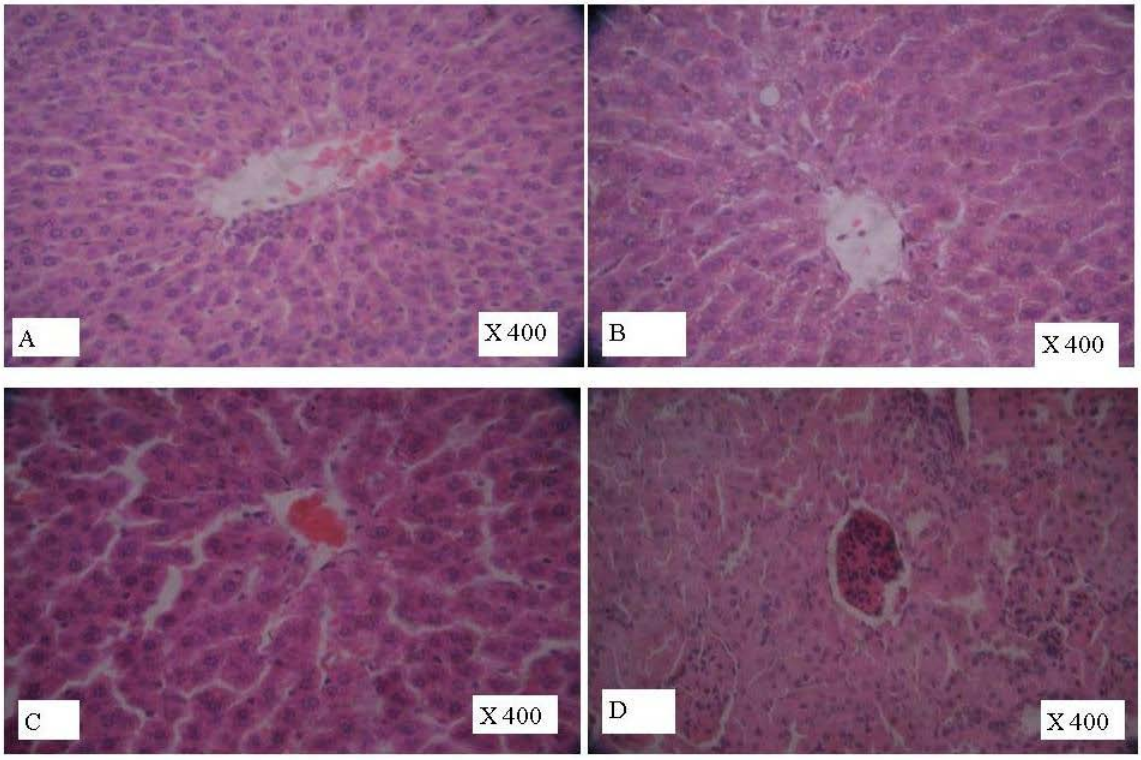


**Plate 3.1. Photomicrograph of the transverse section of the liver of a rat (A) as a control and similar rats treated with a dose 2 g/kg (B), 4 g/kg (C) and 5 g/kg (D) of HIE[A] over a period of 14 days. The liver of the treated rats showed progressive histopathological changes compared to the control including mildly diffused visicular steatosis and focal chronic inflammation with predominantly lymphocytic infiltration.**

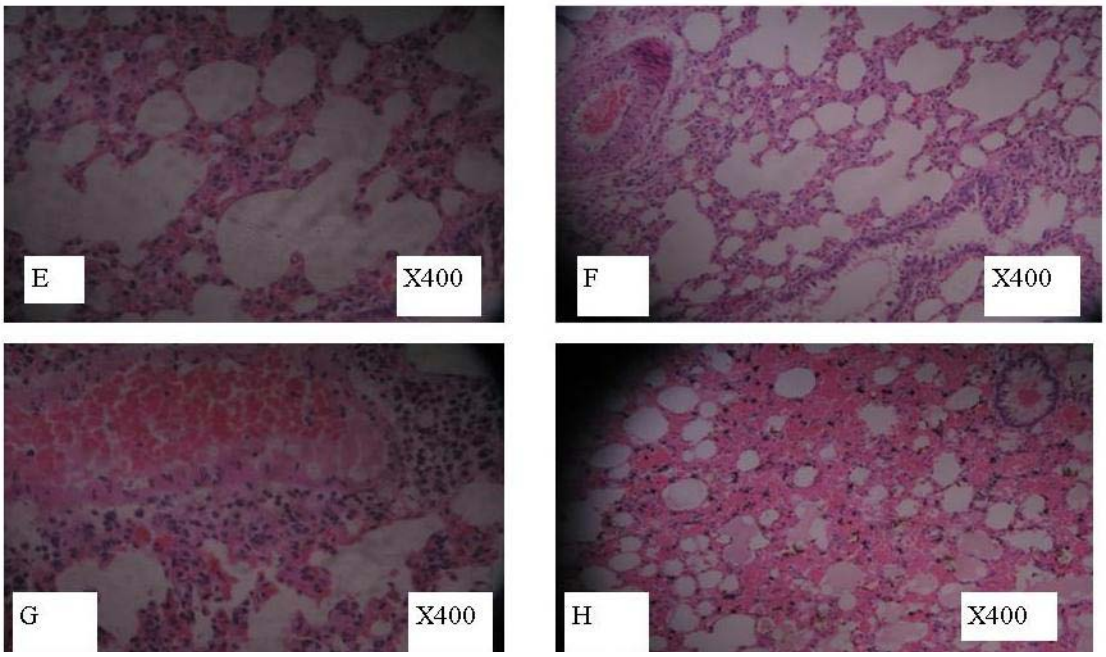


**Plate 3.2. Photomicrograph of the transverse section of the lungs of a rat (E) as a control and similar rats treated with a dose of 2 g/kg (F), 4 g/kg (G) and 5 g/kg (H) of HIE$^{A}$ for a period of 14 days. The photomicrograph of the lungs of the treated rats showed marked progressive changes including diffused alveolar haemorrhage and extensive oedema with rupture of inter alveolar septae, and also bullae formation as compared to the control.**

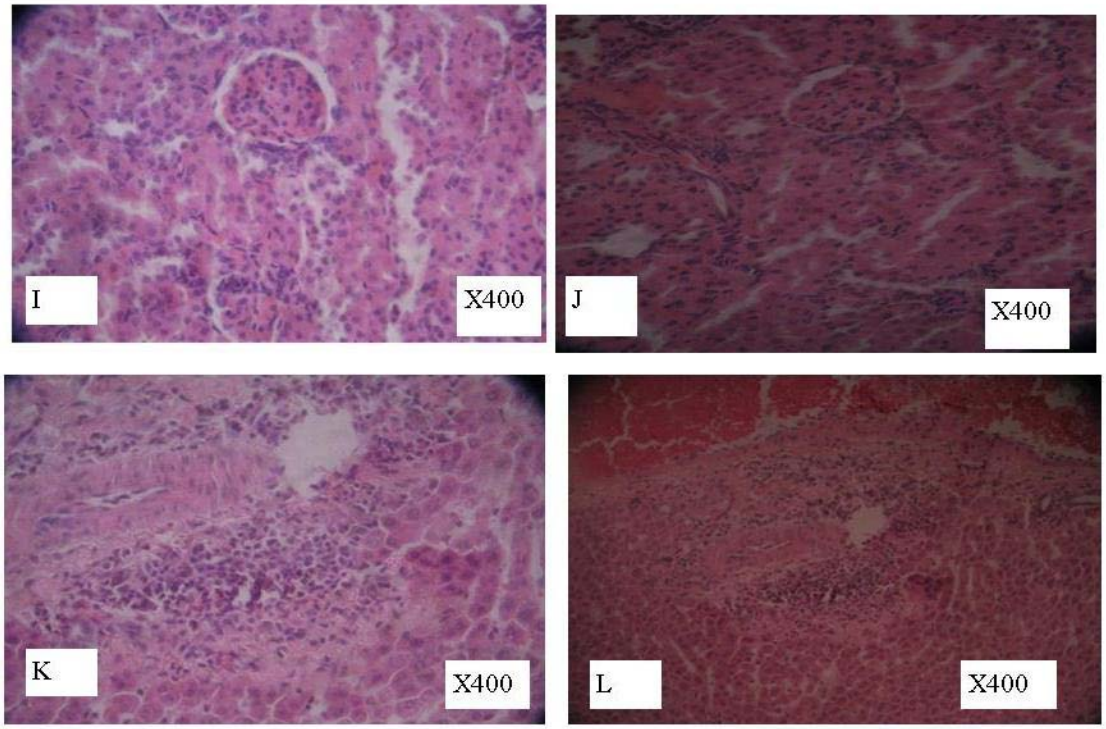


**Plate 3.3. Photomicrograph of the transverse section of the kidney of a rat (I) as a control and that of similar rats treated with a dose of 2 g/kg (J), 4 g/kg (K) and 5 g/kg (L) of HIE[A] for a period of 14 days. The photomicrograph of the treated rat showed progressive histopathological changes including vascular congestion in the glomeruli with patchy focal chronic inflammation compared to the control.**

