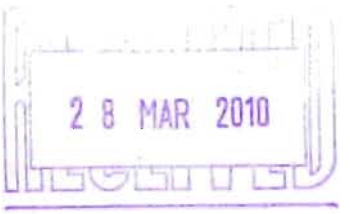
Pharmacological Studies of Brihat Sarvajvar Har Louha (BSH)

A research paper submitted to the Department of Pharmacy, East West University in the partial fulfillment of the requirements for the Degree of Bachelor of Pharmacy.



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Content

List of Table

List of Figure

Abbreviations

The following abbreviations were used throughout this research work.

AA	**Acetic acid**
Con	**Control**
Ctrl	**Control**
Dil	**Dilution**
F	**Female**
CNS	**Central Nervous System**
GI	**Gastrointestinal**
g	**Gram**
Hr	**Hour**
i.p	**Intra-peritoneal**
kg	Kilogram
M	**Male**
Min	**Minute**
ml	Milliliter
p.o.	Per-oral
s	Second
wt	**Weight**
BSH	**Brihat Sarvajvar Har Louha**
BCSIR	**Bangladesh Council of Scientific and Industrial Research**

Abstract

This research work describes the Pharmacological Studies of Brihat Sarvajvar Har Louha (BSH) an ayurvedic drug. Five (5) experiments have been carried out so far at different doses (100 mg/kg, 200 mg/kg and 400 mg/kg) to find out the various degrees of significance of action of the drug Brihat Sarvajvar Har Louha (BSH) on experimental animals. The Hole Cross Test resulted in significant (p=0.042) increase in movement in the close arm which indicates that the drug BSH has potent CNS stimulant activity. Acetic Acid Writhing Test also produced a significant decrease (p=0.046) in writhing response which indicates that the drug BSH has potent analgesic activity. Xylene induced ear edema test has been conceived to study the anti inflammatory effect of a test drug (BSH). Xylene Induced Ear Edema Test showed very highly significant (p=0.000) decrease in inflammation which indicates that the drug has potent anti inflammatory effect. On the other hand Formalin induced paw licking test has been done to study the analgesic and anti-inflammatory effect of a test drug (BSH). Formalin Test also showed significant (p=0.010) decrease in case of analgesic activity. Hole Board test has been done to determine the stimulatory or depressive effect of test drug (BSH). Open field test has been carried out to determine the stimulatory or depressive effect of test drug (BSH), by determining the ambulatory, emotional defecation, central ambulation and standing behavior of mice. These two experiments resulted in no significant observations but there have been overall decreases or increases in activities in different experiments in comparison to respective control groups. From the findings of the experiments we can conclude that this drug (BSH) has analgesic and antipyretic activity and has minimal side effects.

Chapter one

Introduction

1.1 Ayurveda

Ayurveda 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. In Sanskrit, the word Ayurveda consists of the words āyus, meaning 'life', and veda, meaning 'related to knowledge' or 'science'. Evolving throughout its history, Ayurveda remains an influential system of medicine in South Asia. The earliest literature of Ayurveda appeared during the Vedic period in subcontinent. The Sushruta Samhita and the Charaka Samhita were influential works on traditional medicine during this era. Ayurvedic practitioners also identified a number of medicinal preparations and surgical procedures for curing various ailments and diseases.

Ayurveda is considered to be a form of complementary and alternative medicine (CAM) within the western world, where several of its methods, such as the use of herbs, massage, and Yoga as exercise or alternative medicine, are applied on their own as a form of CAM treatment. However, such alternative therapy approaches are not unique to Ayurveda because they are also available under the systems of Unani medicine, Greek medicine and Islamic medicine.

Ayurveda emphasizes prevention of disease, rejuvenation of our body systems, and extension of life span. The profound premise and promise of Ayurveda is that through certain practices, not only can we prevent heart disease and make our headaches go away, but we can also better understand ourselves and the world around us, live a long healthy life in balance and harmony, achieve our fullest potential, and express our true inner nature on a daily basis.

Ayurveda provides an integrated approach to preventing and treating illness through lifestyle interventions and natural therapies. It is based on the view that the elements, forces, and principles that comprise all of nature - and that holds it together and make it function - are also seen in human beings. Laboratory and clinical studies on Ayurvedic herbal preparations and other therapies have shown them to have a range of potentially beneficial effects for preventing and treating certain cancers, treating infectious disease, treating diabetes, promoting health, and treating ageing. Mechanisms underlying these effects may include free-radical scavenging effects, immune system modulation, brain neurotransmitter modulation, and hormonal effects. (Ayerveda, 2009)

1.2 History of Ayurveda

Ayurveda, the science of life, prevention and longevity is the oldest and most holistic medical system available on the planet today. It was placed in written form over 5,000 years ago in India. The professional practice of Ayurveda in the United States began to grow and became more visible in the late 20th century. Recapitulation and adaptation of the older science to modern drug discovery processes can bring renewed interest to the pharmaceutical world and offer unique therapeutic solutions for a wide range of human disorders.

In Bangladesh a huge number of people are living under poverty line and it is hard for them especially for the poor people buying expensive synthetic drug. To getting out of this problem people go for the ayurvedic drug which is less expensive compared to the synthetic one. Drugs essential to the practice are found abundantly in the soil, generally without serious long-term side effects, and effective in certain cases where modern medicine has failed. Here a huge number of ayurvedic products of different manufacturer are available in market for various types of diseases. Officially recognized by the government of Bangladesh shortly following independence, Unani and Ayurvedic drugs were brought under a drug control system in 1982 to provide oversight of manufacturing and marketing. . Given the success and extensive presence of traditional medicine in Bangladesh, the government is considering incorporating it in mainstream primary health care services. Such action is considered a cost-effective, comparatively expedient manner of providing health coverage to large segments of the rural population. In order to implement and institutionalize the Ayurvedic Medical System and also to strengthen and widen the range of services in the District hospitals and Thana Health Complexes, the provision of Alternative Medicine in 30 selected District hospitals have began in 1998 under the 1998-2003 plan of HPSP (Health and Population Service Program). (Ayurvedic-medicines, 2009)

1.3 Safety

Major safety concerns include adulteration of herbal medicines with toxic metals, and intrinsic toxicity of herbal medications. Some traditional Ayurvedic treatments use toxic metals, herbs, and minerals as part of their remedies. Rasa Shastra, the practice of adding metals, minerals or gems to herbs, increases the likelihood of toxic metals such as lead, mercury, or arsenic in the remedy.

Traditionally the toxicity of these materials are believed to be reduced through processes such as samskaras or shodhanas (for metals), which is similar to the Chinese "pao zhi", although the Ayurvedic technique is more complex and may involve prayers as well as physical pharmacy techniques. Rigorous evidence that the metals may be rendered nontoxic is not available, and case reports describe adverse effects to these metals. There is evidence that using some Ayurvedic medicines, especially those involving herbs, metals, minerals, or other materials involves potentially serious risks, including toxicity. Adverse reactions to herbs due their pharmacology are described in traditional Ayurveda texts, but Ayurvedic practitioners are reluctant to admit that herbs could be toxic and the reliable information on herbal toxicity is not easily available. Following concerns about metal toxicity, the Government ruled that Ayurvedic products must specify their metallic content directly on the labels of the product.(Ayerveda, 2009)

1.4 Formulation of BSH

The **Formulation** of Brihat Sarvajvar Har Louha (BSH) is included in the Bangladesh National **Formulary** of Ayurvedic Medicine 1992.

BRIHAT SARVAJVAR HAR LOUHA
(Bhaisajyaratnavali, Jvaradhikara; 980-992.) Ay 58 A 078

1. Parada (suddha)	12 g.
2. Gandhaka (suddha)	12 g.
3. Tamra (bhasma)	12 g.
4. Abhraka (bhasma)	12 g.
5. Maksika (suddha)	12 g.
6. **Hiran**ya (svarna bhasma)	12 g.
7. **Tara** (rajata bhasma)	12 g.
8. **Tala** (haritala bhasma)	12 g.
9. Mrta kanta (lauha bhasma)	48 g.
10. Karavelli (karavellaka) rasa (Pl.)	Q.S. for bhavana 7 days.
11. Dasamula kvatha (Rt.)	Q.S. for bhavana 7 days.
12. Parpata kasaya (Pl.)	Q.S. for bhavana 7 days.
13. Triphala kvatha (Fr.P.)	Q.S. for bhavana 7 days.
14. Guduci svarasa (St.)	Q.S. for bhavana 7 days.
15. Nagavalli rasa (Lf.)	Q.S. for bhavana 7 days.
16. Kakamaci svarasa (Pl.)	Q.S. for bhavana 7 days.
17. Nirgundi svarasa (Lf.)	Q.S. for bhavana 7 days.
18. Punarnava (rakta punarnava) rasa (Pl.)	Q.S. for bhavana 7 days.
19. Ardraka rasa (Rz.)	Q.S. for bhavana 7 days.

