Studies of the effects of Nabayas Louha on different physiological systems of animal model

A research report submitted in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

Submitted by

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This Research paper is dedicated to my Parents

CERTIFICATE

This is to certify that, the thesis "Studies of the effects of Nabayas Louha on different physiological systems of animal model" 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 Ashfaqur Rahman (ID # 2005-2-70-011) 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


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Abstract

Purpose: The research work was carried out to characterize the effect of ayurvedic iron preparation Nabayas Louha on different physiological systems of animal model.

Method: Nabayas Louha was administered by oral route to the animal model (*Swiss albino*) and the effects were determined by comparing with respect to control group which were treated with distilled water. In some experiments positive controls were also used. To investigate the effect of Nabayas Louha different types of experiment models were used which were collected from internationally published publications and journals.

Result: Oral administration of Nabayas Louha was found to increase the % of gastrointestinal emptying and gastrointestinal (GI) motility test. In GI emptying test one of the 2nd hour result was a significant ($p<0.05$). In GI motility tests one result was noticeable ($p<0.099$) for increasing GI emptying. In the forced swimming test two results were noticeable ($p<0.099$) and one result was very significant ($p<0.01$) for depressant effect of Nabayas Louha.

Conclusion: After summarize all the results it can be said that Nabayas Louha may have gastroprokinetic activity but to establish these findings there is a need for a comprehensive study and large scale clinical trial.

Keywords: Nabayas Louha, anemia, gastrointestinal effect, neuropharmacological effect, psychopharmacological effect, analgesic and anti-inflammatory effect.

Chapter - 1

Introduction

Introduction:

1.1 Ayurveda:

Ayurveda, the science of life 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. (Chopra AS, 2003)

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. (Chopra AS, 2003)

1.2 Historical perspective 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. (Ayurveda, 2009)

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). (Ayurveda, 2009)

1.3 Safety concern of Ayurveda:

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 (Saper RB *et al.*, 2008)

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. (Saper RB *et al.*, 2008)

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. (Urmila T *et al.*, 2008)

Following concerns about metal toxicity, the Government ruled that Ayurvedic products must specify their metallic content directly on the labels of the product.

By using Ayurvedic medicine costly and wide-ranging measures of clinical investigations can be avoided in many cases and people in these preferred areas have the option to get cured at a cheaper cost depending on their option. (Ayurveda, 2009)

Taking into consideration the prevalence of Ayurveda as the well accepted in Bangladesh, one can not highlight enough the need for establishing the safety profiles of ayurvedic drugs. Keeping in mind, the current setting this research effort on Ayurvedic formulation, Nabayas Louha explores a range of its toxicological aspects utilizing laboratory animals. The point is to have a better understanding of the likely toxicological profile of the drug under study and, to some level, to come to a decision how acceptable the use of this drug is under the acknowledged type. The task will ultimately result in supplementing and complementing the accessible health care services and, in the long run, will guarantee overall treatment of the population in terms of community wellbeing.

1.4 Ayurveda in Bangladesh:

Bangladesh is rich in biodiversity and has an abundant resource of herbs, plants, and trees. Based on its geographical and seasonal benefits, the country is a potential practitioner of Ayurveda. Indeed, Bangladesh is considered as the home of medicinal plants which have occupied an important position in the socio cultural, spiritual and medicinal arena of rural and tribal lives of Bangladesh. This is of tremendous contemporary relevance because it can on

one hand ensure health security to millions of people and on the other hand it can provide new and safe herbal drugs to the entire world. Relative to allopathic treatment, ayurvedic treatment is easy to access at affordable prices and sometimes is the only source of health care available to the poor. A majority of the population is below the poverty line and for most people the only way to seek medication at an economical rate is by seeking ayurvedic treatment. However, in light of the successful benefits of ayurvedic medicine the demand for such preparations is increasing in both developing and developed countries. In fact, given the success and extensive presence of ayurvedic medicine in Bangladesh, the government is considering incorporating it as one of the mainstream primary health care services. Such action is considered a cost-effective and comparatively expedient manner of providing health coverage to large segments of the rural population. Bangladesh also has high prospect in making footsteps on the global market for medicinal plants and products as nearly 650 medicinal plant species have been identified to be in use in Bangladesh with around 25 plants having high value. In view of this, Ayurvedic preparations were brought under a drug control system in 1982 to provide oversight of manufacturing and marketing. In line with increased national and international demand of Ayurvedic medicines it has become very essential that clinical examination in the extent of safety and efficacy of these formulations be carefully evaluated and their pharmacological profiles established.

1.5 Nabayas Louha:

Nabayas Louha (NBL) is included (page 231-232) 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. Permission to manufacture at industrial scale is printed in page no. 533 (column 1: Product code 12.30). 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)

1.6 Anemia Overview:

Anemia (also spelled anaemia or anæmia; from Ancient Greek "ἀναιμία" anaimia, meaning "lack of blood") is a decrease in normal number of red blood cells (RBCs) or less than the normal quantity of hemoglobin in the blood. A person who has anemia is called anemic.

Blood is comprised of two parts; a liquid part called the plasma and a cellular part. The cellular part contains several different cell types. One of the most important and most numerous cell types is the red blood cell. The purpose of the red blood cell is to deliver oxygen from the lungs to other parts of the body. Red blood cells are produced through a series of complex and specific steps. They are made in the bone marrow (inner part of some bones that make most of the cells in the blood), and when all the proper steps in their maturation are complete, they are released into the blood stream. The hemoglobin molecule is the functional unit of the red blood cells and is the protein structure that is inside the red blood cells.

Even though the red blood cells (RBCs) are made within the bone marrow, many other factors are involved in their production. For example, iron is a very important component of the hemoglobin molecule; erythropoietin, a molecule secreted by the kidneys, promotes the formation of red blood cells in the bone marrow.

In general, there are three major types of anemia, classified according to the size of the red blood cells:

Microcytic anemia: If the red blood cells are smaller than normal, this is called microcytic anemia. The major causes of this type are iron deficiency anemia and thalassemia (inherited disorders of hemoglobin).

Normocytic anemia: If the red blood cells size are normal in size (but low in number), this is called normocytic anemia, such as anemia that accompanies chronic disease or anemia related to kidney disease.

Macrocytic anemia: If red blood cells are larger than normal, then it is called macrocytic anemia. Major causes of this type are pernicious anemia and anemia related to alcoholism. (E-medicine health, 2009)

1.7 Anemia Symptoms:

Because a low red blood cell count decreases oxygen delivery to every tissue in the body, anemia may cause many signs and symptoms. It can also make almost any other underlying medical condition worse. If anemia is mild, it may not cause any symptoms. If anemia is slowly ongoing (chronic), the body may adapt and compensate for the change; in this case there may not be any symptoms until the anemia becomes more severe.

Symptoms of anemia may include fatigue, weakness, shortness of breath, lightheadedness, palpitations (feeling of the heart racing or beating irregularly) and looking pale.

Symptoms of severe anemia may include: chest pain, angina, or heart attack, dizziness, fainting or passing out and rapid heart rate.

Some of the signs that may indicate anemia in an individual may include: Change in stool color, including black and tarry stools (sticky and foul smelling), maroon-colored, or visibly bloody stools if the anemia is due to blood loss through the gastrointestinal tract, rapid heart rate, low blood pressure, rapid breathing, pale or cold skin, yellow skin called jaundice if anemia is due to red blood cell breakdown, heart murmur, enlargement of the spleen with certain causes of anemia. (E-medicine health, 2009)

1.8 Medical Treatment for anemia:

Medical treatment of anemia varies widely and depends on the cause and the severity of anemia. If anemia is mild and associated with no symptoms or minimal symptoms, a thorough investigation by a doctor will be done in the outpatient setting (doctor's office). If any cause is found, then treatment will be started. For example, if anemia is mild and is found to be related to low iron levels, then iron supplements may be given during further investigation to determine the cause of the iron deficiency is carried out.

On the other hand, if anemia is related to sudden blood loss from an injury or a rapidly bleeding stomach ulcer, then hospitalization and transfusion of red blood cells may be required to relieve the symptoms and replace the lost blood. Further measures to control the bleeding may occur at the same time to stop further blood loss.

Blood transfusion may be required in other less critical circumstances as well. For example, an individual who is receiving chemotherapy for a cancer may be expected by the treating physician to have bone marrow problems related to the chemotherapy. Therefore, the doctor may check blood counts routinely, and if the levels get to a low enough level, he or she may order a red blood cell transfusion to help with the symptoms of anemia. (E-medicine health, 2009)

1.9 Medication system for anemia:

Medications and treatments that correct the common underlying causes of anemia include the following:

Iron may be taken during pregnancy and when iron levels are low. It is important to determine the cause of iron deficiency and treat it properly.

Vitamin supplements may replace folate and vitamin B_{12} in people with poor eating habits. In people with pernicious anemia who are unable to absorb sufficient amounts of vitamin B_{12}, monthly injections of vitamin B_{12} are commonly used to replete the vitamin B_{12} levels and correct the anemia.

Epoetin-alfa injection can be used to increase red blood cell production in people with kidney problems. The production of erythropoietin is reduced in people with advanced kidney disease, as described earlier.

Stopping a medication that may be the cause of anemia may also reverse anemia after consultation with a physician.

If alcohol is the cause of anemia, then in addition to taking vitamins and maintaining adequate nutrition, alcohol consumption needs to be stopped. (E-medicine health, 2009)

1.10 Anemia situation in Bangladesh:

Anaemia is a major public health problem in Bangladesh. As well as reducing the survival of mothers and children, anaemia lowers immunity; reduces growth, learning ability, work capacity and productivity; and contributes to low birth weight. (UNICEF Bangladesh)

Over the past three decades a number of studies including four national nutrition surveys (1962/64; 1975/76; 1981/82 and 1995/96) have been carried out to investigate the prevalence of anaemia among different population groups in Bangladesh, and have demonstrated a significant public health problem. Since the 1975/76 survey the average national prevalence of anaemia has not fallen; in 1995/96, 74% were anaemic (64% in urban areas and 77% in rural areas). However, age-specific comparisons suggest that the rates have fallen in most groups except adult men: in preschool children in rural areas it has decreased by about 30%, but the current level (53%) still falls within internationally agreed high risk levels. Among the rural population, the prevalence of anaemia is 43% in adolescent girls, 45% in non-pregnant women and 49% in pregnant women. The rates in the urban population are slightly lower compared with rural areas, but are high enough to pose a considerable problem. It appears that severe anaemia in the Bangladeshi population is less frequent, possibly present among only 2±3% of the population. The data on the etiology of anaemia reveal that iron deficiency may be a substantial cause of anaemia in the Bangladeshi population. Other dietary factors in addition to parasitic infestations may also precipitate the high prevalence of anaemia. (Ahmed, F 2002)

1.11 Laboratory experiment model:

To accomplish the target the research work commonly six types of pharmacological test is carried out - gastrointestinal effect/side-effect, hypothermia/hyperthermia (to check thyroid involvement), analgesic and anti-inflammatory test, neuropharmacological effects/side-effects, psychopharmacological effect and lastly neurotoxicity. Under each type of test, several experiments are carried out in several doses. In maximum case the dose level is 100mg/kg, 200mg/kg, and 400mg/kg per body weight of the animal model (like *Swiss albino*). The experiments are carried out in multiple dose system to find out the accurate dose at which the therapeutic effect, side effect or toxicity shows in the animal model (*Swiss albino*) in the laboratory.

To check the gastrointestinal effect/side-effect of the experimental drug, three experiments are commonly carried out- gastrointestinal motility, gastrointestinal emptying and colon transit time experiment.

Gastrointestinal (GI) motility test is carried out to find the effect of the experimental drug on the peristaltic movement of the gastrointestinal motility tract. Gastrointestinal motility of the solid nutrient meal measured in experimental mice by minor modifications of the two techniques previously described by Martinez V *et al.* in 2002. Gastrointestinal tract is innervated by both the parasympathetic and the sympathetic fibers of the autonomic nervous system. The peristaltic movement of the GI tract is myogenic in character and is mainly initiated by the local reflexes and can occur without any neural connections to the brain or the spinal cord. Extrinsic nerves to the intestine appear to have only a minor role in modulating the peristaltic activity of the organ. (Chatterjee TK, 1993)

If the drug has any effect on the gastric emptying rate then it can be finding out by this GI emptying experiment. Several researchers have used the method to identify the gastric emptying of the solid nutrient meal. (Martinez V *et al.*, 2002)

The colon transit time test is carried out to assess the effect of the experimental drug on colon. Distal colonic transit time is determined by monitoring the time required for expulsion of the glass bead (bead latency). Martinez *et al.* followed this model in 2002 to check the colon transit time in his work title by "Differential actions of peripheral corticotropin-releasing factor (CRF), Urocortin II, and Urocortin III on Gastric Emptying and Colonic transit in mice: Role of CRF receptor subtypes I and II". (Martinez *et al.*, 2002)

Hypoxia experiment is designed to determine the drug's property to modify the survival time of mice under conditions of hypoxia. The hypoxia induced convulsion onset time is inversely proportionate to the brain oxygen demand. In the earlier time, hypoxia experiment use to done to check the thyroid involvement of drug in laboratory animal model. Several researchers have used the method to identify the thyroid involvement and hypoxic effect of experimental drug. Caillard C *et al.* has used this model to test hypoxia of some anticonvulsant drug in 1975. (Caillard C *et al.*, 1975)

To check the analgesic and anti-inflammatory effect of experimental drug, three types experiment use to carry out. These are - formalin induced paw licking test, xylene induced ear edema test and acetic acid (AA) writhing test. The objective of these groups of the experiment is to confirm the presence or absence of analgesic and anti-inflammatory effect of the experimental drug.

The formalin induced paw licking test is very useful for evaluating the mechanism of pain and analgesia. 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. Several researchers have used the method to identify the analgesic and anti-inflammatory effect of test drug. For an example: Santos *et al.* is used this method to test analgesic effects of callus culture extracts from selected species of *Phyllanthus* in mice in year 1994.

Xylene induced ear swelling test is a very effective and easy way to test the anti-inflammatory property of an experimental drug in laboratory animal model especially in mice. It is established model and several researchers have used the method to identify the analgesic and anti-inflammatory effects of test drug. Tang *et al.* describe this method in year 1984 to check the pain & inflammation reducing activities in his project "Anti-inflammatory effect of 3-acetylaconitine."

To test the existence of non-narcotic analgesic property, acetic acid induced writhing test is carried out. The pain sensation is initiated by using acetic acid. The acetic acid induced writhing is inversely proportionate to the non-narcotic analgesic property. Tang *et al.* use this model in year 1984 to check the pain & inflammation reducing activities of 3-acetylaconitine.

To check the neuropharmacological effects or side-effects of drug, two types of experiment is carried out- hole cross test and hole board test. The hole board test has been conceived to study the behavior of the mouse confronted with a new environment (head plunging stereotype) according to the method devised by Boissier and Simon in 1964, Boissier, Simon and Lwoff in 1964 and Boissier and Simon in 1967.

The hole board 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 is carried out to investigate the effect of the test drug on the exploratory behavior of the laboratory animal model (*Swiss albino*). 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).

The purpose of the hole cross test is to determine the stimulatory or depressive effect of test drug. Increased movement indicates stimulatory activity and decreased movement indicate depressive activity. 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 (Robbins *et al.*, 1977 and Takagi *et al.*, 1971).

Climbing out test is a special type CNS test for making a hypothesis of CNS depressing activities of test drug. In this experiment the decrease in the number of animals climbed out of the cage or an increase in time taken to come out of the cage is directly proportionate CNS depressant property. Sandberg F used this method to make a "comparative quantitative study of the central depressant effect on seven clinically used Phenothiazine derivatives" (Sandberg F. *et al.*, 1957)

The forced induced swimming test (FST) was carried out to find out the anti-depressant property of the experimental drug. It is most widely utilized test for antidepressant action of drug. The traditional version of this test was developed by Roger Porsolt and colleagues (Porsolt R *et al.*, 1977)

Motor performance in mice can be assessed with multiple apparatus and protocols. Use of the rota rod is very common, and it is often used with the apparent assumption of the experiments that it is a straightforward and simple assay of coordination. The rota rod is sensitive to drugs that affect motor coordination. The experiment is use to get a clear picture of the effect of the drugs under consideration on the pattern of behavior, characterized by percentage of fall and number of falling. The "Rota-rod" technique has been originated by a 1957 paper of Dunham and Miya and has proved to be of great value in research. (Nakama *et al*., 1972)

1.12 Purpose of the research:

The main objective of this study was to characterize the effect of ayurvedic iron preparation Nabayas Louha on different physiological systems of animal model. It includes find out the gastrointestinal effects of Nabayas Louha by performing gastrointestinal emptying test, gastrointestinal motility test and distal colon transit time test. To find out the analgesic and anti-inflammatory effects of Nabayas Louha by formalin induced paw licking test, xylene induced ear edema test, acetic acid writhing test. To determine neuropharmacological effects of Nabayas Louha by Hole-board test and Hole-cross test. To investigate the psychopharmacological effects of Nabayas Louha by climbing out test, stair case test, forced induce swimming test and to make a hypothesis of possible toxicity of Nabayas Louha by Rotarod test.

Nabayas Louha is an ayurvedic iron preparation and lots of people use this drug in our country and India. All these studies were performed in an effort to ensure the safety of the general patients/ users of the country as a whole.

Chapter - 2

Formulary

Formula of Nabayas Louha

(Caraka samhita, Cikitsasthana Adhyaya 16; 70-71)

Table 2.1: The formulation of Nabayas Louha:

01	Sunthi (Rz.)	1 part
02	Marica (Fr.)	1 part
03	Pippali (Fr.)	1 part
04	Haritaki (Fr.P.)	1 part
05	Bibhitaka (Fr.P.)	1 part
06	Amalaki (Fr.P.)	1 part
07	Musta (musta) (Rz.)	1 part
08	Vidanga (Fr.)	1 part
09	Citraka (Rt.)	1 part
10	Ayoraja (Lauha bhasma)	9 part

Dose: 250 mg/day

***Important therapeutic use of Nabayas Louha*:**

- ✓ Pandu *(anaemia)*;
- ✓ Hrdroga *(heart disease)*;
- ✓ Kustha *(dermatological diseases)*;
- ✓ Arsa *(haemorrhoids)*;
- ✓ Kamala *(jaundice)*.

Chapter - 3

Materials & Methods

3.1. Collection of the Ayurvedic formulation:

The aim of this research was to find out the effects of Nabayas Louha (NBL) on different physiological systems of animal model. For this the drug Nabayas Louha (Batch no 084) was collected from "Sree Kundeswari Aushadhalaya Ltd", Chittagong, Bangladesh.

3.2. Dose of administration:

For the experiments, the tablets of Nabayas Louha were crushed into powder and made into a solution with distilled water. Then the 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. For all the pharmacological studies the drugs were administered at a dose of 100mg/kg, 200mg/kg and 400mg/kg body weight.

3.3. Route of administration:

For all the pharmacological studies, the drug was administered orally. [Per oral (p.o.) route]

3.4. Experimental laboratory animal:

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 and maintained at natural day night cycle. They were fed with "mouse chow" which was prepared according to the formula developed at Bangladesh Council of Scientific and Industrial Research (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.

3.5. Control group:

A group of equal number of mice as the drug treated group was simultaneously employed in the experiment. They were administered with distilled water as par the same volume as the drug treated group and this group served as the control. Six to ten mice were taken for each group for both the control and the experiment group.

3.6. Doses used in different experiments:

- For Gastric emptying test: 100mg/kg body weight.
- For GI motility test: 100mg/kg body weight.
- For colon transit time test: 100mg/kg body weight.
- For hypoxia test: 100, 200 and 400mg/kg body weight.
- For formalin test: 100mg/kg body weight.
- For Xylene induced ear edema in mice: 100mg/kg body weight.
- For Acetic acid writhing test: 100 and 400mg/kg body weight.
- For Hole board test: 100, 200 and 400mg/kg body weight.
- For Hole cross test: 100, 200 and 400mg/kg body weight

- For Climbing out test: 100, 200 and 400mg/kg body weight.
- For Stair case test: 100mg /kg body weight.
- For Forced swim test: 100mg/kg body weight.
- For Rota rod test: 100mg/kg body weight.