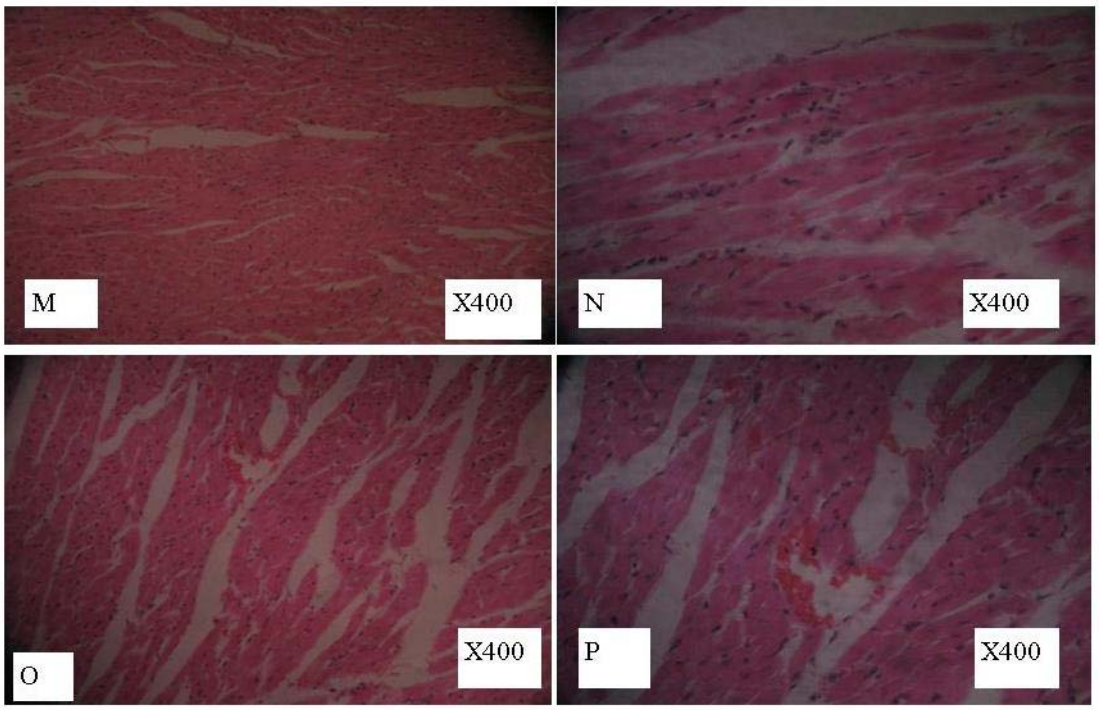


**Plate 3.4. Photomicrograph of the transverse section of the heart of a rat (M) as a control and that of similar rats treated with a dose of 2 g/kg (N), 4 g/kg (O) and 5 g/kg (P) of HIE$^{A}$ for a period of 14 days. With respect to the control the treated rats showed progressive histopathological changes including diffused single cell necrosis with mild diffused chronic inflammation.**

# *Chapter 4*

# DISCUSSION

## 4.1 EFFECT OF HIE ON SMOOTH MUSCLE PREPARATIONS

### *4.1.1 The Guinea-Pig Ileum*

$HIE^A$ produced dose - dependent contractions of all isolated guinea - pig ileum preparations studied in this thesis. The contractions produced by $HIE^A$ were similar to those produced by acetylcholine, histamine and nicotine however the $EC_{50}$ values obtained indicated that the $HIE^A$ was the least potent.

The contractions produced by $HIE^A$ were readily antagonized by atropine and hexamethonium indicating that $HIE^A$ has some muscarinic and nicotinic activities.

Mepyramine did not inhibit the contractions produced by $HIE^A$ however it potentiated the contractions produced by $HIE^A$ on the isolated guinea - pig ileum. As a result of the potentiation the log dose - response curves of $HIE^A$ in the presence of mepyramine were shifted to the left and it also decreased the $EC_{50}$ values considerably indicating that the $HIE^A$ was potent in the presence of mepyramine. These observations could be due to increased sensitivity of the ileum to the effects of the $HIE^A$ since in the absence of mepyramine those observations were not made. This same observation also seems to be at variance with the observation made by Rang and Dale (2003) that many H1 - receptor antagonist also show significant anti - muscarinic effects, though their affinity is much lower for muscarinic receptors than for histaminic receptors. Since the $HIE^A$ had earlier

shown some muscarinic activity, in the view of Rang and Dale's observation it was expected that mepyramine, an H1 - receptor antagonist inhibit effects of $HIE^A$ however minimal. When the antagonism between atropine and $HIE^A$ was studied, it was found that the interaction between the two drugs was non - competitive. This interaction differs from the competitive antagonism between atropine and acetylcholine. Again, the antagonism between hexamethonium and $HIE^A$ was also non - competitive.

Johnson (1998) had observed that contractions and relaxations of smooth muscles are regulated by changes in the amount of cytosolic calcium available to interact with the regulatory proteins. In relaxed muscle, the level of "free" cytosolic calcium (calcium that is not bound to other structures such as sarcoplasmic reticulum (SR), mitochondrion, and nuclei) is low ($< 10^{-7}$M). Upon stimulation of the muscle, the level of calcium ions increases into the micromolar range to initiate contraction. Calcium binds first with calmodulin (one of the calcium binding proteins found in tissues) and then calcium - calmodulin complex binds to and activates the myosin light chain kinase. Once the stimulus for muscle contraction ceases, free calcium levels decrease, and calcium dissociates from the regulatory proteins, then the muscle relaxes again. The above mechanism is attributed to reference agonists (acetylcholine, histamine and nicotine) to produce contractions of smooth muscles like the guinea - pig ileum. For $HIE^A$ to also produce contractions similar to those of the reference agonists on the guinea-pig isolated ileum suggests $HIE^A$ may be using similar mechanisms.

$HIE^A$ showed stability to cholinesterase, similar to those of carbamylcholine and methylcholine. This gives an indication that the $HIE^A$ might have components comparable to carbamylcholine or methylcholine in terms of their activity to plasma cholinesterase.

### *4.1.2 The Isolated Rabbit Jejunum*

$HIE^A$ exhibited biphasic effect on all isolated rabbit jejunum studied. Lower doses of $HIE^A$ completely abolished the pendular movements of the rabbit jejunum and as the doses were increased $HIE^A$ produced contractions of the tissue which were dose - dependent. Though the relaxations were similar to those induced by noradrenaline, they were not inhibited by phentolamine indicating that the relaxations of the rabbit jejunum produced by $HIE^A$ were not α - adrenoceptor mediated. Atropine inhibited effects of $HIE^A$ but the inhibitions were not significant to suggest outright muscarinic receptor stimulation. Hexamethonium did not have any effect on the contractions produced by $HIE^A$ on the rabbit isolated jejunum and this indicates that contractions produced by $HIE^A$ were not ganglion - mediated.

From all indications, it is possible the biphasic effect of $HIE^A$ on the isolated rabbit jejunum could be due to the fact that it is a crude extract and may be containing many compounds of varying physiological and pharmacological effects. It could also, in part be due to the various components of $HIE^A$ acting synergistically to produce varied responses.

### *4.1.3 The Isolated Rat Uterus*

$HIE^A$ produced dose – dependent myometrial contractions of both the pregnant and non – pregnant isolated rat uterus preparations indicating non - specific activity on the isolated pregnant and non-pregnant rat uterus preparations. The myometrial contractions produced by $HIE^A$ were similar to those produced by acetylcholine and oxytocin however the $EC_{50}$ values showed $HIE^A$ to be the least potent.

The myometrial contractions of the pregnant and non – pregnant rat uterus preparations produced by $HIE^A$ do not support the report made by Irvine (1960) that *Heliotropium indicum* is used by Asante women to stop abortion, because drugs that produce myometrial contractions normally are abortifacient.

The myometrial contractions produced by $HIE^A$ were readily inhibited by atropine on both the pregnant and non - pregnant rat uterus preparations indicating a possible muscarinic activity.

Adrenaline antagonized myometrial contractions produced by both acetylcholine and $HIE^A$ but does not lend support to the observed muscarinic activity of $HIE^A$ because the effect of adrenaline was physiological. Rang and Dale (2003) had reported that selective $\beta_2$ - adrenoceptor agonists, for example salbutamol, inhibit spontaneous or oxytocin - induced contractions of the pregnant uterus. Salbutamol which effectively inhibited oxytocin similarly inhibited the effect of $HIE^A$ on the pregnant rat uterus preparation. Though salbutamol is not a specific antagonist for oxytocin however its ability to inhibit both $HIE^A$

and oxytocin only suggests that $HIE^A$ and oxytocin could be acting via similar receptor sites.

Vane and Williams (1973) had reported that the uterine stimulating activity of oxytocic agents might be mediated through uterine prostaglandins release and uterine membrane sensitisation.

Also, Hertelendy (1973) had shown that oxytocin induced parturition in the term pregnant rabbit was blocked by indomethacin, an inhibitor of prostaglandin biosynthesis.

However, Vane (1971) and Ku *et al*., (1975) had both observed that uterine contractions of oxytocic agents are not linked to uterine membrane sensitization by prostaglandin release. Because of the fundamental significance of these observations, it was important that direct evidence be sought to ascertain whether uterine contractions induced by oxytocic agents are linked to prostaglandin release and further sensitization of uterine muscle.

Nevertheless, isolated rat uterus pretreated separately with two kinds of prostaglandin synthetase inhibitors (Indomethacin and Diclofenac Sodium) could not modify contractions produced by oxytocin and $HIE^A$, though in the case of $HIE^A$ diclofenac sodium inhibited the contractions but insignificantly. As much as the observation in this project contrasts with the findings of Vane and Williams (1973) and Hertelendy (1973), it however supports the observation made by Vane (1971) and Ku *et al.,* (1975).

$HIE^A$ may be producing myometrial contractions on isolated pregnant and non - pregnant rat uterus preparations through many receptor sites including muscarinic and oxytocic receptor sites.

## 4.2 EFFECT OF HIE ON THE CARDIOVASCULAR SYSTTEM

### *4.2.1 The Isolated Frog's Heart*

$HIE^A$ produced negative inotropic and chronotropic effects on the isolated frog heart, and these effects were inhibited by atropine but the atropine inhibitions of $HIE^A$ were not significant. It may be possible that the negative inotropic and chronotropic effects of $HIE^A$ on the isolated frog heart were mediated through muscarinic receptor sites as observed by Loffelholz and Papano (1985). The negative inotropic and chronotropic effects of $HIE^A$ were not mediated through histaminic action, since mepyramine did not have inhibitory effect on the depressor effects produced by $HIE^A$ on the frog isolated heart.

### *4.2.2 The Perfused Rabbit Heart*

$HIE^A$ dose – dependently depressed the perfused rabbit heart. Also, the $HIE^A$ dose - dependently reduced the rate of coronary perfusion. This could be due to constriction of coronary vessels by the $HIE^A$, since acetylcholine which also reduced the coronary perfusion does so by producing constriction of coronary vessels mediated through muscarinic receptors (Bowman and Rand 1980a). The rabbit perfused heart in all the

experiments stopped beating when high doses of $HIE^A$ and acetylcholine were administered. This observation could be due to toxic effects of the drugs.