Prepare pills of 125 mg.

Dose: 125 mg 3 times a day.

1.5 Important Therapeutic Uses of BSH

This drug (BSH) is a great remedy of all kinds of acute & chronic fever, malaria and acute pain.

1.6 Antipyretic agents and Fever

Antipyretics (literally "against the fire") are drugs that reduce body temperature in situations such as fever. However, they will not affect the normal body temperature if one does not have a fever.

Fever (also known as pyrexia, or a febrile response, and archaically known as ague), is a medical symptom which describes an increase in internal body temperature to levels which are above normal (37°C, 98.6°F). A fever is most accurately characterized as a temporary elevation in the body's thermoregulatory set-point, which is usually by about 1-2°C. This elevation in thermoregulatory set-point means that the previous "normal body temperature" would be considered hypothermic. Effector mechanisms, such as increased blood pressure, increased heart rate, activation of brown adipose tissue and muscular shivering attempt to counteract the perceived hypothermia, thereby reaching the new thermoregulatory set-point. It is the most common symptom of many diseases.

An adaptive mechanism, fever is the body's reaction to pathogens; it attempts to raise core body temperature to levels which will speed up the actions of the immune system and may also directly denature, debilitate, or kill the pathogen. Most fevers are caused by infections and almost all infectious diseases can cause fever. When a patient has or is suspected of having a fever, that person's body temperature is measured using a thermometer. If successful in ridding the body of an invasive pathogen, fever is an important protective immune mechanism and should generally not be suppressed. However, there are instances when fever escalates to temperatures where the body is at risk of destroying its own cells and must be brought under control with suppressive medication. (Grover., 1978)

1.6.1 Measurement and normal variation

When a patient has or is suspected of having a fever, that person's body temperature is measured using a thermometer. At a first glance, fever is present if:

- Temperature in the anus (rectum/rectal) is at or over 37.8 °C (100.0 °F)
- Temperature in the mouth (oral) is at or over 37.5 °C (99.5 °F)
- Temperature under the arm (axillary) is at or over 37.2 °C (99.0 °F)
- Temperature in the ear (otic) is at or over 37.2 °C (99.0 °F)

1.6.2 Causes of fever

1.6.2.1 Causes of fever according to Ayurveda

Fever occurs when the digestive fire (Agni) and digestive toxins (Ama) which are normally found within the gastrointestinal tract are thrown out of their place by disrupted Doshas and then they overflow into the blood and lymphatic system. Its circulation in the body causes the typical symptoms like high temperature, heaviness etc. Because of this the Three Doshas are further irritated and it spreads throughout the blood stream. When supplemented with its own heat plus the heat of the misplaced Agni, the temperature of the body raises and causes the symptoms of fever.

1.6.2.2 Causes of fever according to modern concept

It can occur independently or as symptom of any diseases. Fever is generally due to the pathological impacts of microbes or traumatic or several other reason.

Temperature is ultimately regulated in the hypothalamus. A trigger of the fever, called pyrogen, causes a release of Prostaglandin E2 (PGE2). PGE2 then in turn acts on the hypothalamus, which generates a systemic response back to the rest of the body, causing heat-creating effects to match a new temperature level.

1.6.3 General symptoms of fever

The main symptoms of fever are a raise in body temperature, chills, sore throat, body stiffness, muscle aches, headache, disturbed digestion, lack of appetite etc. In acute stages delirium and unconsciousness is also there. (Grover., 1978)

1.4.4 Mechanism

Fever is a positive feedback mechanism which acts towards the direction of change. Therefore, fever is the opposite of thermoregulation. Substances which induce fever are called pyrogens. Although external pathogens may be the ultimate reason for a fever, it is the internal or endogenous pyrogens that directly cause the increase in the thermoregulatory set-point.

One model for the mechanism of fever is the detection of lipopolysaccharide (LPS), which is a cell wall component of gram negative bacteria. An immunological protein called Lipopolysaccharide Binding Protein (LBP) binds to LPS. The LBP-LPS complex then binds to the CD14 receptor of a nearby macrophage. This binding results in the synthesis and release of various cytokine factors, such as interleukin 1, 6 and the tumor necrosis factor alpha. These cytokine factors are released into general circulation where they migrate to the circumventricular organs of the brain, where the blood-brain barrier is reduced. The cytokine factors bind with endothelial receptors on vessel walls, or interact with local microglial cells. When these cytokine factors bind, they activate the arachidonic acid pathway. This pathway (as it relates to fever), is mediated by the enzymes phospholipase A2 (PLA2), cyclooxygenase-2 (COX-2) and PGE2 synthase (membrane-associated protein involved in eicosanoid and glutathione metabolism, also known as mPEGS-1). These enzymes ultimately mediate the synthesis and release of prostaglandin E2 (PGE2).

PGE2 is the ultimate mediator of the febrile response. It acts near the ventromedial preopic area (VMPO) of the anterior hypothalamus and the parvocellular portion of the periventricular nucleus (PVH). It is in these areas that the thermal properties of fever emerge. Presumably, the elevation in thermoregulatory set-point is mediated by the VMPO, whereas the neuroendocrine effects of fever are mediated by the PVH, pituitary gland and various endocrine organs. Other heat effector mechanisms are mediated by the brain stem/medullary premotor sympathetic activation to the autonomic nervous system, which ultimately leads to the activation of brown adipose Tissue. The body can also induce shivering, or raise blood pressure through a mechanism of vasoconstriction. The set-point temperature of the body will remain elevated until PGE2 is no longer present. (Furciuele *et al.*, 1961)

1.6.5 Risk of fever

- The main risk of mild or moderate fevers is dehydration. People need more fluids than usual when they have a fever.
- A fever greater than 106 degrees Fahrenheit can result in brain damage and death in some cases
- A person with a fever may have an infection that is contagious. If other conditions are causing the fever, there are no risks to others.

1.6.6 Management of fever according to Ayurveda

Ayurveda, emphasis that fever is due to toxicity in the Rasa Dhatu (the body's basic vital tissue) and manages fever by Fasting (Langhana), Sudation (Swedana), patience (Kala - should not rush for aggressive treatments to make temperature down)), Light diet (e.g. Yavagu), Bitter drugs (Tikta Bheshajam) and Detoxification (ama pachana). The treatment depends on the duration, the cause of the fever and the other symptoms that accompany it.

a) **Fasting** - Strengthens the digestive system and eliminates Ama which in turn cleans the channels of the body. Strong person can fast whereas weak persons can take up a light diet. A lot of liquid diet like vegetable soups or just hot ginger water which alleviates the aggravated Dosha and increases the appetite should be taken.

b) **Sweating** - One can drink a simple spicy tea to induce sweat or he or she can be covered with a blanket so that he/she is made to sweat as this process clears toxins, raises the body temperature that kills the external organisms like viruses or bacteria and normalizes the body temperature.

c) **Light Diet**- After the body achieves the normal temperature one should take light diet of fresh fruits, Khichari and lightly cooked vegetables.

d) **Bitter Ayurvedic drugs** - The medicines which burns Ama, increases the white blood cell count and help the body to fight infections are useful. They are Jwarahara Vati, Shadangapaniya, Guduchi Sattva, Mahasudarshan Vati, Sanshamani Vati and other different bitter herbs and compounds.

e) **Detoxification**- After fever the patient is advised to undergo a detoxification (mild purgative regime Panchakarma) treatment as it expels remaining Ama and strengthens the digestive system. Then one should undergo a preventive regime which will reduce the chances of recurrence of fever.

