Pharmacological Evaluation of an Ayurvedic Preparation, Icchabhedi Rasa

DEPARTMRNT OF PHARMACY

A research report submitted in partial fulfillment of the requirements

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CERTIFICATION

This is to certify that, the thesis "Pharmacological Evaluation of an Ayurvedic Preparation, Icchabhedi Rasa" submitted to the Department of Pharmacy, East West University, Mohakhali, Dhaka, in partial fulfillment of the requirements for the Degree of Bachelor of Pharmacy (B. Pharm) was carried out by Mehnaz Islam Ferdousi (ID # 2006-1-70-043) under our guidance and supervision and that no part of the thesis has been submitted for any other degree. We further certify that all the sources of information and other facilities availed of in this connection is duly acknowledged.

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ABSTRACT

Purpose – The present study was undertaken to explore the spectrum of pharmacological aspects of a marketed Ayurvedic preparation, Icchabhedi Rasa, by utilising experimental animals (*Swiss albino).*

Method – The pharmacological action of Icchabhedi Rasa was evaluated using five previously established animal models. Three aspects of the drug were studied: gastrointestinal effect/side effect, analgesic and anti-inflammatory effect and neuropharmacological effect/side effect. The experiments were carried out at different doses – 100 mg/kg, 200 mg/kg, and 400 mg/kg of the animal's body weight.

Result – At dose 100 mg/kg, Icchabhedi Rasa was found to increase gastrointestinal motility in 2nd hour 15 minutes study which is significant ($p = 0.046^*$). At the same dose, it exerted significant ($p = 0.022^*$) analgesic action in the first phase of formalin induced paw licking test. In acetic acid induced writhing test at dose 100 mg/kg, writhing response decreased significantly ($p = 0.022^*$) in the first minute of the five minutes study. In open field test, at dose 200 mg/kg, Icchabhedi Rasa significantly increased ambulatory activity at two time intervals – minute 60 ($p = 0.017^*$) and minute 240 ($p = 0.039^*$). Also, at the same dose, there was a significant increase in standing up response at two time intervals – minute 30 ($p = 0.044^*$) and minute 240 ($p = 0.040^*$).

Conclusion – The results obtained show that Icchabhedi Rasa can increase gastric peristalsis and may be useful as a purgative. It may also possess some analgesic property. At higher doses, it may induce anxiogenic effects. However, further investigations must be carried out to confirm these results and hence establish a complete safety profile for Icchabhedi Rasa.

Keywords – Ayurvedic medicine, Icchabhedi Rasa (ICB), constipation, pharmacological aspects, animal models.

Chapter One

Introduction

1.1. The Ayurvedic System of Medicine

Ayurveda, the "science of life", or longevity, is a system of traditional medicine native to the Indian Subcontinent and practiced in other parts of the world as a form of alternative medicine.[3] It is believed to be the oldest healing science or system of medicine in existence dealing with both the preventive and curative aspects of life in a most comprehensive way.[1,5] The origin of Ayurveda has been lost in prehistoric antiquity, but its concepts and approaches have been perfected between 2500 and 500 B.C. This system of using natural resources for betterment of health was developed through the experimentation and experiences of day-to-day lifestyle of people.[2]

Ayurveda is a holistic system of health care which aims to integrate and balance the body, mind, and spirit leading to good health and happiness.[4] According to the Ayurvedic concepts, good health is, in turn, based on the equilibrium of *dosha* (humour), *agni* (digestive fire), *dhatu* (seven body tissues: lymph, blood, muscle, adipose tissue, bone, bone marrow, semen), and *mala* (feces, urine, and other waste products).[43] It is believed that any imbalance or disturbance in these basic body principles will cause disease. In Ayurveda, the disease treatment is employed to regain the harmony and balance of basic elements and functional principles of the body.[2] This is done by cleansing the body of substances that can cause disease and strengthening the body's defense mechanism (by various herbal formulae, lifestyle changes, and diet) so that the body will resist a disease with the goal of eliminating it.[4,10] Thus, Ayurveda is based on theories of health maintenance and illness and on ways to prevent, manage, or treat health problems.[4] In contrast, conventional medicine is primarily oriented toward the treatment of disease only; here, drugs are developed based on the concept that the elimination of specific causes of a disease, such as microorganisms, will cure a disease.[10]

Ayurveda relates humankind to nature.[2] That is why, Ayurvedic formulations and dosage forms are derived from natural resources only. These formulations are generally multi-component mixtures, containing plant and animal-derived products, minerals and metals, where each component has a wide diversity in its therapeutic potentials. However, Ayurvedic medicines are mostly dominated by plant-derived products including oils and common spices.[2,4] Currently, more than 600 herbal formulae and 250 single plant drugs are included in the "pharmacy" of Ayurvedic treatments.[4]

1.2 Present Status

Since its origin about 5000 years ago, Ayurveda has been practiced extensively and successfully in South Asia. It remains the main medical system in India as well as in Pakistan, Sri Lanka, Nepal, Tibet, and Bangladesh.[6]

The holistic approach of Ayurvedic medicine is consistent with the World Health Organisation's (WHO) definition of 'health' – a state of complete physical, mental, and social well-being and not merely an absence of disease or infirmity.[7,43] As a result, the use of traditional herbal medicines is increasing gradually in the developed and industrialized countries, in connection with disease prevention and the maintenance of health.[7] For instance, in the United States Ayurvedic medicine is considered complementary and alternative medicine (CAM) and more than 200,000 adults in the U.S. used Ayurveda in 2006, according to the National Center for Complementary and Alternative Medicine.[4]

WHO estimates that one-third of the world's population has no regular access to essential modern medicines; in some parts of Africa, Asia, and Latin America, as much as half of the population faces these persistent shortages. However, in these same situations, the rich resources of traditional remedies and practitioners are available and accessible. In addition,

traditional medicines are available at a relatively low cost compared to most essential modern medicines.[7]

In this context, the use of Ayurvedic medicines is considered cost-effective in developing countries like Bangladesh where, despite reasonable economic growth, about half of the population still lives in poverty and about a third in extreme poverty.[9] Hence, it is hard and sometimes unfeasible for the poor people to buy expensive synthetic drugs. Moreover, at present many Ayurvedic products of different manufacturers are available in the market for various types of diseases. Hence, the accessibility and, more importantly, affordability of Ayurvedic medicines make this traditional system of medicine very popular in Bangladesh, especially in rural areas where the average poverty level is 53% (as compared with 37% in urban areas).[7,9,45]

In line with increased national and international demand of Ayurvedic medicines, the safety, efficacy, and quality of the products used have become important concerns for both health authorities and the public.[7] Primary concern relates to the safety of the formulation because herbal medicines are perceived as "safe", although in reality there are potential risks, such as side effects, in use of all medicines.[7] Therefore, it is of utmost importance to characterize and establish the pharmacological and safety profiles of Ayurvedic drugs.

Keeping in mind the present scenario, this research work on an Ayurvedic formulation known as Icchabhedi Rasa (ICB) explores a spectrum of its pharmacological aspects utilising experimental animals.

1.3 Icchabhedi Rasa

Rasa-yoga or simply rasa are medicinal preparations described in Ayurveda that contain mineral drugs as their main ingredients, in the form of powder or pills. One example of such dosage form is Icchabhedi Rasa.[8]

Icchabhedi Rasa (ICB) is included in the Bangladesh National Formulary of Ayurvedic Medicine 1992 (Approved by the Government of Bangladesh vide Ministry of Health and Family Welfare Memo No. Health-1/Unani-2/89/(Part-1) 116 dated 03 June 1991). Bangladesh National Formulary of Ayurvedic Medicine is compiled by the National Unani and Ayurvedic Formulary Committee and published by the Bangladesh Board of Unani and Ayurvedic Systems of Medicine, 38, Bangabandhu Avenue, Dhaka-1000 under the authority vested in the Board vide section 13(j) of the Bangladesh Unani and Ayurvedic practitioners Ordinance, 1983 in collaboration with the World Health Organisation (WHO). Directorate of Drug Administration (DA) has issued Notification, DA/Admin/1-10/96/6212 dated 19 October 1996, and license under Drug Act, 1940 and Rules there under and Drug (Control) Ordinance 1982 for local manufacture and sale in Bangladesh (published in Bangladesh Gazette # 24, Part VI, dated Thursday, 11 June 1998). At present, a good number of Ayurvedic manufacturers are manufacturing and marketing this classical Ayurvedic medicinal preparation.

1.3.1 Ingredients

Like any typical Ayurvedic preparation, Icchabhedi Rasa is a multi-component mixture consisting of several plant extracts, minerals, and metals. Each of the components is discussed in the following section:

1.3.1.1 *Sunthi* –

Botanical Name: *Zingiber officinale* - *Rhizoma* (Zingiberaceae)

Sangskrit: Sunthi (dry), Adrakha (fresh)

Common Name: Ginger

Constituents: The active ingredients in ginger are thought to reside in its volatile oils, which comprise approximately 1-3% of its weight. These include zingiberene, zingerone, zingiberol, bisapolene, camphene, borneol, phellandrene, citral, etc. The pungent principles are gingerol and shogaols both of which are phenolic compounds.[13,14]

Commonly used part(s): Bulb[11]

Therapeutic Uses: Carminative, coolant; abdominal disorders such as indigestion, flatulence, disorders of gallbladder, hyperacidity; respiratory disorders such as cold and cough; joint disorders like arthritis; and sexual diseases.[11,12]

1.3.1.2 *Marica* –

Botanical Name: *Piper nigrum – Fructus* (Piperacea)

Sangskrit: Marich, Marica.

Common Name: Black pepper

Constituents: Piperine is the active principle of black pepper. This is also the principal alkaloid of these plants. The piperine content is about 3 to 9 % (on dry weight basis). Other alkaloids present are piperidine, piperanine, piperettine and chavicine. These are responsible for the pungent taste that black pepper possesses. It contains 1 to 2.3 % essential oils such as sabinene, camphene, limonene, myrcene, and piperonal; fixed oil and chromium.[15,16,17]

Commonly used part(s): Fruit[18]

Therapeutic Uses: Carminative; gastrointestinal disorders including dyspepsia, flatulence, constipation, hemorrhoids, low appetite (increases saliva and bile secretion), sluggish digestion, and abdominal pain; intermittent fevers; and respiratory disorders such as cold with productive cough, asthma, bronchitis, pneumonia and sore throats.[16,17,18]

1.3.1.3 *Rasa (parada)* –

Common Name: Mercury, quick silver

Sangskrit: Rasa, parada, rasendra

Uses: Mercury is the most important metal used in Ayurveda both for *deha siddhi* (maintenance as well as promotion of positive health and prevention as well as cure of obstinate and otherwise incurable diseases) and *lauha siddhi* (alchemy or transmutation of ordinary metals into noble metals like gold and silver). It is of course poisonous to the human body in is normal form. Therefore, before the metal is administered to a patient, it is made digestible, assimilable and acceptable to the tissues by making it free of the defects. This is carried out by the process of *sodhana* or purification. During this process, the metal acquires a different property which is useful therapeutically and overcomes the original harmful effects.[19]

Mercury serves to heighten the medicinal properties of anything with which it is compounded. It nourishes all the vital parts of the body. It is claimed to cure all sorts of diseases, especially leprosy.[20]