### *4.2.3 The Anaesthetized Cat Blood Pressure*

In heart pacemaker cells , stimulation of muscarinic acetylcholine receptors (mAChRs) leads to activation of special class of $K^+$ channels (Sakmann *et al.*, 1983), and this possibly inhibits the slow inward $Ca^{2+}$ current (Giles and Nobles, 1976) resulting in decreased heart rhythmicity. Exogenous acetylcholine confirms it by exhibiting negative inotropic and chronotropic effects on the anaesthetized cat and these effects were inhibited by atropine, as described by Hilf *et al* (1989) that agonist actions at muscarinic $M_2$ receptor subtypes are competitively inhibited by mAChR antagonist atropine. $HIE^A$ also had similar effects on the anaesthetized cat reducing the systemic blood pressure dose - dependently. Though these effects of $HIE^A$ were inhibited by atropine, the inhibitions were not significant enough to suggest outright muscarinic mediation. $HIE^A$ could be eliciting depressor effect on the anaesthetized cat possibly by muscarinic receptor activation or probably through an unknown mechanism.

## 4.3 ANALGESIA

Many models have been used to evaluate the analgesic effects of drugs depending on the source of pain. The stimulus may be thermal (tail flick, and hot plate tests), mechanical (tail or paw pressure tests), electrical (stimulation of paw, tail or dental pulp) or chemical

(writhing and formalin tests) (Chau *et al*., 1989; Ferreira *et al*., 1978; and Le Bars *et al*., 2001). However, the formalin test first described by Dubuisson and Dennis (1977) has been shown as the most predictive of acute pain Le Bars *et al*., (2001) and a valid model of clinical pain (Costa - Lotufo *et al*., 2004; Vasconcelos *et al*., 2003; Vissers *et al*., 2003). The formalin test is a well characterized and accepted method in pre-clinical screening of analgesics (Vissers *et al*., 2003; Abbott *et al*., 1988). Intradermal injection of formalin into the rat paw resulted in a biphasic nociceptive response shown by flinching, licking, or biting of the injected paw. An analgesic drug would decrease the nociceptive responses (flinching, licking or biting of the infected paw) (Courteix *et al*., 1998). It has been suggested that the first phase of the formalin induced pain is due to a direct stimulation of nociceptors by formalin and this is characteristic of centrally acting analgesics (Chau *et al*., 1989; Le Bars *et al*., 2001; Szolcsanyi *et al*., 2004) whereas the second phase involves inflammatory components with release of different pain mediating substances that possibly activate afferent nerves (Malmberg *et al*., 1992; Le Bars *et al*., 2001; Yashal *et al*., 1998), hence the formalin test is appropriate for testing both centrally and peripherally acting analgesics, NSAIDs and corticosteroids Vasconcelos *et al*., (2003). Diclofenac sodium, an NSAID was much effective in decreasing the nociceptive responses in the second phase as previously reported (Bukhari *et al*., 2007; Yano *et al*., 2006; Ortiz *et al*., 2002). This observation lends further support to the earlier reports (Malmberg *et al*., 1992; Bukhari *et al*., 2007; Mino *et al*., 2004; Rosland *et al*., 1990) characterizing cyclo - oxygenase inhibitors in decreasing pain perception in the second phase of formalin induced pain nociception. The analgesic activities produced by morphine, $HIE^{E}$ and $HIE^{A}$ in both the

first and second phases were similar and characteristic of centrally acting analgesics. The anti - nociceptive activity of the HIE could be due to its phyto - constituents as previously reported (Betanabhatla *et al*., 2007) and also by the reported anti – inflammatory activity of *Heliotropium indicum*. Though the HIE showed analgesic activity, the observed activities were not significant perhaps due to the low doses used (30 - 300 mg/kg *p.o*).

## 4.4 TOXICITY TESTS

### *4.4.1 Acute Toxicity Study*

Symptoms associated with acute toxicity such as diarrhoea, convulsions, sedation, fatigue, neuromuscular incoordination, polyuria, writhing and mortality particularly when a high dose of a drug is taken were not observed. The fact that none of the above symptoms were observed in $HIE^A$ treated rats (1, 2, 4 and 5 g/kg *p.o*) and mice (1, 2 and 3 g/kg *p.o*) in this thesis suggest that $HIE^A$ is well tolerated by rats and mice. Beyond these dose ranges the safety of $HIE^A$ cannot be guaranteed for rats and mice.

### *4.4.2 Sub-Acute Toxicity Study*

No animal in $HIE^A$ treated groups died over the experimental period however rats receiving high doses of $HIE^A$ (4 g/kg and 5 g/kg *p.o*) exhibited prostration and also gathered at the corners of their cages. These observations could be attributed to $HIE^A$, since similar effects were not seen in the animals in the control group and those animals that received lower

doses of $HIE^A$. Amissah (2007) had reported a possible link between hypotension and prostration in rats it could be possible that hypotensive effect of $HIE^A$ might have caused the prostration in the rats that received high doses.

With respect to the following haematological parameters: R B C count, W B C count haemoglobin concentration and platelet concentration, $HIE^A$ treated rats showed insignificant changes from those of the control group over the experimental period.

This observation suggests perhaps that the $HIE^A$ has insignificant haemopoietic activity so far as the rats are concerned.

Saponins, one of the phytoconstituents of $HIE^A$, have been reported to have haemolytic activity (Onning *et al.*, 1996; Casarett and Doull, 1996) and could be responsible for the virtually insignificant changes in the haemoglobin concentration and R B C count of $HIE^A$ treated rats.

### *4.4.3 Histopathology*

Several reports have implicated pyrrolizidine alkaloids isolated from *Heliotropium indicum* in liver damage (Casarett and Doull, 1996; Tandon *et al.*, 1990; and McDermott and Ridker, 1978).

In this study, $HIE^A$ treated rats showed histopathological changes in their liver, Kidney, lungs and heart as compared to those of the control group.

This observation confirms the earlier reports and also suggests the possibility of $HIE^A$ having some cumulative toxic effects since in both the acute and sub-acute toxicity tests none of the animals died yet histopathological examination of some internal organs revealed marked organ damages. Also this observation lends support to earlier works by Castles *et al.,* 1976; Rakieten *et al.,* 1974 whose work had implicated indicine – N – oxide, an isolate from *Heliotropium indicum,* in liver damage but could not support it with any histological evidence.

The toxic effects of $HIE^A$ may not be due to only its alkaloidal components but could also be in part due to tannin*s* and saponins. After all, tannins and saponins have already been reported to have some toxic effects including haemolysis (Gatachew *et al.,* 2000; Waterman and Cole, 1994; Mahanato *et al.*, 1982).

$HIE^A$ treated rats did not show significant changes in body weight over the experimental period.

However, rats that received doses of $HIE^A$ (2 g/kg and 4 g/kg *p.o*), gained slight weight increase during the last seven days.

This observation correlates with the haematological values obtained which also showed insignificant changes in the blood parameters of $HIE^A$ treated rats compared to control rats. There were also no changes in the mean of the organ/body weight ratio of $HIE^A$ treated rats as compared to those of the control rats.

## 4.5 GENERAL DISCUSSION

The spasmogenic activity of $HIE^A$ on smooth muscles, particularly the guinea - pig ileum was mediated via muscarinic $M_3$ - receptor subtype and nicotinic cholinergic receptors which were readily inhibited by atropine and hexamethonium.

The muscarinic activity of $HIE^A$ on the guinea - pig isolated ileum was replicated on the rabbit isolated jejunum and rat isolated uterus as atropine competitively inhibited contractions of $HIE^A$ on these preparations. Results from the effects of prostaglandin synthetase inhibitors (indomethacin and diclofenac) on the contractile responses of the isolated rat uterus preparation to $HIE^A$ seem to suggest that the possible mechanism of action of $HIE^A$ on both the pregnant and non – pregnant rat uterus preparations does not involve prostaglandin release and subsequent sensitization of uterine muscle to drugs. It may therefore be possible that $HIE^A$ stimulate contractions on the isolated rat uterus independently

$HIE^A$ produced "biphasic" effect on the rabbit isolated jejunum, possibly due to the fact that it is a crude extract and therefore contain many components which have varied pharmacological and physiological activities. It should also be noted that a single drug may elicit different effects by reacting with different receptor systems as pointed out by Van Rossum (1962).

$HIE^A$ showed stability to cholinesterase, suggesting that perhaps the cholinomimetic component of the extract is of the carbamylcholine, methylcholine or pilocarpine type.

Results gathered from the studies of the effects of $HIE^A$ on the cardiovascular system indicate $HIE^A$ has negative inotropic and chronotropic effects on the frog isolated heart, perfused rabbit heart and the anaesthetized cat. It must be emphasized that all these effects were dose-dependent.

Though the above cardiovascular effects of $HIE^A$ were inhibited by atropine, the inhibitions were not significant to suggest possible exclusive muscarinic activity. It may be possible the $HIE^A$ exerts its effects on the cardiovascular system through an unknown mechanism. The possible site of action of $HIE^A$ on the cardiovascular system remains open requiring further investigations.

The analgesic activity of $HIE^A$ was similar to that of morphine particularly in the first phase which perhaps suggests $HIE^A$ could be centrally acting, since morphine is a known centrally acting analgesic. The analgesic activity of $HIE^A$ could also be attributed to some of its constituent phyto - compounds (Alkaloids,steroids).

The histopathological changes observed in the liver, kidney, lungs, and the heart of $HIE^A$ treated rats confirm earlier reports implicating alkaloidal components isolated from $HIE^A$ including pyrrolizidine and indicine - N - oxide alkaloids Casarett and Doull, (1996). Also, saponins are reported to have considerable haemolytic effects (Gatachew *et al.,* 1976; Rakieten *et al*., 1974; Mahanato *et al.*, 1982) and could play a role in the organ damage produced by $HIE^A$. The possible mechanism of action of these toxic agents as suggested by Carballo *et al*., (1992) has it that formation of pyrrole metabolites from PAs by liver

microsomal oxidation and cross linking of DNA strands by pyrrole metabolites are responsible for the liver damage.

*Chapter 5*

# CONCLUSIONS

Results obtained from experiments performed in this thesis have led to the following conclusions:

- $HIE^A$ have some muscarinic and nicotinic activities.
- $HIE^A$ shows significant stability to plasma cholinesterase activity
- $HIE^A$ produced biphasic effect on the isolated rabbit jejunum.
- $HIE^A$ has contractile effect on the uterus.
- Effect of $HIE^A$ on the isolated rat uterus may not be linked to prostaglandin biosynthesis.
- $HIE^A$ produced negative inotropic and chronotropic effects.
- $HIE^A$ has depressor effect in the anaesthetized cat.
- $HIE^A$ could have centrally acting analgesic activity.
- $HIE^A$ was well tolerated up to oral doses of 3 g/kg in mice and 5 g/kg in rats.
- $HIE^A$ has cumulative toxic effects on the liver, kidney, lungs, and the heart.