Thus **Ayurveda** manages fever by restoring the Agni (fire) in the body and enhancing the proper metabolism. (Eddy *et al.*, 1953)

1.6.7 Management of fever according to modern concept

Treatment of fever is normally done by two ways-

- Lowering the set-point
- Facilitating heat loss

The former is accomplished with antipyretics such as ibuprofen or paracetamol (acetaminophen). Aspirin can also be given to adults. They block prostaglandin synthesis at the thermo regulating centers in the hypothalamus and at peripheral target sites.

Heat removal is generally by wet cloth or pads, usually applied to the forehead, but also through bathing the body in tepid water. Using water that is too cold can induce vasoconstriction, and reduce effective heat loss.

Heat loss may also be accomplished by heat conduction, convection, radiation, or **evaporation** (sweating, perspiration), or a combination of these.

1.6.8 Types of Fever

- Continued Fever - the temperature remains above normal for long period of time.
- Intermittent Fever - type of fever wherein body temperature periodically rises and falls.
- Relapsing Fever - type that recurs sometimes a number of times, several days after the temperature has returned to normal.

1.7 Analgesic Agents

Analgesics are one kind of drug or medicine that reduces the perception of pain by raising the patient's pain threshold. They are not cures for pain; they simply mask it.

Pain can be defined as an unpleasant sensation that can range from mild, localized discomfort to agony. Pain has both physical and emotional components. The physical part of pain results from nerve stimulation. Pain may be contained to a discrete area, as in an injury, or it can be more diffuse, as in disorders like fibromyalgia. Pain is mediated by specific nerve fibers that carry the pain impulses to the brain where their conscious appreciation may be modified by many factors. Pain has been classified as "productive" pain and "non-productive" pain. While this distinction has no physiologic meaning, it may serve as a guide to treatment (Sinatra RS, 1998).

- "Productive" pain has been described as a warning of injury, and so may be both an indication of need for treatment and a guide to diagnosis.
- "Non-productive" pain by definition serves no purpose either as a warning or diagnostic tool.

Traditionally, pain has been divided into two classes, acute and chronic. (Devor M *et al.*, 1998).

I. **Acute Pain:** Acute pain is the normal, predicted physiological response to an adverse chemical, thermal or mechanical stimulus associated with surgery, trauma and acute illness. Acute pain is a vital, protective mechanism that permits us to live in an environment fraught with potential dangers. Its importance is most clearly illustrated in the rare cases of congenital absence of nociceptors in which babies and children are prone to self mutilation and continuous environmental injuries, usually resulting in death at a very young age. (Daniel *et al*, 1999)

II. **Chronic Pain:** Chronic pain is continuous, long-term pain of more than 12 weeks or after the time that healing would have been thought to have occurred in pain after trauma or surgery. Chronic pain serves no physiologic role and is itself not a symptom, but a disease state. (Zahid *et al.*, 2009)

1.8 Physiological classification of pain

Physiologically pain can be classified into the following two categories:

1.8.1 Nociceptive pain

Nociception is the physiological sense for perception of physiological pain. Nociception does not describe psychological pain. Nociceptors are the free nerves endings of neurons that have their cell bodies outside the spinal column in the dorsal root ganglion and are named based upon their appearance at their sensory ends. These sensory endings look like the branches of small bushes. The interpretation of pain occurs when the nociceptors are stimulated and subsequently transmit signals through sensory neurons in the spinal cord, which releases glutamate, a major exicitory neurotransmitter that relays signals from one neuron to another and ultimately to the thalamus, in which pain perception occurs. From the thalamus, the signal travels to the cerebrum, at which point the individual becomes fully aware of the pain (Keats AS. 1959).

It is two types: - I. Somatic pain and II. Visceral pain.

I. **Somatic pain** originates from ligaments, tendons, bones, blood vessels, and even nerves themselves and is detected with somatic nociceptors. The scarcity of pain receptors in these areas produces a dull, poorly-localized pain of longer duration than cutaneous pain for examples include sprained ankle and broken bones.

II. **Visceral pain** originates from body organs. Visceral nociceptors are located within body organs and internal cavities. The even greater scarcity of nociceptors in these areas produces a pain usually greater and of a longer duration than somatic pain. Visceral pain is extremely difficult to localize and several injuries to visceral tissue exhibit "referred" pain, where the sensation is localized to an area completely unrelated to the site of injury. Myocardial ischaemia (the loss of blood flow to a part of the heart muscle tissue) is possibly the best-known example of referred pain; the sensation can occur in the upper chest as a restricted feeling, or as an ache in the left shoulder, arm or even hand.

1.8.2 Neuropathic pain

It results from irritation or damage to the nervous. As compared to acute pain less is known about the etiology of chronic pain. Chronic pain often occurs in the absence of ongoing illness or after healing is completed. A fundamental difference between inflammatory pain with tissue hypersensitivity and neuropathic pain is that in the former, the pain is relieved when inflammation has resolved and latter, it may persist after healing of the primary event. In summary, nociceptive pain is greatly relieved when healing is completed, while neuropathic pain persists after healing is completed (McQuay H *et al.*, 1998).

1.9 Research on herbal drugs in terms of Bangladesh

Brihat Sarvajvar Har Louha (BSH) 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 3-6-1991). 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 Organization. Directorate of Drug Administration has issued Notification DA/Admin/1-10/96/6212 dated 19th October 1996 has issued license under Drug Act, 1940 and Rules there under and Drug (Control) Ordinance 1982 for local manufacture and sale in Bangladesh. (Published Bangladesh Gazette #24 Part VI dated Thursday, June 11th 1998.) At present a good number of Ayurvedic manufacturers are manufacturing and marketing the Classical Ayurvedic Medicinal Preparation.

Aims & Objective

2.1 Aims of Study

The main objective of this study includes:

- To find out the analgesic and anti-inflammatory effects of BSH by Formalin Induced Paw Licking Test, Xylene induced Ear Edema Test, Acetic acid writhing test.
- To determine neuropharmacological effects of BSH by Open field test, Hole-board test and Hole-cross test.
- To monitor the psychopharmacological effects of BSH by climbing out test.

Chapter Two

Materials & Methods

2.1 Administration of Drug

For the pharmacological experiment, the powdered tablets were made into a solution and administered at a volume such that it would permit optimal dosage accuracy without contributing much to the total increase in the body fluid. For all the pharmacological studies the drugs were administered per oral route.

Table 2.1 Doses Used In Different Experiments

SL. No.	Experiment Name	Dose
01	Hole Cross Test	100 mg/kg, 200 mg/kg, 400 mg/kg Body Wt
02	Hole Board Test	100 mg/kg, 200 mg/kg, 400 mg/kg Body Wt
03	Open Field Test	100 mg/kg, 200 mg/kg, 400 mg/kg Body Wt
04	Climbing Out Test	100 mg/kg and 200 mg/kg Body Wt
05	Acetic Acid Induced Writhing Test	100 mg/kg and 200 mg/k Body Wt
06	Formalin Induced Paw Licking Test	100 mg/kg Body Wt
07	Xylene Induced Ear Edema Test	100mg/kg body wt

2.2 Experimental Animals

Male and Female mice (Swiss-Webster strain, 20-40 gm body weight) bred in the Animal House of the Department of Pharmacy, Jahangirnagar University, were used for the pharmacological experiments. They were kept in cages having dimensions of 30 x 20 x 13 cm and 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" (prepared according to the formula developed at BCSIR, Dhaka).

Before starting an experiment the animals were carefully marked on different parts of their body, 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.

2.3 Controls

Two groups of equal number of mice were simultaneously employed in the experiment. Six to ten mice were taken for each group for both the control and the experiment group. As the drug treated group, other group is treated with distilled water and this group served as the control.

2.4 Pharmacological Study with Animal Models

2.4.1 Formalin Induced Paw Licking Test

Principle

Formalin induced paw licking test has been done to study the analgesic and anti-inflammatory effect of a test drug. If the paw licking count is decreased in the first phase of experiment then the drug has analgesic activity. If the number of paw licking decrease in the second phase then test drug has anti-inflammatory activity.

Reagent

Not applicable

Procedure

Formalin 1% was administered to mice and immediately the licking time was registered for 5 min (first phase, neurogenic). Twenty minutes after the beginning of the experiment (second phase, inflammatory) the licking time was registered for other 5 min. Experimental drug was administered 60 min (p.o.) before the formalin injection. (Tjolsen *et al.*, 1992).