3.7 Gastric emptying measurement:

Materials: 40 mice, 40 plastic cases for observation, electronic balance (Shimadzu, Japan), distilled water, feeding needle, experimental drug, cotton and tissue paper, scissor and forceps etc.

Method: Forty Swiss-Webster male mice were fasted for 18 hours prior to the experiment. Out of the 40, 20 were randomly chosen as the test drug group and the remaining 20 as the control group. Fasted animals had free access to water and pre-weighed solid food (solid: water ratio being 60:40) for a period of 1 hour. At the end of the 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. Immediately after the 1 hour feeding period, test drug was orally administered to the mice of drug group at 100mg/kg (1x doses) while their control group counterparts were fed distilled water. 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 with tap water and the remaining gastric wall was 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. Percent gastric emptying (% GE) was calculated as-

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

3.8. Gastro-intestinal motility test:

Materials: $BaSO_4$ (Merck, India) for preparing $BaSO_4$ milk suspension, Sodium CMC (Merck, India), 128 male mice, feeding needle, a large scale for measuring the intestine dissecting tool box etc.

Method: $BaSO_4$ milk was prepared by adding $BaSO_4$ at 15% w/v in 0.5% CMC suspension. The milk was given to a group of 12 mice 15 minutes after the administration of the Nabayas Louha. The treated mice were divided into two sub-groups and were sacrificed after 15 and 30 minutes after the administration of the milk. The distance traversed by $BaSO_4$ milk were measured and expressed as a percentage of the total length of small intestine (from pylorus to the ileoceccal junction). The test drug was compared with the control group administered with distilled water.

3.9. Colon Transit Time test:

Materials: Glass bead, rat feeding needle, stopwatch, 40 female mice, 20 plastic mice cases, marker etc.

Method: One hour after drug administration, a single glass bead, 2 mm in diameter was inserted into the distal colon of each mouse at 2 cm from the anus, after which the mice were returned to their respective cages and observed closely. Distal colonic transit time was determined by monitoring the time required for expulsion of the glass bead (bead latency).

3.10. Hypoxia test:

Materials: 20 empty glass gar of 300 ml capacity, paraflim paper, grease, stopwatch, 20 mice, feeding needle, distilled water etc.

Method: Two set of ten mice per groups were used for hypoxia experiment. 2 hr after the treatment, the hypoxia time was recorded individually for all the animals. The animals (mice) were placed in an empty glass jar of 300 ml capacity jar and the jars were made air tight with greased glass stoppers and the time until the onset of convulsion was recorded with the help of stopwatch.

3.11. Formalin induced paw licking test:

Materials: 20 female mice, distilled water, feeding needle, 10 glass jar, electronic balance (Shimadzu, Japan), micro litter syringe (Hamilton, Switzerland), formalin (BDH, England), methanol (VWR, England), measuring cylinder, stopwatch.

Method: Formalin 1% was administered to mice by intraplantar route (IP), and immediately the licking time was registered for 5 minute (first phase, neurogenic). 15 (fifteen) minutes after the beginning of the experiment (second phase, inflammatory) the licking time was

registered for other 5 min. Experimental drug was administered orally 120 minute (p.o.) before the formalin injection.

3.12. Xylene induced ear edema test:

Materials: 20 female mice, xylene (BDH, England), electronic balance (Shimadzu, Japan), micro liter syringe (Hamilton, Switzerland), methanol (VWR, England), distilled water, feeding needle, scissor, experimental drug, measuring cylinder, and stopwatch.

Method: Male Swiss mice were divided into two groups of ten mice each. After 30 min of the p.o. of Nabayas Louha, xylene (0.03 ml) was applied to the anterior and posterior surfaces of the right ear. Mice were sacrificed 2 hour after xylene application and both ears were removed. 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.

3.13. Acetic Acid induced writhing test:

Materials: Acetic acid (COO, Germany), methanol (VWR, England), 20 mice, 10 separate mice case for observation, sterile syringe, feeding needle, injection needle, counter.

Method: Acetic acid (AA) induced abdominal writhing assay (non-narcotic analgesic activity) muscular contraction was induced by the intraperitonial injection of 0.6% acetic acid (0.25ml/animal). The test preparations were administered orally 45 minutes before the intraperitonial injection of 0.6% 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. The population of control group was 10 and drug group is also 10

3.14. Hole Board test:

Materials: 12 mice, hole board (a board contain total of 16 holes, each 3 cm in diameter, were presented to the mouse in a flat space of 25 square centimeters.), stopwatch, cotton, methanol (VWR, England), feeding needle, counter.

Method: 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.

3.15. Hole Cross test:

Materials: 12 mice, hole cross instrument (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), stopwatch, cotton, methanol (BDH, England), feeding needle, counter.

Method: 12 mice ware taken for the experiment. 6 mice were for control and 6 mice were for drug group. 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.

3.16. Climbing Out Test:

Materials: 20 mice, a cage with dimension of 60 X 50 X 30 cm and having dark walls, feeding needle, stopwatch.

Method: 20 mice ware taken for the experiment. 10 mice were for control and 10 mice were for drug group. The animals were put in climbing out cage. Animals were supplied with a ladder and the time taken to climbs out of the cage was recorded for a maximum period of 10 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.

3.17. Staircase Test:

Materials: 20 mice, methanol (VWR, England), stopwatch, feeding needle, cotton, and staircase (The apparatus consists of a white PVC enclosure with a five-step staircase. The box is placed in a room with constant lighting, isolated from external noise, and thermostatically controlled).

Method: Male mice weighing 21± 3g were used in these studies. The day before the test, the animals were randomly divided into groups of 20 mice in plastic cages. All the animals for a single experiment where placed at the same height in the animal house. They were transferred to the laboratory at least 1 hour before the start of the test. Each animal was used only once.

The animal was placed singly on the floor of the box with its back to the staircase. The number of steps climbed and the number of rears were counted over a 3-min period. A step was considered to be climbed only if the mouse had placed all four paws on the step. The number of steps descended was not taken into account, in order to simplify the observations. After each animal had been tested, the box was rapidly cleaned to eliminate any olfactory cue which might modify the next animal's behavior. Experimental drugs were administered orally (p.o.) (100mg/kg) 60 min before the test to groups of 10 mice. In each experiment, a control group received only distilled water. The treatments were randomized, and the observer was unaware of the treatment given to each group (blind method). All studies were carried out between 8 a.m. and 5 p.m.

3.18. Forced induce swim Test:

Materials: 20 mice, stopwatch, cotton, glass case, feeding needle, and a large box made by glass for swimming.

Methods: The most widely utilized animal model of antidepressant action is the forced swim test (FST). The traditional version of this test was developed by Roger Porsolt and colleagues and comprises exposing mice to a 15-min pre-swim 24 h before a 5-min test exposure in 15–18 cm of 25oC water. Following an initial period in which the mice produces escape-directed behaviors, it will adopt an immobile posture, which is believed to reflect either a failure to persist with escape-directed behavior or a passive behavior to cease active forms of coping to the stressful stimuli. A wide range of clinically effective antidepressants have been shown to increase the time that the rat spends in active escape behaviors.

3.19. Rotarod test with constant speed model:

Materials: 20 mice, Rotarod apparatus (Techno, Lucknow, India), counter, feeding needle.

Method: It basically consist of five 3 cm diam. drums, suitably machined to provide grip. The above drums, whose angular speed can be varied by a simple belt gear, turn on ball bearings. They are driven by a heavy duty D.C. motor which sets the rotors in motion via the belt gear at the speed selected (16-20-24-28-32 r.p.m.). Six flanges divide the drums, enabling five mice to be on the treadmill simultaneously. When a mouse falls off its cylinder section on to the plate below, the plate trips (trip at one second intervals) and the corresponding magnetic static-switch is activated thus the counter is disconnected, thereby recording the animal's endurance time in seconds. At the end of a run, the display shows for each animal the running time and the instrument rotation speed at the time that animal fell off.

3.20. 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™ (Version 14) was applied for the analysis of data. $p \leq 0.05$ was taken to be the level of significance, $p \leq 0.01$ was taken to be the level of highly significance, $p \leq 0.001$ was taken to be the level of very highly significance.

P-value determines the appropriateness of rejecting the null hypothesis in a hypothesis test. P-values range from 0 to 1. Smaller the p-value, the smaller the probability that rejecting the null hypothesis is a mistake.

Chapter -4

Result & Discussion

4.1 Gastric Emptying test:

Statistical findings and Discussion:

Nabayas Louha (NBL) treated male mice at dose 100mg/kg exerted increase in Gastric emptying at 2nd hour compare to respective control group which is statically significant (p=0.018*).

But in case of 4th hour NBL treated mice exerted negligible decrease in GI emptying compare to control group. But this result was not statistically significant ($p<0.05$)

The difference in % of Gastric emptying between the NBL treated group and the control group with the time lapsed is summarized in a numerical form as follows:

After 2nd hour = (92.22-86.68) = 7.53 % Increase

After 4th hour = (91.31- 92.86) = -1.55 % Decrease

Here

(-) =Decrease (+) =Increase

Tabular and graphical presentation of the effect of NBL (100mg/kg) on the gastric emptying test utilizing male mice:

Table 4.1.1: Effect of NBL (100mg/kg) on gastric emptying test after 2nd hour study:

Group		% GE (Mean ± SEM)
Control (n=10)		86.6830 ± 2.44775
NBL(n=10)		94.2170 ± 1.36399
t/p		-2.689 / **0 .018***
95% confidence interval	Lower	-13.54006
	Upper	-1.52794

N.B: *(≤ 0.05) = Significant

Figure 4.1.1: Graphical presentation of the effect of NBL (100mg/kg) on gastric emptying test after 2nd hour study

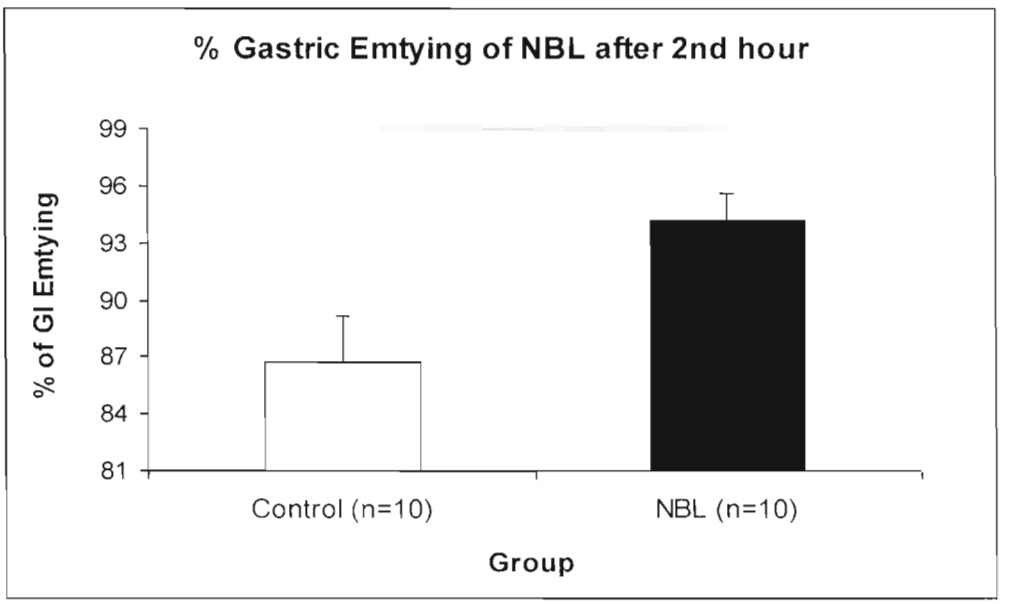


Table 4.1.2: Effect of NBL (100mg/kg) on gastric emptying test after 4th hour Study:

Group		% GE (Mean ± SEM)
Control (n=10)		92.8600 ± 1.05541
NBL(n=10)		91.3130 ± 1.46776
t/p		0.856 / 0 .403
95% confidence interval	Lower	-2.25108
	Upper	5.34508

Figure 4.1.2: Graphical presentation of the effect of NBL (100mg/kg) on gastric emptying test after 4th hour study:

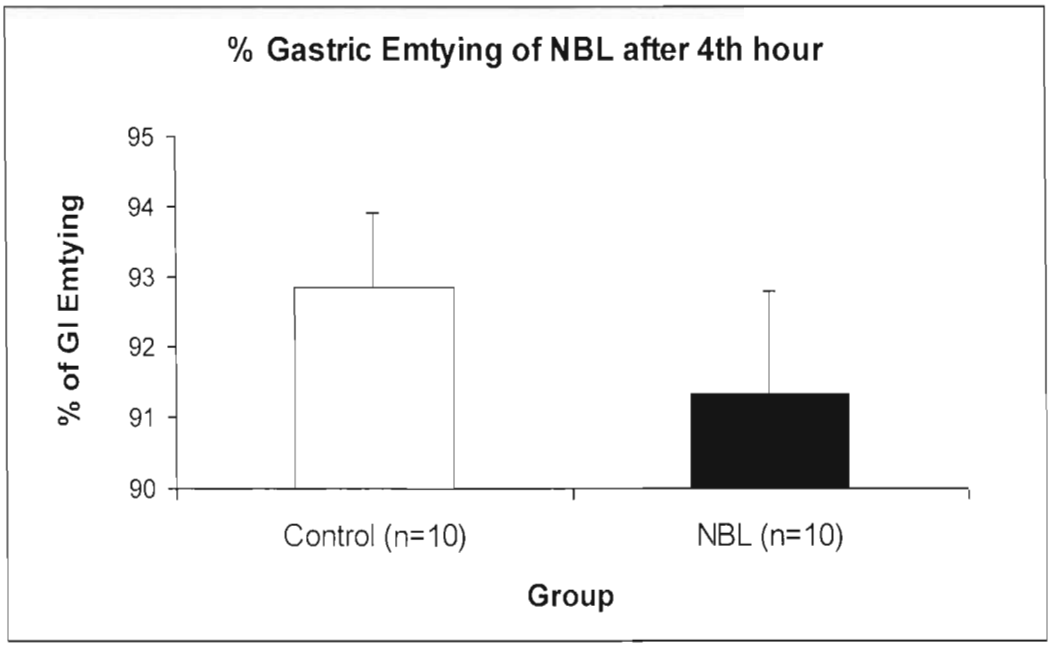


Figure 4.1.3: Graphical presentation of over all effect of NBL (100mg/kg) on Gastric emptying test from 2nd hour and 4th hour study

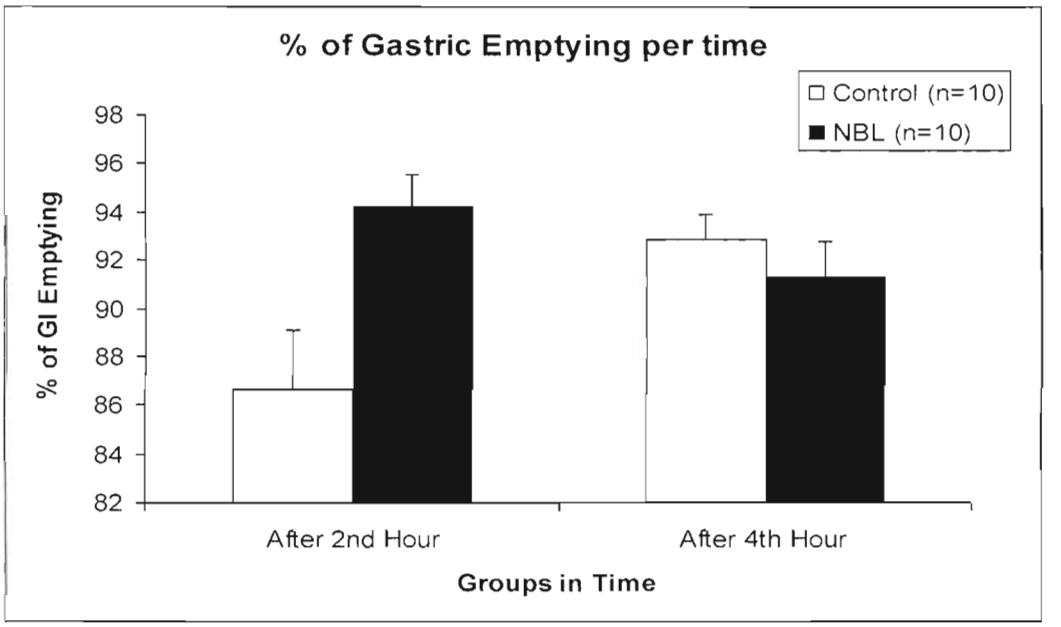
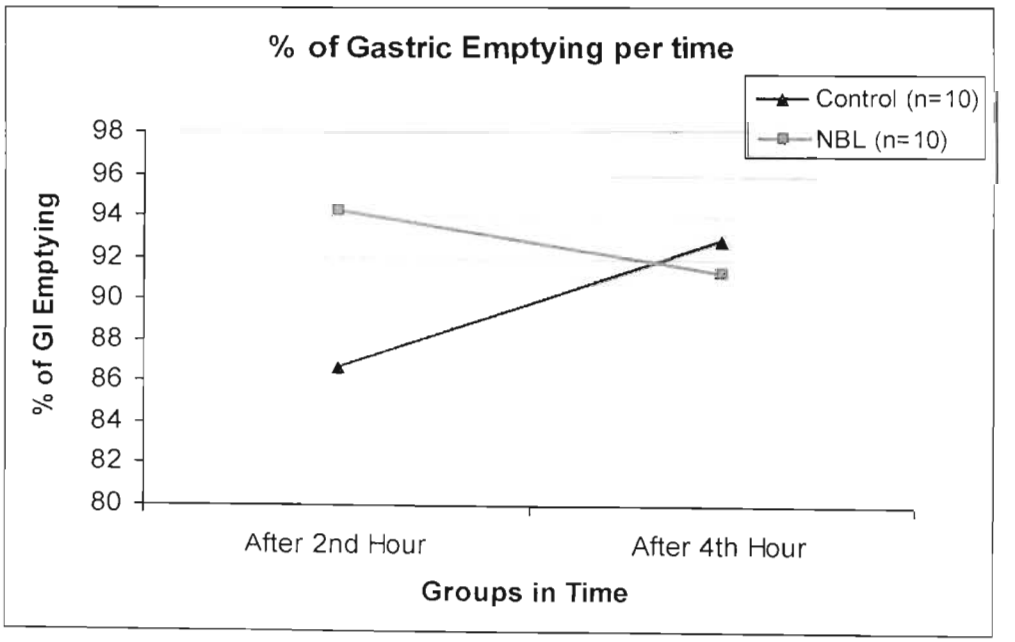


Figure 4.1.4: Line chart of over all effect of NBL (100mg/kg) on gastric emptying test from 2nd hour and 4th hour study



4.2 Gastrointestinal Motility Test:

***Statistical findings and Discussion*:**

In the Gastro intestinal motility test NBL treated male mice at dose 100mg/kg, showed an mixed gut motility in 1st hour, decreased in GI motility effect in 2nd hour followed by an increase in the 3rd and 4th hour.

At 1st hour

After 15 minutes study:

As evident from the table below, NBL is found to increase the gut motility of the experimental male mice in the 15 minutes study, but the increase (p=0.868) was statistically insignificant.

After 30 minutes study:

In the 30 minutes study, NBL treated male mice showed almost same intensity of gut motility which was statistically insignificant.