# RECOMMENDATIONS

- The mechanism of action of $HIE^A$ on the cardiovascular system needs to be investigated further in order to establish the possible site of action.
- The component of the $HIE^A$ responsible for its depressor effect needs to be identified.
- Though the analgesic activity of $HIE^A$ was insignificant in this particular work it still needs to be evaluated further using the formalin test and other pain models and also higher doses of the extract must be used in the next study.
- The effect of $HIE^A$ needs to be investigated on skeletal muscles.
- The effect of $HIE^A$ on the uterus must be investigated further to establish its specific role on the physiology of the uterus and also to verify whether it has teratogenic effect on pregnancy.

# REFERENCES

**ABBIW, D.K** (1990). Useful plants of Ghana. West Africa use of wild and cultivated plants, Intermediate technology Publications and the Royal Botanic Garden. KEW, UK. 52. 156.

**ABBOT, F.V.** (1988). Peripheral and central antinociceptive actions of ethylketocyclazocine in the formalin test. *Eur. J. Pharmacol*. **152**: 93-100.doi: 10.1016/0014-299(88)90899-4: PMID: 2905269.

**ABDULAZEEZ, A. A.,** Ayoola, M. D., Otulana, J. O., Akinola, O. B., Olayinola, Abimbola, Ejiwunmi, A. B. (2008). Evaluation of the Histo - gastroprotective and anti - microbial activities of *Heliotropium indicum* Linn (Boraginaceae), *Malaysian Journal of Medical Sciences* 15(3).

**ABUBAKAR, M. S**., Musa, A. M., Ahmed, A., Hussaini, I. M. (2007). The perception and practice of traditional medicine in the treatment of cancers and inflammations by the Hausa and Fulani tribes of Northern Nigeria. *Journal of Ethnopharmacology*, Vol. 111, Issue 3. Pp 625 – 629.

**ADIARATOU, T.,** Drissa, D., Seydou, D., Hilde, B., Berit, S.P. (2005). Ethnopharmacological Survey of different uses of seven medicinal plants from Mali

(West Africa) in the region Doila, Kokokani and Siby. *Journal of Ethnobiology and Ethnomedicine*. 1: p7.

**AMISSAH, F.,** Fleicher, T. C., Ishmael, K.S., Wood, E. (2007). Cardiovascular Effects of an Alcoholic Extract of Tetrapleura tetraptera (Taub) fruit in vitro and in anaesthetized rats. The *Ghana Pharmaceutical Journal*, Vol. 32 No. 2: 30 – 38.

**BETANABHATLA, K. S**., Jasmin Sajni, R., Karthik, R., Raamamurthy, J., Christina, A. J. M., Sasikumar, S. (2007), Antiinflammatory and Antinociceptive activities of *Heliotropium indicum* Linn in experimental animal models.

**BIRECKA, H**., DiNolfo, T.E., Martin, W.B., Frohlich, M.W. (1984). Polyamines and leaf senescence in pyrrolizidine alkaloid bearing Heliotropium plant. *J. Phytochemistry*. **23(5):** 991 – 998.

**BOLTON, T.** (1979). Mechanisms of action of transmitters and other substances on smooth muscle. *Physiological Reviews*, **59**: 606 – 718.

**BOUQUET, A. AND DEBRAY, M,** (1974). Plantes medicinales de la Cote d'Ivoire. Travaux et Documents de I'O.R.S.T.O.M., Paris.Pp 32, 232.

**BOWMAN, W.C., RAND, M.J**. (1980a). The heart and drugs affecting cardiac function. In textbook of pharmacology, 2nd Edition, Blackwell Scientific Publications, Oxford, London, Edinburgh, Boston Melbourne, P 22. 1 – 22.86.

**BRAGA, R.** (1976). Plantas do Nordeste, Especialmente do Ceara, 3rd Edition. Coleção Mossoroense: RN, p. 249.

**BUKHARI, I. A.,** Khan, R. A., Gilani, A. H., Shah, A. J., Hussain, J., Ahmed, V. U. (2007). The analgesic, anti - inflammatory and calcium antagonist potential of *Tenacetum artemisiodes. Arch Pharm Res* **30 (3):** 303 – 308.

**BULL, L. B**., Culvenor, C. C. J., Dick, A. T. (1968). The pyrrolizidine Alkaloids : Their Chemistry, Pathogenicity, and Other Biological Properties in Neuberger A., Tatum, E.L ,eds. Frontiers of Biology, Vol 9. New York; John Wiley and Sons. Pp 1 – 284.

**BURKILL, H.M**. (1985). The useful plants of West Tropical Africa. Great Britain: Royal Botanic Gardens Kew. White Friar Press Limited.

**CARBALLO, M.,** Mudry, M.D., Larripa, I.B., Villam, Y.E., d'Aquino, M. (1992). Gentoxic action of an aqueous extract of *Heliotropium curassavicum* var. Argentinum. *Mutation Research* **279**: 245 – 253.

**CASTLES, T. R.,** Snyder, J. L., Lee, C., Folk, R. M., Cooney, D. A. (1976). Toxicity of Indicine – N – Oxide (NSC132319) in mice, dogs, and monkeys. *Chem Abstr*; **84**: 54233.

**CHADHA, Y.K**. (1981). The wealth of India. A dictionary of Indian raw materials and industrial products. New Dehli, Council of Scientific and Industrial Research: 5, 28.

**CHANDLER, R.F**., Hooper, S.N., Freeman, L. (1979). Herbal Remedies of the Maritime Indians. *J. Ethnopharmacology*, **1 (1)**: 49 – 68.

**CHAU, T.T**. (1989). Analgesic Testing in Animals. Models in Pharmacological Methods in the Control of Inflammation. Edited by Chang, J.Y and Lewis, A.J. New York: Alan, R. Liss Inc, pp: 195-212. ISBN-10: 047151764X.

**COCKS, M., MOLLER, V.** (2002). Use of indigenous and indigenised medicines to enhance personal well – being: A South African case study. *Social Science and Medicine*, **54 (3)**: 387 – 398.

**COSTA-LOTUFO, L.V**., de Lucena, D.F., Andrade-Neto, J., Bezerra, J.N.S., Leal, L.K.A.M., de Sousa, F.C.F., de Barrosviana, G.S. (2004). Analgesic, anti-inflammatory and central depressor effects of the Hydroalcoholic extract and fractions from *Aeolanthus suaveolens*. *Biol. Pharm. Bull*. **27**: 821-824. Doi: 10.1248/bpb.27.821; PMID: 15187426. http://www.jstage.jst.go.jp/article/bpb/27/6/821/pdf.

**COURTEIX, C**., Bourget, P., Caussade, F., Bardin, M., Coudore, F., Fialip, J., Eschalier, A. (1998). Is the reduced efficacy of morphine in diabetic rats caused by alterations of opiate receptors or morphine pharmacokinetics*? J. Pharmacol. Exp. Ther*. **285**: 63- 70. PMID: 9535995. http://jpet.aspetjournals.org/egi/reprint/285/1/63.

**CULVENOR, C.C.J.** (1968). Tumor Inhibitory Activity of Pyrrolizidine Alkaloids. *J Pharm Sci;* **57**: 1112 – 1117.

**DALZIEL, J. M**. (1937). The Useful Plants of West Africa. Royal Botanic Gardens, Kew, **2 (1):** 64 – 69.

**DATTA, A.**, Narula, A., Bansal, K. C., Srivasta, P.S. (2003). Enhanced alkaloid production in callus cultures of *Heliotropium indicum* L and regeneration of plantlets. *J Plant Biochem. Biotechnol*. **12**: 139 – 142.

**DERIANIYAGALA, S. A**., Udugamasooriya, D. G., De Silva, E. D., Williams, D. E., Andersen, R. J. (2002). Rosmarinic acid and methyl rosmarinate from *Heliotropium indicum*. *Journal of Tropical Medicinal Plants*. **3 (1**): 25 – 28.

**DESHMUKH, P.,** Gupta, P., Shankar, R. (2009). *Heliotropium indicum, Coldenia procumbens, Boraginaceae,* Antimicrobial Activity, Phytochemicals. *Journal of pure and applied microbiology,* Vol. 3, No. 1. Pp 199 – 204.

**DIWAN, P. V**., Tillo, L. D., Kulkarni, D. R. (1982). Influence of *Tridax procumbens* on wound healing. *Indian Journal of Medical Research*, **75**: 460 – 464.

**DUBUISSON, D., DENNIS, S.G**. (1977). The formalin test: a quantitative study of the analgesic effects of morphine, meperidine and brain stem stimulation in rats and cats. *Pain*, 4, 161 – 174.

**FERREIRA, S. H**., Lorrenzetti, B. B., Correa, F. M. A. (1978). Central and peripheral anti - analgesic action of aspirin - like drugs. *Eur J Pharmacol* **53**: 39 – 48.

**GATACHEW, G**., Makhar, H. and Becker, K. (2000). Effect of polyethylene glycol on *in vitro* degradability of nitrogen from tannin rich browse and herbaceous legumes. *Brit J. of Nutri*; **84**: 73 – 83.

**GHANA HERBAL PHARMACOPOEIA**. (1992). Accra. Advent Press. Pp 63 – 71.

**GILES, W.R**., Noble, S.J. (1976). Changes in the membrane currents in bullfrog atrium produced by acetylcholine. *J Physiol*, 261, 103.

**GHANA HERBAL PHARMACOPOIEA** (2007). Science and technology policy research (STEPRI), Council for scientific and industrial research (CSIR). Pp 122 – 125.

**HARTMAN, T.** (1999). Chemical ecology of pyrrolizidine alkaloids. *Planta* **207(4):** 483 – 495.

**HAYASHIDA, K.,** Takeuchi, T.,Shimizu, H., Ando, K., Harada, E. (2003). Lactoferrin enhances opioid - mediated analgesia via nitric oxide in the rat spinal cord. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **285**: R306-R312. PMID: 12702487. http://ajpregu.physiology.org/egi/reprint/285/2/R306.

**HERTELENDY, F.** (1973). Block of oxytocin-induced parturition and oviposition by prostaglandin inhibitors. Life Science: **13 (11**): 1581 – 9.