2.4.2 Xylene Induced Ear Edema Test

Principle

Xylene induced ear edema test has been conceived to study the anti inflammatory effect of a test drug. If the difference of weight between the two ears is less or minimal then test drug has anti-inflammatory effect.

Reagent

Not applicable

Procedure

Male Swiss mice were divided into groups of ten mice each. After 30 min of the administration of test drug, xylene (0.03 ml) was applied to the anterior and posterior surfaces of the right ear. Both ears of each mice were removed, 2 h after xylene application. Circular sections of both treated and untreated ears were taken using a 7 mm diameter cork borer and weighed. The difference in weight between left untreated ear sections and right treated ear section was calculated (Tang *et al.*, 1984).

2.4.3 Acetic Acid Induced Writhing Test

Principle

Acetic acid induced writhing test has been done to study the analgesic effect of a test drug. If the number of writhing is decreased then test drug has analgesic activity.

Reagent

Not applicable

Procedure

Muscular contraction was induced by the intraperitonial injection of 0.6% acetic acid (AA) (0.25ml/animal). The test preparations were administered orally 30 minutes before the intraperitonial injection of acetic acid. Mice were cased individually to count number of writhes (painful muscular contraction) after 15 minutes of AA injection for 5 minutes. The average number of writhes and the percent protection were calculated and then compared between the animals of the experimental groups and the animals of the Control group.

Percent protection was calculated as follows: -

% Protection= [100-(treated mean/control mean)] X 100%

2.4.4 Hole Cross Test

Principle

Hole cross test was carried out to determine the stimulatory or depressive effect of test drug. Increased movement indicates stimulatory activity and decreased movement indicate depressive activity.

Reagent

Not applicable

Procedure

In this experiment, the method of Takagi et al (1971) was employed. In a box having dimension of 30 X 20 X 14 cm, a hole of 3 cm in diameter at a height of 4.5 cm from the floor was constructed on the dividing wall. Spontaneous movement of the animals through the hole from one chamber to the other was counted for a period of 2 minutes. The observation was conducted 30, 60, 120 and 240 minutes after oral administration of test drugs and was compared with control animal administered with normal saline. (Takagi *et al.*, 1971)

2.4.5 Hole Board Test

Principle

The Hole-Board test (head plunging stereotype) according to the method devised by Boissier and Simon (1964), Boissier, Simon and Lwoff (1964) and Boissier and Simon (1967). The test enables the initial exploratory activity of the animal and its variations brought about by psychotropic elements of a drug to be unmistakably assessed. The hole board test was carried out to investigate the effect of the drug on the exploratory behavior of the animals. Exploration can be defined as a broad category of behavior, the consequences of which are to provide the organism with information about the exteroceptive environment. The principle of the test is that a novel situation of open field evokes in the animals a pattern of behavior characterized by exploration (head dipping through the holes), locomotion (ambulation past the holes) and emotional defecation. It has been considered that the exploration evoked under an unfamiliar environment is modified with physiological factors such as curiosity, fear and anxiety and the modulation of these factors after the administration of a drug (Nakama *et al.*, 1972).

Reagent

Not applicable

Procedure

This experiment was carried out by the following method of Nakama et al, 1972. A total of 16 holes, each 3 cm in diameter, were presented to the mouse in a flat space of 25 square centimeters. Each of the animal was transferred carefully to one corner of the field and the number of ambulation (expressed as the number of holes passed), head dipping and number of fecal boluses excretion was recorded for a period of 2 minutes at pre 30 minutes and post 30, 60, 120 and 240 minutes intervals and were compared with the control animals administered with distilled water (Nakama *et al.*, 1972)

2.4.6 Open Field Test

Principle

Open field test was carried out to determine the stimulatory or depressive effect of test drug. Increased ambulatory, emotional defecation, central ambulation and standing behavior indicate stimulatory property and decreased number of these parameters indicates depressive effect of test drug.

Reagent

Not applicable

Procedure

In this experiment, the method of Gupta (1971) was employed. The floor of an open field of half square meter was divided in to a series of squares, each alternatively colored black and white. The apparatus had a wall of 40 cm. The number of squares, traveled by the animal, was recorded for a period of two minutes. The open field test is designed to measure behavioral responses such as locomotor activity, hyperactivity, and exploratory behaviors. Open field is also used as a measure of anxiety. (Gupta *et al.*, 1971)

2.4.7 Statistical Analysis

Data were presented as Mean ± SEM (Standard Error of the Mean). Unpaired "t" tests were done for statistical significance tests. SPSS (Statistical Package for Social Science) for WINDOWS (Ver. 12) was applied for the analysis of data. $p = 0.05$ was taken as the level of significance.

Chapter Three

Results & Discussion

3.1 Formalin Induced Paw Test

The formalin pain test is very useful for evaluating the mechanism of pain and analgesia (Tjolsen *et al.*, 1992). Drugs which act mainly centrally, such as narcotic analgesics, inhibit both phases of pain in this model while peripherally acting drugs such as aspirin are indomethacin, only inhibit the late phase. (Santos *et al.*, 1994).

BSH treated male group exerted a decrease in paw licking effect in the formalin induced paw licking test when compared to the corresponding control group. (Table 3.1, Figure 3.1)

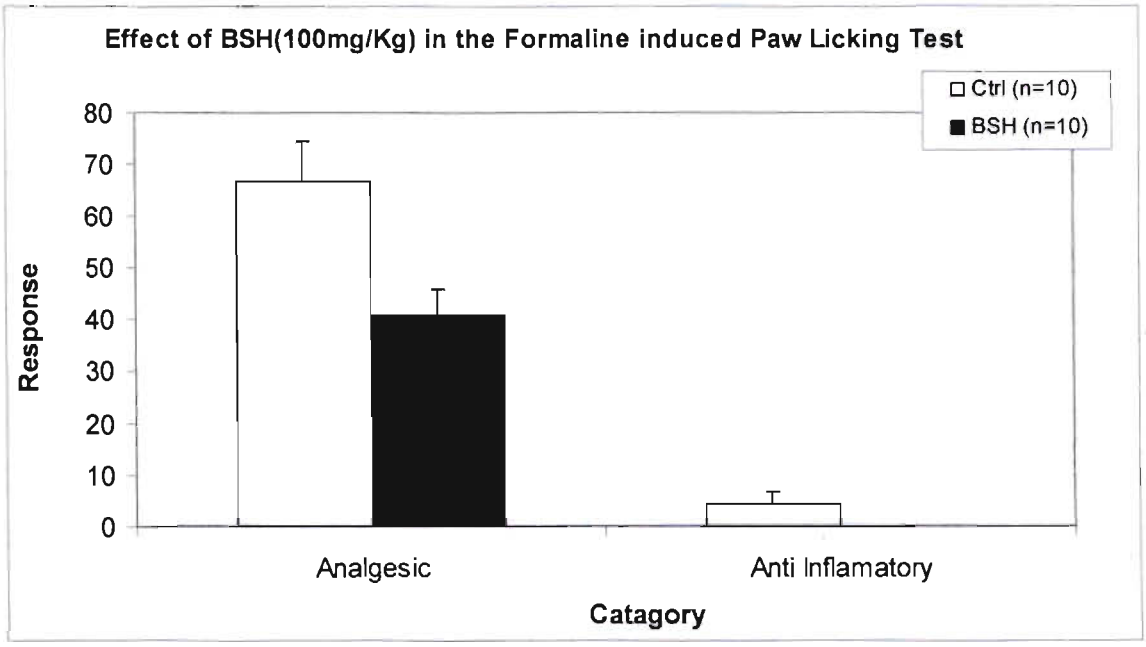
The decrease in paw licking effect was found to be significant (***p*=0.010**)

Table 3.1 The effect of BSH (100 mg/kg) in the Formalin Induced Paw licking (Analgesic + Inflammation) Test

Group		Analgesic (1st Phase)	Inflammation (2nd Phase)
Ctrl (n=10)		66.70±7.545	4.40±2.455
BSH (n=10)		40.70±5.079	0.00±0.000
t/p		2.858/**0.010***	1.792/0.107
95% confidence interval	**Lower**	6.890	-1.153
	Upper	45.110	9.953

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

Figure 3.1 The effect of BSH (100mg/Kg) in the Formalin Induced Paw licking (Analgesic + Inflammation) Test



3.2 Xyline Induced Ear Edema Test

Xylene induced ear swelling in mice, was selected to represent model of acute (exudative phase) inflammation.