1.3.1.4 *Gandhaka* –

Common Name: Sulphur

Sangskrit: Gandhaka, rasa gandhaka

Uses: Sulphur is of four different kinds, according to its colour. Of these, the yellow variety is best suited to the requirements of mercury and of medicines prepared with a view to cure physical decay and senility.[21] It is pungent in taste, hot in potency and sweet after digestion and during the process of metabolism. It is a rejuvenating agent, carminative, stimulant of digestion and aphrodisiac. It enhances the properties of drugs to which it is added. Sulphur is used on the treatment of various kinds of skin diseases (like scabies, eczema, leprosy, and ringworm) and respiratory diseases (bronchitis, asthma, and tuberculosis).[19]

Sulphur contains two foreign matters - particles of stone and poison. It is, therefore, to be purified very carefully before use. If used without purification, sulphur causes burning sensation in the body, giddiness and vitiation of blood.[19,21]

1.3.1.5 *Tankana* –

Common Name: Borax

Sangskrit: Tankana, tanka

Uses: *Tankana* is generally available in the banks of lakes containing saline water. Therefore, it should be cleaned of physical impurities like sand, mud, stone, or pieces of wood before use. It is pungent in taste, hot in potency, ununctuous, laxative and expectorant. Borax is also useful in the treatment of amenorrhoea, nervous disorders, chronic fever, chronic bronchitis and asthma.[19]

1.3.1.6 *Jaipala* –

Botanical Name: Croton tiglium (Euphorbiaceae)

Sangskrit: Jayapala, dandibeeja, mukula

Common Name: Croton, purging croton

Constituents: Croton seeds contain 30 to 45 % stable oil that has about 1 to 3 % poisonous alkaloids and glycerides of stearic, palmitic, myristic, lauric and oleic acids. Glycerine ethers and some volatile acids like formic, acetic, isobutyric and isovalleric acids are also present. The active part of croton is crotonic acid that is freely soluble in alcohol.[22]

Commonly used part(s): Seeds

Therapeutic Uses: Chronic constipation (It is a good drastic purgative and acts especially upon the mucous lining of the intestinal tract, producing a transudation of the watery portions of the blood, causing copious watery diarrhea); indigestion; poisoning; worms; inflammation; eczema.[22,23,24,25]

In larger dose or without purification, croton is toxic.[24]

1.3.2 Formulation of Icchabhedi Rasa

The formulation[26] of Icchabhedi Rasa is given in the following table : -

Table 1.1: **Formulation of Icchabhedi Rasa (ICB)**

20:5 ICCHABHEDI RASA (Bhaisajyaratnavali, Udararogadhikara; 84)	
Ingredients	*Amount*
Sunthi (rhizome)	1 part
Marica (fruit)	1 part
Rasa (parada) – suddha	1 part
Gandhaka – suddha	1 part
Tankana – suddha	1 part
Jaipala – suddha	3 parts

1.3.2.1 Method of Preparation

Rasa (mercury), *gandhaka* (sulphur), *tankana* (borax) and *jaipala* (croton seeds) must be used in the purified form. First, a *kajjali* (amalgum) is prepared with rasa and gandhaka. Then other drugs are added in small quantities at a time and ground in the *khalva* (stone mortar) and mixed well.[26]

Commercially, the powder is then dispensed as tablets or pills. It is administered orally and the usual dose is 120-240 mg to be taken on an empty stomach in early morning with the *anupana* (adjuvant) water.[26,44]

1.4 Indication(s) of Icchabhedi Rasa

ICB is a drastic purgative and is primarily indicated for *anaha* meaning constipation. It is a common disorder, often frustrating and difficult to manage especially in the elderly. Women suffer from constipation more often than men.[27]

1.4.1 Definition

Constipation can be defined as the infrequent and difficult passage of stools. The frequency of normal bowel movements among healthy people varies greatly, ranging from three movements per day to three times per week. As a rule, if more than three days pass without a bowel movement, the intestinal contents may harden and dry out, and a person may have difficulty or even pain during elimination. Sometimes stools may harden and be painful to pass even after shorter intervals between bowel movements. [27,44]

1.4.2 Ayurvedic Concept and Definition

Most people develop constipation after years of either an imbalanced lifestyle (e.g. lack of exercise) or diet (e.g. insufficient dietary fibre, inadequate fluid intake). [27] In general, there are some contributing factors. The most common is the suppression of natural urges resulting in the subsequent disappearance of the normal eliminative urges. This habitual suppression can lead to a kind of psychosocial form of constipation whereby elimination may be regular but not complete resulting in a constipated colon. [44]

According to Ayurveda, normal stool has a definite consistency and hardness, which is called well-formed stool. During digestion, food undergoes an acidic state in which it is in semi-

liquid form in the small intestines. As it travels further in the large intestine, this digested food (called *ahara rasa*) is absorbed and the waste is left in the large intestine. The caecum, the first portion of this part of the gastrointestinal tract, has the dominance of *vata* (movement / propulsion) and *pitta* (digestive fire) *doshas* that helps in drying of waste and giving it a form. When these *doshas* are in a pathological state, it results in excess drying of the faecal matter. This stool becomes dry and hard and is expelled in small quantities with difficulty. In such situations, if an individual holds the urge to defecate for various reasons, there will be a possibility of collection of faecal matter that becomes very hard; this is a result of too much absorption of water from the stool. In many diseases, constipation occurs as a symptom. [44]

In Ayurveda, constipation is called *vibandha*. The word is derived from "vi" prefixed to "bandh," meaning that which is especially bound (in the intestine) or obstructed. Its synonyms are *purishasanga*, *purishanaha* (both meaning the accumulation of faeces), *vishtambha* (obstruction), *kricchravitka*, *alpavitka* (both meaning the passing of a small quantity of stool), and *anaha* (bloated abdomen due to accumulation of stools). [26,44]

1.4.3 Clinical Description

The clinical features[44] of the disease as described in conventional medicine are when a person experiences the following:

- Passes a hard stool fewer than three times per week
- Strains more than one of four times
- Has abdominal bloating or discomfort

Ayurvedic literature[44] describes the clinical features of the disease as the condition that is manifested with the following signs and symptoms:

- Pain in the abdomen (left hypochondriac, iliac, and umbilical regions)

- Reduced bowel movement
- Painful defaecation
- Dry stools
- Indigestion
- Headache
- Churning pain in rectum
- Pain in the sacral region

1.4.4 Aetiology

According to Ayurveda, constipation is mainly caused by aggravation of *vata*, though it can sometimes be caused by aggravation of *pitta* and *kapha doshas*. The following are some of the most common causes of constipation, according to conventional medicine: [27,44]

✓ Improper diet — The most common cause of constipation may be a diet high in animal fats and refined sugar but low in fiber found in vegetables, fruits, and whole grains.

✓ Inadequate fluid intake — Liquids like water and juice add fluid to the colon and bulk to stools, making bowel movements softer and easier to pass. People who have problems with constipation should drink enough of these liquids every day, about eight 8-oz glasses. Other liquids that contain caffeine (e.g., coffee and cola) seem to have a dehydrating effect.

✓ Lack of exercise

✓ Changes in life or routine — During pregnancy, women may be constipated because of hormonal changes or because the heavy uterus compresses the intestine. Aging may also affect bowel regularity, because a slower metabolism results in less intestinal activity and muscle tone.

✓ Ignoring the urge to have a bowel movement — People who ignore the urge to have a bowel movement may eventually stop feeling the urge, which can lead to constipation.

✓ Laxative abuse — People who habitually take laxatives become dependent upon them and may require increasing dosages until the intestine becomes insensitive and fails to work properly.

✓ Travel — People often experience constipation when traveling long distances, which may relate to changes in lifestyle, schedule, diet, and drinking water.

✓ Fissures and hemorrhoids — Painful conditions of the anus can produce a spasm of the anal sphincter muscle, which can delay a bowel movement.

✓ Specific diseases — Diseases that cause constipation are neurological disorders metabolic and endocrine disorders, and systemic conditions that affect organ systems. These disorders can slow the movement of stool through the colon, rectum, or anus.

✓ Mechanical compression — Scarring, inflammation around diverticula, tumors, and cancer can produce mechanical compression of the intestine and result in constipation.

✓ Irritable bowel syndrome (IBS) — Also known as spastic colon, IBS is one of the most common causes of constipation. Some people develop spasms of the colon that delay the speed with which the contents of the intestine move through the digestive tract, leading to constipation.

✓ Nerve damage — Injuries to the spinal cord and tumors pressing on the spinal cord can produce constipation by affecting the nerves that lead to the intestine.

✓ Medications — Many medications can cause constipation. These include pain medications (especially narcotics), antacids containing aluminum, antispasmodic drugs, antidepressant drugs, tranquilizers, iron supplements, anticonvulsants for epilepsy, antiparkinsonism drugs, and antihypertensive calcium channel blockers.

✓ Problems with colon and rectum — The peristaltic activity of the intestine may be ineffective and result in colonic inertia or outlet obstruction. Intestinal obstruction, scar tissue

(adhesions), diverticulosis, tumors, colorectal stricture, Hirschsprung's disease, or cancer can compress, squeeze, or narrow the intestine and rectum and cause constipation.

1.4.5 Pathogenesis and Pathology

Normally muscle contractions propel the waste products of digestion through the intestines. In the large intestine, reabsorption of up to 90% of the water and salt takes place because they are essential for many of our body's functions. If too much water is absorbed or if the waste moves too slowly, one may become constipated. [44]

According to Ayurveda, the *apana vata* affected (due to various etiological factors) dries up the stools, which obstructs the bowel movements and results in constipation. In short, the pathology of the disease is described as a result of the obstruction or reduced motility in the large intestine, the part of the excretory system, and due to the pathological changes in *apana vata* involving the faecal matter. [44]

1.4.6 Clinical Features

The Ayurvedic literature describes two types of constipation – [44]

- Constipation due to *ama* (*amaja anaha*) — Constipation caused by *ama* presents with the following symptoms: thirst, burning sensation in the head, pain in the abdomen, and suppression of eructation and coryza.
- Constipation due to faeces (*purishaja anaha*) — In this type of constipation, retention of faeces and urine, acute abdominal pain, and fainting are seen. Vomiting of undigested material and pedal oedema may also occur in severe cases.

Constipation can also be classified in the following way based on *dosha* dominance in a constitution – [44]

- ***Vata* Constipation** – In Ayurveda, excretory process is controlled by *vata*, the principle that governs all kinds of movement in the body. The particular subdosha of *vata* involved in constipation is called *apana vata* which controls the movements in the pelvis and elimination and reproduction. Typically, when *apana vata* gets out of balance, it will first cause dryness in the colon where the stool can become hard and impacted.
- ***Pitta* Constipation** – The dominance of *pitta*, whose property is heat, causes this form of constipation. An increased heat in the colon can also dry out the colon, aggravating *apana vata* and leading to constipation.
- ***Kapha* Constipation** – When there is excess *vata* or dryness in the colon, the body will defend itself by producing more colonic mucus to combat dryness. When this happens in excess, the clogged colon with mucus causes a *kapha*-based constipation. This imbalance combined with a mucus forming diet will result in a condition that could become chronic.