**HEVI, J.** (1989). In Ghana, conflict and complementarity. Journal Hastings Cent Rep, SPEC Suppl pp 5 - 7.

**HILF, G.,** Gierschik, P., Jakobs, K.H. (1989). Muscarinic acetylcholine receptor-stimualted binding of guanosine 5 – O - (3-thiotriphosphate) to guanine-nucleotide-protein-binding proteins in cardiac membranes. *Eur J Biochem*, **186**: 725.

**HOLM, L. G**.,Pluknett, D. L., Pancho, J. V., Herberger, J. P. (1977). The world's worst weeds: Distribution and Biology. Honolulu, HI University of Hawaii Press. P 609.

IRVINE, F. R. (1930). Plants of the Gold Coast. London : Oxford University Press.

**IRVINE, F.R**. (196). Woody plants of Ghana with special reference to their uses. Oxford University Press, London. P 58.

**JOHNSON, L.R**. (1998). Essential Medical Physiology. 2nd edition, Lippincott – Raven Publishers, 227 East Washington Square, Philadelphia, PA19106 – 3780. Pp 129 – 134.

**JOSE, R. T** (2000). The Chemistry of Antipredesor defense by Secondary Compounds in Neotropical Lepidptera: Facts,Perspectives and Caveats. *J Braz Chem Soc*. **11(6, Suppl)**: 551 – 561.

**KALYAN, S.B**., Jasmin, S.R., Karthik, R., Rooma, M. J., Christina, A.J.M., Sasikumar, S. (2007). Anti-inflammatory and antinociceptive activities of *Heliotropium indicum* Linn in experimental animal models.

**KERHARO, J. AND BOUQUET, A**. (1950). Plantes medicinales et toxiques de la Cote d'Ivoire – Haute – Volta. Mission d'etude de la pharmacopee indigene en A.O.F. Editions Vigot Freres, Paris. 300.

**KERHARO, J., AND ADAM, J.G.** (1974). La pharmacopee Senegalaise traditionelle. Plantes medicinales et toxiques. Edition Vogot Freres, Paris. P 403.

**KINGSBURY, J.M**. (1964). Poisonous plants of the USA and Canada. Prentice. Hall, Inc., Englewood Cliffs, NJ, USA.

**KLOSS, J.** (1939). Back to Eden. The classical guide to herbal medicine, natural foods and home remedies. Merck and Co. Inc, Royway, N.J. Pp 47 – 50.

**KU, E.C**., Wsvary, J.M., Cash, W.D. (1975). Diclofenac sodium, the sodium salt of O-(2, 6-dichlorophenylamino)-phenylacetic acid (GP45840, Voltaren), is a potent inhibitor of sheep seminal vesicle prostaglandin synthetase in vitro. Like indomethacin, aspirin and certain other NSAIDS. Biochemistry Pharmacology.

**KUGELMAN, M**., Lui, W.C., Axelrod, M., McBride, T.J., Rao, K.V. (1976). Indicine-N-oxide: The antitumour principle of *Heliotropium indicum*. *Lloydia,* **39**:125 – 128.

**LE BARS, P.L**., Katz, M.M., Berman, N et al., (1997). A placebo controlled, double-blind, randomized trial of an extract of *Gingko biloba* for dementia. *Journal of the American Med Assoc*. **278 (16):** 1327 – 1332.

**LETENDRE, L**., Smithson, W. A., Gilchrist, G.S. *et a.l*., (1981). Activity of indicine N – oxide in refractory acute leukemia. Cancer; **47**: 437 – 441.

**LI, C.P**. (1974). Chinese Herbal Medicine. USDHEN Publication (NH) 75 – 732. Washington D.C: Public Health Service.

**LOFFELHOLZ, K**., PAPANO, A. J. (1985). The Parasympathetic Neuro - Effector junction of the heart. *Pharmacol. Rev*. **37**: 1.

**MAHANATO, S. B**., Ganguly, A. N. Sadhu, N. P. (1982). Review – Steroidal Saponins. *Phytochemistry*, **21 (5**): 959 – 978.

**MALMBERG, A.B**., and Yaksh, T.L, (1992). Anti - nociceptive actions of spinal non-steroidal anti-inflammatory agents on the formalin test in the rat. *J pharmacol, Exp Ther*, **263**: 136 – 46.

**MATTOCK, A.C**. (1961). Minor alkaloids of *Heliotropium indicum* Linn. *Journal of chemical Soc*. Pp 329 - 331.

**McDERMOTT, W. V.,** Ridker, P. M. (1990). The Budd – Chiarri Syndrome and Hepatic Veno – Occlussive Disease. *Arch Surg*, **125**: 525 – 527.

**McLEAN, E.K** (1970). The toxic reactions of pyrrolizidine (Senecio) alkaloids. *Pharmacol Res Commun*; **22**: 429 – 483.

**MINO, J.,** Moscatelli, V., Hnatyszyn, O., Govzakzany, S., Aceredo, C., Ferraro, G. (2004). Antinociceptive and anti-inflammatory activities of *Artemisia copa* extracts. *Pharmacol. Res*. **50**: 59 – 63. Doi: 10.1016/j.phrs.2003.12.016; PMID: 15082030.

**MISER, J.S.**, Smithson, W.A., William Krivit, Carla Hughes, Dianne Davis, Mark Krailo and Denman Hammond (1991). Phase 11 Trial of Indicine N – oxide in Relapsed Pediatric Solid Tumors. *Investigational New Drugs*, 9 (4): 339 – 342.

**MONTGOMERY, R. D**. (1980). Cyanogens. In toxic constituents of plant food stuffs. Liener S. E. (ed), 2nd Edition. Acad. Press New York.

**MUTHU, C**., Ayyanar, M., Raja, Ignacimuthu, S. (2006). Medical Plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. *Journal of Ethnobiology and Ethnomedicine*, **2**: 43.

**NETHAJI, S., MANOKARAN, C.** (2009). Phytochemical Analysis and Antimicrobial Activity of *Heliotropium indicum* L and *Coldenia procumbens* L. *Journal of pure and applied microbiology*,Vol. 3, No. 1. Pp 195 – 198.

**OAKENFUL, D. G.** and Sidhu, G. S. (1990). Could Saponins be a useful Treatment for Hypercholesterolaemia? *Eur. J. of Clin. Nutri*. **44**: 79 – 88.

**OKPAKO, D. T. and TAIWO, Y. O. O.** (1984). Cyclo – oxyginase inhibitors antagonize indirectly evoked contractions of the guinea – pig isolated ileum by inhibiting acetylcholine release. *Br. J. Pharm*., **82, 577 – 585.**

**ONNING, G.,** Wang, Q., Westrom, B., ASP, N. G. and Karlsson, B. W. (1996). Influence of oat saponins on intestinal permeability *in vitro* in the rat. *Brit. J. of Nutri*. **74**: 141 – 151.

**ORTIZ, M.I.,** Granados-Soto, V., Castañeda-Hernández. (2002). The NO - cGMP-$K^+$ channel pathway participates in the antinociceptive effect of diclofenac, but not of indomethacin. *Pharmcol. Biochem. Behar* **76(1):** 187 - 95. Doi: 10.1016/S0091 - 3057(03)00214 - 4; PMID: 13679232.

**ORWA, J.A**. (2002). Editorial: Herbal Medicine in Kenya: Evidence of Safety and Efficacy. *East African Medical Journal*. **79(7):** 341 – 2.

**PATON, D.M.** (1968). The contractile response of the isolated rat uterus to noradrenaline and 5 - hydroxytryptamine. *Eur. J. Pharmac*. **3**. 310 – 315.

**PATTERSON, L.** (2001). Second Annual International Conference on Traditional Medicine in Ghana, Africa. Available at http:// www.ccnh.edu/newsletr/holtimesv8n4/conference.html.

**PENSO, G.** (1980). The role of W.H.O. in the selection and characterization of medicinal plants (Vegetable drugs*). J. Ethnopharmacology*, **2 (2)**: 183 – 184.

**PEREZ MALDONADO, R. A. AND NORTON, B. W**. (1996). Digestion of $^{14}C$ – labeled condensed tannins from Desmodium intortum in sheep and goats. *Brit. J. of Nutri.* **76**: 501 – 513.

**PHILIPSON, S. D**., Handa, S. S. (1978). Alkaloid N – oxides. A review of recent developments. *Lloydia*; **41**; 385 – 431.

**PRICE, K. R.,** Johnson, I. T. and Fenwick, G. R. (1987). The Chemistry and Biological Significance of Saponins in foods and feeding stuffs. *CRC Critical Reviews in Food Science and Nutrition*, **26**: 27 – 135.

**RADANACHALESS, T.** Maxwell, J. F. (1994). Weeds of soybeans fields in Thailand. Multiple Cropping, Center Publications. Thailand.

**RAKIETEN, N**., Cooney, D.A., Davis, R.D. (1974). Toxicity Studies of NSC132319, Indicine N – oxide, following single IV (Intravenous) administration to beagle dogs. *Chem Abstr*, 81: 114824.

**RANG, H. P**., Dale, M. M., Ritter, J. M., Moore, P. K. (2003). Pharmacology. 5th edition. Elsevier, Churchill Livingstone. Pp 144, 443 – 448.

**REDDY, J.S**., Rao, P.R., Reddy, M.S. (2002). Wound healing effects of *Heliotropium indicum, Plumbago zeylanicum and Acalypha indica* in rats. *Journal of Ethnopharmacology*, **79**: 249 – 251.

**REINA, M.,** Merich, A.H., Cabrora, R., Gonzalez – Coloma, A. (1996). Pyrrolizidine alkaloids from *Heliotropium bovei. Phytochemistry*, **38**: 355.

**RIDKER, P. M.,** Okhuma, S., McDermott, *et al* (1985). Hepatic veno – occlusive disease associated with the consumption of pyrrolizidine containing dietary supplements. *Gastroentorology*. **88:** 1050 – 1054.

**ROBBERS, J.E., TYLER, V.E**. (1999). Tyler's herbs of choice: The therapeutic use of phytomedicinals. New York: Haworth Herbal Press. Pp. 63 – 68.

**ROSLAND, S.H**., Tjølsen, A., Machle, B., Hole, D.K., (1990). The formalin test in mice. Effect of formalin concentration. *Pain* **42**:235 – 242. Doi: 10.1016/0304-3959(90)91167-H. PMID: 2247320.