At 2nd hour

After 15 minutes study:

As evident from the table below, NBL is found to decrease (p=0.335) the gut motility of the experimental male mice in the 15 minutes study and the increase was statistically not significant.

After 30 minutes study:

Followed by the 15 minutes study, in 30 minutes study, NBL treated male mice also showed decreased gut motility effect compare to the corresponding control group. The results was not statistically significant but was **statistically noticeable *(p=0.081)***

At 3rd hour

After 15 minutes study:

In the 15 minutes study, NBL treated male mice showed almost same intensity of gut motility effect of slightly increased (p=0.658) effect which was not statistically significant.

After 30 minutes study:

In the 30 minutes study, the drug NBL was found to increase (p=0.448) the gastrointestinal motility of the experimental male mice compare to the corresponding control group. But the result was not statistically significant.

At 4th hour

After 15 minutes study:

In the 15 minutes study, NBL treated male mice showed almost same intensity (p=0.918) of gut motility which was not statistically significant.

After 30 minutes study:

In the 30 minutes study, the drug NBL was found to increase (p=0.499) the gastrointestinal motility of the experimental male mice compare to the corresponding control group. But the result was not statistically significant.

Tabular and graphical presentation of the effect of NBL (100 mg/kg) on the gastrointestinal motility test utilizing male mice

Table 4.2.1: The effect of NBL (100 mg/kg) on gastrointestinal motility test after 1st hour 15 minutes study period

Group		% Traversed ± S.E.M.
Ctrl (n=8)		55.1385±4.12379
NBL(n=8)		59.2901±2.96072
t/p value		-0.818/0.427
95% confidence interval	Lower	-15.03966
	Upper	6.73661

Figure 4.2.1: Graphical presentation of the effect of NBL (100 mg/Kg) on gastrointestinal motility test after 1st hour 15 minutes study.

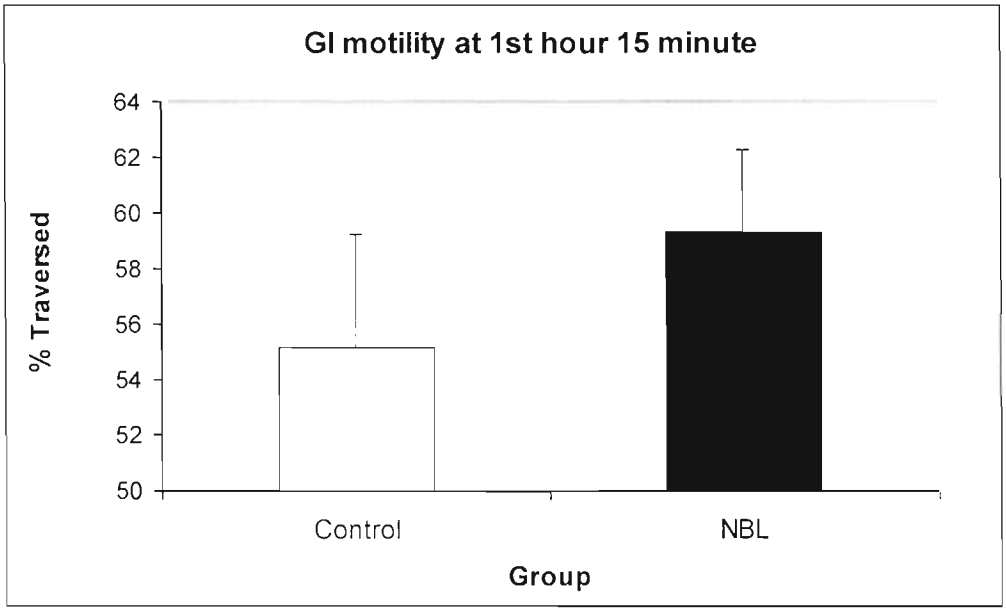


Table 4.2.2: The effect of NBL (100 mg/kg) on gastrointestinal motility test after 1st hour 30 minutes study period

Group		% Traversed ± S.E.M.
Ctrl (n=8)		74.5253±2.76326
NBL(n=8)		73.7748±3.46462
t/p value		0.169/0.868
95% confidence interval	Lower	-8.79869
	Upper	10.29964

Figure: 4.2.2: Graphical presentation of the effect of NBL (100 mg/Kg) on gastrointestinal motility test after 1st hour 30 minutes study.

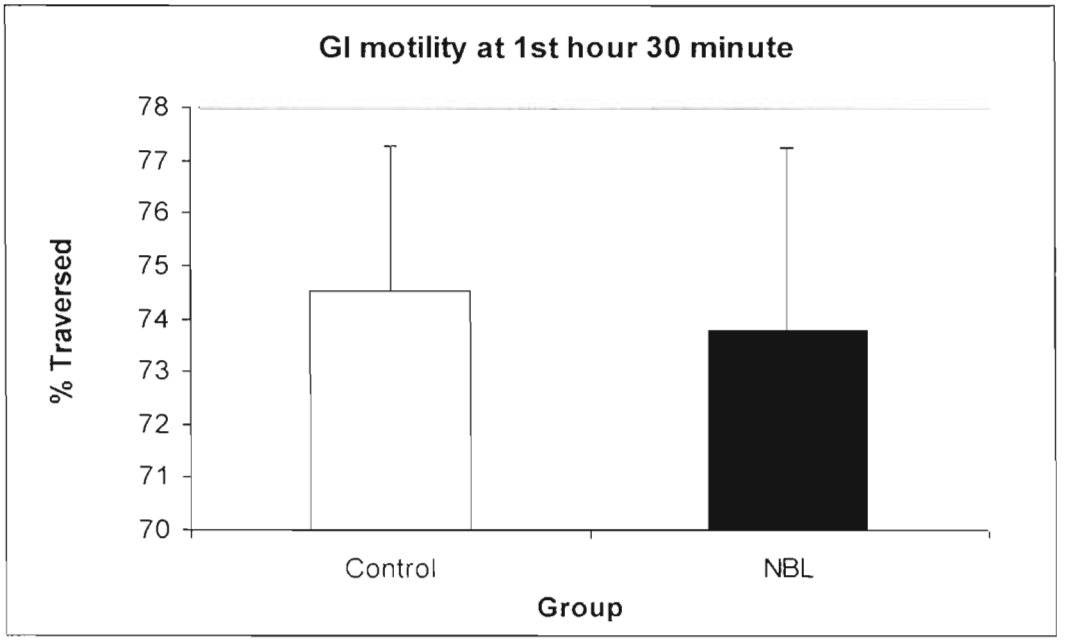


Table 4.2.3: The effect of NBL (100 mg/kg) on gastrointestinal motility test after 2nd 15 minutes study period

Group		% Traversed ± S.E.M.
Ctrl (n=8)		59.3751±3.91100
NBL(n=8)		54.5533±2.83897
t/p value		0.998/0.335
95% confidence interval	Lower	-5.54347
	Upper	15.18703

Figure 4.2.3: Graphical presentation of the effect of NBL (100 mg/Kg) on gastrointestinal motility test after 2nd hour 15 minutes study

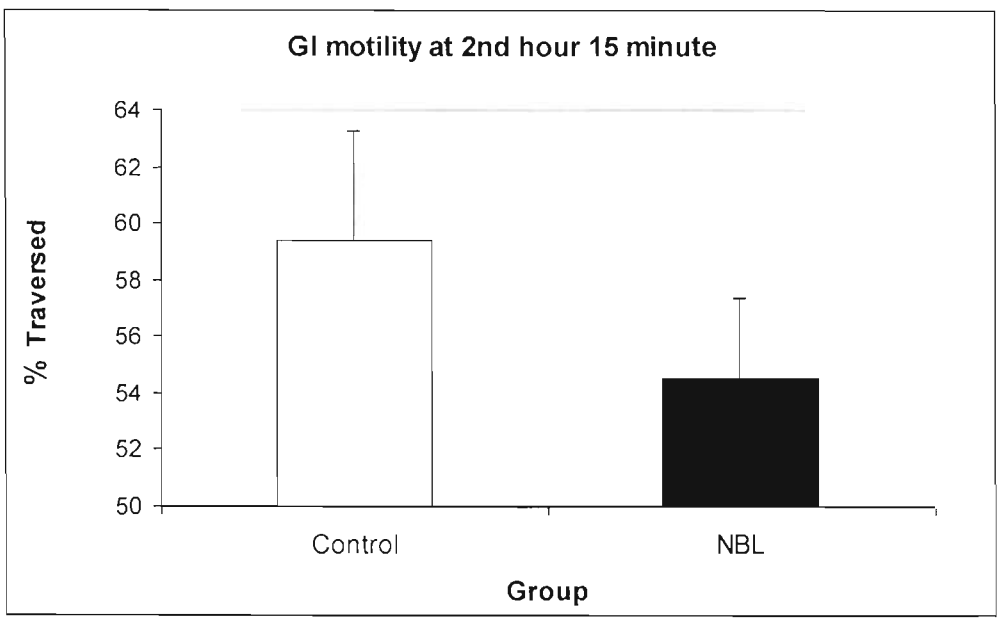


Table 4.2.4: The effect of NBL (100 mg/kg) on gastrointestinal motility test after 2nd hour 30 minutes study period

Group		% Traversed ± S.E.M.
Ctrl (n=8)		74.6946±2.24268
NBL(n=8)		69.1180±1.94187
t/p value		1.880/**0.081**
95% confidence interval	Lower	-0.78608
	Upper	11.93921

Figure: 4.2.4: Graphical presentation of the effect of NBL (100 mg/Kg) on gastrointestinal motility test after 2nd hour 30 minutes study.

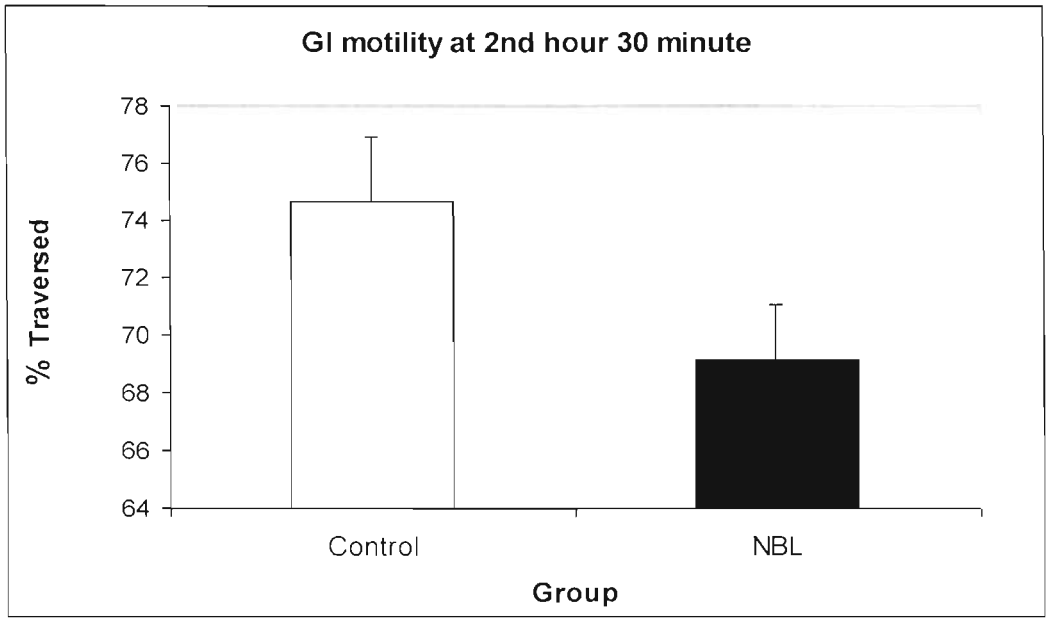


Table 4.2.5: The effect of NBL (100 mg/kg) on gastrointestinal motility test after 3rd hour 15 minutes study period

Group		% Traversed ± S.E.M.
Ctrl (n=8)		57.1993±2.33576
NBL(n=8)		58.4188±1.34400
t/p value		-0.453/0.658
95% confidence interval	Lower	-6.99929
	Upper	4.56039

Figure 4.2.5: Graphical presentation of the effect of NBL (100 mg/Kg) on gastrointestinal motility test after 3rd hour 15 minutes study.

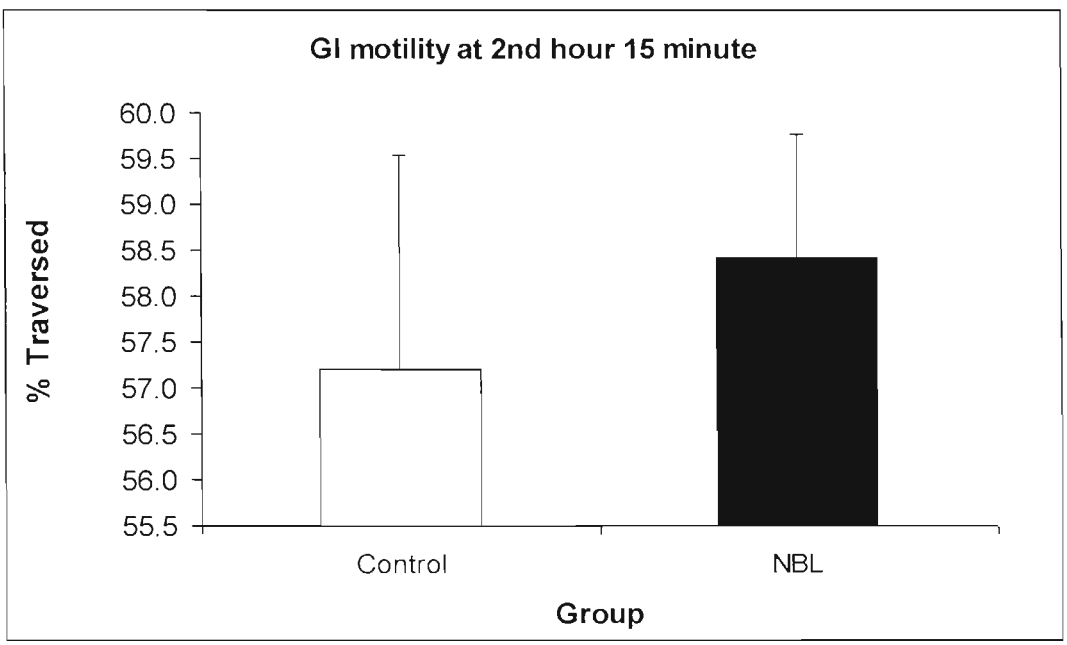


Table 4.2.6: The effect of NBL (100 mg/kg) on gastrointestinal motility test after 3rd hour 30 minutes study period

Group		% Traversed ± S.E.M.
Ctrl (n=8)		73.9856±3.63145
NBL(n=8)		77.8904±3.44709
t/p value		-0.780/0.448
95% confidence interval	Lower	-14.64646
	Upper	6.83680

Figure 4.2.6: Graphical presentation of the effect of NBL (100 mg/Kg) on gastrointestinal motility test after 3rd hour 30 minutes study.

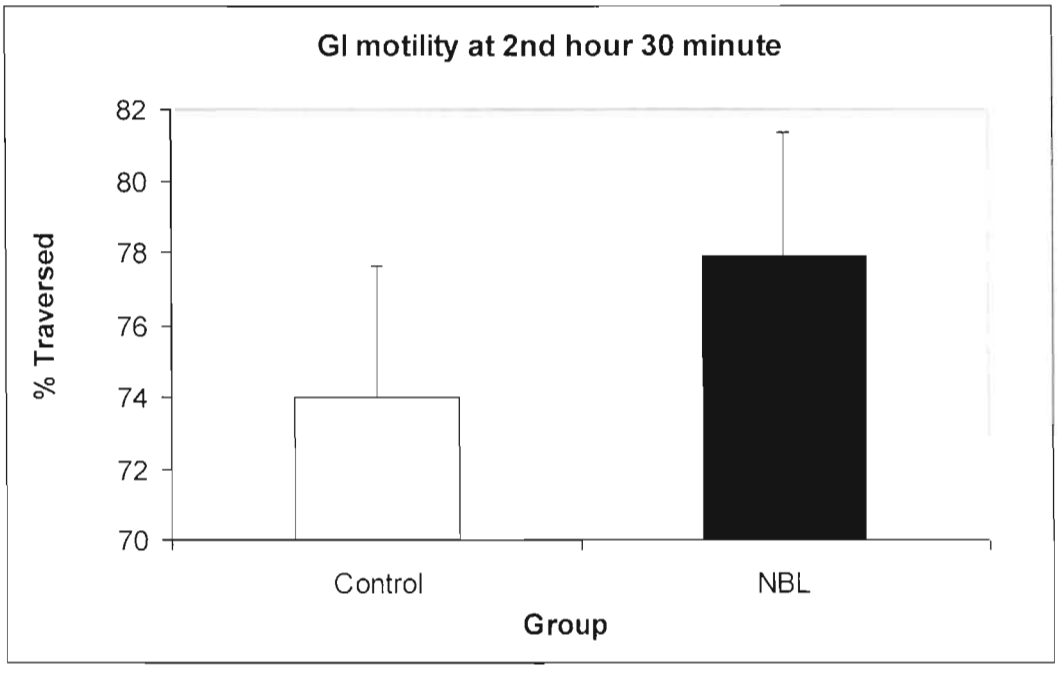


Table 4.2.7: The effect of NBL (100 mg/kg) on gastrointestinal motility test after 4th hour 15 minutes study period

Group		% Traversed ± S.E.M.
Ctrl (n=8)		63.9822±4.37869
NBL(n=8)		64.6695±4.88466
t/p value		-0.105/.918
95% confidence interval	Lower	-14.75698
	Upper	13.38239

Figure: 4.2.7: Graphical presentation of the effect of NBL (100 mg/Kg) on gastrointestinal motility test after 4th hour 15 minutes study.

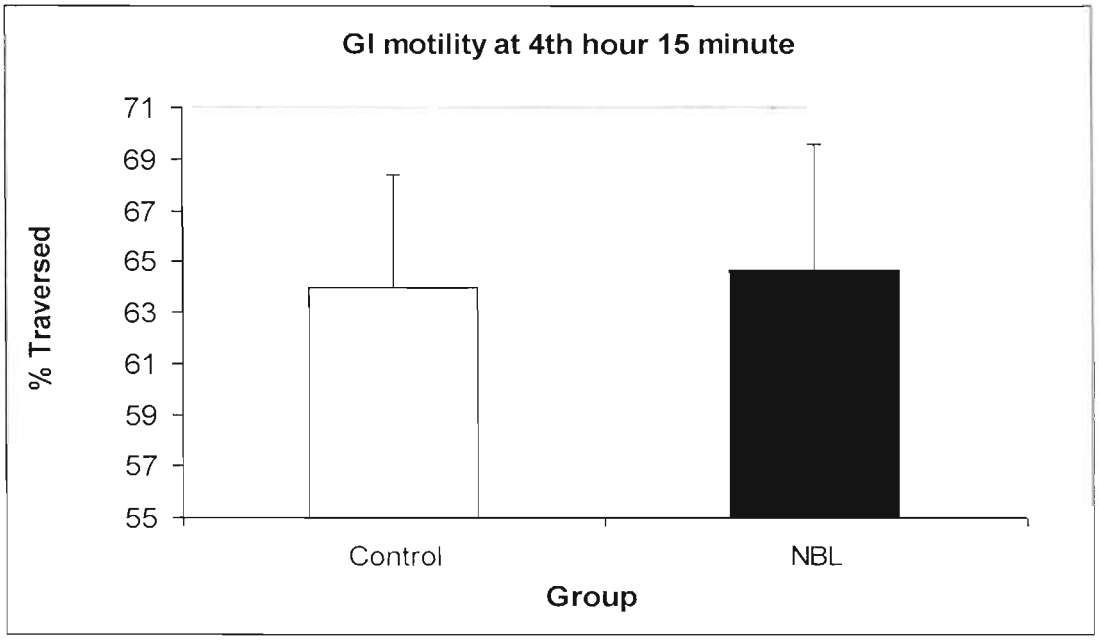


Table 4.2.8: The effect of NBL (100 mg/kg) on gastrointestinal motility test after 4th hour 30 minutes study period

Group		% Traversed
Ctrl (n=8)		80.4209±2.63495
NBL(n=7)		84.2941±4.83977
t/p value		-0.703/0.499
95% confidence interval	Lower	-16.26287
	Upper	8.51637

Figure 4.2.8: Graphical presentation of the effect of NBL (100 mg/Kg) on gastrointestinal motility test after 4th hour 30 minutes study.