1.4.7 Therapy for Constipation – Purgation Therapy (*Virecana*)

Although treatment depends on the cause, severity, and duration, in most cases non-pharmacological approaches like dietary and lifestyle changes will help relieve symptoms and help prevent constipation. [27] One of the general lines of treatment for constipation is purgation therapy.

Virecana, or purgation, is administered for cleansing of *pitta* and stools in the intestine. It cleanses both the small intestine and colon. Many herbs and other ingredients are used as laxatives or purgatives. The dose and the selection of herbs also depend on the nature of one's bowel, called *koshta* in Ayurveda. If the bowel is extremely harsh (condition termed as

krura koshtha) where it is controlled by more *vata* and *kapha*, the person fails to purge and therefore needs drastic purgatives like ICB in higher doses.[44]

ICB causes brisk and drastic purgation and is useful in the treatment of chronic constipation where the patient does not respond to the general laxatives. It is contraindicated for general use in constipation and is employed for *virecana* therapy only.[44]

Administration of ICB results in loss of huge quantity of watery stool which may not only solve the problem of constipation but will also aid loosing abdominal fluid through severe purgation. This may be helpful in other abdominal diseases (*udararoga*) particularly in ascites where fluid accumulates in the abdominal cavity.[26,28]

1.5 Prevalence of Constipation in Bangladesh

Constipation is a highly common and bothersome disorder that negatively affects patients' social and professional lives and places a great economic burden on both patients and national health services. [29] An accurate determination of the prevalence of constipation in Bangladesh is difficult owing to absence of any national statistics. However, an extrapolated statistical data given by US Census Bureau, International Data Base, 2004 shows the prevalence rate of constipation in Bangladesh is approximately about 1.6 %. This only provides a general indication and not the actual prevalence of the disease in this region. [30]

Nevertheless, the lifestyle and dietary intake trend of Bangladeshi people makes constipation one of the most frequent gastrointestinal complaints. This is more prominent in older adults due to decreased mobility and other co-morbid medical conditions. [29] In addition, most

people do not seek any medical help[44] to treat themselves due to embarrassment especially in rural areas where patients may have some unusual beliefs about their bowel habits.

1.6 Pharmacological Models

1.6.1 Gastrointestinal effects / side effects –

Gastrointestinal effect/side effect can be studied using the two commonly used experiments given below:

- *Gastrointestinal (GI) motility test* – This experiment is carried out by the method previously described by Chatterjee (1993) in order to find the effect of the drug under study on the peristaltic movement of the GI tract.[40] The GI tract is innervated by both the parasympathetic and the sympathetic fibers of the autonomic nervous system. Its peristaltic movement is myogenic in character which is mainly initiated by the local reflexes and can occur without any neural connections to the brain or the spinal cord.[31] Extrinsic nerves to the intestine appear to have only a minor role in modulating the peristaltic activity of the organ.[32]

- *Gastric emptying (GE) test* – To study the effect of an experimental drug on gastric emptying time the two techniques described previously by Martinez *et al* is performed with some minor modifications.[33]

1.6.2 Analgesic and Anti-inflammatory studies –

Two experimental models are commonly employed to carry out analgesic and/or anti-inflammatory studies:

- *Formalin induced paw licking test* – This popular experiment, formalin induced paw licking test, is a very useful method for evaluating the mechanism of pain and analgesia of an experimental drug.[34] Drugs which act mainly centrally, such as narcotic analgesics, inhibit both phases of pain in this model while peripherally acting drugs, such as aspirin and indomethacin, only inhibit the late phase.[35]

Acetic acid induced writhing test – To test the existence of non-narcotic analgesic property acetic acid induced writhing test is performed. The pain sensation or algesia is initiated by using acetic acid. The acetic acid induced writhing is inversely proportional to the non-narcotic analgesic property, if present, of the drug.[36,42]

1.6.3 Neuropharmacological effects / side effects –

Neuropharmacological effect/side effect of an experimental drug is using the open field model described by Gupta (1971). It is a widely used procedure for examining the effect of the drug under consideration on the pattern of behaviour – locomotion, exploration and anxiety.[37,38] A different and more complex environment is presented for exploration to the animal in this experiment.[39,41]

1.7 Aim of the Present Study

The traditional system of Ayurvedic medicines can thus provide a cost-effective treatment solution of preventing this commonly occurring abdominal disorder in this region to all strata of population. Hence, the importance of establishment of pharmacological and safety profiles of Icchabhedi Rasa is a demand of time and cannot be overlooked.

This research work on the marketed Ayurvedic preparation, Icchabhedi Rasa (ICB), is aimed to have a better understanding of its possible pharmacological profile by utilizing experimental animals and to some extent, to decide how reasonable the use of this drug is under the stated circumstances. The project will ultimately result in supplementing and complementing the existing health care facilities and in the long run, will ensure total coverage of the population in terms of public health.

Chapter Two

Materials and Methods

2.1 Collection of the Ayurvedic Formulation

To study the pharmacological effects of the drug utilising animal models, the commercial pack of Ichhabhedi Rasa (Batch# 003) was collected from "Sree Kundeswari Aushadhalaya Ltd.", Chittagong, Bangladesh.

2.2 Dose of Administration

The tablets were first crushed into powder in a mortar and pestle and then a solution of ICB is prepared using distilled water. This solution was administered at a volume such that it would permit optimal dosage accuracy without contributing much to the total increase in the body fluid.

To carry out the various pharmacological studies the drug was administered at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg of the body weight. The different doses used in the experiments performed are given in the following table:

Table 2.1: **Doses used in different experiments**

Serial number	*Name of the experiment*	*Dose*
01.	Gastrointestinal motility test	100 mg/kg
02.	Gastric emptying test	100 mg/kg
03.	Formalin induced paw licking test	100 mg/kg
04.	Acetic acid induced writhing test	100 mg/kg, 200 mg/kg, 400 mg/kg
05.	Open field test	100 mg/kg, 200 mg/kg

2.3 Route of Administration

For all the pharmacological studies the solution of the drug was administered to the experimental animals orally by means of a long feeding needle with a ball-shaped end.

2.4 Experimental Animals

Female mice (Swiss-Webster strain, 20-40 g body weight) bred in the animal house of the Department of Pharmacy, Jahangirnagar University, were used for the various pharmacological experiments performed in this study. They were kept in cages having dimensions of 30 x 20 x 13 cm^3. Soft wood shavings were employed as bedding in the cages.

The animals were provided with standard laboratory food and tap water 'ad libitum' and maintained at natural day night cycle. They were fed with "mouse chow" which is prepared according to the formula developed at Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. Before starting an experiment, the animals were weighed using an electronic balance (Shimadzu, Japan) and carefully marked at the base of their tails, which was later used as identification mark for a particular animal, so that the response of a particular mouse prior to and after the administration could be noted separately.

For each experiment there were two groups of mice – control group and drug treated group. Each group consisted of equal number (about six to ten depending on the experiment) of mice of the same sex.

The mice in drug group were administered ICB solution orally of a specified dose and those in the control group were administered with distilled water as par the same volume as the drug treated group.

2.5 Common Materials Employed in Different Experiments

The general materials used in all the experiments include female mice within a specific body weight range, plastic cages, distilled water, ICB solution, feeding needle, electronic balance, stopwatch, counter, etc.

2.6 Pharmacological Study with Animal Models

2.6.1 Gastrointestinal (GI) Motility Test –

Additional material(s): $BaSO_4$ (Merck, India), sodium carboxymethylcellulose (CMC) (Merck, India), dissection tools.

Method: The experiment was carried out by the method previously described by Chatterjee (1993). 128 female mice were weighed (body weight ranging from 30 to 40 g) and divided into four sets to represent the action of the drug at four different hours (1^{st}, 2^{nd}, 3^{rd}, and 4^{th} hour) with respect to drug administration.

$BaSO_4$ milk was prepared by adding $BaSO_4$ at 15% w/v in 0.5% sodium CMC suspension. In this experiment, $BaSO_4$ acts as a non-absorbable marker to measure GI motility.

$BaSO_4$ milk was given to a group of 12 mice 15 minutes after administration of the test drug (ICB). The drug treated mice were subdivided into two groups and sacrificed by cervical dislocation after 15 and 30 minutes after the administration of the milk. The distance traversed by $BaSO_4$ milk through the small intestine was measured and expressed as a percentage of the total length of small intestine (from pylorus to the ileocaeccal junction). The test drug was compared with the control group that was administered with distilled water. The experiment was performed in the same manner for all the sets of mice to study the effect of ICB on gastric peristalsis at four different hours.

2.6.2 Gastric Emptying (GE) Test –

Method: Fourty female mice were fasted for 18 hours prior to the experiment. Out of the 40, 20 were randomly chosen as the drug (ICB) group and the remaining 20 as the control group. Fasted animals had free access to water and preweighed solid food (solid : water ratio being 60:40) for a period of 1 hour. At the end of 1 hour period, the remaining food was weighed, and adjustment for spillage was taken into consideration. The difference between the initial and final food weights gives the total food intake by each mouse.

Immediately after the 1 hour feeding period, ICB was orally administered to the mice of drug group at a dose of 100 mg/kg while their control group counterparts were administered with distilled water at the same dose level.

The percentage of the gastric emptying of the ingested food was assessed 2 hours after the administration of the drug. The mice were sacrificed by cervical dislocation and the stomach removed by cutting off the cardiac and pyloric ends. The stomach was weighed in an electronic balance and opened; the gastric content was washed thoroughly with tap water and the remaining gastric wall was carefully blotted dry and weighed. The gastric content was calculated as the difference between the total weight of the stomach with contents and the weight of the gastric wall after the contents were washed out.

Percentage gastric emptying (% GE) was calculated as-

$$\%\ of\ GE = 1 - \frac{Gastric\ Content}{Total\ Food\ Intake} \times 100$$

The same procedure was repeated to study the effect of ICB after 4 hours of drug administration.

2.6.3 Formalin Induced Paw Licking Test –

Additional material(s): Glass jars, microlitre syringe (Hamilton, Switzerland), 1% formalin (BDH, England), stopwatch.

Method: 20 female mice were weighed (body weight ranging from 30 to 35 g). Out of 20, 10 were randomly chosen for the drug (ICB) group and the remaining 10 for the control group.

Algesia was induced by injecting 1% aqueous formalin solution to each mouse by the intraplantar route and immediately the paw licking time was registered for five minutes. This is the first phase of the experiment termed as neurogenic phase. Twenty minutes after the beginning of the experiment, i.e. after injecting formalin, the paw licking time was registered for another five minutes. This comprises the second or inflammatory phase of the experiment. ICB was administered orally 2 hours prior to formalin injection in the drug group. Similarly the control group was given distilled water.

2.6.4 Acetic Acid (AA) Induced Writhing Test –

Additional material(s): 0.6% aqueous acetic acid solution (COO, Germany), sterile injection syringe, counter.

Method: 20 female mice were weighed (body weight ranging from 30 to 35 g). Out of 20, 10 were randomly chosen for the drug (ICB) group and the remaining 10 for the control group.

ICB was administered orally to the mice of drug group. Fourty-five minutes interval was given to ensure proper absorption of the administered substance. In order to induce abdominal muscular contraction, each mouse was then injected 0.6% acetic acid (0.25 ml/animal) by the intraperitoneal route. It was placed in an observation box and number of

writhes (painful abdominal muscular contractions and stretches) produced was counted for five minutes after fifteen minutes of acetic acid injection.