**SAKMANN, B**., Noma, A., Trautivein, W. (1983). Acetylcholine activation of single potassium channel in isolated pacemaker cell of mammalian heart. *Nature*, 303, 250.

**SAMMY, J.N.S**., Luciana, L., Machado, Otilia., D.L.P., Raimundo Brac-Fillo, Cassia, R.O., Ping Yao., Geoffrey, A.C., Telma, L.G.L. (2005). Pyrrolizidine alkaloids from *Heliotropium indicum. J. Braz. Chem. Soc*. Vol. 16, No. 6b, Sao Paulo.

**SCHAUENBERGER, P**., Paris, F. (1977). Guide to medicinal plants. New Canaan, CT, Keats Publishing. Pp 13 – 14.

**SODI, T**. (1996). Towards Recognition of indigenous Healing: Prospects and Constraints. *Journal of Comprehensive Health*, **7 (1)**:5 – 7.

**SOERJANI, M**., Kostermans, A. J. G. H., Tjitrosoepomo, G. (1987). Weeds of rice in Indonesia. Balai Pustaka. Jakarta.

**SOFOWORA, A**. (1993). Screening plants for Bioactive Agents, In: Medicinal plants and Traditional Medicinals in Africa. 2nd edition .Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria. Pp 134 – 156.

**SRIKUMAR, R**., Murugaian, P., and Thangaraj, R. (2009). Antiinflammatory Activity of *Heliotropium indicum* against *Aspergillus flavus*. *Journal of Natural Science and Technology – Life Sciences and Bioinformatics*. Pp 1, 35 – 36.

**SRINIVAS, K**., Rao, M.E.B., Rao, S.S. (2000). Anti-inflammatory activity of *Heliotropium indicum* Linn and Leucas aspersa spreng in albino rats. *Indian Journal of Pharmacology*, **32(1**): 37 – 8.

**SRIVASTAVA, S.C**., Mitra, S., Bandopadhyay, S. (2003). Some plants of ethnomedicinal importance from Darjeeling District , West Bengal. *Journal of Economic and Taxanomic Botany*, 27: 972 – 977.

**SURESH, J. R.,** Rao, P. R., Reddy, M. S. (2002). Wound healing effects of *Heliotropium indicum, Plumbago zeylanicum* and *Acalpha indica* in rats. *Journal of Ethnopharmacology*, Vol. 79, Issue 2. Pp 249 – 251.

**SZOLCSANYI, J.,** Böleskei, K., Ā Szabó, Pintér, E., Elekes, K., Börzsei, R., Almási, R., Szüts, T., Kéri, G., Helyes, Z., (2004). Analgesic effect of TT-232, a heptapeptide somatostatin analogue, in acute pain models of the rat and the mouse and in streptozotocin-induced diabetic mechanical allodynia. *Eur. J. Pharmacol*. **498 (1 – 3):** 103 – 109. Doi:10.1016/j.ejphar.2004.07.085.

**TABI, M.** (1994). Nursing in Ghana. *Journal of Nursing Administration*, **24 (9**): 17 – 18.

**TABI, M. AND FRIMPONG, S.** (2003). HIV Infection of Women in Developing Countries. *International Nursing Review*, **50**: 40 – 50.

**TANDON, H. D**., Tandon, B. N., Mattocks, A. R. (1978). An epidemic of veno – occlusive disease of the liver in Afganistan. *Am J Gastroenterology*. **70**: 607 – 613.

**THEERAPHAN, M**., John, K., Boonsom, L., Saisunee, L., Stephen, G., Pyne. (2005). Composition and antituberculosis activity of the volatile oil of *Heliotropium indicum* Linn growing in Phitsanulok, Thailand. *Flavour and Fragrance Journal*, vol 21, issue 2, pp 265 – 267.

**THOMSON, A.B.R.,** Shaffer, E.A. (1994). First Principles of Gastroenterology: The basis of disease and an approach to management. 2nd edition, Canadian Association of Gastroenterology. Pp 130 – 172.

**TREASE, G.E., EVANS, W.C**. (1989). A textbook of pharmacognosy. London: Bailliere Tindall Ltd. P 71 – 98.

**UDUPA, X. L**., Rao, S. G., Kulkarni, D.R. (1989). Wound Healing Profile of Septilin. *Indian Journal of Physiology and Pharmacology*, **33**: 39 – 42.

**VAN EEDEN, A**. (1993). The Traditional Healer and our Health System. *South African Medical Journal*, **8**:441 – 442.

**VAN ROSSUM, J.M**. (1963). Cumulative dose-response curves. *Arch Int Pharmacodyn*, **143**: 299 – 330.

**VANE, J. R**. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin - like drugs. *Nature. New Biol*., **231**. 232 – 235.

**VANE, J.R., WILLIAMS, K.I**. (1973). The contribution of prostaglandin production to contractions of the isolated uterus of the rat. *Br. J. Pharmac*. **48**: 629 – 639.

**VASCONCELOS, S.M.M.**, Oliveira, G.R., de Carvalho, M.M., Rodriguez, A.C.P., Silveira, E.R., Fonteles, M.M.F., Sousa, F.C.F., Briana, G.S. (2003). Antinociceptive Activities of the Hydroalcoholic extracts from Erythrina velutina and Erythrina Mulungu in mice. *Biol. Pharm. Bull*. **26:** 946 – 949. PMID: 12843615. http://www.jstage.jst.go.jp/article/bpb/26/7/946pdf.

**VISSERS, K.V.,** Hoffmann, F., Geenan, R., Biermans, Meert, T., (2003). Is the second phase of formalin test useful to predict activity in chronic constriction injury models? A pharmacological comparison in different species. *Pain practice* **3**: 298 – 309. PMID: 17166125.

**WALKER, R**. (1953). Usages Pharmaceutiques des plantes spontanees du Gabon. Bull. Inst. Etudes Centrafricaines, from the data bank PHARMEL 2 (ref. HP 10). 4 – 6.

**WALKER, R. AND SILLANS, A. R**. (1961). Les plantes utiles du Gabon. Encyclopedie Biologique. Editions Paul Lechevalier, 12 rue de Tournon, Paris, 1: 478.

**WALL, M. E.,** Eddy, C. R., McClenna, M. L., Klump, M. E. (1952). Detection and estimation of steroid and sapogenins in plant tissue. *Anal. Chem*. *24*: 1337 – 1342.

**WANG, Y.X**., Bowersox, S.S., Pettus, M., Gao, D. (1999). Anti - nociceptive properties of Ferfluramine, a serotonin re-uptake inhibitor, in a rat model of neuropathy. *J. Pharmacol. Exp. Ther*. **291(3)** 1008-1016. PMID: 10565818. http://jpet.aaspetjournals.org/egi/reprint/291/3/1008.

**WATERMAN, P. G. AND COLE, S**. (1994). Analysis of Phenolic Plant Metabolites, London, Blackwell Scientific Publications.

**WHO.** (2003). FactSheet No. 134. Traditional Medicine. URL: http://www.who.int/mediacentre/factssheets/fs134/en/.

WORLD BANK (2002). 2002 World Health Indicators, at: http://devdata.worldbank.org/genderstats/rhealth.pdf.

**WORLD HEALTH ORGANISATION (WHO)** (2002). Traditional Medicine Report by the Secretariat. Available at: http:// www.who.int/gb/EB_WHA/PDF/EB111?eeb1119.pdf.

**YANO, S**., Suzuki, M., Yuzurihara, Y., Kase, S., Takeda, S., Wantanabe, M., Aburada, Miyamoto, K. (2006). Antinociceptive effect of methylleugenol on formalin-induced hyperalgesia in mice. *Eur. J. Pharmcol* **553**: 99 – 103.

**YASHPAL, K**., Coderre, T.J., (1998). Influence o f formalin concentration on the antinociceptive effects of anti-inflammatory drugs in the formalin test in rats: Separate mechanisms underlying the nociceptive effects of low and high formalin concentration. *Eur. J. Pain*: **2**: 63 – 68. Doi: 10.1016/s1090-3801(98)90047-7: PMID: 10700302

**ZHAO, X.L**., Chan, M.Y., Ogle, C.W. (1989). The identification of pyrrolizidine alkaloid containing plants. *American Journal of Chinese Medicine*. **17 (1-2)**: 71 – 78.

# APPENDIX

## 5.1 THE COMPOSITION OF TYRODE PHYSIOLOGICAL SOLUTION

This physiological solution was used to maintain the isolated guinea - pig ileum preparation.

The Tyrode solution was made up of:

| | |
|---|---|
| Calcium chloride ($CaCl_2$) | 1.0 g/L |
| Glucose ($C_6H_{12}O_6$) | 1.0 g/L |
| Pottasium chloride (KCl) | 0.2 g/L |
| Magnesium chloride ($MgCl_2$) | 0.01g/L |
| Sodium hydrogen carbonate ($NaHCO_3$) | 1.0 g/L |
| Sodium chloride (NaCl) | 8.0 g/L |
| Sodium bisphosphate ($NaH_2PO_4$) | 0.05 g/L |

## 5.2 THE COMPOSITION OF RINGER - LOCKE'S PHYSIOLOGICAL SOLUTION

The above physiological solution was used to maintain the isolated rabbit jejunum preparation.

The solution comprises:

| | |
|---|---|
| Sodium chloride (NaCl) | 9.0 g/L |
| Sodium hydrogen carbonate ($NaHCO_3$) | 0.5 g/L |
| Pottasium chloride (KCl) | 0.42 g/L |
| Calcium chloride ($CaCl_2$) | 0.24 g/L |
| Glucose ($C_6H_{12}O_6$) | 1.0 g/L |

## 5.3 THE COMPOSITION OF DE JALON'S PHYSIOLOGICAL SOLUTION

The above physiological solution was used to maintain the isolated rat uterus preparation.

The solution was made up of:

| | |
|---|---|
| Sodium chloride (NaCl) | 9.0 g/L |
| Sodium hydrogen carbonate ($NaHCO_3$) | 0.5 g/L |
| Pottasium chloride (KCl) | 0.42 g/L |
| Calcium chloride ($CaCl_2$) | 0.06 g/L |
| Glucose ($C_6H_{12}O_6$) | 0.5 g/L |

## 5.4 THE COMPOSITION OF FROG - RINGER SOLUTION

This solution was used to maintain the isolated frog heart preparation.