Statistical Findings:

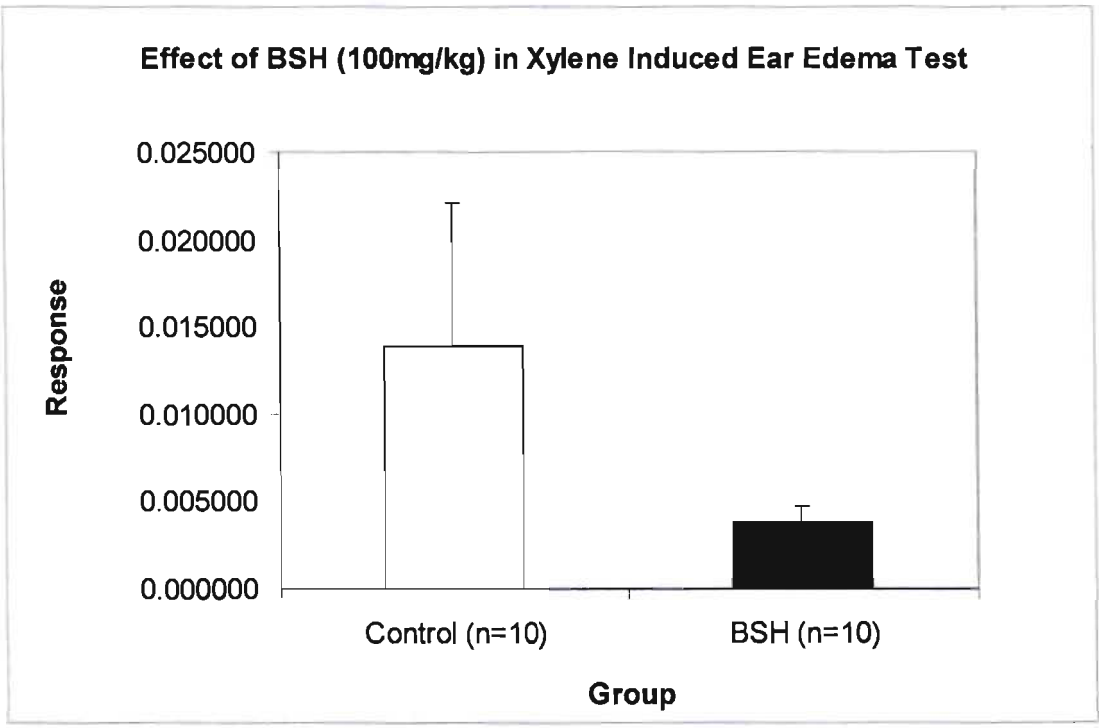
BSH treated male group exerted a decrease in inflammation in the xylene induced ear edema test when compared to the corresponding control group. (Table 3.2, figure 3.2) The decrease in inflammation was found to be very highly significant (***p*=0.000**)

Table 3.2: The effect of BSH (100 mg/kg) in the Xylene Induced Ear Edema Test.

Group		Inflammation	% of Inflammation
Ctrl (n=10)		0.01390±0.000823	101.012%
BSH (n=10)		0.00378±0.000954	
t/p		-8.077/**0.000*****	
95% Confidence Interval	**Lower**	0.007478	
	Upper	0.012766	

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

Figure 3.2 The effect of BSH (100 mg/Kg) in the Xylene Induced Ear Edema Test.



3.3 Acetic Acid Induced Writhing Test

The experiment was carried out to find out the existence of non-narcotic analgesic property. The pain sensation was initiated by using Acetic acid. The acetic acid induced writhing is inversely proportionate to the non-narcotic analgesic property. BSH treated male group exerted a decrease in writhing effect in the acetic acid induced writhing test in 1st minute when compared to the corresponding control group.

The decrease in writhing was found to be significant **(p=0.046)**

The percent of protection was 25.62%, which means this drug (BSH) decreases the pain. (Table 3.3, Figure 3.3.1)

At dose, 200mg/kg, **BSH** treated male mice showed **increasing** response compare to the control group from the initial at min 1st to min 5th. The results of the response were statistically insignificant.

The percent of protection was -19.00%, which means this drug could not decrease the pain whether pain is more than the control group. (Table 3.3.2, Figure 3.3.2)

Table 3.3.1 The effect of BSH (100 mg/kg) in the Acetic Acid Induced Writhing Test

Group		Min1	Min2	Min3	Min4	Min5
Control (n=10)		5.30±0.667	5.00±0.667	4.60±0.542	4.60±0.933	4.70±0.857
BSH (n=10)		3.70±0.335	3.80±0.389	4.00±0.632	3.30±0.367	3.20±0.291
t/p		2.142/**0.046***	1.555/0.137	0.721/0.480	1.296/0.211	1.658/0.115
95% Confidence interval	**Lower**	0.031	-0.421	-1.149	-0.807	-0.401
	Upper	3.169	2.821	2.349	3.407	3.401

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

Figure 3.3.1 The effect of BSH (100mg/Kg) in the Acetic Acid Induced writhing test.

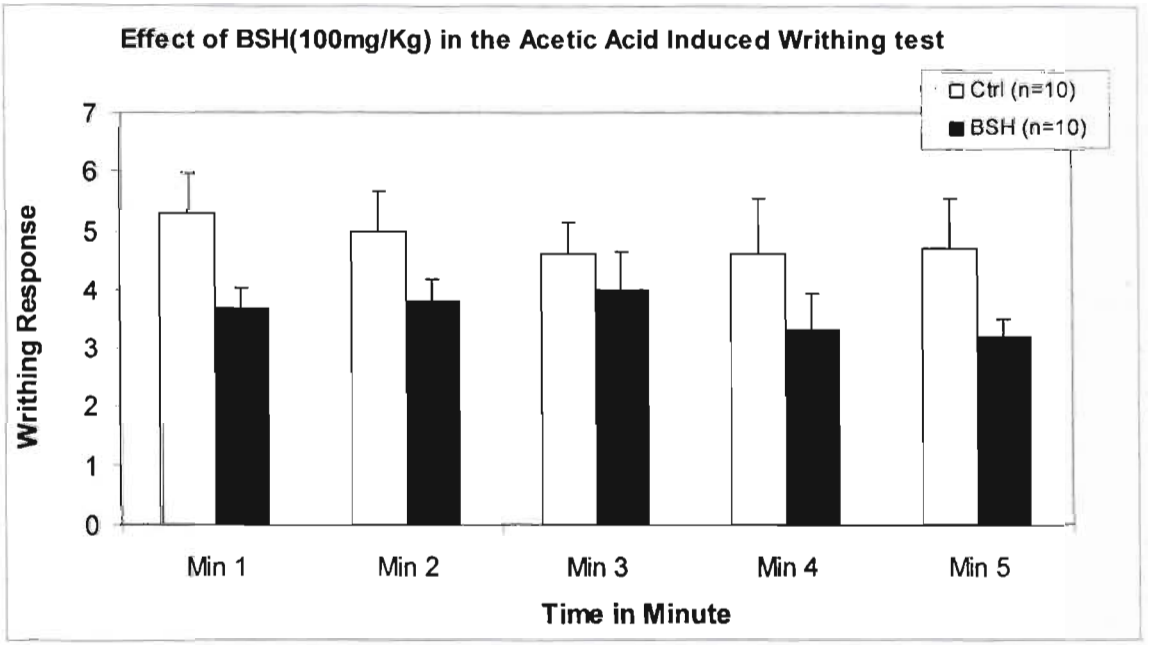
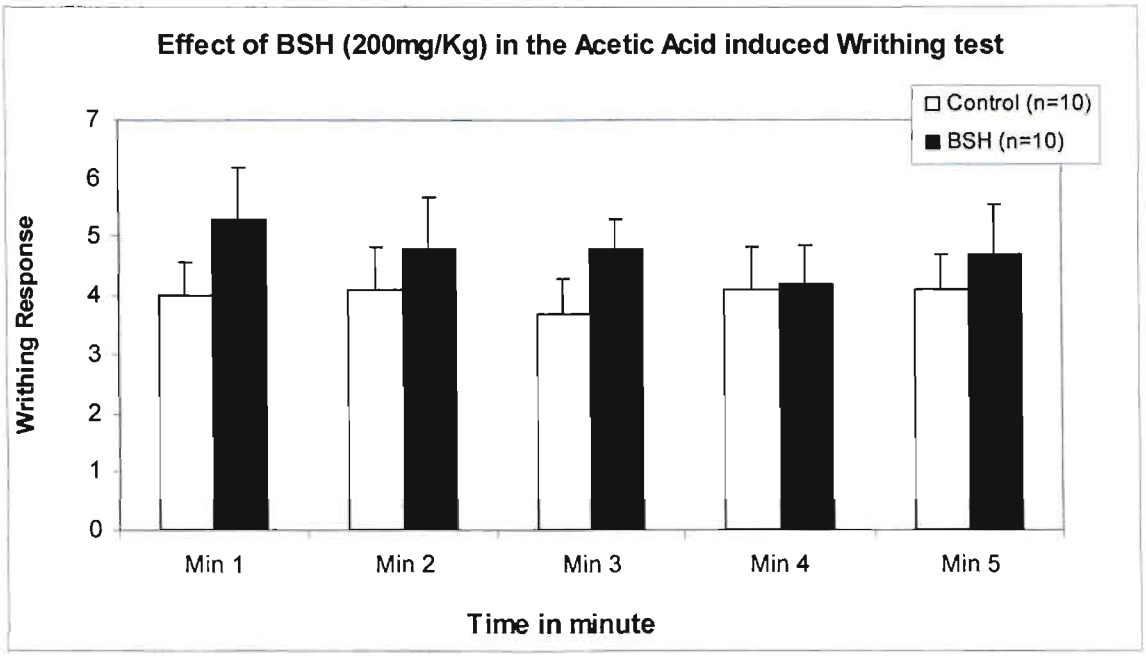