The average number of writhes was calculated and the percent protection was calculated as –

$$\% \text{ Protection} = 100 - [(\text{treated mean/control mean}) \times 100]$$

The readings were then compared to that of the distilled water treated control group.

2.6.5 Open Field Test –

Additional material(s): Open field apparatus

Method: 12 mice were weighed (body weight ranging from 20 to 25 g) and divided into two groups: 6 for the drug (ICB) group and 6 for the control group. Each mouse was placed on one corner of the open field apparatus (a box consisting of a half square meter floor divided into a series of squares alternatively coloured black and white and enclosed by walls 40 cm in height) and four parameters were recorded for a period of two minutes: (i) the number of ambulation (expressed as the number of squares traveled); (ii) the number of times it entered the center region; (iii) the number of stand ups and (iv) the number of faecal boluses it expelled. Readings were taken at pre 30 minutes and post 30, 60, 120 and 240 minutes interval and were compared with those of the control group.

2.7 Statistical Analysis

For each experiment, data were stated as mean values of the groups ± standard error of the mean of the individual values (mean ± SEM). Unpaired t-tests were done for testing the statistical significance. SPSS (Statistical Package for Social Science) for WINDOWS™ (Ver. 12) was applied for the analysis of data.

p values were used to indicate the level of significance in the difference in response between control and drug treated groups. It determines the appropriateness of rejecting the null hypothesis in a hypothesis test. The value of p ranges from 0 to 1. Smaller the p-value, smaller is the probability of rejecting the null hypothesis. In this study,

- $p \leq 0.05$ was taken to be significant,
- $p \leq 0.01$ was taken to be highly significant, and
- $p \leq 0.001$ was taken to be very highly significant.

Chapter Three

Results and Discussion

3.1 GASTROINTESTINAL (GI) MOTILITY TEST

Statistical Findings

The pattern in the change in GI motility of ICB treated female mice at dose 100 mg/kg is depicted below:

1st hour – GI motility of the drug (ICB) group found to exhibit an increase in the 15 minutes study. In 30 minutes study, ICB was found to decrease GI motility slightly when compared to the respective control group. The results obtained were not statistically significant ($p > 0.05$).

2nd hour – GI motility of ICB treated mice was found to increase after the 15 minutes study period compared to the corresponding control group. This increase in peristaltic movement (as measured by percentage $BaSO_4$ traversed) was statistically significant ($p = 0.046^*$).

In contrast, there was a reduction in the GI motility in the drug (ICB) group after the 30 minutes study period in comparison to the control group.

3rd hour – After 15 minutes study it was observed that the gut motility of the mice in drug (ICB) group increased than those in control. On the contrary, gut motility was found to decrease in drug group than the corresponding control in 30 minutes study.

None of the results of this study period were statistically significant ($p > 0.05$).

4th hour – In contrast to the other hours, GI motility was found to decrease in the 15 minutes study followed by an increase in the 30 minutes study period in the SMB treated mice compared to the control group. The results obtained were not statistically significant ($p > 0.05$).

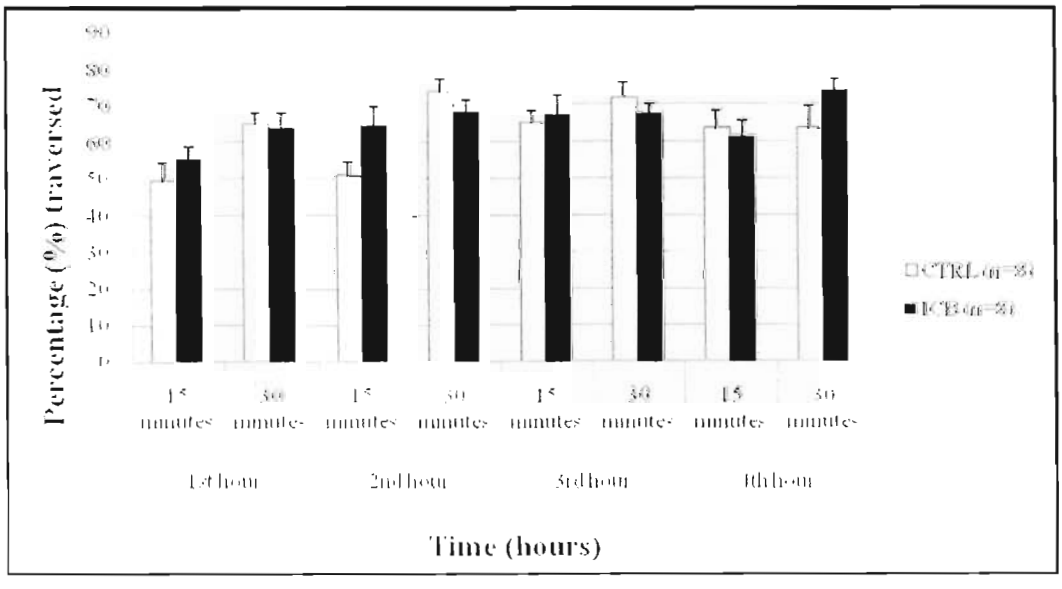
Tabular and graphical presentation of the effect of ICB (100 mg/kg) in gastrointestinal (GI) motility test using female mice

Table 3.1: **The effect of ICB (100 mg/kg) in gastrointestinal (GI) motility test**

Group		Percentage (%) Traversed							
		1st hour		*2nd hour*		*3rd hour*		*4th hour*	
		15 min	*30 min*	*15 min*	*30 min*	*15 min*	*30 min*	*15 min*	*30 min*
CTRL (n = 8)		49.506± 4.991	64.884± 3.169	50.718± 4.104	73.712± 3.525	65.041± 3.458	71.872± 4.530	63.572± 5.009	63.651± 6.498
ICB (n = 8)		55.633± 3.153	64.105± 3.760	64.812± 4.960	68.225± 3.287	67.744± 5.249	68.091± 2.528	61.928± 4.025	74.488± 2.920
t/p		-1.038/ 0.317	0.158/ 0.876	-2.189/ **0.046***	1.139/ 0.274	- 0.430/ 0.674	0.729/ 0.478	0.256/ 0.802	-1.521/ 0.150
95% confidence interval	Lower	-18.790	-9.768	-27.902	- 4.849	-16.185	-7.346	-12.137	-26.117
	Upper	6.535	11.325	- 0.2865	15.824	10.779	14.909	15.425	4.443

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.1: **The effect of ICB (100 mg/kg) in gastrointestinal (GI) motility test**



3.2 GASTRIC EMPTYING (GE) TEST

Statistical Findings

At dose 100 mg/kg, percent gastric emptying of ICB treated female mice was similar to that of the control group in the 2nd hour of the experiment. In the 4th hour, there was a negligible increase in percent gastric emptying in drug (ICB) group when compared to the corresponding control group.

However, the results were not statistically significant ($p > 0.05$).

With lapse of time the difference in percent gastric emptying between drug (ICB) and control can be summarized in a numerical form as:

- ***2nd hour***

  (77.135 - 76.460) % = 0.675 % (increase)

- ***4th hour***

  (88.305 - 83.708) % = 4.597 % (increase)

[Here (-) = Decrease and (+) = Increase]

It is noteworthy to mention that with the lapse of time gastric emptying slightly increased in the drug (ICB) group than the corresponding control group.

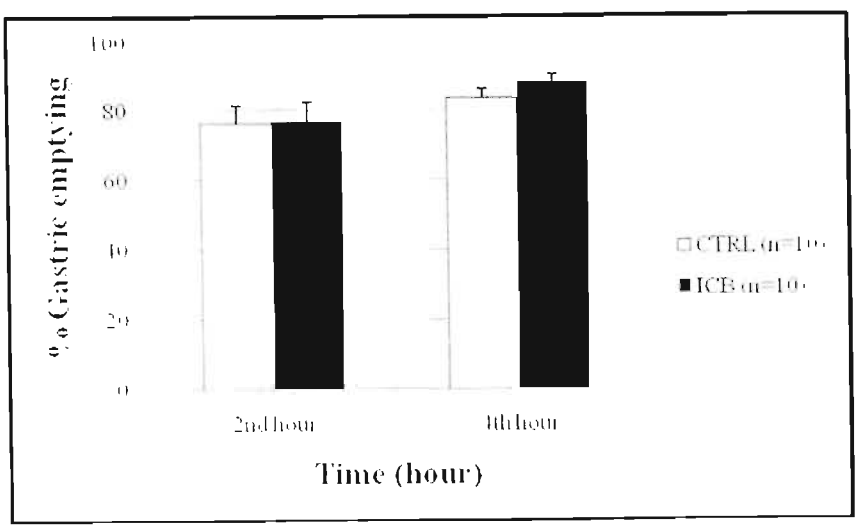
Tabular and graphical presentation of the effect of ICB (100 mg/kg) in gastric emptying (GE) test using female mice

Table 3.2: **The effect of ICB (100 mg/kg) in gastric emptying (GE) test**

Group		Percent (%) gastric emptying	
		2nd hour	4th hour
CTRL(n=10)		76.460±5.181	83.708±2.595
ICB(n=10)		77.135±5.249	88.305±2.334
t/p		-0.092/0.928	-1.317/0.204
95% confidence interval	Lower	-16.171	-11.930
	Upper	14.821	2.736

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.2: **The effect of ICB (100 mg/kg) in gastric emptying (GE) test**



3.3 FORMALIN INDUCED PAW LICKING TEST

Statistical Findings

ICB treated female mice at dose 100 mg/kg showed a decrease in mean paw licking time compared to the respective control group in the first five minutes of the experimental study period. Hence, ICB exerted analgesic activity and the result obtained was statistically significant ($p = 0.022^*$).

In contrast, the mice in drug (ICB) group exhibited an increase in mean paw licking time than the corresponding control group in the last five minutes of the experimental study period. So ICB may not exert any anti-inflammatory activity. However, the result was statistically insignificant ($p > 0.05$).