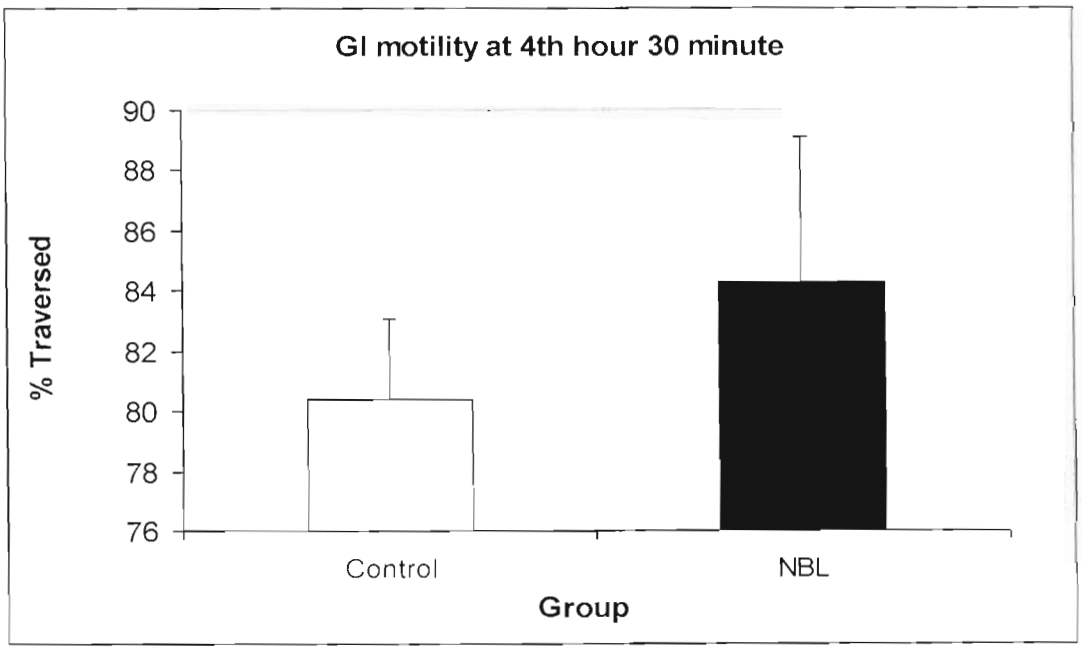


Figure 4.2.9: Line Graphical presentation of the effect of NBL (100mg/Kg) on gastrointestinal motility test from 1st to 4th hour study period

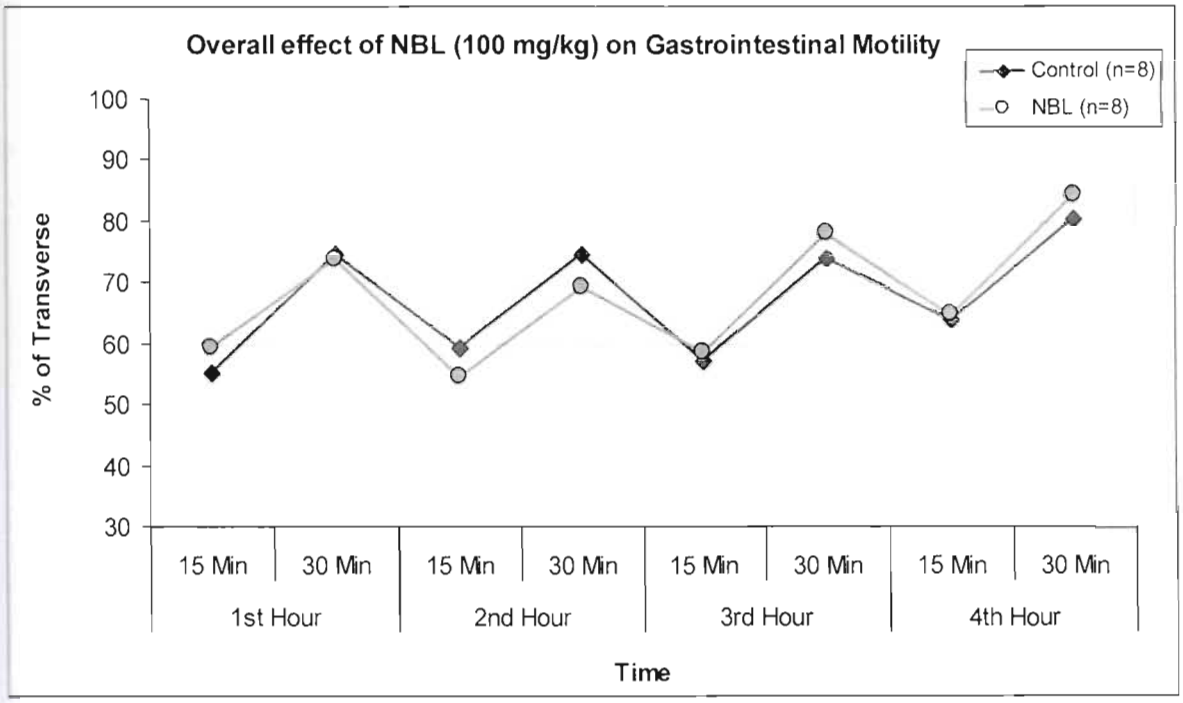
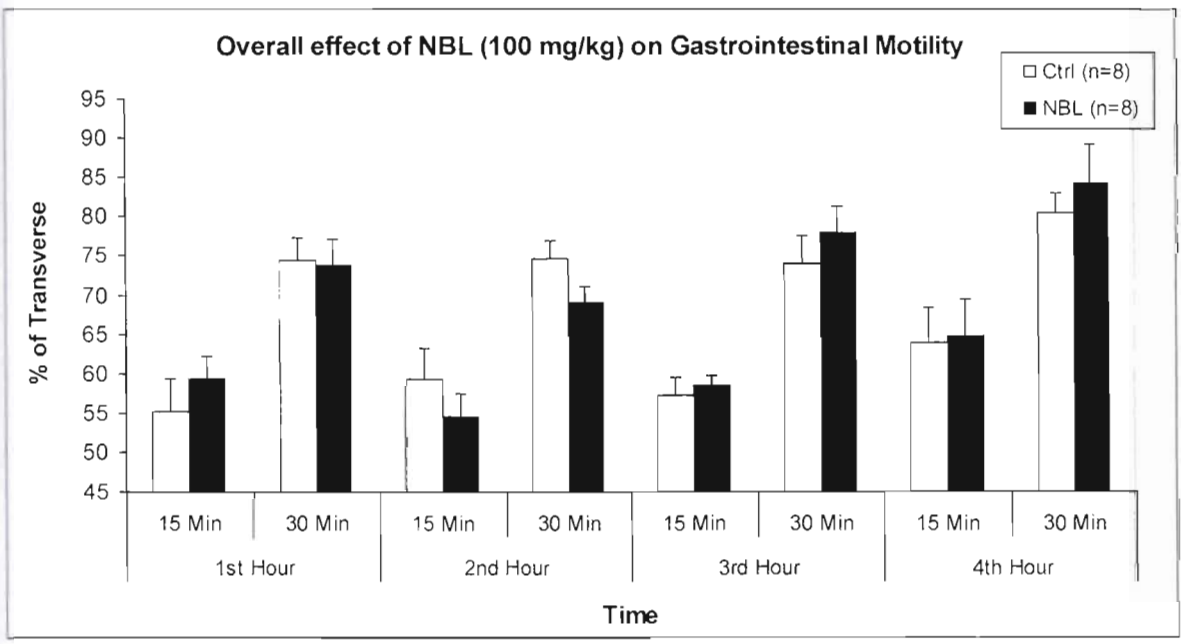


Figure 4.2.10: The overall effect of NBL (100 mg/kg) on gastrointestinal motility test from 1st to 4th hour study period.



4.3 Colon Transit time test:

***Statistical findings and Discussion*:**

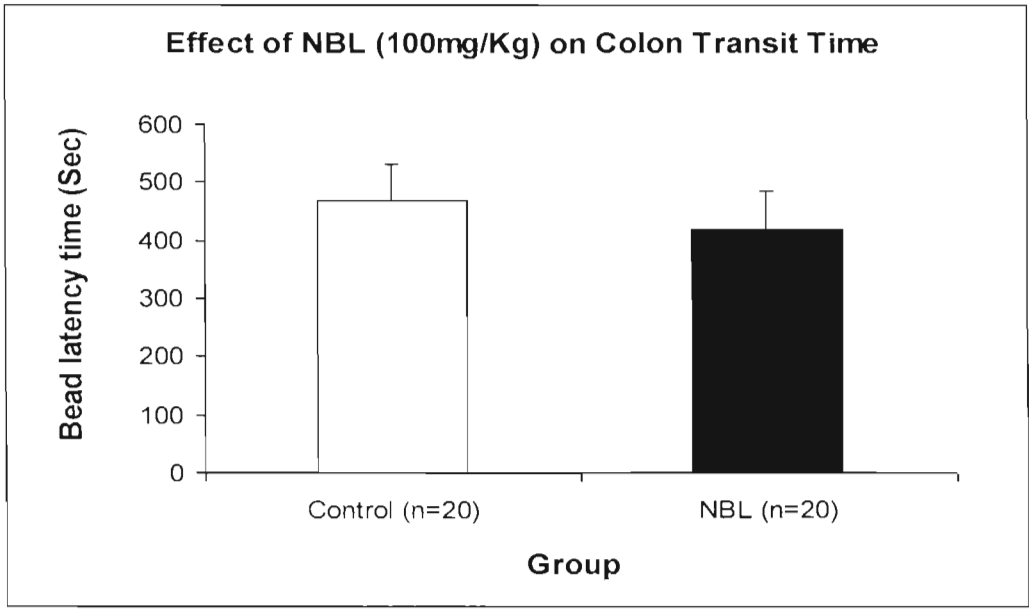
The drug NBL treated male mice (25-30g) at dose level of 100mg/kg showed decrease in bead latency time (p=0.581) compared to the control group which is not statistically significant.

Tabular and graphical presentation of the effect of NBL (100mg/kg) on the colon transit time test utilizing male mice

Table 4.3.1: The effect of NBL (100mg/kg) on colon transit time Test.

Group		Bead latency time (Second) ± SEM
Control (n=20)		468.83±62.443
NBL (n=20)		418.20±66.216
t/p		0.556/0.581
95% confidence interval	Lower	-133.953
	Upper	235.219

Figure 4.3.1: Graphical presentation of the effect of NBL (100mg/Kg) on Colon transit time Test.



4.4 Hypoxia Test:

***Statistical findings and Discussion*:**

NBL treated male mice at three dose levels (100 mg/kg, 200 mg/kg, and 400 mg /Kg) to check is there is an effect on Hypoxia time.

At dose 100mg/Kg and 400mg/Kg, NBL treated male mice exerted an increase (p=0.134 and 0.249 respectively) in the survival time compare with the control group and decrease (p=0.341) in the survival time compare with the control group.

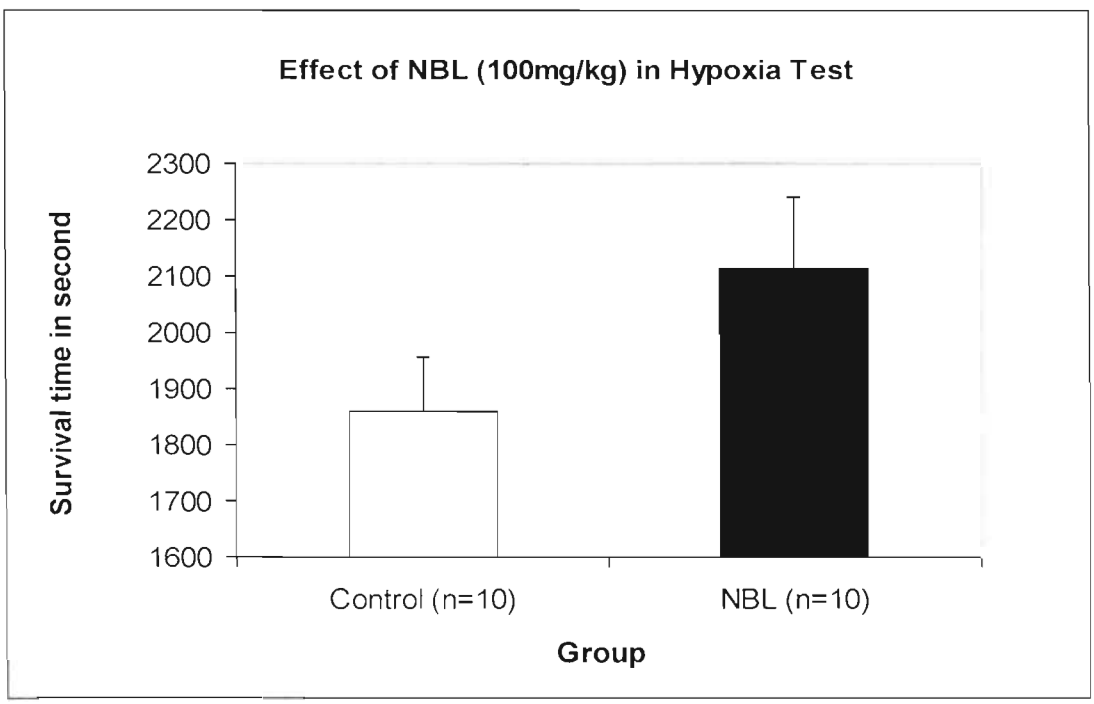
But the result was not statistically significant.

Tabular and Graphical presentation of the effect of NBL (100mg/kg) on the hypoxia test utilizing male mice.

Table 4.4.1: The effect of NBL (100mg/kg) in the hypoxia test

Group		Mean Survival Time (sec) ± SEM
Control (n=10)		1858.80±98.563
NBL(n=10)		2111.80±127.811
t/p		-1.568/0.134
95% confidence interval	Lower	-592.090
	Upper	86.090

Figure 4.4.1: Graphical presentation of the effect of NBL (100mg/kg) on the Hypoxia Test

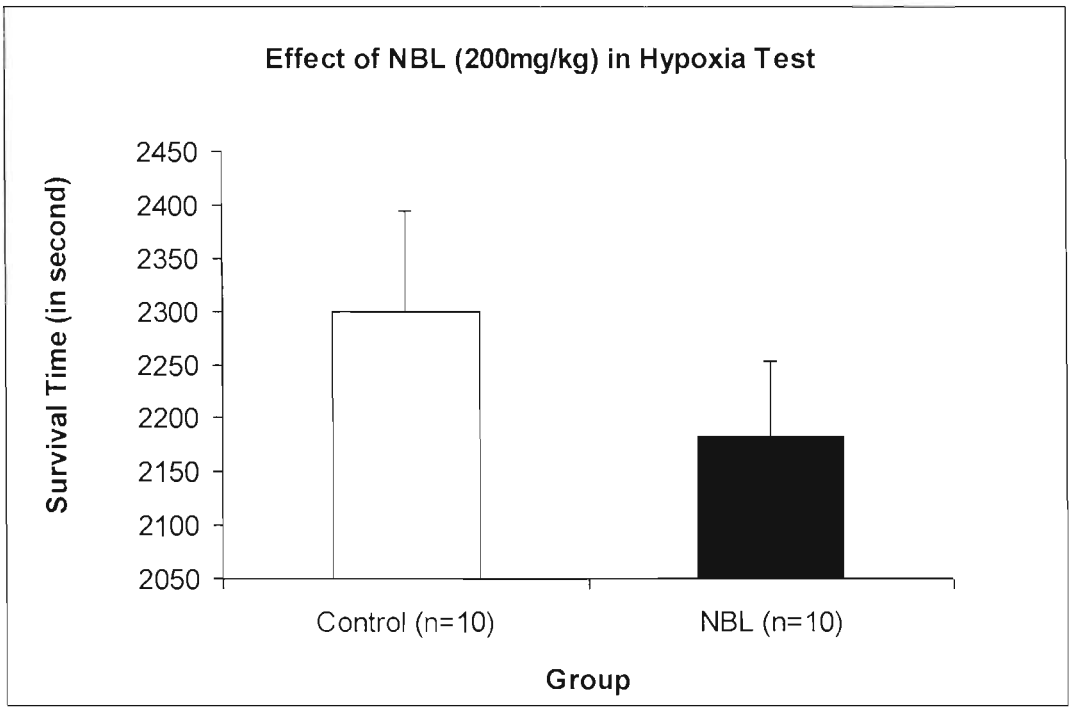


Tabular and graphical presentation of the effect of NBL (200mg/kg) on the Hypoxia Test utilizing Male mice

Table 4.4.2: The effect of NBL (200mg/kg) in the Hypoxia Test

Group		Mean Survival Time (sec) ± SEM
Control (n=10)		2299.00±95.027
NBL(n=10)		2183.40±69.673
t/p		0.981/0.341
95% confidence interval	Lower	-133.571
	Upper	364.771

Figure 4.4.2: Graphical presentation of the effect of NBL (200mg/kg) in Hypoxia Test



Tabular and graphical presentation of the effect of NBL (400mg/kg) on the Hypoxia test utilizing male mice

Table 4.4.3: The effect of NBL (400mg/kg) in the hypoxia test

Group		Mean Survival Time (sec) ± SEM
Ctrl(n=10)		1669.20±68.8
NBL(n=10)		1781.80±64.812
t/p		-1.191/0.249
95% confidence interval	Lower	-311.179
	Upper	85.979

Figure 4.4.3: Graphical presentation of the effect of NBL (400mg/kg) in Hypoxia Test

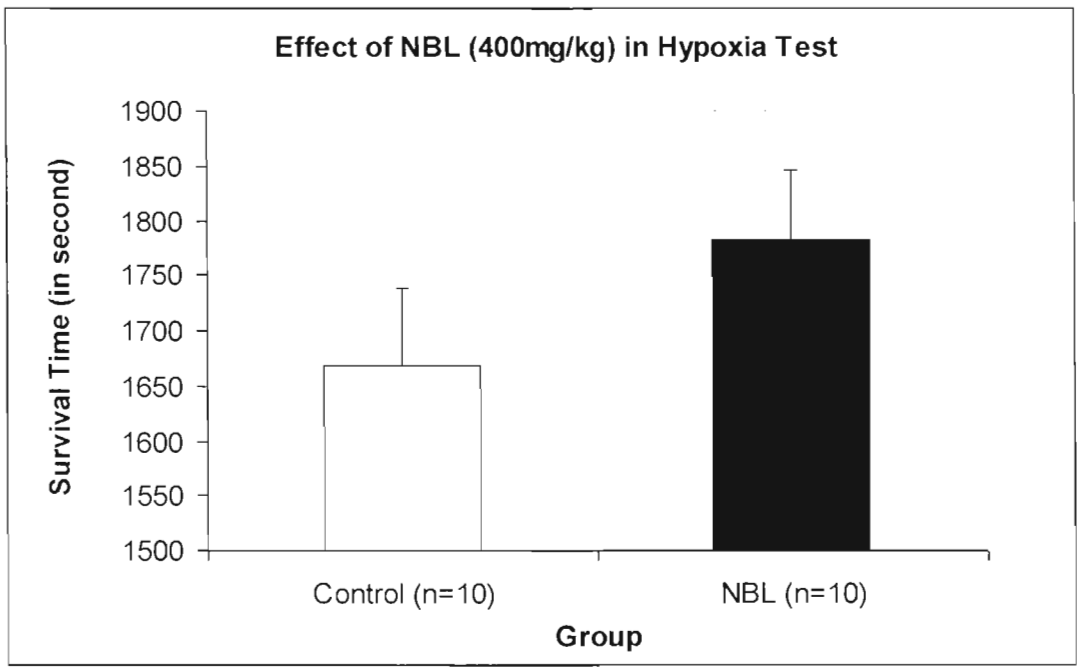


Figure 4.4.4: Graphical presentation of the overall effect of NBL (100mg/Kg, 200mg/Kg, 400mg/Kg) Hypoxia Test