Table 3.3.2The effect of BSH (200 mg/kg) in the Acetic Acid Induced Writhing Test

Group		Min1	Min2	Min3	Min4	Min5
Control (n=10)		4.00±0.577	4.10±0.722	3.70±0.578	4.10±0.722	4.10±0.605
BSH(n=10)		5.30±0.895	4.80±0.867	4.80±0.512	4.20±0.663	4.70±0.844
t/p Value		-1.221/0.238	-.621/0.543	-1.424/0.172	-0.102/0.920	-0.578/0.570
95% Confidence interval	**Lower**	-3.538	-3.070	-2.723	-2.160	-2.781
	Upper	0.938	1.670	0.523	1.960	1.581

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

Figure 3.3.2 The effect of BSH (200mg/Kg) in the Acetic Acid Induced writhing test.



3.4 Hole Cross Test

As spontaneous movements of the animals include, by definition, both the propulsive and non-propulsive movements of the animal, and as the fluctuating and multifarious nature of many overt movements patterns impossible, to accurately measure the effects of a drug on the spontaneous motor activity of animals by using a single experimental procedure, the hole cross test was performed (Robbing, 1977).

BSH treated mice at 100 mg/kg dose exerted decrease during min 0 to till min 60. There was an increased effect at min 120 and min 180. (Table 3.4.1, Figure 3.4.1)

BSH treated mice at 100 mg/kg dose exerted increased response during min 0 to till min 60. There was a decreased effect at min 120.(Table 3.4.2, Figure 3.4.2)

BSH treated mice at 100 mg/kg dose exerted decreased response at min 0, min30, min 120 and min 180. There was an increased effect at min 60 and min 240.

The increase in ambulation was found to be significant **(p=0.042).** (Table 3.4.3, Figure 3.4.3)

Table3.4.1 : The effect of BSH (100mg/kg) in the Hole Cross Test.

Group		Min 0	+30	+60	+120	+180	+240
Control (n=6)		2.17±0.654	3.67±1.909	4.00±1.461	3.00±0.683	2.67±0.494	5.17±2.040
BSH(n=6)		1.50±0.671	1.17±0.401	3.67±0.667	3.17±0.401	4.00±1.317	2.33± 0.422
t/p Value		0.712/0.493	1.282/0.229	0.208/0.840	- 0.210/0.838	- 0.948/0.365	1.360/0.228
95% Confidence level	Lower	-1.421	-1.847	-3.244	-1.932	-4.467	-2.397
	Upper	2.754	6.847	3.911	1.599	1.800	8.064

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

Figure 3.4.1: The effect of BSH (100mg/Kg) in Hole Cross Test

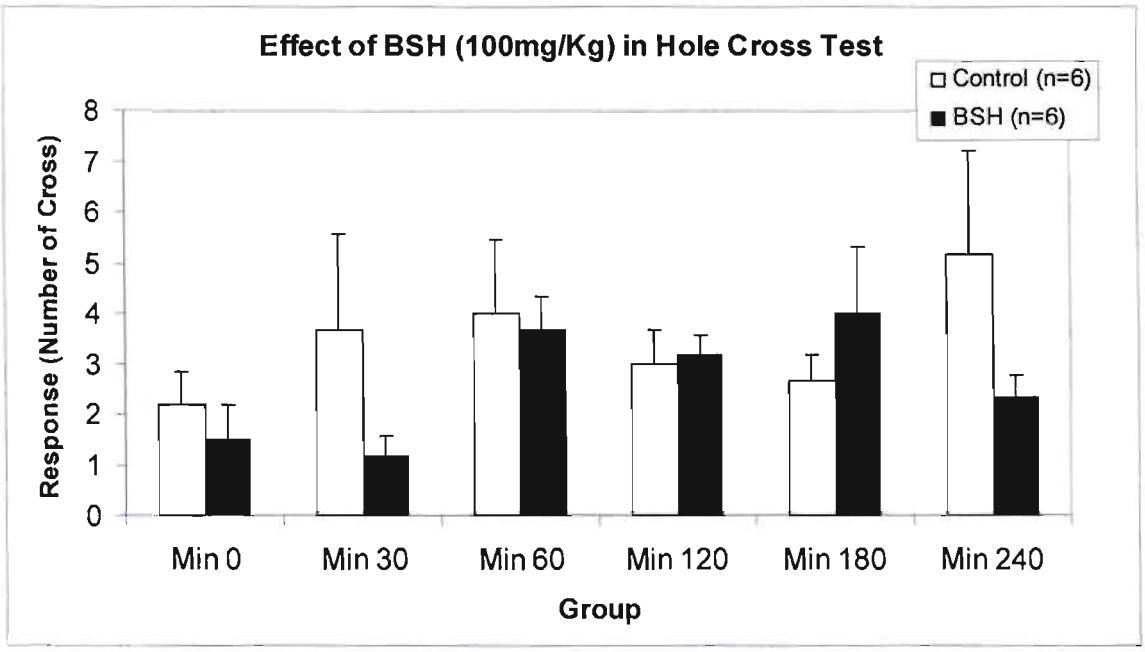


Table 3.4.2: The effect of BSH (200mg/kg) in the Hole Cross Test.

Group		Min 0	+30	+60	+120	+180	+240
Control (n=6)		0.33±0.211	1.50±1.025	2.50±0.885	4.00±1.291	2.50±0.885	1.33±0.422
BSH(n=6)		2.17±0.946	2.33±1.054	3.17±1.195	2.67±1.308	2.50±0.619	4.00± 1.528
t/p Value		- 1.892/0.112	- 0.567/0.583	- 0.448/0.663	0.725/0.485	0.000/1.000	- 1.683/0.145
95% Confidence level	Lower	-4.258	-4.109	-3.980	-2.762	-2.407	-6.584
	Upper	0.591	2.442	2.647	5.428	2.407	1.251