The solution was made up of:

| | |
|---|---|
| Sodium chloride (NaCl) | 6.5 g/L |
| Sodium hydrogen carbonate ($NaHCO_3$) | 0.02 g/L |
| Pottasium chloride (KCl) | 0.14 g/L |
| Sodium bis phosphate ($NaH_2PO_4$) | 0.5 g/L |
| Calcium chloride ($CaCl_2$) | 0.12 g/L |
| Glucose ($C_6H_{12}O_6$) | 2.0 g/L |

## 5.5 DRUGS

- Adrenaline hydrogen tartrate (BDH)
- Acetyl – β - methylcholine (SIGMA)
- Nicotine (SIGMA)
- Acetylcholine bromide (SIGMA)
- Histamine disphosphate (SIGMA)
- Hexamethonium bromide (MAY & BAKER LTD, Dagenham, England)
- Atropine sulphate (E-MERCK AG-DARMSTAD - Germany)
- Oxytocin(Pharm - Inter Sprl,Brusela,Belgica)
- Ritrodine (salbutamol)(Ernest Chemist Ltd,Ghana)

- Nifedipine (Denk Pharma, Germany)
- Diclofenac sodium (Torrent Pharmaceutical Ltd, India).

## 5.6 APPARATUS

- Harvard Research Tissue Bath System (50 - 2153, 230V, 50/60Hz), with the following specifications:
  Bath dimensions: 21.5cm x 29.0cm x18.0cm
  Bath Capacity: 11.2Litres
  Coil capacity: 400ml
  Heaters: 250W total
  Water temperature: 15 to $45^0$C
- Lagendorff's perfusion apparatus
  CAT No. 5035
  200-250V AC
  Dimension: 17.8mm x 16.5mm x21mm
  Scientific & Research Instruments Ltd, Fircroft Way Edenbridge Kent (SRI), England
- Harvard Respirator Pump, Kent, England
- Pressure Transducer (Model P23Gb, Statham)
- Universal Oscillograph (Model 50-8622, Harvard Apparatus Ltd, Kent, England)
- Carotid Artery Cannula (Polyethylene tubing, 1.02 - 1.27mm)
- Femoral vein Cannula (Polyethylene tubing, 1.00 - 1.02mm).

## 5.7 RESULTS OF THE RESPONSES OF THE ISOLATED GUINEA – PIG ILEUM TO ACH, HISTAMINE, NICOTINE AND HIE[A]

| Dose (mg/ml) | ACh | | | Histamine | | | Nicotine | | | HIE[A] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.001 | 3 | 5.5 | 3 | 2.5 | 1.5 | 2 | 0 | 0 | 0 | | | |
| 0.002 | 10 | 12 | 10 | 5.5 | 2.5 | 4 | 0 | 0 | 0 | | | |
| 0.004 | 14 | 14 | 15 | 9.5 | 4.5 | 4.5 | 0 | 0 | 0 | | | |
| 0.008 | 18 | 17 | 21 | 14.5 | 15 | 13.5 | 0.1 | 0.2 | 0.3 | | | |
| 0.016 | 22 | 20.5 | 24.5 | 19 | 20.5 | 20 | 3 | 1 | 0.9 | | | |
| 0.032 | 27.5 | 25 | 26.5 | 21 | 21 | 21.5 | 10 | 2 | 3 | | | |
| 0.064 | 31 | 30 | 28 | 23.5 | 23.5 | 22 | 14 | 5.5 | 4 | | | |
| 0.128 | 31.5 | 31.5 | 30 | 24 | 24.5 | 24 | 16 | 7 | 6.5 | | | |
| 0.256 | 31 | 30 | 30 | 26 | 25 | 25 | 19 | 11.5 | 10.5 | | | |
| 0.8 | | | | | | | | | | 1 | 1.5 | 1 |
| 1.6 | | | | | | | | | | 1 | 2 | 1.5 |
| 3.2 | | | | | | | | | | 2 | 3 | 2.5 |
| 6.4 | | | | | | | | | | 2.5 | 3.5 | 4 |
| 12.8 | | | | | | | | | | 5.5 | 5 | 5 |
| 25.6 | | | | | | | | | | 13 | 12.5 | 11.5 |
| 51.2 | | | | | | | | | | 16.5 | 16 | 15.5 |
| 102.4 | | | | | | | | | | 18 | 16.5 | 16 |
| 204.8 | | | | | | | | | | 18.5 | 16.5 | 16 |

## 5.8 $HIE^A$ IN THE PRESENCE OF ATROPINE

| Dose (mg/ml) | $HIE^A$ only | Atropine( 5 x $10^{-9}$ mg/ml) + $HIE^A$ | Atropine ( 2 x $10^{-8}$ mg/ml) + $HIE^A$ | Atropine ( 1 x $10^{-7}$ mg/ml) + $HIE^A$ |
|---|---|---|---|---|
| 0.028 | 0 | 0 | 0 | 0 |
| 0.047 | 0.01 | 0 | 0 | 0 |
| 0.083 | 0.015 | 0 | 0 | 0 |
| 0.166 | 0.515 | 0 | 0 | 0 |
| 0.333 | 2 | 0.01 | 0.01 | 0 |
| 0.625 | 4.5 | 2 | 2.5 | 0 |
| 1.25 | 9 | 3.5 | 5 | 3 |
| 2.5 | 12.5 | 2 | 3 | 6.5 |
| 5 | 14.25 | 7 | 10 | 5.5 |
| 10 | 17.5 | 11.5 | 9.5 | 10.5 |
| 20 | 19.25 | 16.5 | 20.5 | 13.5 |
| 40 | 22.25 | 19.5 | 20 | 20 |
| 80 | 24.5 | 21 | 22 | 24 |

## 5.9 ANTAGONISM OF NICOTINE AND HIE$^{A}$ BY HEXAMETHONIUM

| Nicotine alone | Hexamethonium (0.025mg/ml) + Nicotine | HIE$^{A}$ Alone | Hexamethonium (0.025mg/ml) + HIE$^{A}$ |
|---|---|---|---|
| 14 | 2 | 12 | 6.5 |
| 14.5 | 1.5 | 12.8 | 7 |
| 14.25 | 1.75 | 12.4 | 6.75 |

## 5.10 ANTAGONISM OF ACH AND HIE$^{A}$ BY ATROPINE

| ACh alone | Atropine ( 1 x $10^{-6}$ mg/ml) | HIE$^{A}$ Alone | Atropine( 1 x $10^{-6}$ mg/ml) + HIE$^{A}$ |
|---|---|---|---|
| 10.0 | 1.50 | 11.00 | 7.00 |
| 10.2 | 2.00 | 11.50 | 7.50 |
| 10.1 | 1.75 | 11.25 | 7.25 |

## 5.11 $HIE^A$ IN THE PRESENCE OF HEXAMETHONIUM ON THE ISOLATED GUINEA – PIG ILEUM.

| Dose (mg/ml) | $HIE^A$ Only | | Hexamethonium( 0.005 mg/ml) + $HIE^A$ | | Hexamethonium (0.05 mg/ml) + $HIE^A$ | |
|---|---|---|---|---|---|---|
| 3.200 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| 6.400 | 0.2 | 0.5 | 0.0 | 0.0 | 0.0 | 0.0 |
| 12.800 | 0.4 | 1.0 | 0.0 | 0.5 | 0.0 | 0.0 |
| 25.600 | 2.0 | 2.5 | 0.1 | 0.9 | 0.0 | 0.0 |
| 51.200 | 7.0 | 6.5 | 4.5 | 3.0 | 0.1 | 0.2 |
| 102.400 | 9.5 | 10.0 | 9.0 | 8.0 | 9.0 | 9.0 |
| 409.600 | 10.0 | 11.0 | 10.0 | 10.0 | 9.0 | 9.5 |

## 5.12 $HIE^A$ IN THE PRESENCE OF MEPYRAMINE ON THE ISOLATED GUINEA – PIG ILEUM.

| Dose (mg/ml) | $HIE^A$ Alone | | Mepyramine ( $5 \times 10^{-9}$ mg/ml) + $HIE^A$ | | Mepyramine ( $1 \times 10^{-7}$mg/ml) + $HIE^A$ | |
|---|---|---|---|---|---|---|
| 0.02 | 0.0 | 0.0 | 1.5 | 1.5 | 1.5 | 2.0 |
| 0.04 | 0.1 | 0.2 | 1.5 | 2.0 | 1.5 | 2.0 |
| 0.08 | 1.0 | 1.5 | 1.5 | 2.0 | 2.0 | 2.5 |
| 0.16 | 1.5 | 2.0 | 2.0 | 2.5 | 5.5 | 6.0 |
| 0.32 | 2.0 | 2.5 | 3.5 | 3.0 | 11.0 | 11.0 |
| 0.64 | 4.0 | 3.5 | 5.0 | 5.0 | 14.5 | 15.0 |
| 1.28 | 9.0 | 8.0 | 10.0 | 11.0 | 22.5 | 22.5 |
| 2.56 | 18.5 | 17.0 | 20.5 | 21.0 | 28.0 | 28.0 |
| 5.12 | 25.0 | 24.0 | 26.0 | 26.5 | 29.0 | 30.0 |

## 5.13 STABILITY OF HIE$^{A}$ AND CHOLINE ESTERS (ACH, CARBACHOLE, AMECHOLE) TO PLASMA CHOLINESTERASE.

| Enzyme (cholinesterase) + ACh | Enzyme (cholinesterase) + Amechole | Enzyme (cholinesterase) + Carbachole | Enzyme (cholinesterase) + HIE$^{A}$ |
|---|---|---|---|
| 5 | 100 | 91.25 | 67.5 |
| 2.5 | 97.5 | 81.25 | 72.5 |

## 5.14 RESPONSES OF THE ISOLATED RABBIT JEJUNUM TO HIE$^{A}$

| Dose (mg/ml) | % Maximum Responses | | |
|---|---|---|---|
| 3.2 | -10.5 | -6.1 | -7.5 |
| 6.4 | -13.2 | -16.7 | -8.8 |
| 12.8 | -15.8 | -18.2 | -11.3 |
| 25.6 | 18.4 | 19.7 | 12.5 |
| 51.2 | 68.4 | 78.8 | 47.5 |
| 102.4 | 84.2 | 87.9 | 87.5 |
| 204.8 | 100.0 | 93.9 | 100.0 |
| 409.6 | 94.7 | 100.0 | 92.5 |