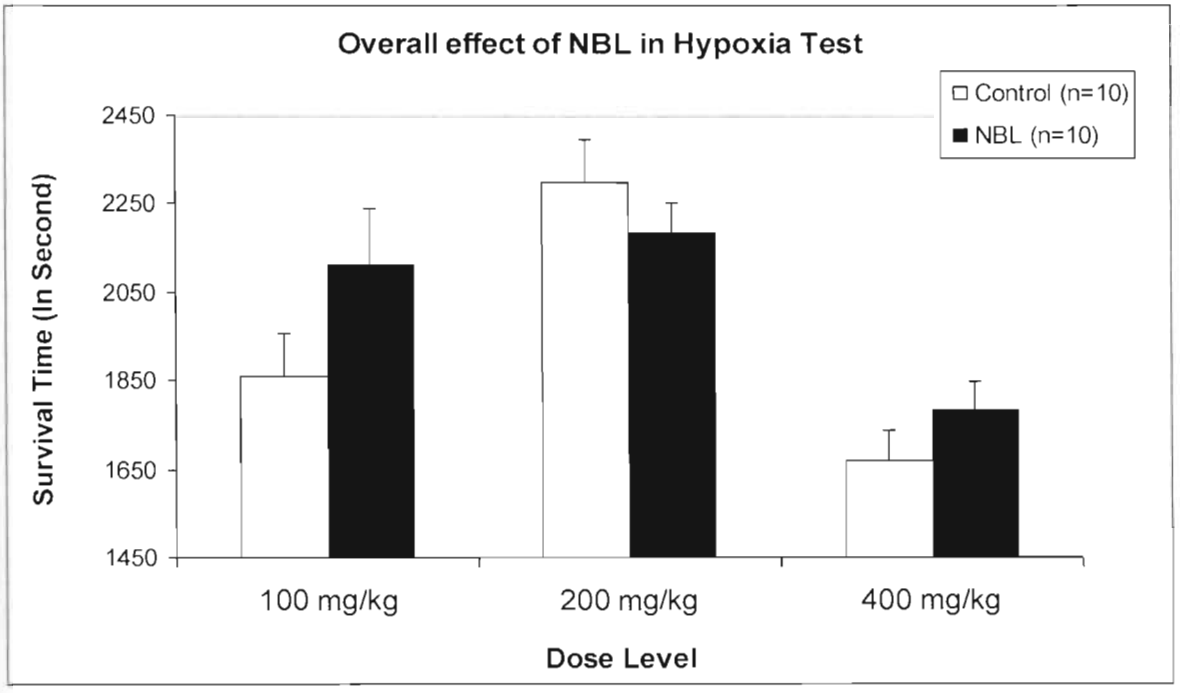
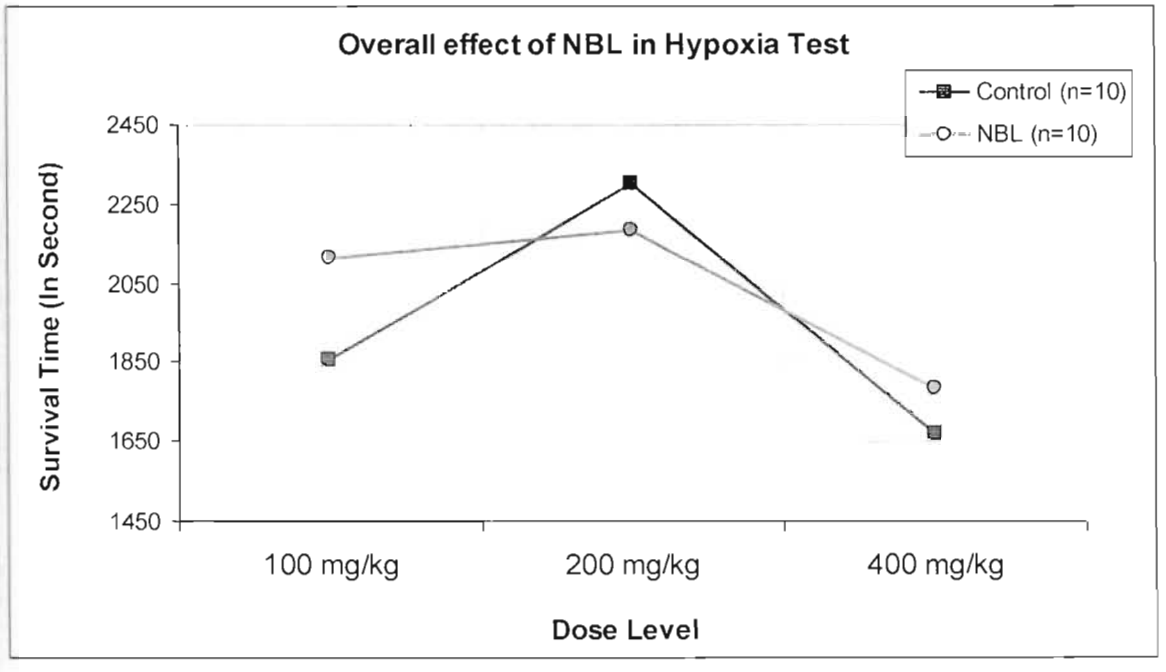


Figure 4.4.5: Line graphical presentation of the effect of NBL (100mg/Kg, 200mg/Kg. 400mg/Kg) Hypoxia Test in different dose.



4.5 Formalin induced paw licking test:

***Statistical findings and Discussion*:**

NBL at dose 100 mg/kg exerted an increase in analgesic activity (p=0.765) and very mildly exerted anti- inflammatory activity (p=0.807) in male mice compared to the respective control group but none of the results were statistically significant.

Tabular and Graphical presentation of the effect of NBL (100mg/kg) on the Formalin Induced Paw licking (Analgesic + Inflammation) Test utilizing Male mice

Table 4.5.1: The effect of NBL (100 mg/kg) in the Formalin Induced Paw licking Test

Group		Analgesic (1st Phase)	Inflammation (2nd Phase)
Ctrl (n=10)		66.70±7.545	4.40±2.455
NBL(n=10)		69.80±6.905	3.60±2.083
t/p		-0.303/0.765	0.927/0.807
95% confidence interval	Lower	-24.589	-5.964
	Upper	18.389	7.564

Figure 4.5.1: Graphical presentation of the effect of NBL (100mg/Kg) in the Formalin Induced Paw licking (Analgesic) Test.

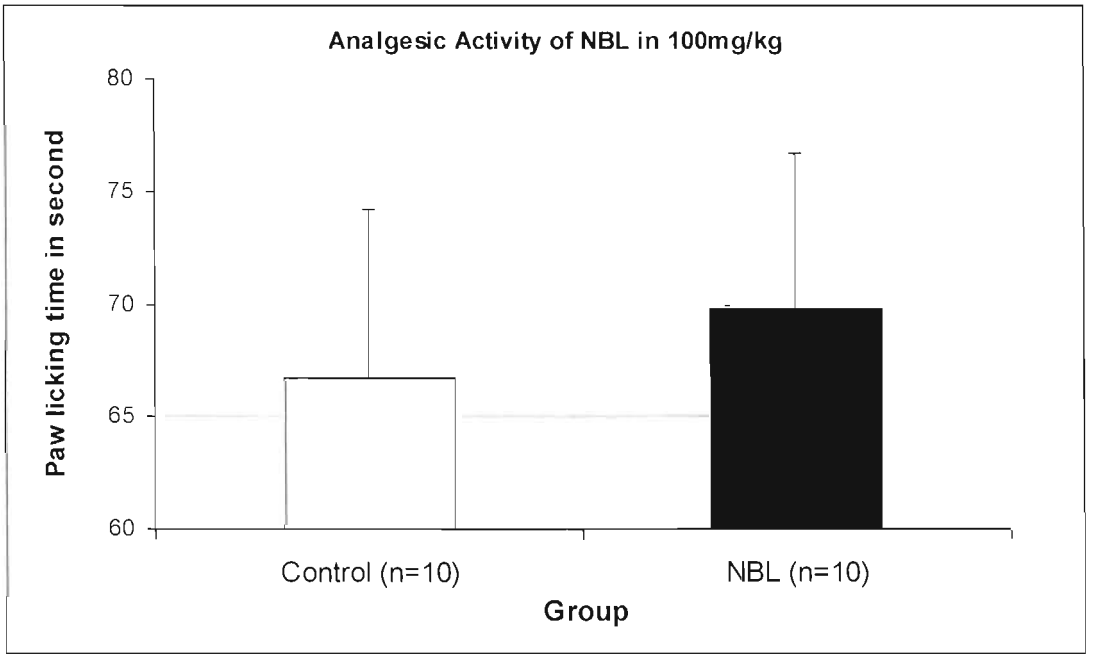


Figure 4.5.2: Graphical presentation of the effect of NBL (100mg /Kg) in the Formalin Induced Paw licking (Inflammation) Test

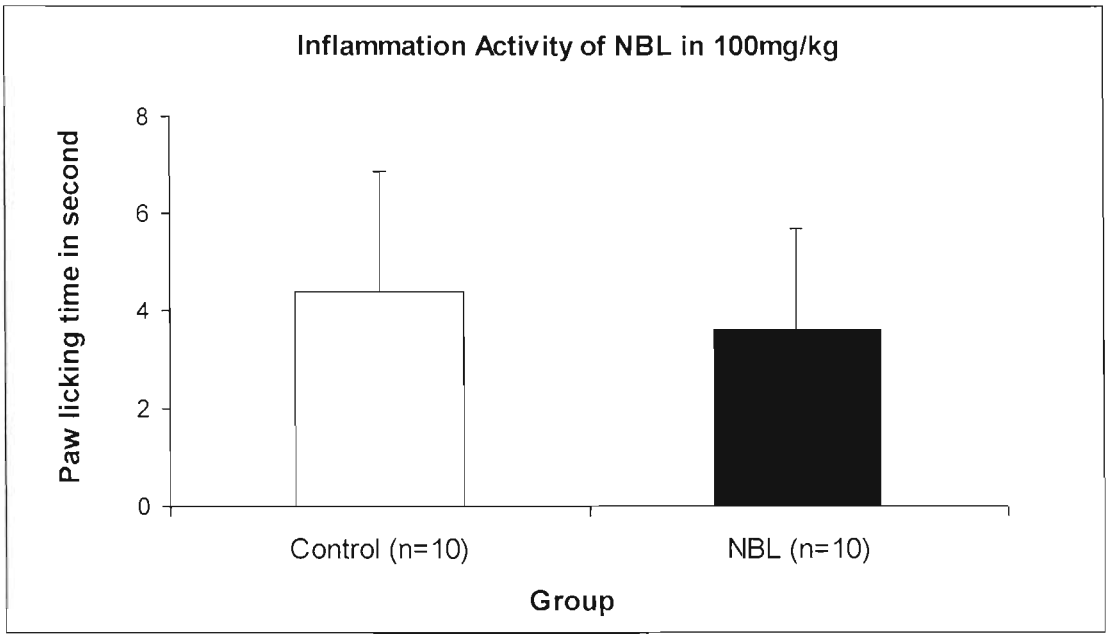
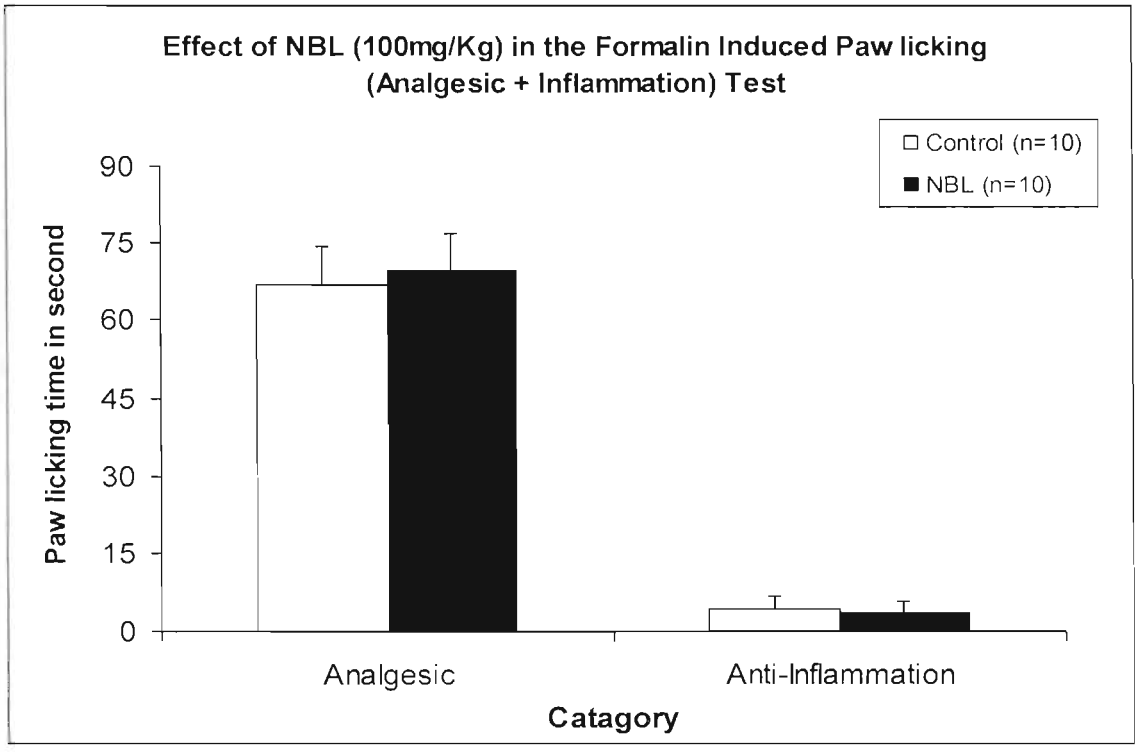


Figure 4.5.3: Graphical presentation of the effect of NBL (100mg/Kg) in the Formalin Induced Paw licking (Analgesic + Inflammation) Test



4.6 Xylene Induced Ear Edema Test:

***Statistical findings and Discussion*:**

At dose 100mg/kg, NBL treated male mice exerted a decrease (p=0.294) in inflammation in the Xylene induced ear edema test when compared to the control group. This decrease was not statistically significant.

From the findings of this experiment, it can be suggested that NBL has mild anti-inflammatory activity.

% of Inflammation = *100-(treated mean/control mean) x 100*

= 100-(0.01350/0.01450) ×100

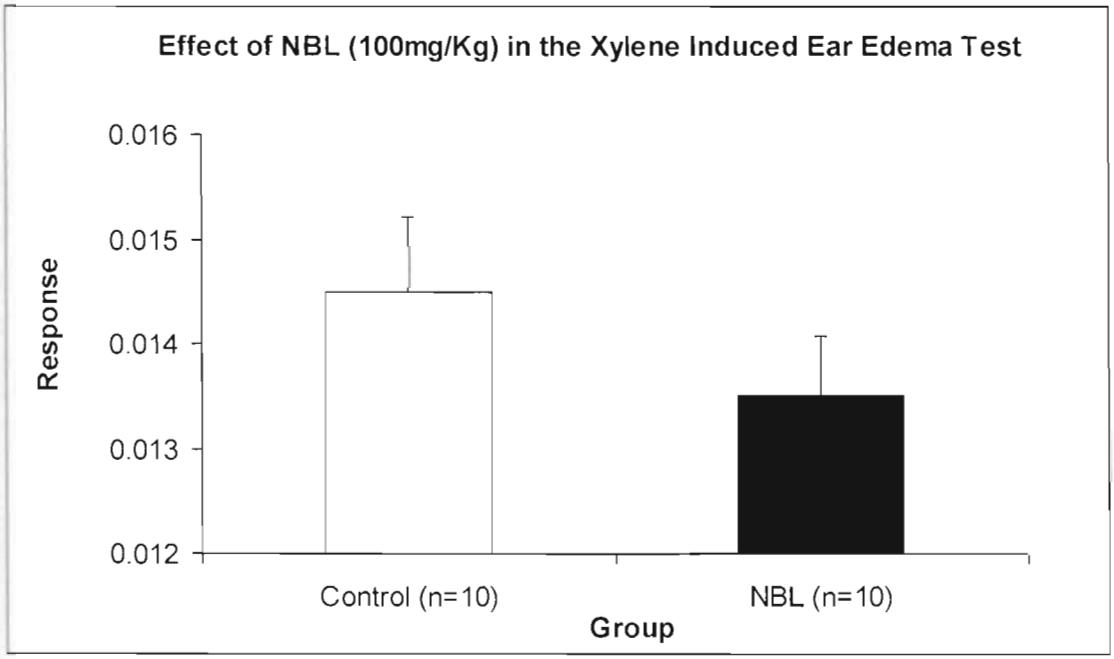
= 6.897%

Tabular and Graphical presentation of the effect of NBL (100mg/kg) on the Xylene induced ear edema test utilizing male mice

Table 4.6.1: The effect of NBL (100mg/kg) in the Xylene Induced Ear Edema Test

Group		Inflammation	% of Inflammation reduced
Ctrl (n=10)		0.01450±0.000719	6.897%
NBL(n=10)		0.01350±0.000582	
t/p		1.081/0.294	
95% confidence interval	Lower	-0.000943	
	Upper	0.002943	

Figure 4.6.1: Graphical presentation of the effect of NBL (100mg/Kg) in the Xylene Induced Ear Edema Test.



4.7 Acetic Acid Induced Writhing Test:

***Statistical findings and Discussion*:**

Writhing Response:

Nabayas Louha (NBL) (100mg/kg) treated male mice exerted a decrease in writhing response compare to the control group from the initial 1st min to 3rd min (p=0.467, 0.656, 0.630 respectively) and increase in writhing response compare to the control group from the 4th min (p=0.567) and 5th min (p=0.897).

At dose, 200mg/kg, NBL treated male mice showed increasing response compare to the control group from the initial at min 1st to 5th min. p values are 0.375, 0.245, 0.450, 0.425, 0.288, 0.209 respectively. All values are > 0.05.