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

Figure 3.4.2: The effect of BSH (200mg/Kg) in Hole Cross Test

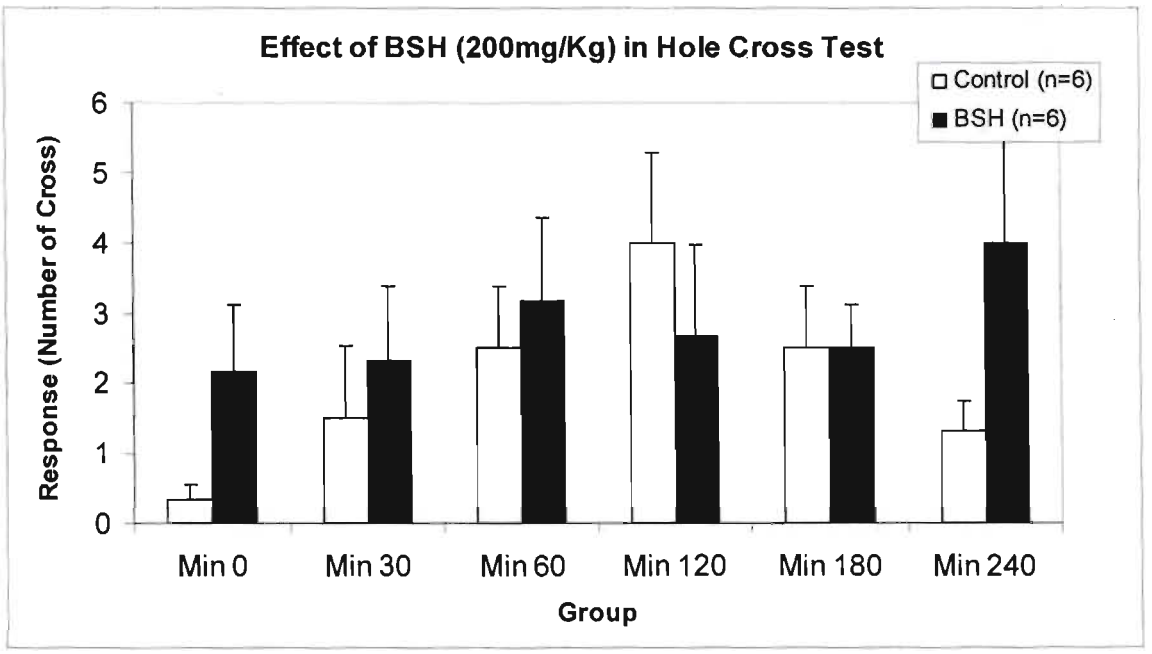
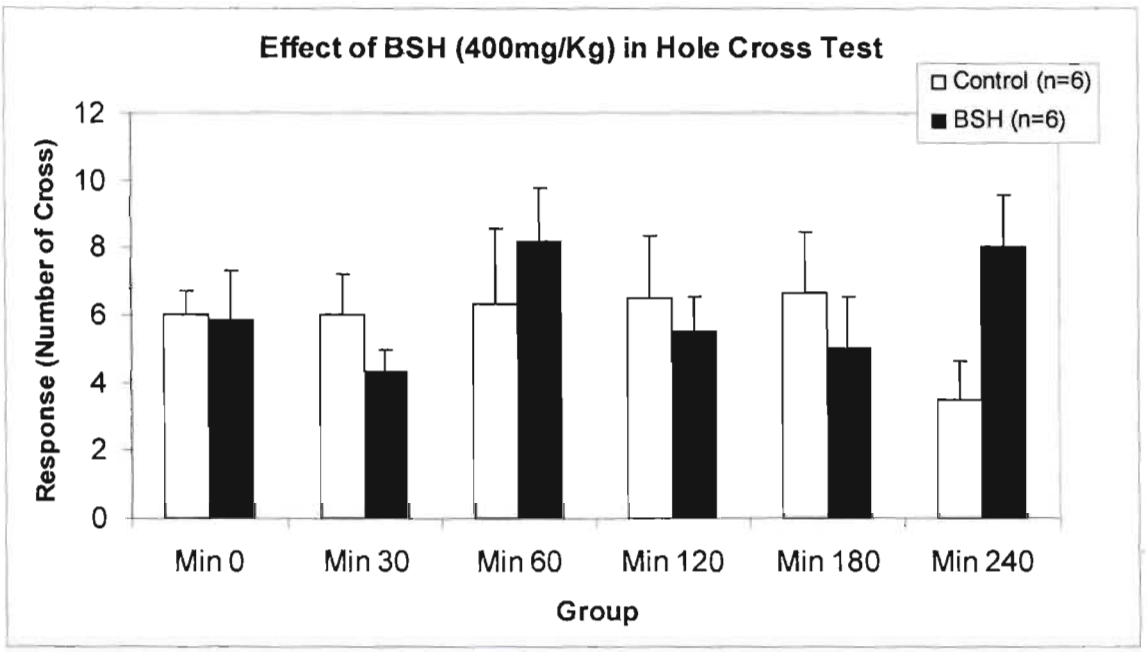


Table 3.4.3: The effect of BSH (400mg/kg) in the Hole Cross Test.

Group		Min 0	+30	+60	+120	+180	+240
Control (n=6)		6.00±0.730	6.00±1.211	6.33±2.246	6.50±1.821	6.67±1.764	3.50±1.118
BSH(n=6)		5.83±1.493	4.33±0.615	8.17±1.621	5.50±1.057	5.00±1.528	8.00± 1.571
t/p Value		0.100/0.922	1.227/0.248	-0.662/0.523	0.475/1.00	0.714/0.491	-2.334/**0.042***
95% Confidence level	Lower	-3.536	-1.359	-8.005	-3.691	-3.532	-8.796
	Upper	3.869	4.693	4.338	5.691	6.866	-0.204

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

Figure 3.4.3: The effect of BSH (400mg/Kg) in Hole Cross Test



3.5 Hole Board Test

The experiment was carried out to get a clear picture of the effect of the drugs under consideration on the pattern of behavior characterized by spontaneous ambulatory activity, exploratory activity and emotional defecation of the animals. This experiment presents with a different and more complex environment to explore. For this experiment male mice weight range 22- 30 g was used.

3.5.1 Ambulation

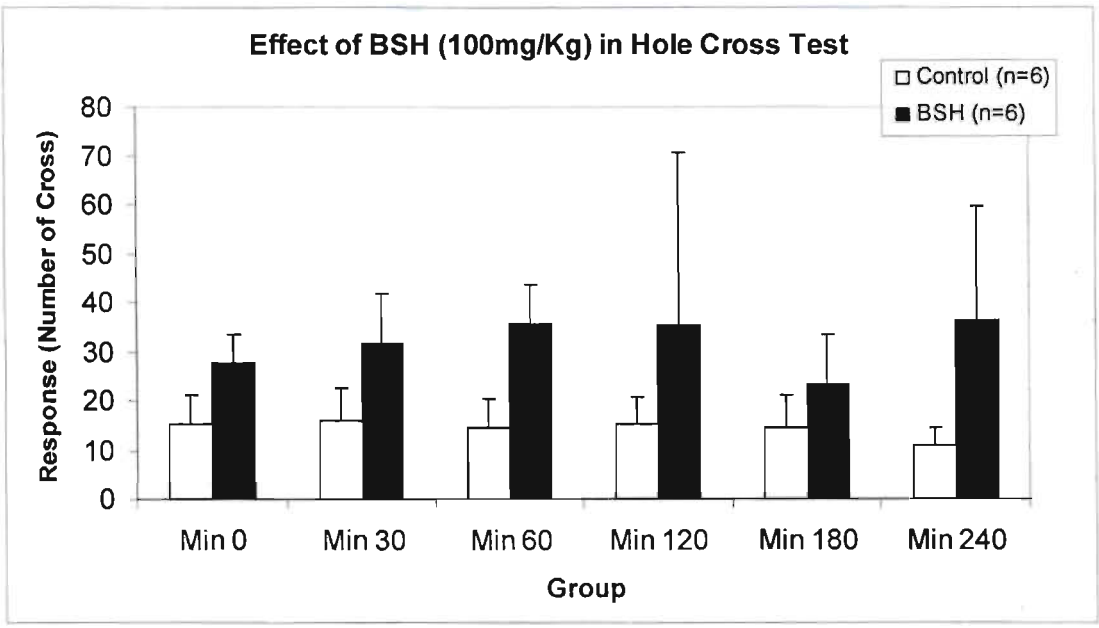
BSH treated mice at 100 mg/kg dose exerted increased response during min 0 to till min 240. (Table 3.5.1, Figure 3.5.1)

Table 3.5.1:The effect of BSH (100 mg/kg) on the Ambulation in Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min 180	Min240
Ctrl(n=6)		15.17±6.024	15.83±6.580	14.50±5.937	15.33±5.512	14.67±6.275	11.00±3.40
BSH (n=6)		27.67±5.909	31.50±10.497	35.67±7.809	35.33±10.246	23.17±5.930	36.50±10.739
t/p		-1.48/0.169	-1.26/0.235	-2.158/ **0.056**	-1.719/ 0.116	-0.985/ 0.348	-2.264/ **0.064**
95% confidence interval	lower	-31.302	-43.270	-43.024	-45.923	-27.737	-50.595
	Upper	6.302	11.937	0.690	5.923	10.737	-0.405

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

Figure 3.5.1: The effect of BSH (100 mg/Kg) in Ambulation of Hole Board Test.