Tabular and graphical presentation of the effect of ICB (100 mg/kg) in formalin induced paw licking (analgesic + inflammation) test using female mice

Table 3.3: **The effect of ICB (100 mg/kg) in formalin induced paw licking (analgesic + inflammation) test**

Group		Mean paw licking time (seconds)	
		Analgesic (1st phase)	Inflammation (2nd phase)
CTRL(n=10)		82.600±6.964	13.000±6.347
ICB(n=10)		54.111±9.001	20.111±6.255
t/p		2.532/**0.022***	-0.795/0.438
95% confidence interval	Lower	4.747	-25.983
	Upper	52.231	11.761

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.3.1: **The effect of ICB (100 mg/kg) in the formalin induced paw licking (analgesic) Test**

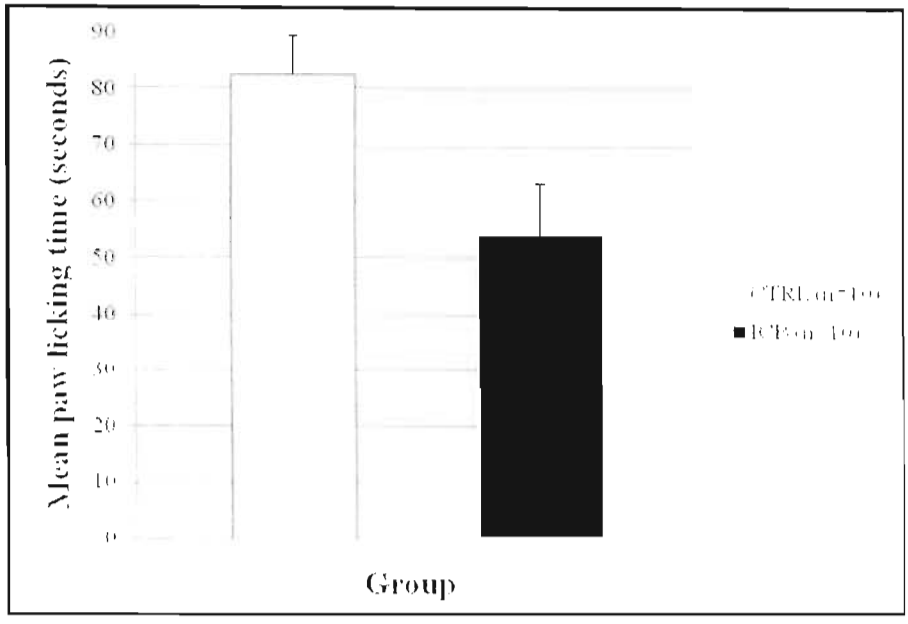
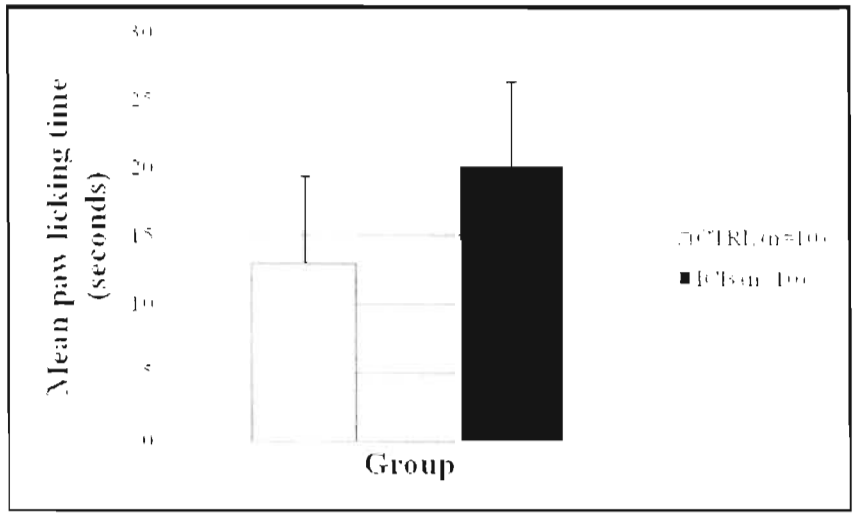


Figure 3.3.2: **The effect of ICB (100 mg/kg) in the formalin induced paw licking (inflammation) test**



3.4 ACETIC ACID (AA) INDUCED WRITHING TEST

Statistical Findings

Writhing Response:

ICB treated female mice at dose 100 mg/kg showed a decrease in writhing response from 1st to 3 rd minute and an increase in writhing response in the 4th and 5th minute when compared to the corresponding control group. The decrease in writhing response in the first minute was found to be statistically significant ($p = 0.022^*$). The overall pain perception decreased during the five minutes study period.

At dose 200 mg/kg, the mice in drug group showed an increase in writhing in 1st, 2nd, and 5th minutes whereas in 3rd and 4th minutes writhing response was less than the corresponding control group. Mean writhing in five minutes of ICB treated mice was similar to the control group. None of the results were statistically significant ($p > 0.05$).

At dose 400 mg/kg, the mice in drug group exerted an overall decrease in writhing response compared to the control group throughout the five minutes study period. However, the result was not significant statistically ($p > 0.05$).

The percentage (%) protection provided by ICB at different doses is as follows:

- 15.77 % at 100 mg/kg
- 2.05 % at 200 mg/kg
- 20.33 % at 400 mg/kg

Tabular and graphical presentation of the effect of ICB (100 mg/kg) in acetic acid (AA) induced writhing test using female mice

Table 3.4.1: **The effect of ICB (100 mg/kg) in acetic acid (AA) induced writhing test**

Group		1st min	2nd min	3rd min	4th min	5th min
CTRL(n=10)		5.714±0.680	5.429±0.481	4.429±0.369	3.429±0.369	3.714±0.421
ICB(n=10)		3.333±0.624	3.700±0.883	3.900±0.809	3.900±0.823	4.300±0.651
t/p		2.567/**0.022***	1.521/0.149	0.595/0.563	- 0.523/0.610	- 0.682/0.506
95% confidence interval	Lower	0.392	- 0.694	-1.403	-2.431	-2.416
	Upper	4.370	4.152	2.460	1.489	1.244

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.4.1: **The effect of ICB (100 mg/kg) in acetic acid (AA) induced writhing test**

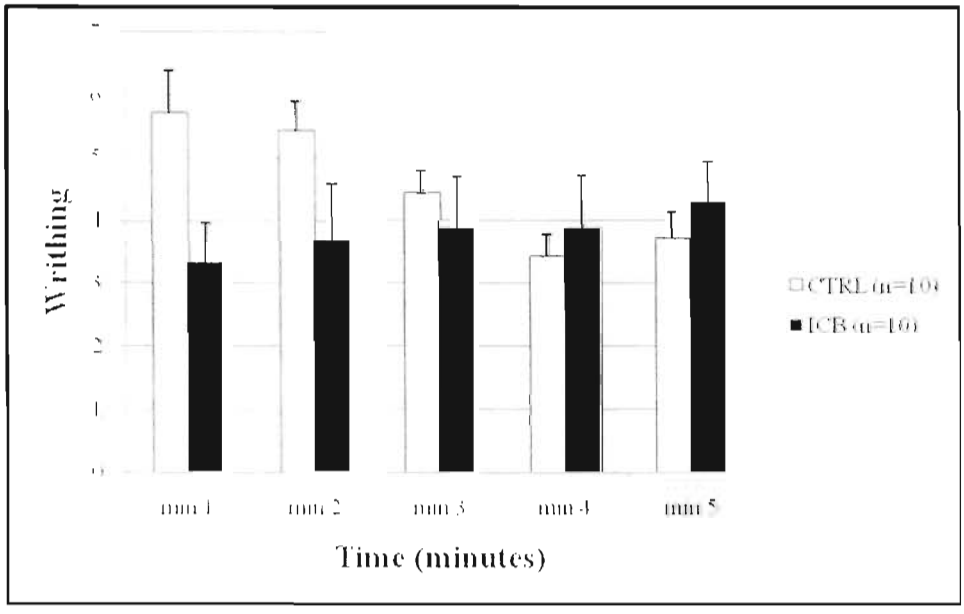
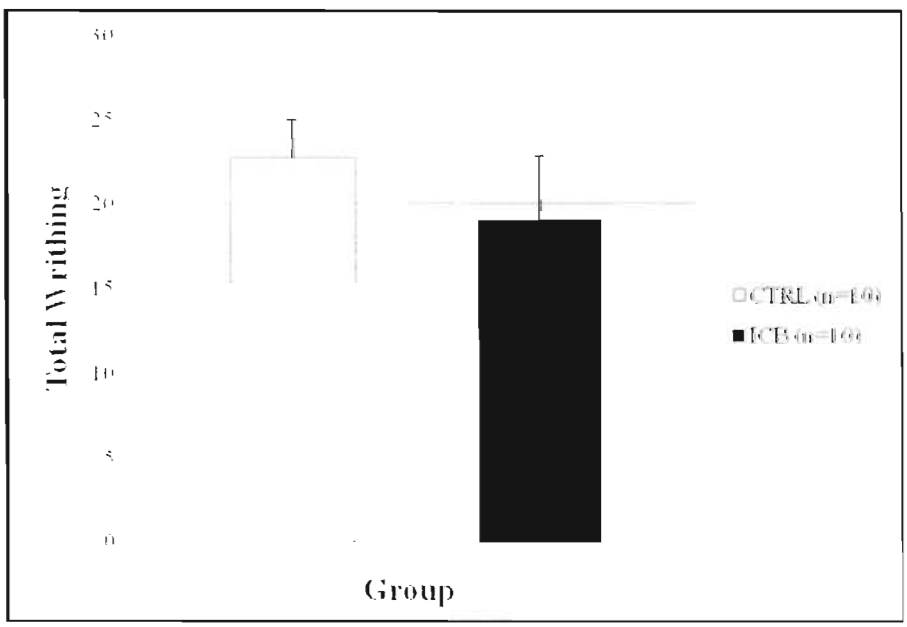


Table 3.4.2: **The effect of ICB (100mg/kg) in acetic acid (AA) induced writhing test in five minutes study period**

Group		Mean of total writhing	% Protection
CTRL(n=10)		22.715±2.320	
ICB(n=10)		19.133±3.790	
t/p		-0.591/0.562	15.77 %
95% confidence interval	Lower	-13.208	
	Upper	7.408	

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.4.2: **The effect of ICB (100mg/kg) in acetic acid (AA) induced writhing test in five minutes study period**



Tabular and graphical presentation of the effect of ICB (200 mg/kg) in acetic acid (AA) induced writhing test using female mice

Table 3.4.3: **The effect of ICB (200 mg/kg) in acetic acid (AA) induced writhing test**

Group		1st min	2nd min	3rd min	4th min	5th min
CTRL(n=10)		3.400±0.499	3.900±0.482	4.700±0.578	4.200±0.727	3.300±0.597
ICB(n=10)		4.100±0.809	4.800±0.663	3.400±0.542	2.800±0.593	4.000±0.615
t/p		-0.737/0.471	-1.098/0.287	1.641/0.118	1.492/0.153	-0.817/0.425
95% confidence interval	Lower	-2.697	-2.623	-0.365	-0.571	-2.500
	Upper	1.297	0.823	2.965	3.371	1.100

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.4.3: **The effect of ICB (200 mg/kg) in acetic acid (AA) induced writhing test**