Percent protection:

Percent protection was calculated as follows: -

% Protection=100-(treated mean/control mean) x 100

The percent of protection by Nabayas Louha was:

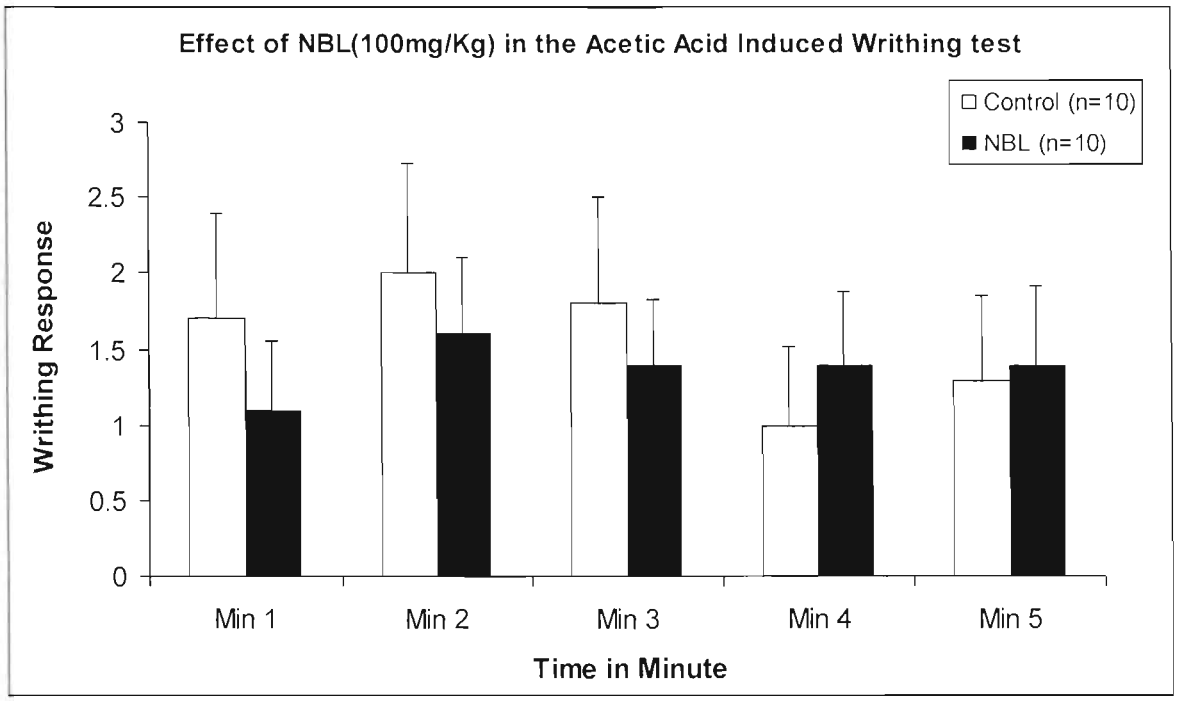
- 11.54% (100 mg/kg)
- -53.03% (200 mg/kg) (pain perception increased)

Tabular and Graphical presentation of the effect of NBL (100mg/kg) on the Acetic Acid Induced Writhing Test utilizing Male mice

Table 4.7.1: The effect of NBL (100mg/kg) in the Acetic Acid Induced Writhing Test

Group		Mean ± Standard Error Mean				
		1st Min	2nd Min	3rd Min	4th Min	5th Min
Control (n=10)		1.70± 0.684	2.00± 0.730	1.80± 0.696	1.00± 0.516	1.30± 0.559
NBL(n=10)		1.10± 0.458	1.60± 0.499	1.40± 0.427	1.40± 0.476	1.40± 0.521
t/p		0.729/ 0.467	0.452/ 0.656	0.490/ 0.630	-0.569/ 0.567	-0.131/ 0.897
95% confidence interval	lower	-1.130	-1.458	-1.315	-1.876	-1.705
	Upper	2.330	2.254	2.115	1.076	1.505

Figure 4.7.1: Graphical presentation of the effect of NBL (100mg/Kg) in the Acetic Acid Induced writhing test.



Tabular and graphical presentation of the effect of NBL (200mg/kg) on the acetic Acid induced writhing test utilizing male mice.

Table 4.7.2: The effect of NBL (200mg/kg) in the Acetic Acid induced writhing test

Group		Mean ± Standard Error Mean				
		1st Min	2nd Min	3rd Min	4th Min	5th Min
Control (n=10)		1.40± 0.562	1.30± 0.496	1.30± 0.367	1.20± 0.442	1.40± 0.521
NBL(n=10)		2.10± 0.526	2.30± 0.667	1.80± 0.533	1.70± 0.423	2.20± 0.512
t/p		-0.910/ 0.375	-1.203/ 0245	-0.773/ 0.450	-0.817/ 0.425	-1.098/ 0.288
95% confidence interval	lower	-2.317	-2.747	-1.860	-1.786	-2.334
	Upper	0.917	0.747	0.860	0.786	0.734

Figure 4.7.2: Graphical presentation of the effect of NBL (200mg/Kg) in the Acetic Acid induced writhing test.

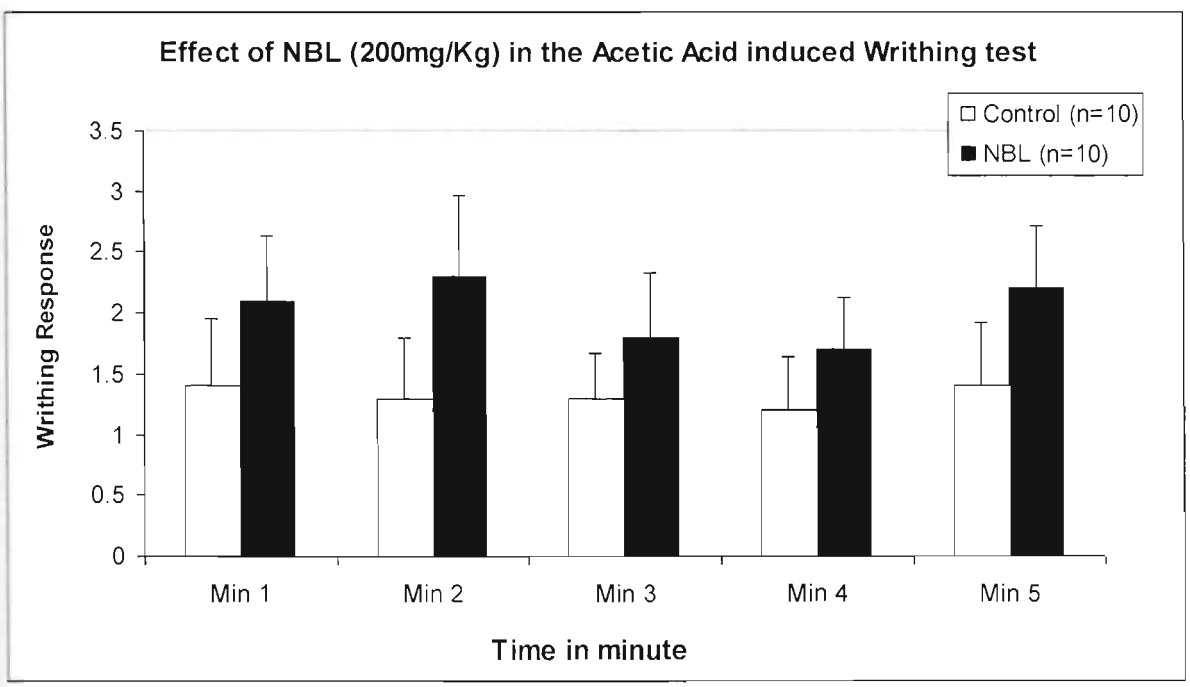


Table 4.7.3: The effect of NBL (100mg/kg) in the acetic acid induced writhing test from min 01- 05 study period.

Group	Parameter	
Male mice	Min 0-5 (Mean ± Standard Error Mean)	% Protection
Control (n=10)	7.80±3.19	11.54%
NBL (n=10)	6.90±2.38	

Figure 4.7.3: Graphical presentation of the effect of NBL (100mg/Kg) in the Acetic Acid Writhing Test from min 01-05 study period.

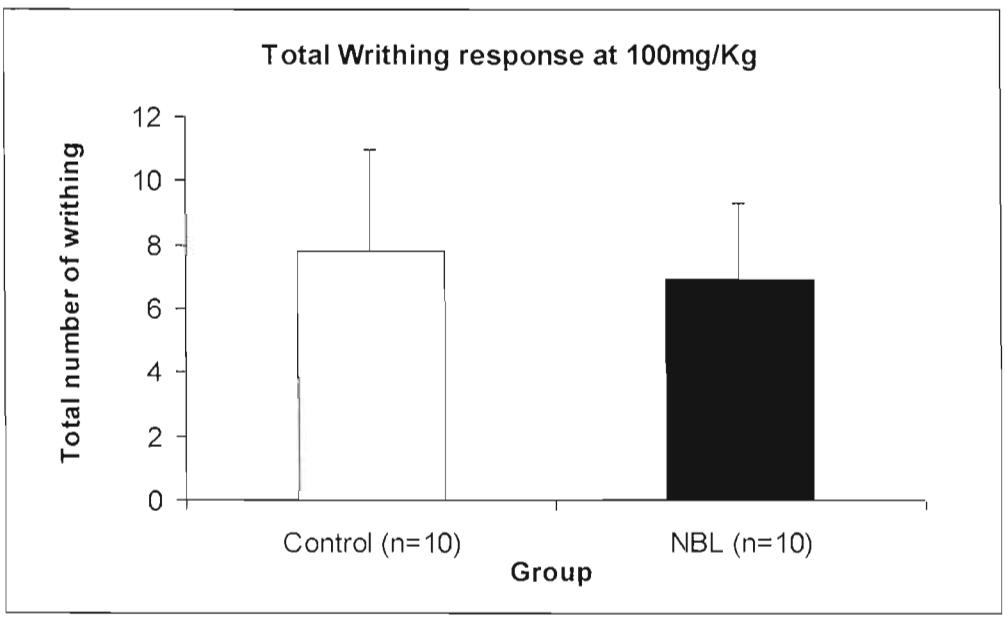
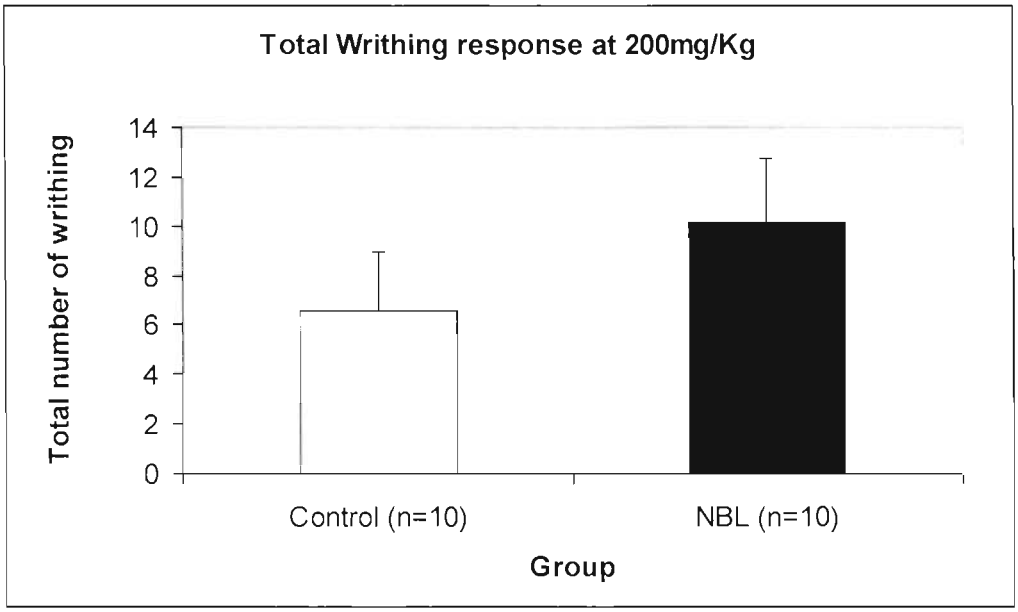


Table 4.7.4: The effect of NBL (200mg/kg) in the acetic acid induced writhing test from min 01- 05 study period

Group	Parameter	
Male mice	Min 0-5 (Mean ± Standard Error Mean)	% Protection
Control(n=10)	6.60±2.39	-53.03%
NBL (n=10)	10.10±2.66	

Figure 4.7.4: Graphical presentation of the effect of NBL (200mg/Kg) in the Acetic Acid Writhing Test from min 01-05 study period



4.8 Hole Board Test

***Statistical findings and Discussion*:**

Ambulation

At the dose 100mg/Kg, NBL treated male mice exerted an overall decrease in the ambulatory effect through out the experimental study period when compared to the corresponding control group except in min180.

In 180 minute the ambulatory effect is slightly decrease compared to the corresponding control group.

But, none of the results were statistically significant.

At dose 200mg/Kg, NBL group exerted an increase in ambulatory activity at min 0, min 30, min 60 and min 120. But the ambulatory activity was almost similar at min 180 and at min 240 it decreased.

But, none of these effects of ambulation was statistically significant.

At dose 400mg/Kg also, NBL treated mice showed an overall increase in the ambulation except in min60 the ambulatory activity was almost similar to the compared to the corresponding control group.

But, none of the results were statistically significant.

Head Dipping

At dose 100mg/Kg, NBL treated group male mice showed an overall similar type of in head dipping activity in all through out the experimental study period when compared to the corresponding control group. But in the min 60 the number of head deep of control group is higher compared to the corresponding control group.

But none of the results were statistically significant.

At dose 200mg/Kg, the head dipping activity was similar at min0 and then gradually increases from min 30 to min 180 and then decreased at min 240. In this experiment the result was found statistically significant at min30 *(p=0.043*) and* noticeable at min180 *(p=0.094).*

At dose 400mg/Kg, NBL treated Male mice showed increased head dipping activity from min30 to min 180 and then decreased at min240 but none of the results were statistically significant.

Emotional Defecation

At dose 100mg/kg, NBL treated male mice showed an overall increase in defecation compare with the corresponding control group except in min0. In min0 the intensity of decreased level of defecation was found statistically significant *(p=0.018*)*

At dose 200mg/Kg, NBL treated mice showed mixed activity. In min 0 min, min30, min180, min240 defecations are decreased and in min60 and min120, defecations are increased. But none of the results are significant

At a higher dose of 400mg/Kg, NBL treated mice also showed mixed activity. In min 30 intensity of defecation was similar and from min120 to min 240 the defecations are decreased. But none of the results are significant

Tabular and Graphical presentation of the effect of NBL (100 mg/kg, 200 mg/kg, 400 mg/kg) on the Hole Board Test utilizing Male mice.

Table 4.8.1 The effect of NBL (100 mg/kg) in the ambulation of hole board test.

Group		Min0	Min30	Min60	Min120	Min 180	Min240
Ctrl(n=6)		33.50± 6.825	34.17± 5.437	32.17± 7.897	20.33± 5.970	19.00± 3.483	16.00± 3.454
NBL(n=6)		25.33± 7.693	27.33± 9.106	24.17± 8.448	18.50± 7.060	20.33± 5.766	13.83± 5.735
t/p		0.794/ 0.446	0.644/ 0.537	0.692/ 0.505	0.198/ 0.847	-0.198/ 0.847	0.324/ 0.753
95% confidence interval	lower	-14.748	-17.538	-17.766	-18.769	-16.343	-12.752
	Upper	31.081	31.204	33.766	22.435	13.676	17.085

Figure 4.8.1: Graphical presentation of the effect of NBL (100 mg/Kg) in Ambulation of Hole Board Test.

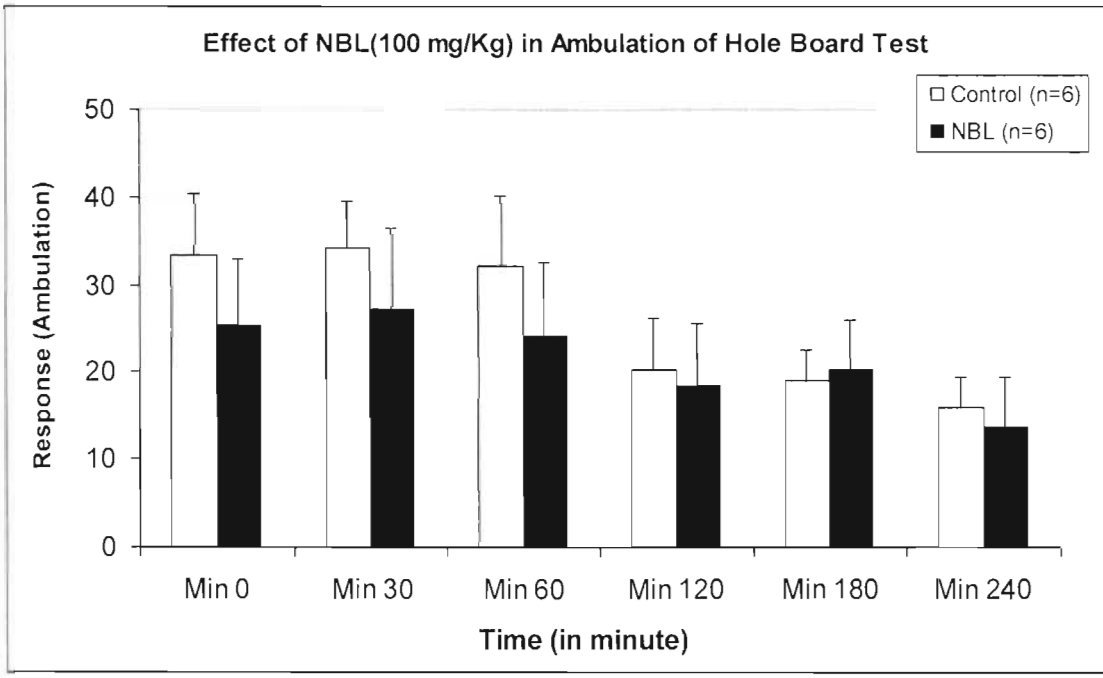


Table 4.8.2: The effect of NBL (200 mg/kg) in the ambulation of hole board Test.