## 5.15 ANTAGONISM OF ACH AND HIE$^{A}$ BY ATROPINE AND THOSE OF NICOTINE AND HIE$^{A}$ BY HEXAMETHONIUM ON THE ISOLATED RABBIT JEJUNUM.

| Acetylcholine alone | Atropine + acetylcho | HIE$^{A}$ alo | Atropine + HIE$^{A}$ | Nicotine alone | Hexamethonium + nicotine | HIE alo | Hexamethonium + HIE |
|---|---|---|---|---|---|---|---|

| | **line** | **ne** | | | | **ne** | |
|---|---|---|---|---|---|---|---|
| 3.0 | 1.0 | 2.5 | 2.0 | 1.8 | 1.5 | 2.6 | 1.5 |
| 3.5 | 1.5 | 2.0 | 1.9 | 2.0 | 1.0 | 2.2 | 2.0 |
| 4.0 | 1.2 | 3.0 | | 2.5 | 1.2 | 1.8 | 2.5 |
| | | | | 1.0 | | 1.5 | |
| | | | | 1.7 | | | |

## 5.16 RESPONSES OF THE ISOLATED RAT UTERUS TO OXYTOCIN, ACH, AND HIE[A].

| Dose (mg/ml) | Oxytocin | | Acetylcholine | | HIE[A] | |
|---|---|---|---|---|---|---|
| 0.000083 | 33.3 | 35. | | | | |
| 0.000167 | 46.7 | 45. | | | | |
| 0.000330 | 60.0 | 50. | | | | |
| 0.000667 | 66.7 | 60. | | | | |
| 0.001300 | 86.7 | 75. | | | | |
| 0.002670 | 93.3 | 95. | | | | |
| 0.005300 | 100.0 | 100. | | | | |
| 0.001000 | | | 48. | 50.0 | | |
| 0.002000 | | | 76. | 66.7 | | |
| 0.004000 | | | 88. | 76.7 | | |
| 0.008000 | | | 96. | 83.0 | | |
| 0.016000 | | | 100. | 86.7 | | |
| 0.032000 | | | 100. | 100.0 | | |
| 0.130000 | | | | | 0.0 | 0.0 |
| 0.270000 | | | | | 0.0 | 0.0 |
| 0.530000 | | | | | 47.6 | 42.9 |
| 1.100000 | | | | | 85.7 | 66.7 |
| 4.300000 | | | | | 100.0 | 85.7 |
| 8.500000 | | | | | 100.0 | 100.0 |
| 17.100000 | | | | | 100.0 | 100.0 |

## 5.17 EFFECT OF ADRENALINE ON THE RESPONSES OF THE ISOLATED RAT UTERUS TO ACH, AND $HIE^A$.

| ACh Alone | Adrenaline + ACh | $HIE^A$ alone | Adrenaline + $HIE^A$ |
|---|---|---|---|
| 27. | 7.5 | 27.5 | 7.0 |
| 27. | 5.5 | 27.5 | 6.5 |
| 27. | 6.0 | 27.0 | 7.5 |

## 5.18 EFFECT OF ATROPINE ON THE RESPONSES OF THE ISOLATED RAT UTERUS TO ACH, AND $HIE^A$.

| Ach alone | Atropine + ACh | $HIE^A$ alone | Atropine + $HIE^A$ |
|---|---|---|---|
| 24.5 | 15.0 | 23.0 | 21.0 |
| 24.5 | 14.5 | 23.5 | 14.0 |
| 22.0 | 14.0 | 25.0 | 11.0 |
| 22.5 | 12.0 | 23.0 | 16.0 |
| 23.5 | | 23.0 | 18.5 |
| 22.0 | | 24.0 | |

## 5.19 EFFECT OF SALBUTAMOL ON THE RESPONSES OF THE ISOLATED RAT UTERUS TO OXYTOCIN AND $HIE^A$.

| oxytocin alone | Salbutamol + Oxytocin | $HIE^A$ alone | Salbutamol + $HIE^A$ |
|---|---|---|---|
| 36.0 | 20.0000 | 38.0000 | 22.0000 |
| 38.0 | 20.0001 | 39.5000 | 23.5000 |
| 37.0 | 20.5000 | 38.0000 | 22.5000 |

## 5.20 5.20 EFFECT OF INDOMETHACIN ON THE RESPONSES OF THE ISOLATED RAT UTERUS TO OXYTOCIN AND HIE$^{A}$.

| Oxytocin alone | Indomethacin + Oxytocin | HIE$^{A}$ alone | Indomethacin + HIE$^{A}$ |
|---|---|---|---|
| 50.1 | 48.0 | 46.5 | 47.5 |
| 49.0 | 49.0 | 46.5 | 45.5 |
| 50.2 | 50.0 | 47.0 | 46.0 |

## 5.21 EFFECT OF DICLOFENAC ON THE RESPONSES OF THE ISOLATED RAT UTERUS TO OXYTOCIN AND HIE$^{A}$.

| *Oxytocin alone* | *Diclofenac + Oxytocin* | *HIE$^{A}$ alone* | *Diclofenac + HIE$^{A}$* |
|---|---|---|---|
| 48.0 | 50.0 | | 29.0 |
| 48.5 | 49.5 | 39.0 | 21.0 |
| 50.0 | 48.5 | 42.0 | 29.0 |
| 50.0 | | 40.0 | |
| | | 39.0 | |
| | | 41.0 | |
| | | 41.0 | |

## 5.22 EFFECTS OF ACH, HISTAMINE, ADRENALINE AND HIE$^{A}$ ON THE ISOLATED FROG HEART PREPARATION.

| Dose ( mg/ml) | Acetylcholine | | Histamine | | Adrenaline | | *HIE$^{A}$* | |
|---|---|---|---|---|---|---|---|---|
| 0.001 | 88.89 | 100.00 | 83.33 | 100.00 | 52.1 | 43.1 | 100.00 | 100.00 |
| 0.002 | 66.66 | 88.89 | 66.67 | 58.35 | 63.0 | 64.1 | 85.70 | 64.70 |
| 0.004 | 44.44 | 44.44 | 50.01 | 41.68 | 73.5 | 84.0 | 71.50 | 58.80 |
| 0.008 | 33.33 | 22.22 | 33.34 | 33.34 | 72.5 | 88.2 | 64.35 | 47.04 |
| 0.016 | 22.22 | 11.11 | 16.67 | 16.67 | 84.0 | 94.5 | 71.50 | 41.16 |
| 0.032 | 11.11 | 0.00 | 16.67 | 8.34 | 100.0 | 98.7 | 57.20 | 35.28 |
| | | | | | | | 42.90 | 29.40 |
| | | | | | | | 14.30 | 11.76 |

## 5.23 EFFECT OF HIE$^{A}$ ON THE RATE OF HEART BEAT OF THE ISOLATED FROG HEART.

| Dose (mg/ml) | % Decrease in Frog heart beat | | |
|---|---|---|---|
| 2. | 100.00 | 100.00 | 100.00 |
| 4. | 90.47 | 86.36 | 75.00 |
| 8. | 71.42 | 72.27 | 70.83 |
| 16. | 57.14 | 68.18 | 63.63 |
| 32. | 42.85 | 45.45 | 54.54 |
| 64. | 33.33 | 36.36 | 45.45 |
| 128. | 23.80 | 27.27 | 31.81 |
| 256. | 14.28 | 18.18 | 13.63 |

## 5.24 EFFECT OF HIE$^{A}$ ON THE FORCE OF CONTRACTION OF THE ISOLATED FROG HEART.

| Dose (mg/ml) | % Decrease in Force of Contrac | | |
|---|---|---|---|
| 2. | 100.00 | 100.00 | 100.00 |
| 4. | 94.73 | 89.47 | 70.00 |
| 8. | 86.84 | 84.21 | 65.00 |
| 16. | 84.21 | 73.68 | 50.00 |
| 32. | 81.57 | 68.42 | 35.00 |
| 64. | 68.42 | 57.89 | 25.00 |
| 128. | 73.68 | 47.36 | 20.00 |
| 256. | 5.26 | 10.52 | 5.00 |

## 5.25 EFFECT OF ATROPINE ON THE RESPONSES OF THE ISOLATED FROG HEART TO ACH.

| ACh( 4 x $10^{-6}$ mg/ml) | Atropine( 5 x $10^{-6}$ mg/ml) + ACh | Atropine (8 x $10^{-6}$ mg/ml) + ACh |
|---|---|---|
| 100. | 90.90 | 80.0 |
| 95. | 85.70 | 81.9 |

## 5.26 EFFECT OF ATROPINE ON THE RESPONSES OF THE ISOLATED FROG HEART TO $HIE^A$.

| $HIE^A$ (50 mg/ml) | Atropine + $HIE^A$ | Atropine + $HIE^A$ |
|---|---|---|
| 100. | 99.00 | 90.9 |
| 99. | 98.00 | 85.7 |

## 5.27 EFFECT OF $HIE^A$ ON THE CORONARY FLOW RATE OF THE PERFUSED RABBIT HEART.

| Dose (mg/ml) | flow rate / 30 seconds | |
|---|---|---|
| 0.8 | 97.50 | 98.7 |
| 1.6 | 94.70 | 87.5 |
| 3.2 | 81.60 | 75.0 |
| 6.4 | 57.89 | 50.0 |
| 12.8 | 42.08 | 40.0 |
| 25.6 | 31.56 | 25.0 |

## 5.28 DEPRESSOR EFFECT OF HIE$^{A}$ ON THE ANAESTHETIZED CAT.

| Dose (mg/ml) | Depressor effect | | |
|---|---|---|---|
| 0.3 | 100.00 | 88.46 | 100.00 |
| 0.9 | 90.91 | 100.00 | 100.00 |
| 3.0 | 83.64 | 86.54 | 94.00 |
| 9.0 | 58.18 | 61.53 | 62.00 |
| 30.0 | 63.63 | 57.69 | 60.00 |
| 90.0 | 36.36 | 44.23 | 46.00 |

## 5.29 ANTAGONISM OF THE EFFECTS OF ACH, AND HIE$^{A}$ ON THE ANAESTHETIZED CAT.

| Acetylcholine alone | Atropine + acetylcholine | HIE$^{A}$ alone | Atropine + HIE$^{A}$ |
|---|---|---|---|
| 45. | 15. | 0.8 | 0.8 |
| 43. | 13. | 0.6 | 0.6 |
| | | 1.0 | |