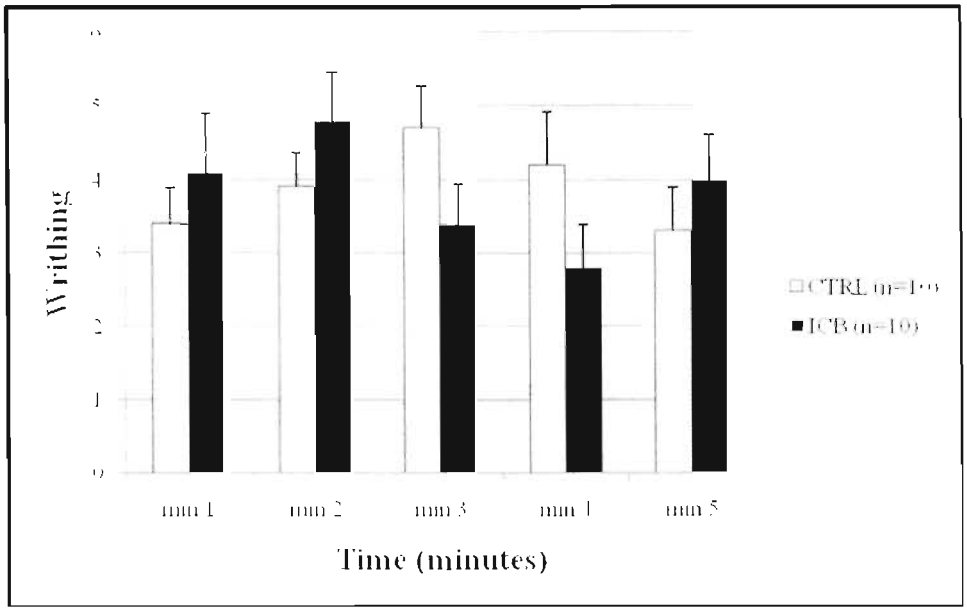
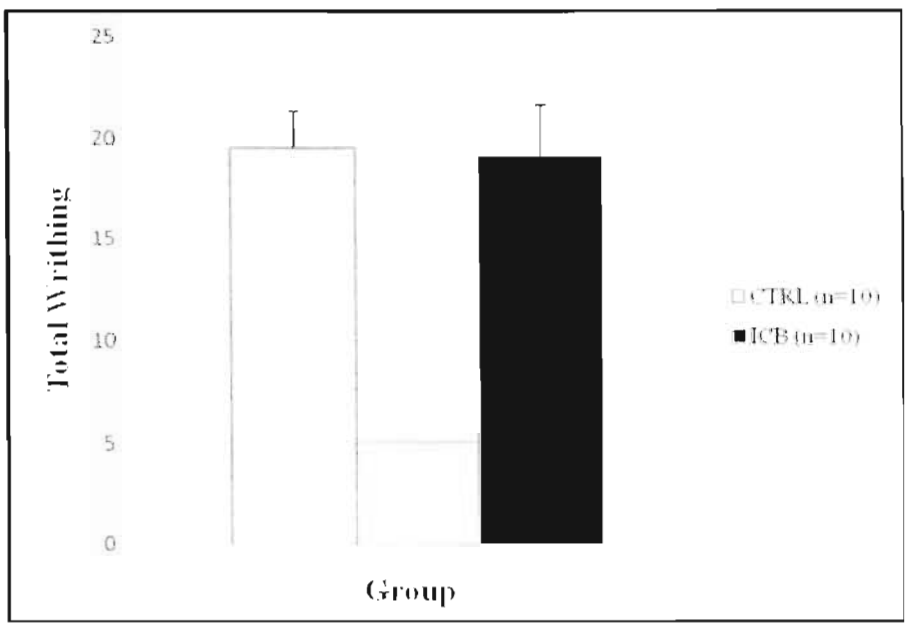


Table 3.4.4: **The effect of ICB (200mg/kg) in acetic acid (AA) induced writhing test in five minutes study period**

Group		Mean of total writhing	% Protection
CTRL(n=10)		19.500±1.821	
ICB(n=10)		19.100±2.549	
t/p		0.128/0.900	2.05 %
95% confidence interval	Lower	- 6.182	
	Upper	6.982	

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.4.4: **The effect of ICB (200mg/kg) in acetic acid (AA) induced writhing test in five minutes study period**



Tabular and graphical presentation of the effect of ICB (400 mg/kg) in acetic acid (AA) induced writhing test using female mice

Table 3.4.5: **The effect of ICB (400 mg/kg) in acetic acid (AA) induced writhing test**

Group		1st min	2nd min	3rd min	4th min	5th min
CTRL(n=10)		3.800±0.467	4.100±0.674	3.400±0.499	3.400±0.400	3.500±0.453
ICB(n=10)		2.700±0.473	3.500±0.637	2.100±0.526	3.300±0.597	2.900±0.504
t/p		1.656/0.115	0.647/0.526	1.793/0.090	0.139/0.891	0.885/0.388
95% confidence interval	Lower	-0.295	-1.348	-0.223	-1.410	-0.825
	Upper	2.495	2.548	2.823	1.610	2.025

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.4.5: **The effect of ICB (400 mg/kg) in acetic acid (AA) induced writhing test**

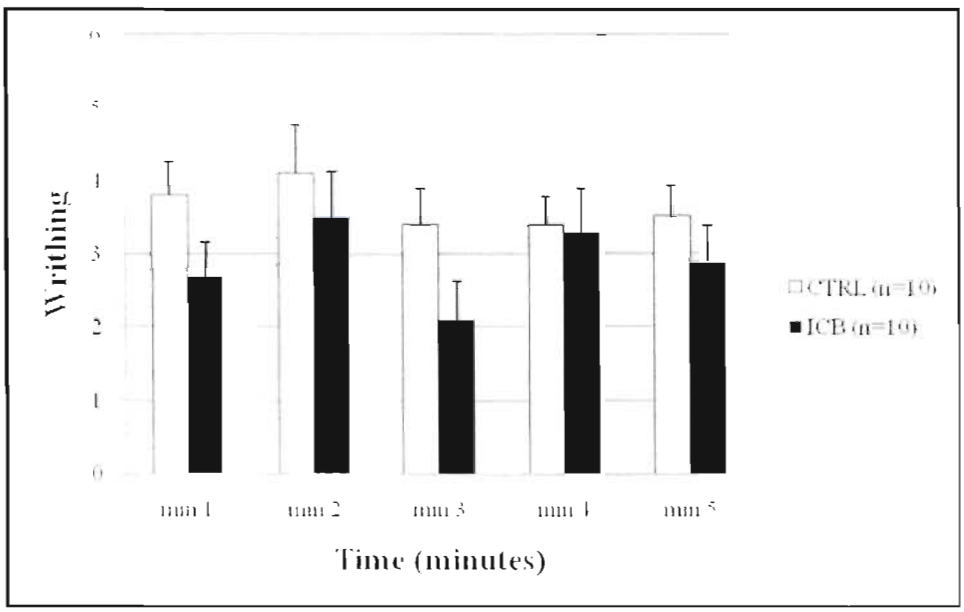
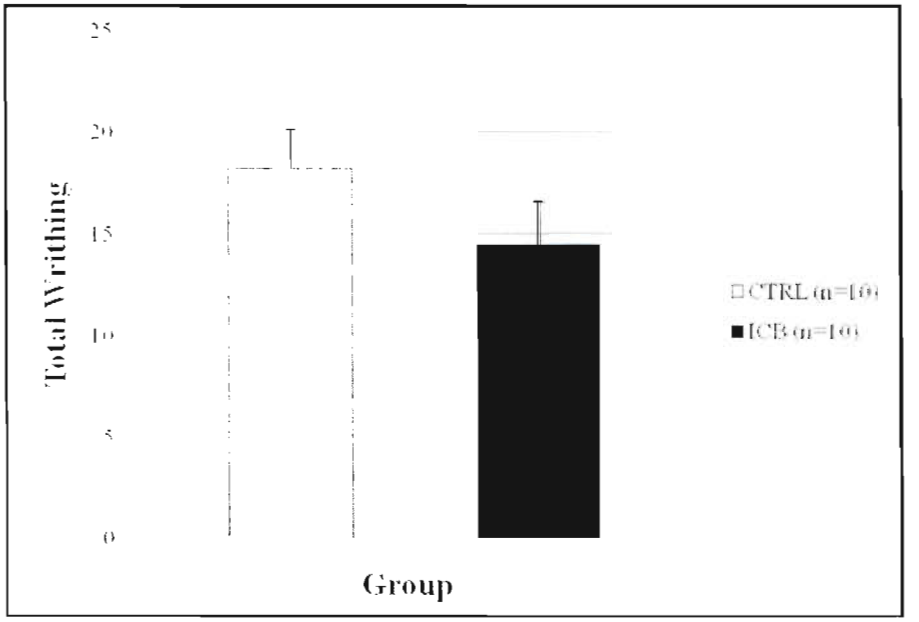


Table 3.4.6: **The effect of ICB (400mg/kg) in acetic acid (AA) induced writhing test in five minutes study period**

Group		Mean of total writhing	% Protection
CTRL(n=10)		18.200±1.931	
ICB(n=10)		14.500±2.125	
t/p		1.289/0.214	20.33 %
95% confidence interval	Lower	-2.333	
	Upper	9.733	

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.4.6: **The effect of ICB (400mg/kg) in acetic acid (AA) induced writhing test in five minutes study period**



3.5 OPEN FIELD TEST

Statistical Findings

Total Ambulation:

At dose 100 mg/kg, ICB treated female mice showed an overall increase in ambulatory activity compared to the control group at all time intervals. Nevertheless, none of the results were statistically significant ($p > 0.05$).

Similarly, at dose 200 mg/kg, ambulatory activity of female mice in drug (ICB) group was more than the corresponding control group at all time intervals. Increase in ambulation was found to be significant at two time intervals – minute 60 and minute 240 – where $p = 0.017^*$ and 0.039^* respectively.

Total standing up behaviour:

ICB treated female mice at dose 100 mg/kg exhibited an overall increase in standing up response when compared to the control group throughout the experiment.

Also, at dose 200 mg/kg there was increased standing up response in drug (ICB) treated mice than the control group at all time intervals. Increase in standing up behavior was found to be significant at two time intervals – minute 30 and minute 240 – with p values 0.044^* and 0.040^* respectively.

Total centre ambulation:

At both 100 mg/kg and 200 mg/kg doses, total centre ambulation in ICB treated mice increased than the corresponding control group at all time intervals throughout the experiment.

The results obtained were statistically not significant ($p > 0.05$).

Emotional defaecation:

At dose 100 mg/kg, defaecation in drug group was less than control at minute 30. At minute 60 defaecatory response was found to be similar in both drug and control groups. From minute 120 onwards, defaecation increased in drug treated mice. However, at minute 240 mice in drug group showed decrease faeces production than the control group.

At dose 200 mg/kg, defaecation in drug treated mice was less than the corresponding control group till minute 60. From minute 120 onwards, defaecation increased in drug treated mice than the control.

However, the results obtained were not found to be statistically significant ($p > 0.05$).