3.5.2: Head Dipping:

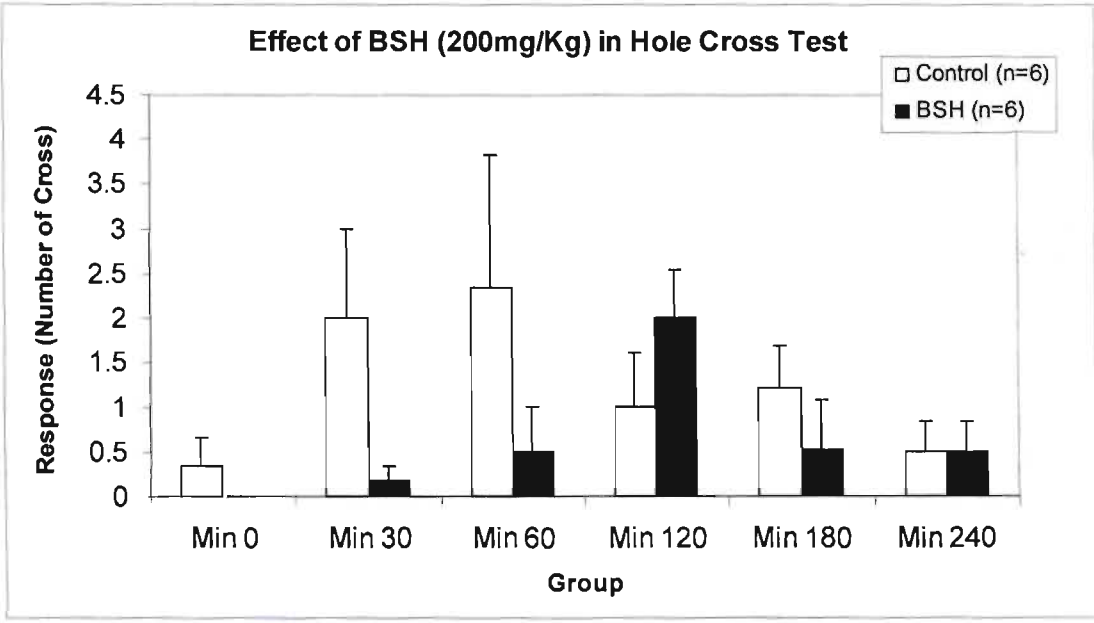
BSH treated mice at 100 mg/kg dose exerted decreased response during min 0 to till min 240. (Table 3.5.2, Figure 3.5.2)

Table3.5.2:The effect of BSH (100 mg/kg) on the Head Dipping in Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min 180	Min240
Ctrl(n=6)		0.33±0.333	2.00±1.00	2.33±1.563	1.00±0.632	1.211±0.494	0.50±0.342
BSH (n=6)		0.00±0.000	0.17±0.167	0.5±0.500	2.00±1.438	0.516±0.211	0.50±0.342
t/p		1.00/0.363	1.808/0.127	1.117/0.290	-0.637/ 0.539	0.620/0.549	0.000/1.00
95% confidence interval	lower	-0.524	-0.732	-1.824	-4.499	-0.864	-1.076
	Upper	1.190	4.399	5.491	2.499	1.531	1.076

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

Figure 3.5.2: The effect of BSH (100 mg/Kg) on Head Dipping in Hole Board Test.



3.5.3 Defecation

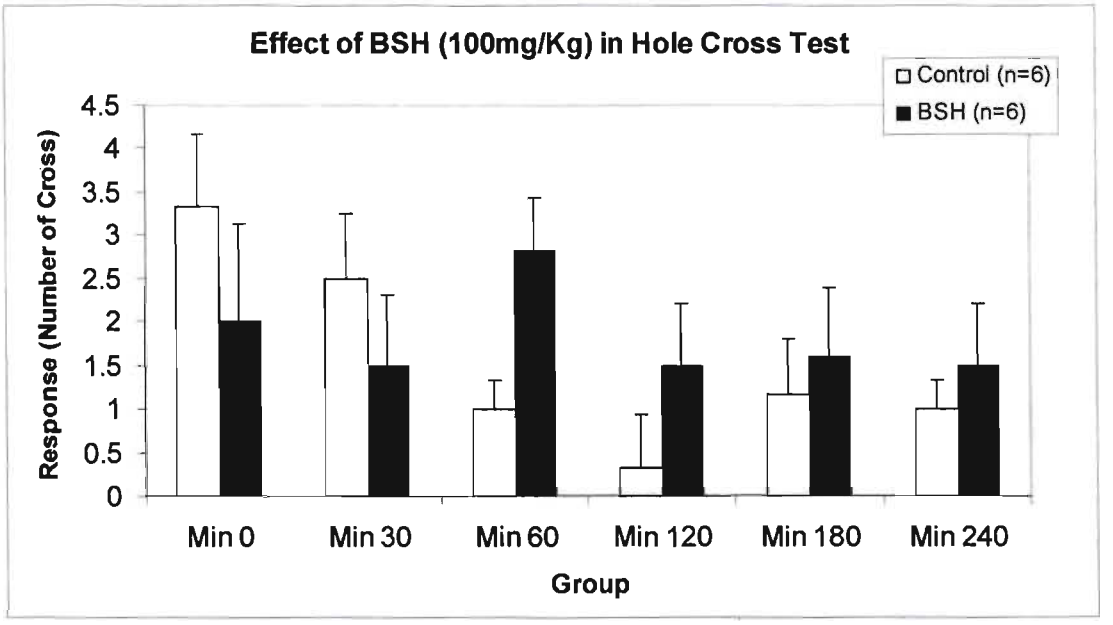
BSH treated mice at 100 mg/kg dose exerted decreased response during min 0 to till min 30. There was an increased effect during min 60 to till min 240. (Table 3.5.3, Figure 3.5.3)

Table 3.5.3: The effect of BSH (100 mg/kg) in the Defecation of Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min 180	Min240
Ctrl(n=6)		3.33±0.843	2.50±0.764	1.00±0.683	0.33±0.333	1.17±0.601	1.00±0.632
BSH (n=6)		2.00±1.125	1.50±0.806	2.83±0.601	1.50±0.719	1.67±0.803	1.50±0.719
t/p		0.948/0.365	0.900/0.389	-2.015/ 0.072	-1.472/ 0.172	-0.499/-0.5	-0.522/-0.5
95% confidence interval	lower	-1.80	-1.474	-3.865	-2.932	-2.734	-2.633
	Upper	4.467	3.474	0.198	0.599	1.734	1.633

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

Figure 3.5.3:The effect of BSH (100 mg/Kg) on Defecation in Hole Board Test.



Chapter Four

Conclusion

4.1 Conclusion

The formalin induced paw licking test of BSH shows significant (p=0.010) analgesic activity and anti-inflammatory activity.

The Xylene induced ear edema test also shows highly significant (p=0.000) anti-inflammatory activity.

The Acetic Acid induced writhing test also shows significant (p=0.046) analgesic activity.

From the above three experiments (Formaline induced paw licking test, Xylene induced ear edema test and Acetic acid induced writhing test) we can say that the drug BSH have analgesic and anti inflammatory activity.

The Hole Cross Test resulted in significant (p=0.042) increase in movement in the close arm which indicates that the drug BSH has potent CNS stimulant activity.

The Hole Board Test did not produce significant results throughout the experiment in all the 3 doses (100 mg/kg, 200 mg/kg, 400 mg/kg Body Wt).

From the above two (Hole cross and Hole Board) test we can conclude that the drug BSH have little stimulatory activity.

From the results of above experiments we can suggest that the drug can be prescribed to the patients suffering from acute & chronic fever, malaria and acute pain.

All these experiment were executed in an attempt to confirm the safety of the general patients or users of the society and country as a whole.

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