Group		Min0	Min30	Min60	Min120	Min 180	Min240
Ctrl(n=6)		29.00± 5.733	39.67± 7.834	33.17± 6.231	25.17± 3.341	35.33± 5.103	53.17± 13.639
NBL(n=6)		30.67± 9.247	45.83± 13.0	61.83± 18.510	41.17± 9.509	34.83± 7.236	32.00± 5.465
t/p		-0.153/ 0.881	0.404/ 0.695	-1.468/ 0.173	-1.587/ 0.143	0.056/ 0.956	1.441/ 0.196
95% confidence interval	lower	-25.909	-40.159	-72.184	-38.458	-19.229	-14.050
	Upper	22.576	27.825	14.851	6.458	20.229	56.383

Figure 4.8.2: Graphical presentation of the effect of NBL (200 mg/Kg) in Ambulation of Hole Board Test

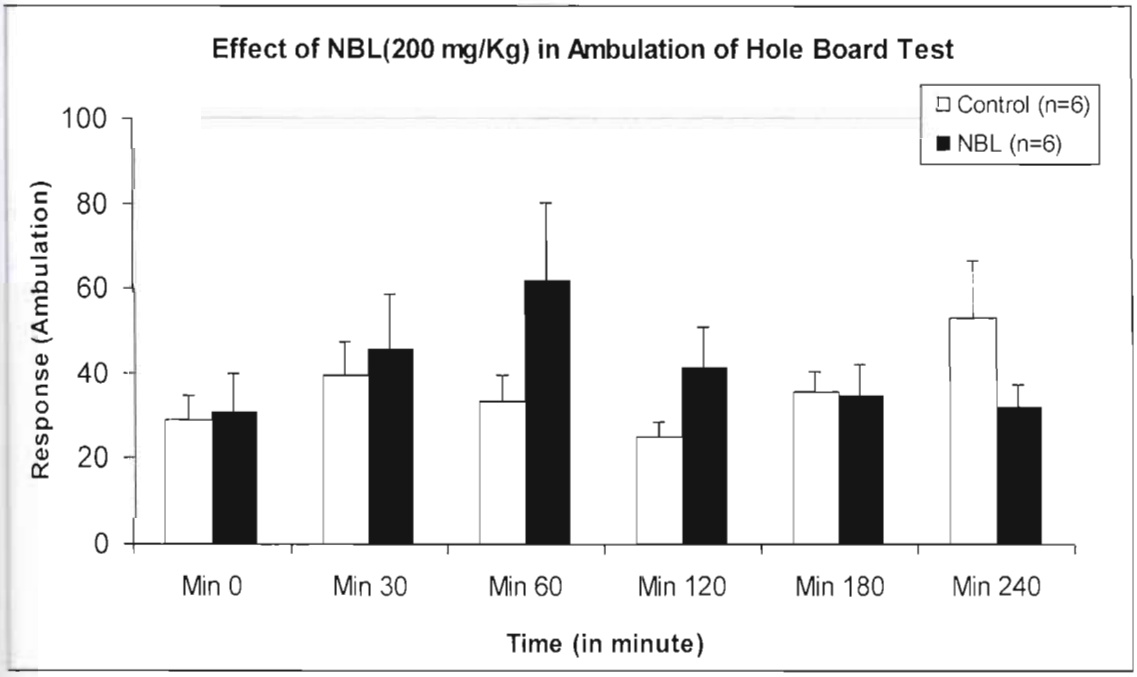
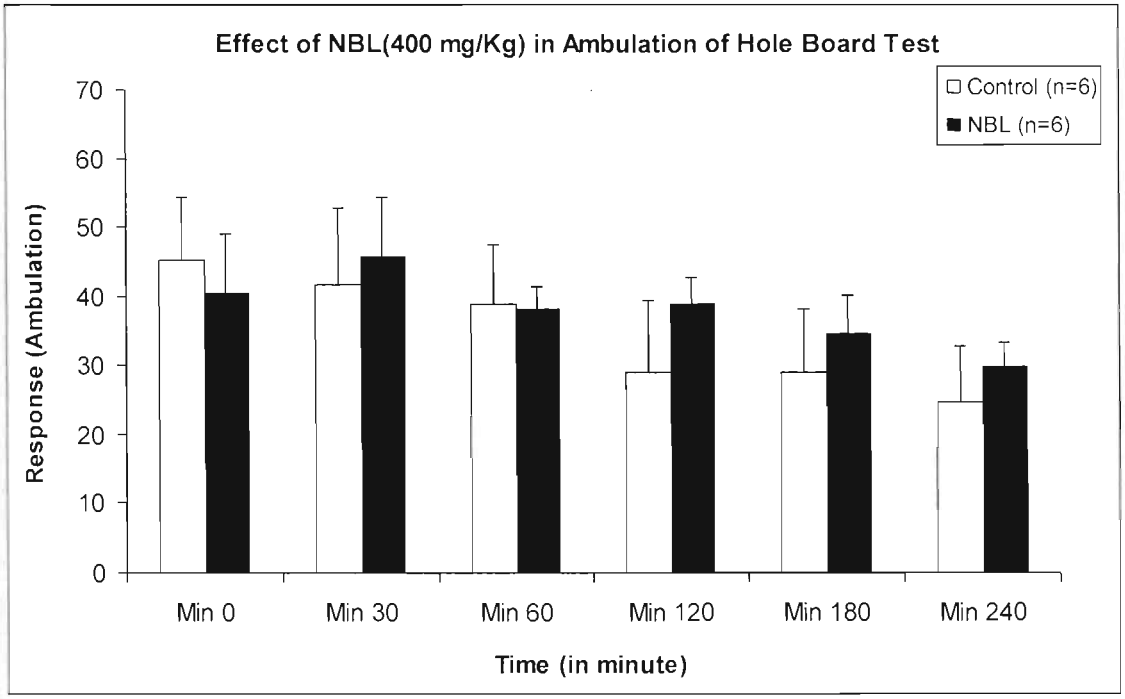


Table 4.8.3: The effect of NBL (400 mg/kg) in the Ambulation of Hole Board Test.

Group		Time					
		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		45.17± 9.228	41.67± 11.087	38.83± 8.696	29.17± 10.222	29.00± 9.147	24.67± 8.123
NBL(n=6)		40.33± 8.578	45.83± 8.384	38.17± 3.361	39.00± 3.624	34.50± 5.731	29.83± 3.487
t/p		0.384/ 0.709	-0.300/ 0.770	0.072/ 0.945	-0.907/ 0.386	-0.510/ 0.621	-0.584/ 0.572
95% confidence interval	lower	-23.239	-35.137	-21.758	-33.999	-29.551	-24.863
	Upper	32.906	26.804	23.091	14.332	18.551	14.529

Figure 4.8.3: Graphical presentation of the effect of NBL (400 mg/Kg) in Ambulation of Hole Board Test.



Head dipping

4.8.4 Table: The effect of NBL (100 mg/kg) in the Head dipping of Hole Board Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		2.67± 1.406	2.83± 2.286	7.33± 3.138	2.67± 1.116	2.00± 0.816	2.00± 0.516
NBL(n=6)		2.33± 1.476	1.67± 1.308	2.50± 1.360	2.17± 1.222	2.17± 1.249	2.17± 1.376
t/p		0.164/ 0.873	0.443/ 0.667	1.413/ 0.188	0.302/ 0769	-.112/ .913	-.113/ -.167
95% confidence interval	lower	-4.209	-4.703	-2.786	-3.187	-3.492	-3.712
	Upper	4.875	7.036	12.453	4.187	3.159	3.379

Figure 4.8.4: Graphical presentation of the effect of NBL (100 mg/Kg) in Head dipping of Hole Board Test.

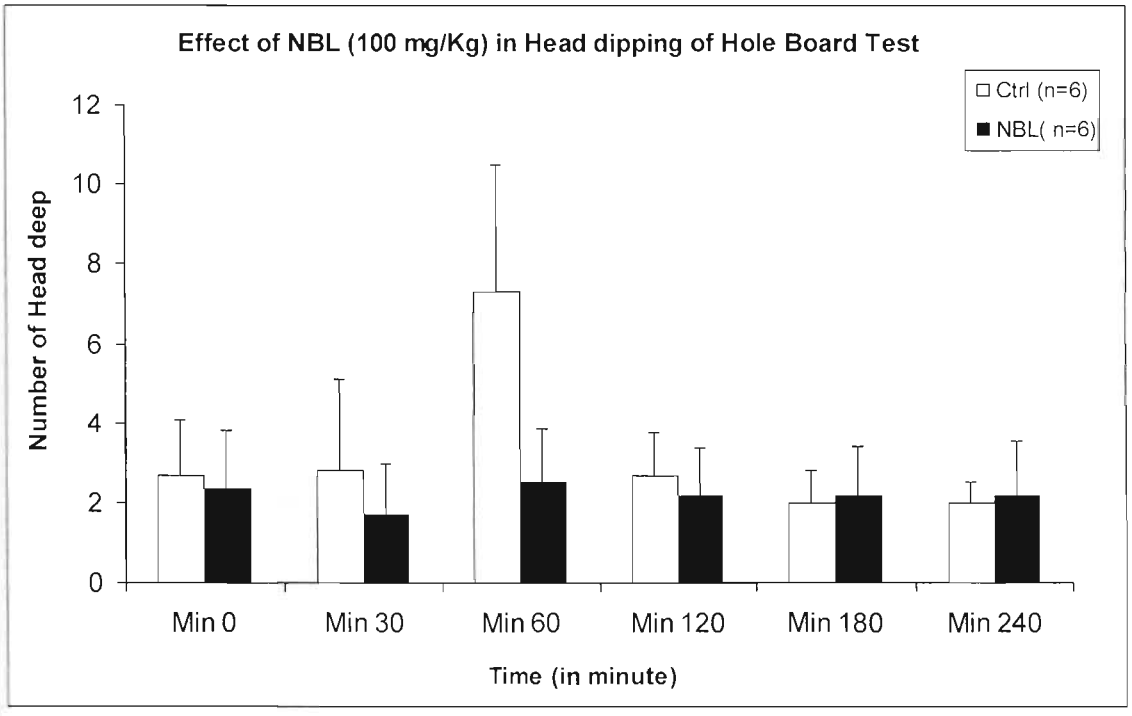


Table 4.8.5: The effect of NBL (200 mg/kg) in the Head dipping of Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		0.17± 0.167	2.00± 0.683	1.33± 0.494	3.00± 1.713	5.50± 1.648	0.67± 0.494
NBL(n=6)		0.17± 0.167	0.17± 0.167	0.50± 0.500	0.83± 0.543	2.00± 0.931	1.83± 1.276
t/p		0.000/ 1.000	2.607/ **0.043***	1.185/ 0.263	0.113/ 0.256	1.849/ **0.094**	-.0853/ 0.414
95% confidence interval	lower	-0.525	0.082	-0.733	-1.836	-0.718	-4.215
	Upper	0.525	3.585	2.400	6.170	7.718	1.882

Figure 4.8.5: Graphical presentation of the effect of NBL (200 mg/Kg) in Head dipping of Hole Board Test

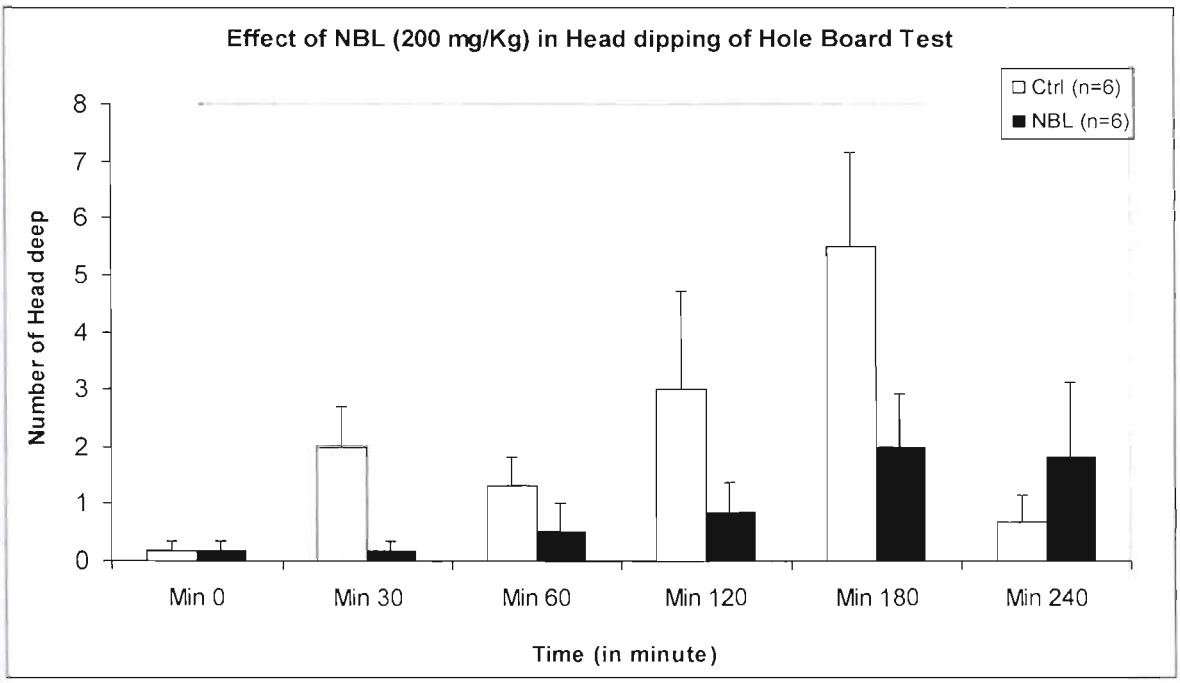
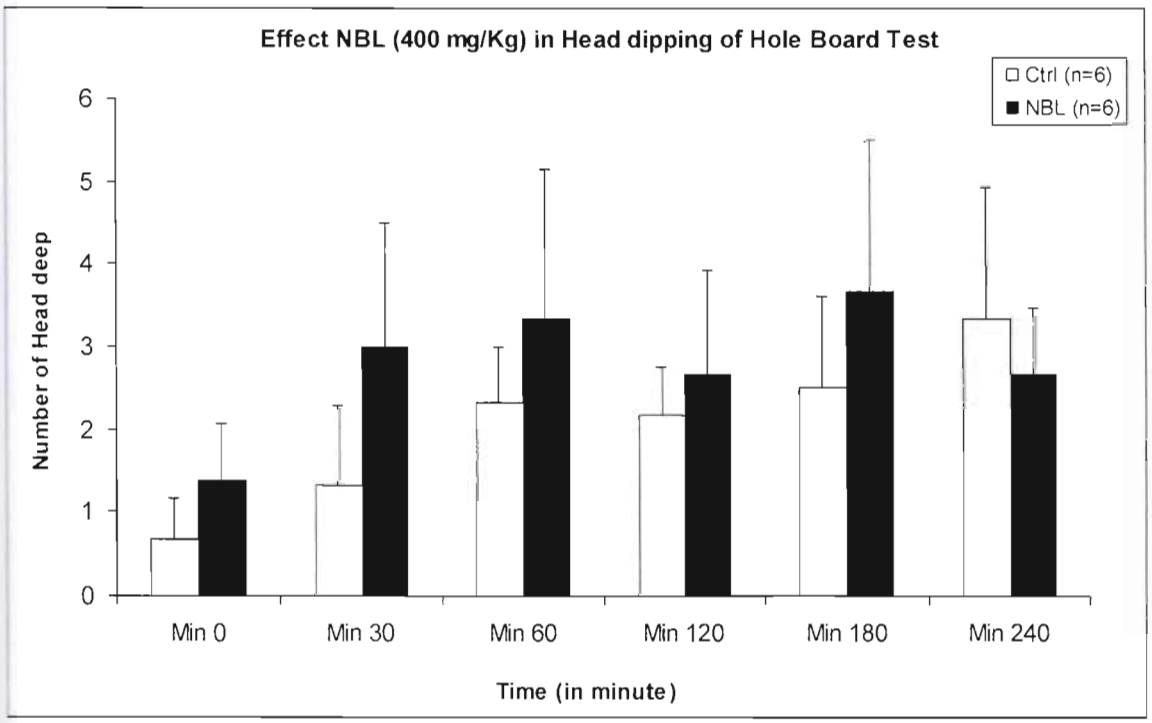


Table 4.8.6: The effect of NBL (400 mg/kg) in the Head dipping of Hole Board Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		0.67± 0.494	1.33± 0.955	2.33± 0.667	2.17± 0.601	2.50± 1.118	3.33± 1.606
NBL(n=6)		1.38± 0.698	3.00± 1.506	3.33± 1.82	2.67± 1.256	3.67± 1.82	2.67± 0.803
t/p		-0.838/ 0.422	-0.935/ 0.372	-0.516/ 0.617	-0.359/ 0.730	-0.546/ 0.597	0.371/ 0.718
95% confidence interval	lower	-2.622	-5.639	-5.318	-3.776	-5.925	-3.333
	Upper	1.189	2.305	3.318	2.776	3.592	4.666

Figure 4.8.6: Graphical presentation of the effect of NBL (400 mg/Kg) in Head dipping of Hole Board Test



Defecation

Table 4.8.7: The effect of NBL (100 mg/kg) in the Defecation of Hole Board Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		0.83± 0.167	0.50± 0.500	0.00± 0.000	0.33± 0.333	0.00± 0.000	0.00± 0.000
NBL(n=6)		0.17± 0.167	1.00± 0.516	0.17± 0.167	2.17± 1.222	0.17± 0.167	0.17± 0.167
t/p		2.828/ 0.018*	-0.696/ 0.503	-1.00/ 0.36	-1.447/ 0.179	-1.00/ 0.363	-1.00/ 0.363
95% confidence interval	lower	0.141	-2.102	-0.595	-4.968	-0.595	-0.595
	Upper	1.192	1.102	0.262	1.302	0.262	0.262

Figure 4.8.7: Graphical presentation of the effect of NBL (100 mg/Kg) in Defecation of Hole Board Test

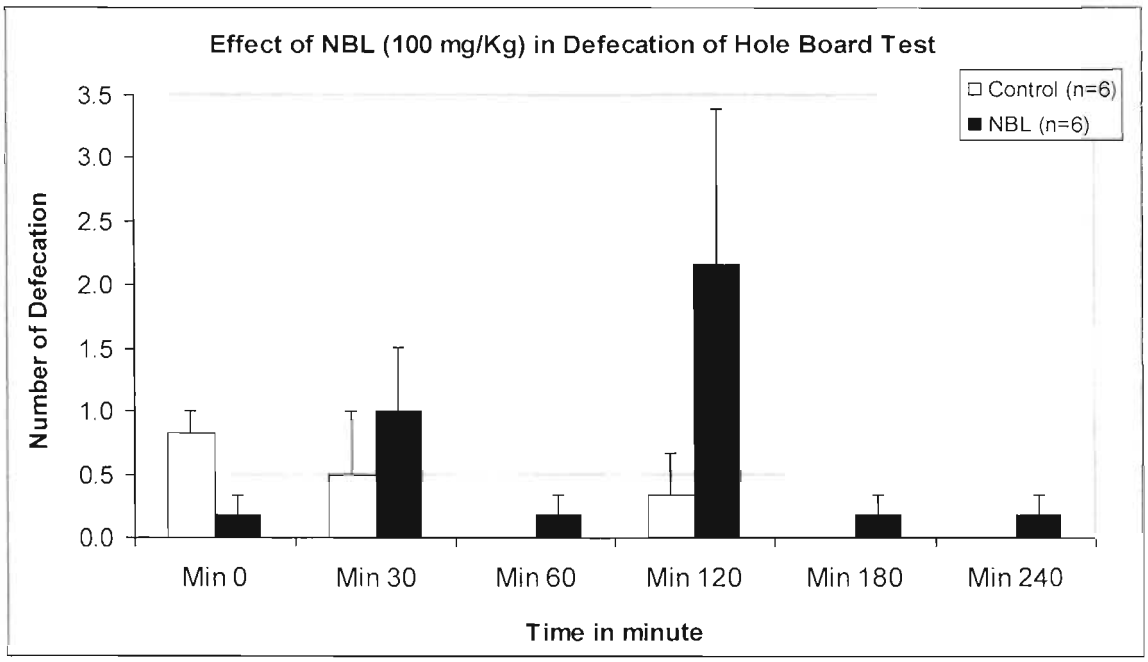


Table 4.8.9: The effect of NBL (200 mg/kg) in the Defecation of Hole Board Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		1.67± 0.615	0.33± 0.333	0.17± 0.167	0.33± 0.333	0.50± 0.342	0.33± 0.211
NBL(n=6)		1.17± 0.601	0.17± 0.167	0.67± 0.333	0.50± 0.342	0.00± 0.000	0.00± 0.000
t/p		0.582/ 0.574	0.447/ 0.664	-1.342/ 0.209	-0.349/ 0.734	1.464 / .0203	1.581/ 0.175
95% confidence interval	lower	-1.415	-0.664	-1.330	-1.230	-0.378	-0.209
	Upper	2.415	0.997	0.330	0.897	1.378	0.875

Figure 4.8.9: Graphical presentation of the effect of NBL (200 mg/Kg) in Defecation of Hole Board Test.