Tabular and graphical presentation of the effect of ICB (100 mg/kg) in open field test using female mice

Table 3.5.1: **The effect of ICB (100 mg/kg) in total ambulation in open field test**

Group		Min0	Min30	Min60	Min120	Min180	Min240
CTRL (n=6)		90.667±19.578	67.000±6.121	51.167±8.023	18.833±8.171	21.833±6.539	22.000±5.955
ICB (n=6)		103.833±10.025	71.333±7.566	56.000±6.393	30.833±6.279	25.000±5.756	26.500±4.972
t/p		-0.599/ 0.563	-0.445/ 0.666	-0.471/ 0.648	-1.165/ 0.271	-0.363/ 0.724	-0.580/ 0.575
95% confidence interval	Lower	- 62.176	-26.018	-27.690	-34.961	-22.578	-21.785
	Upper	35.843	17.351	18.023	10.961	16.244	12.785

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.5.1: **The effect of ICB (100 mg/kg) in Ambulation in Open Field Test**

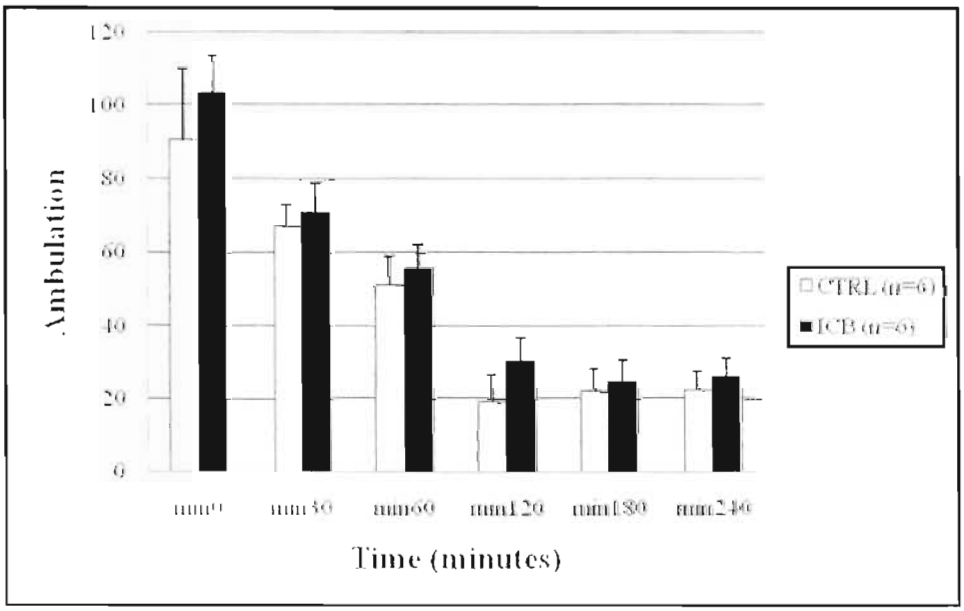


Table 3.5.2: **The effect of ICB (100 mg/kg) in total standing up behaviour in open field test**

Group		Min0	Min30	Min60	Min120	Min180	Min240
CTRL (n=6)		6.667±2.155	4.500±1.408	3.833±1.014	0.167±0.167	0.833±0.654	1.333±0.615
ICB (n=6)		7.333±1.606	7.333±1.174	7.333±1.994	1.500±0.957	2.000±1.000	2.500±1.057
t/p		-0.248/0.809	-1.545/0.153	-1.564/0.149	-1.372/0.225	- 0.976/0.352	- 0.954/0.362
95% confidence interval	Lower	- 6.655	- 6.918	- 8.485	- 3.789	- 3.829	- 3.891
	Upper	5.321	1.252	1.485	1.123	1.496	1.557

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.5.2: **The effect of ICB (100 mg/kg) in total standing up behaviour in open field test**

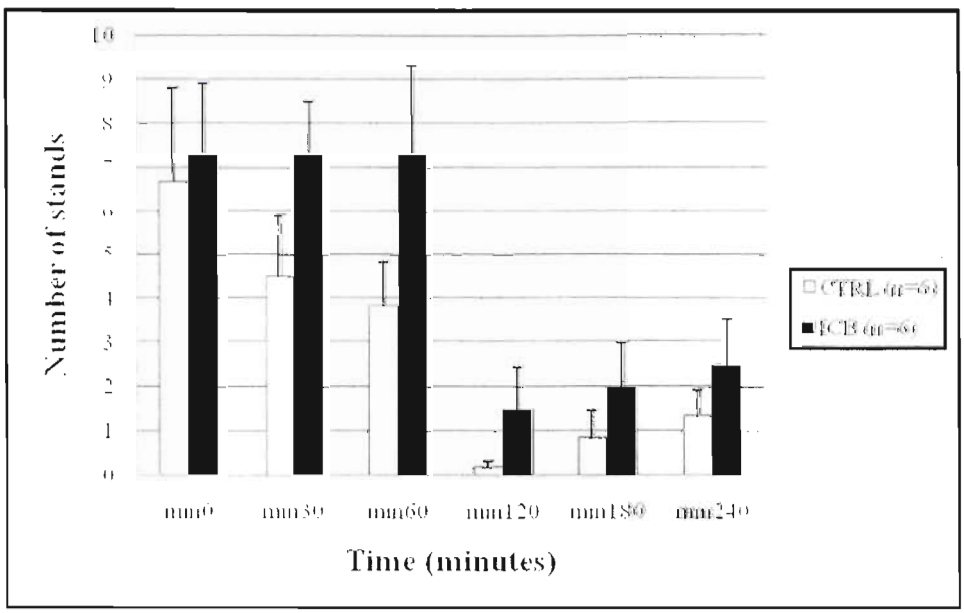


Table 3.5.3: **The effect of ICB (100 mg/kg) in total centre ambulation in open field test**

Group		Min0	Min30	Min60	Min120	Min180	Min240
CTRL(n=6)		1.000±0.516	0.167±0.167	0.167±0.167	0.000±0.000	0.000±0.000	0.000±0.000
ICB(n=6)		0.667±0.211	0.333±0.211	0.833±0.401	0.333±0.333	0.167±0.167	0.333±0.211
t/p		0.598/0.563	-0.620/0.549	-1.534/0.171	-1.000/0.363	-1.000/0.363	-1.581/0.175
95% confidence interval	Lower	-0.909	-0.765	-1.705	-1.190	-0.595	-0.875
	Upper	1.576	0.432	0.371	0.524	0.262	0.209

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.5.3: **The effect of ICB (100 mg/kg) in total centre ambulation in open field test**

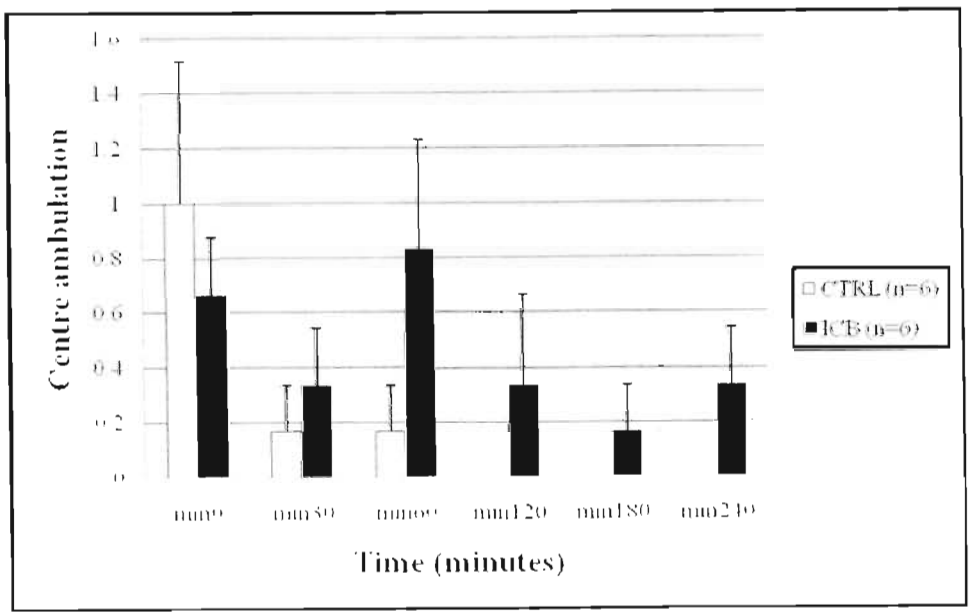
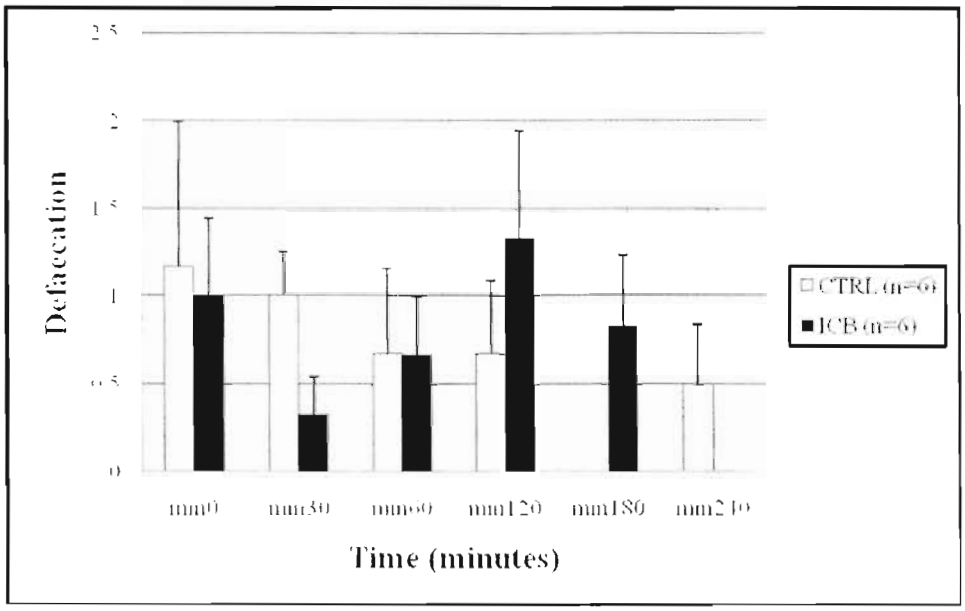


Table 3.5.4: **The effect of ICB (100 mg/kg) in emotional defaecation in open field test**

Group		Min0	Min30	Min60	Min120	Min180	Min240
CTRL(n=6)		1.167±0.833	1.000±0.258	0.667±0.494	0.667±0.422	0.000±0.000	0.500±0.342
ICB(n=6)		1.000±0.447	0.333±0.211	0.667±0.333	1.333±0.615	0.833±0.401	0.000±0.000
t/p		0.176/0.864	2.000/0.073	0.000/1.000	-0.894/0.392	-2.076/0.093	1.464/0.203
95% confidence interval	Lower	- 1.941	- 0.076	- 1.329	- 2.327	- 1.865	-0.378
	Upper	2.274	1.409	1.329	0.994	0.198	1.378

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.5.4: **The effect of ICB (100 mg/kg) in emotional defaecation in open field test**



Tabular and graphical presentation of the effect of ICB (200 mg/kg) in open field test using female mice

Table 3.5.5: **The effect of ICB (200 mg/kg) in total ambulation in open field test**

Group		Min0	Min30	Min60	Min120	Min180	Min240
CTRL (n=6)		124.833±13.159	73.000±8.783	46.500±12.449	48.000±10.841	51.667±13.781	24.667±8.409
ICB (n=6)		158.333±11.721	96.500±5.987	89.333±6.323	59.167±17.910	65.667±14.291	65.500±15.053
t/p		-1.901/0.086	-2.211/0.051	-3.068/**0.017***	-0.533/0.605	-0.705/0.497	-2.368/**0.039***
95% confidence interval	Lower	-72.765	-47.184	-75.476	-57.814	-58.236	-79.251
	Upper	5.765	0.184	-10.190	35.481	30.236	-2.415

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.5.5: **The effect of ICB (200 mg/kg) in total ambulation in open field test**

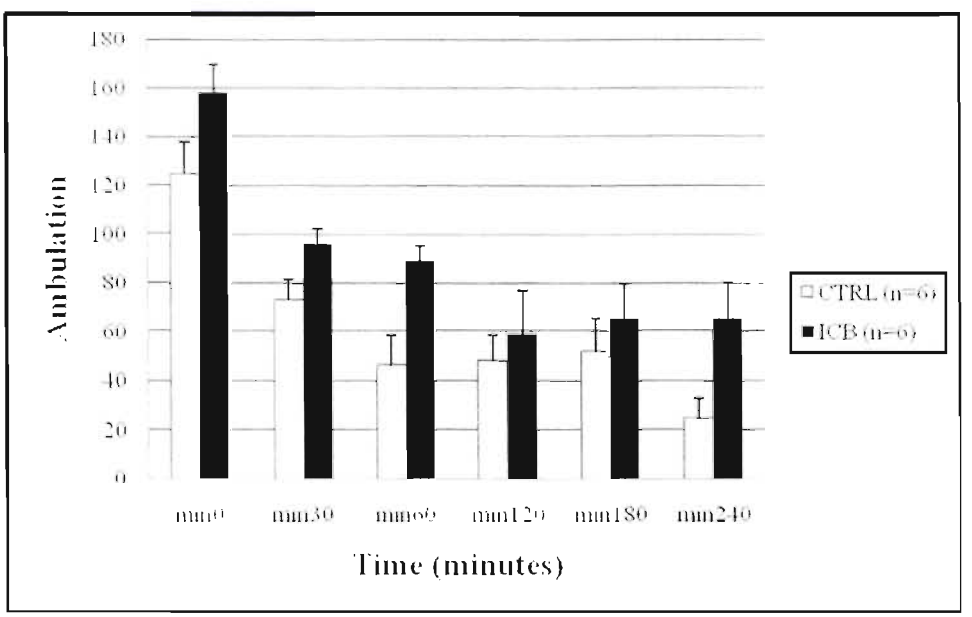


Table 3.5.6: **The effect of ICB (200 mg/kg) in total standing up behaviour in open field test**

Group		Min0	Min30	Min60	Min120	Min180	Min240
CTRL (n=6)		8.667±2.231	8.500±1.057	8.667±2.848	6.667±1.542	6.000±2.266	2.667±0.615
ICB (n=6)		14.667±2.894	14.000±2.145	15.167±3.092	10.833±4.159	8.167±2.574	5.833±1.195
t/p		-1.642/0.132	-2.300/**0.044***	-1.546/0.153	-0.939/0.370	-0.632/0.542	-2.357/**0.040***
95% confidence interval	Lower	-14.143	-10.827	-15.867	-14.049	-9.808	-6.161
	Upper	2.143	- 0.173	2.867	5.716	5.475	-0.173

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.5.6: **The effect of ICB (200 mg/kg) in total standing up behaviour in open field test**

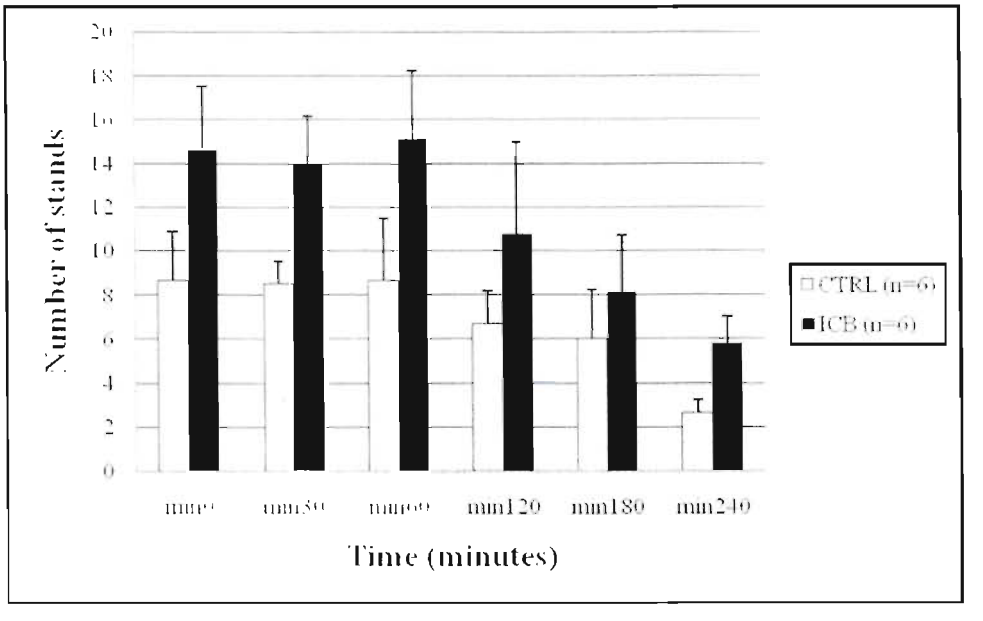


Table 3.5.7: **The effect of ICB (200 mg/kg) in total centre ambulation in open field test**

Group		Min0	Min30	Min60	Min120	Min180	Min240
CTRL(n=6)		1.167±0.307	0.667±0.333	0.167±0.167	0.000±0.000	0.500±0.224	0.000±0.000
ICB(n=6)		2.000±0.447	1.500±0.500	1.667±0.615	1.000±0.516	1.000±0.447	1.000±0.632
t/p		-1.536/0.156	-1.387/0.196	-2.355/0.059	-1.936/0.111	-1.000/0.341	-1.581/0.175
95% confidence interval	Lower	-2.042	-2.172	-3.076	-2.327	-1.614	-2.626
	Upper	0.376	0.506	0.076	0.327	0.614	0.626

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.5.7: **The effect of ICB (200 mg/kg) in total centre ambulation in open field test**

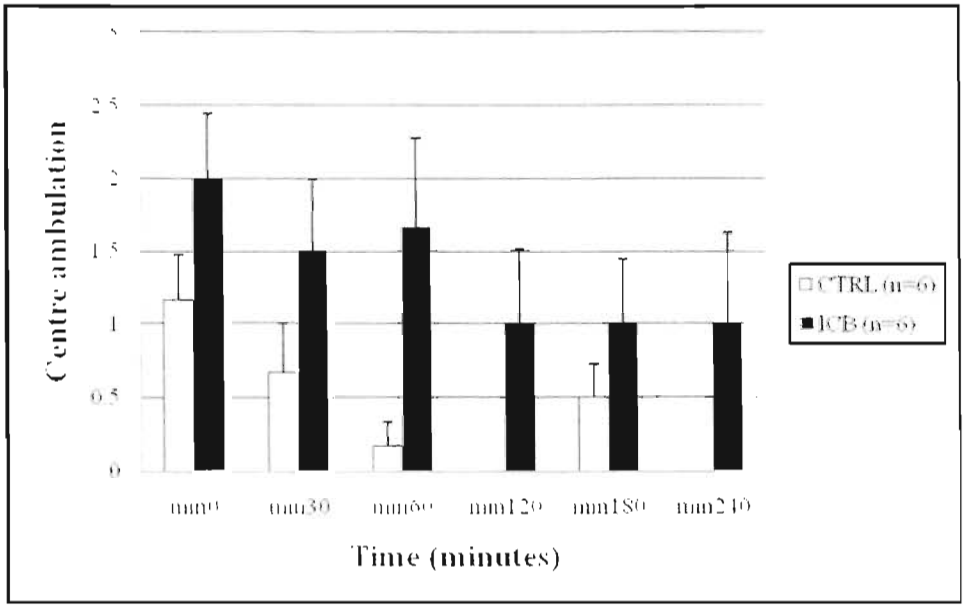
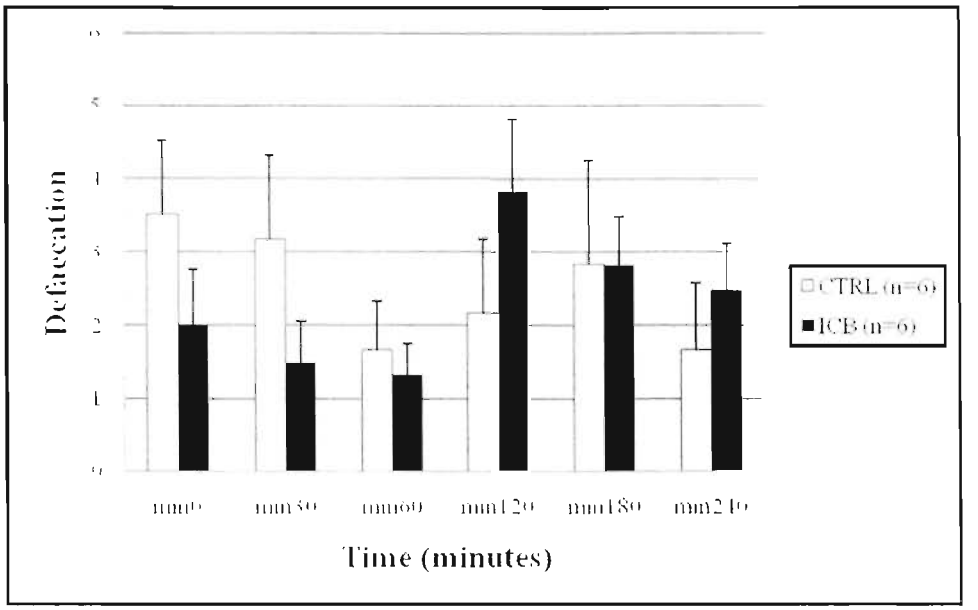


Table 3.5.8: **The effect of ICB (200 mg/kg) in emotional defaecation in open field test**

Group		Min0	Min30	Min60	Min120	Min180	Min240
CTRL(n=6)		3.500±1.025	3.167±1.167	1.667±0.667	2.167±1.014	2.833±1.424	1.667±0.919
ICB(n=6)		2.000±0.775	1.500±0.563	1.333±0.422	3.833±0.980	2.833±0.654	2.500±0.619
t/p		1.168/0.270	1.287/0.238	0.423/0.682	-1.182/0.265	0.000/1.000	-0.752/0.469
95% confidence interval	Lower	-1.362	-1.378	-1.424	-4.809	-3.492	-3.302
	Upper	4.362	4.712	2.091	1.476	3.492	1.636

N.B :*(< 0.05) = Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure 3.5.8: **The effect of ICB (200 mg/kg) in emotional defaecation in open field test**



Chapter Four

Conclusion

The pharmacological action of Icchabhedi Rasa was investigated from three aspects: gastrointestinal effect/side effect, analgesic and/or anti-inflammatory effect, and neuropharmacological effect/side effect. The results obtained after scrupulous statistical analysis show that ICB increases gastric peristalsis significantly in gastrointestinal motility test in 2nd hour 15 minutes study. Therefore, ICB may have purgative property and this finding also lends pharmacological support to its folkloric use in the treatment of constipation.

The significant results obtained from formalin induced paw licking test (neurogenic phase) and acetic acid induced writhing test indicate that ICB may possess some analgesic property.

In the open field test at higher dose (200 mg/kg) ICB was found to increase total ambulatory and standing up behavoiurs significantly. Thus, at higher doses, ICB may induce anxiety as neuropharmacological side effect.

However, in order to confirm these results other sophisticated and modern pharmacological methods must be employed accompanied by properly conducted clinical trial to finally establish a comprehensive safety profile for the marketed Ayurvedic preparation, Icchabhedi Rasa.

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ABBREVIATIONS

The following abbreviations were used throughout this paper:

Acetic acid	AA
Control	CTRL
Female	F
Gastric Emptying	GE
Gastrointestinal	GI
Gram	g
Hour	h
Icchabhedi Rasa	ICB
Intraperitoneal	i.p.
Kilogram	kg
Minute	min
Milligram	mg
Millilitre	ml
Standard error of mean	SEM
Second	s
Weight	wt