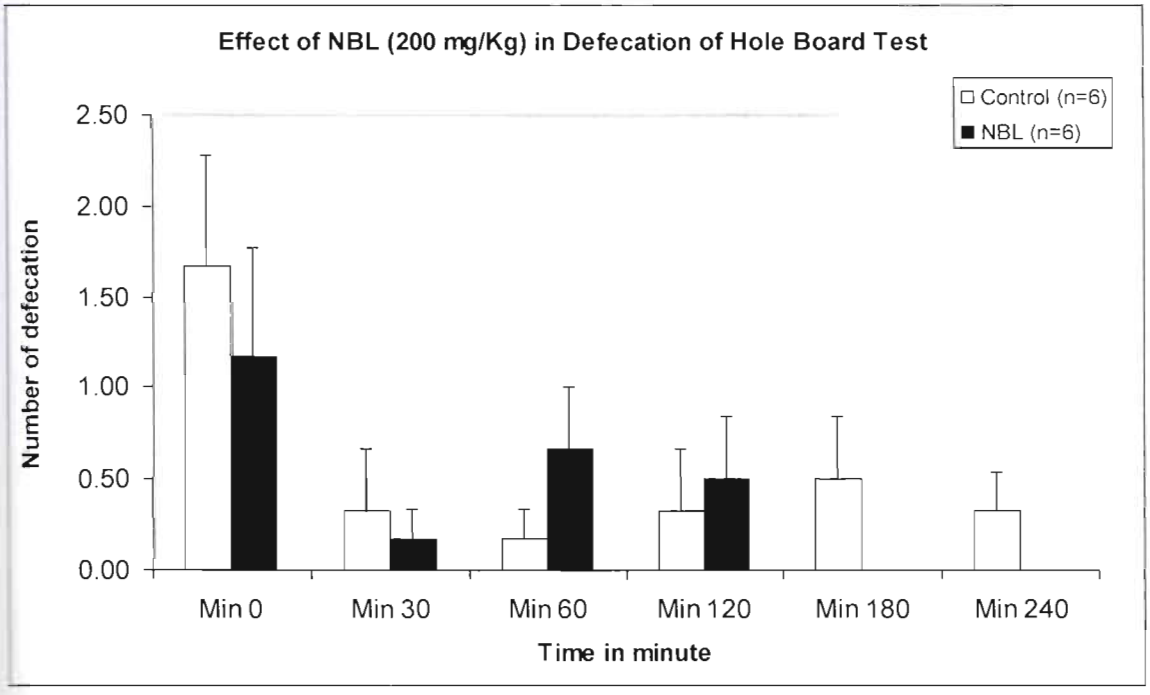
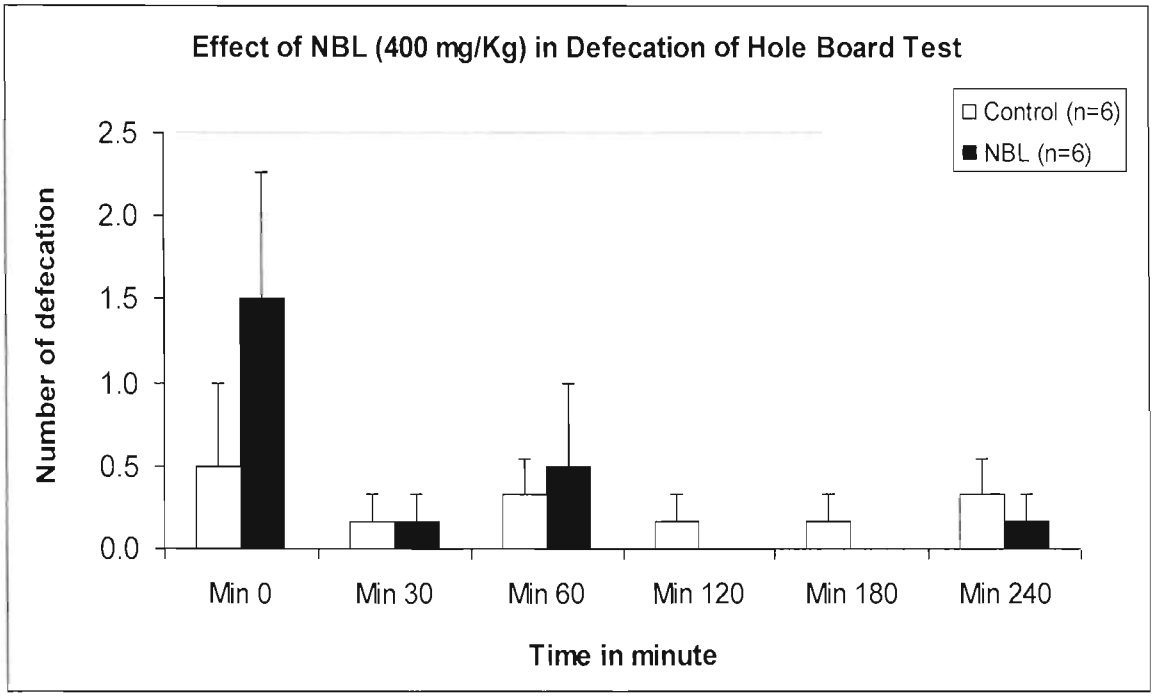


Table 4.8.10: The effect of NBL (400 mg/kg) in the Defecation of Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		0.50± 0.500	0.17± 0.167	0.33± 0.211	0.17± 0.167	0.17± 0.167	0.33± 0.211
NBL(n=6)		1.50± 0.764	0.17± 0.167	0.50± 0.500	0.00± 0.000	0.00± 0.00	0.17± 0.167
t/p		-1.095/ 0.299	0.000/ 1.000	-0.307/ 0.765	1.000/ 0.363	1.000/ 0.363	0.620/ 0.549
95% confidence interval	lower	-3.034	-0.525	-1.376	-0.262	-0.262	-0.432
	Upper	1.034	0.525	1.042	0.595	0.595	0.765

Figure 4.8.10: Graphical presentation of the effect of NBL (400 mg/Kg) in Defecation of Hole Board Test.



5.9 Hole Cross test

***Statistical findings and Discussion*:**

Nabayas Louha (NBL) treated Male mice at three dose levels (100 mg/kg, 200 mg/kg, and 400 mg /Kg) exerted overall mixed activity in hole cross test.

At dose 100mg/Kg, NBL treated male mice exerted a mixed Hole cross activity. In min 0, min 60 and min 120 the hole cross activity was less then respected control group and in min 180 and min 240 the hole cross activity was greater then respective control group and in min 30 the effect was similar.

But none of these results are significant.

At dose 200mg/Kg, NBL treated male mice exerted an overall increase activity in the Hole cross test from min 0 to min 180 and than in min 240 it slightly decreased.

But none of these results are significant.

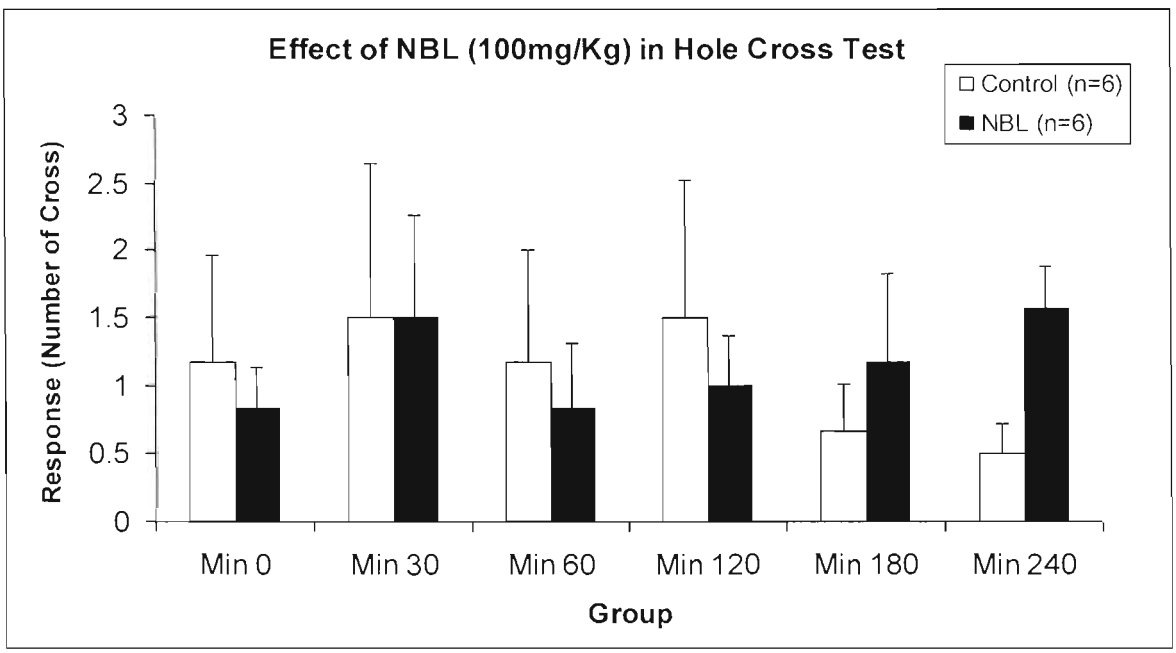
At a higher dose of 400mg/Kg, NBL treated male mice exerted an overall decrease activity in the Hole cross test from min 0 to min 180 and than in min 240 it slightly increased.
But none of these results are significant.

Tabular and Graphical presentation of the effect of NBL (100 mg/kg) on the Hole Cross Test utilizing Male mice

Table 4.9.1: The effect of NBL (100mg/kg) in the Hole Cross Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Control (n=10)		1.17± 0.792	1.50± 1.147	1.17± 0.833	1.50± 1.025	0.67± 0.333	0.50± 0.224
NBL(n=10)		0.83± 0.307	1.50± 0.764	0.83± 0.477	1.0± 0.365	1.17± 0.654	1.57± 0.307
t/p		0.392/ 0.703	.000/ 1.000	0.347/ 0.736	0.0460/ 0.661	-0.681/ .511	-0.877/ 0.401
95% confidence interval	Lower	-1.560	-3.071	-1.806	-2.136	-2.136	-1.180
	Upper	2.227	3.071	2.473	3.136	1.136	0.513

Figure 4.9.1: Graphical presentation of the effect of NBL (100mg/Kg) in Hole Cross Test

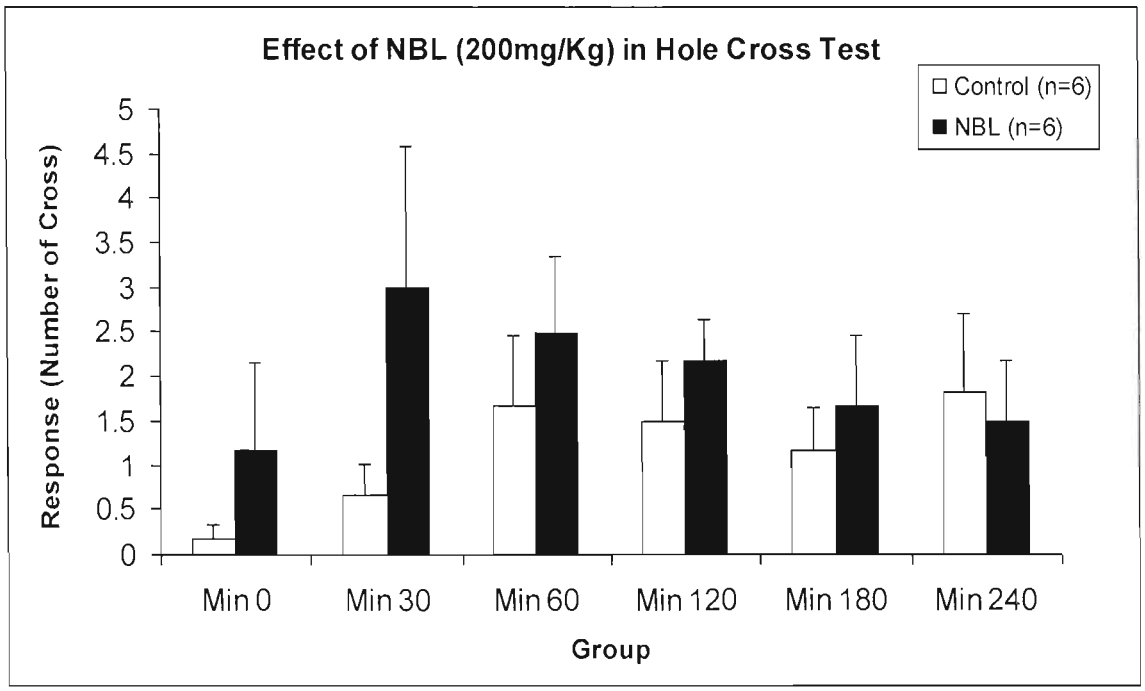


Tabular and Graphical presentation of the effect of NBL (200 mg/kg) on the Hole Cross Test utilizing Male mice

Table 4.9.2: The effect of NBL (200mg/kg) in the Hole Cross Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Control (n=10)		0.17± 0.167	0.67± 0.333	1.67± 0.803	1.50± 0.671	1.17± 0.477	1.83± 0.872
NBL(n=10)		1.17± 0.980	3.00± 1.571	2.5± 0.847	2.17± 0.477	1.67± 0.803	1.50± 0.671
t/p		-1.006/ 0.338	-1.453/ 0.201	-0.714/ 0.491	-0.810/ 0.437	-0.535/ 0.604	0.303/ 0.768
95% confidence interval	Lower	-3.216	-6.360	-3.433	-2.501	-2.581	-2.119
	Upper	1.216	1.694	1.766	1.168	1.581	2.785

Figure 4.9.2: Graphical presentation of the effect of NBL (200mg/Kg) in Hole Cross Test.

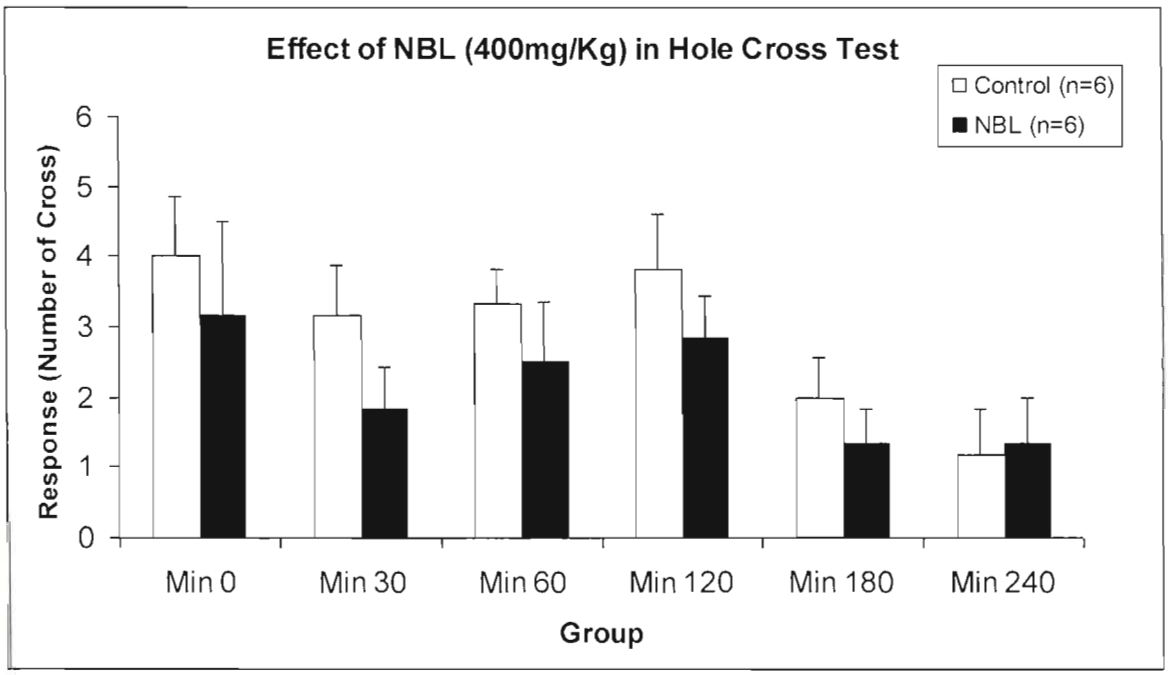


Tabular and Graphical presentation of the effect of NBL (400 mg/kg) on the Hole Cross Test utilizing Male mice

Table 4.9.3: The effect of NBL (400mg /kg) in the Hole Cross Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Control (n=10)		4.00± 0.856	3.17± 0.703	3.33± 0.494	3.83± 0.792	2.00± 0.577	1.17± 0.654
NBL(n=10)		3.17± 1.327	1.83± 0.601	2.50± 0.847	2.83± 0.601	1.33± 0.494	1.33± 0.667
t/p		0.528/ 0.609	1.441/ 0.180	0.850/ 0.420	1.006/ 0.338	0.877/ 0.401	-0.178/ 0.862
95% confidence interval	Lower	-2.686	-.728	-1.425	-1.216	-1.027	-2.248
	Upper	4.352	3.394	3.091	3.216	2.360	1.914

Figure 4.9.3: Graphical presentation of the effect of NBL (400mg/Kg) in Hole Cross Test.



4.10 The stair case Test:

***Statistical findings and Discussion*:**

NBL (male mice) group, at dose 100mg/Kg, was studied in the stair case test. NBL decreased (p=0.781) the number of steps and increased (p=0.705) the number of rearing in comparison to the respective control group but none of these are statically significant.

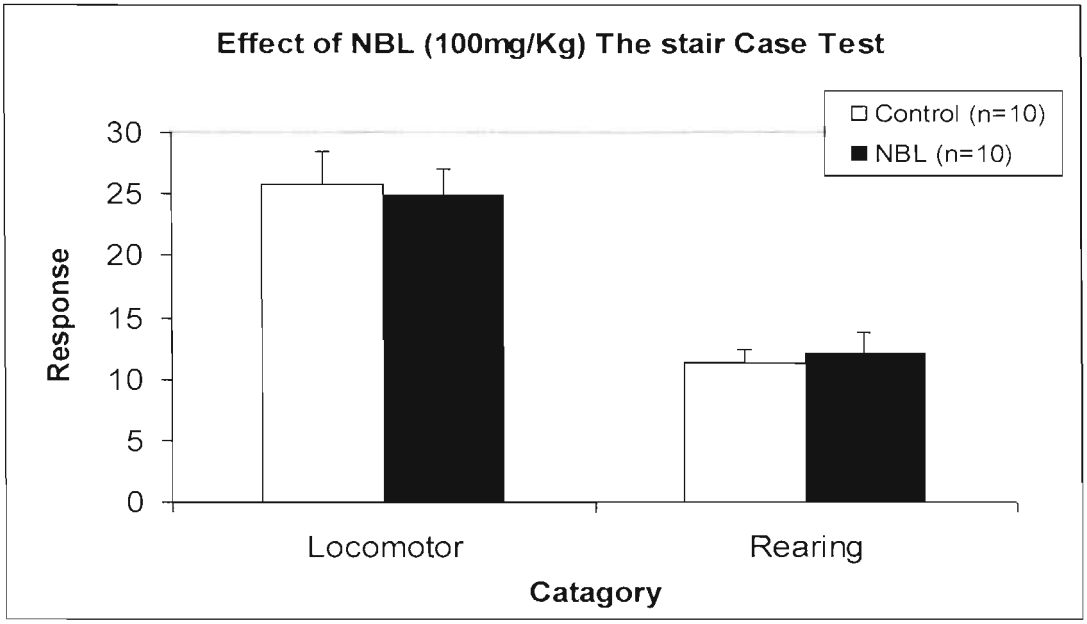
Thus NBL probably don't have any effect like anxiolytic activity.

Tabular and Graphical presentation of the effect of NBL (100mg/kg) on the Stair Case Test utilizing Male mice

Table 4.10.1: The effect of NBL (100mg/kg) in the Stair Case Test on male mice

Group		Steps Climbed Out (Locomotor)	Number of Rearing
Control (n=10)		25.80±2.719	11.30±1.174
NBL(n=10)		24.80±2.265	12.10±1.716
t/p		0.283/0.781	-0.385/0.705
95% confidence interval	Lower	-6.435	-5.168
	Upper	8.435	3.568

Figure 4.10.2: Graphical presentation of the effect of NBL (100mg/Kg) in the Stair Case Test on male mice.



4.11 Climbing Out Test

***Statistical findings and Discussion*:**

NBL treated male mice at three dose levels (100 mg/kg, 200 mg/kg, and 400 mg /Kg) exerted overall increase in hole cross activity.

NBL treated male mice at dose levels (100 mg/Kg) exerted increase in time taken to come out of the cage in min 60, min120 and min 180. The exceptions were in min 30 and in min 240 time required for the drug treated mice to come out the cage was decreased then the control group.

But no results are statically significant.

NBL treated male mice at dose levels (200 mg/Kg) exerted decrease in time taken to come out of the cage in min 30, min120 and min 240. The exceptions were in min 60 and in min 180 time required for the drug treated mice to come out the cage was increased then the control group.

NBL treated male mice at dose levels (400 mg/Kg) exerted increase in time taken to come out of the cage in min 30, min 60, min120 and min 240. The exceptions was in min 180 time required for the drug treated mice to come out the cage was decreased then the control group.

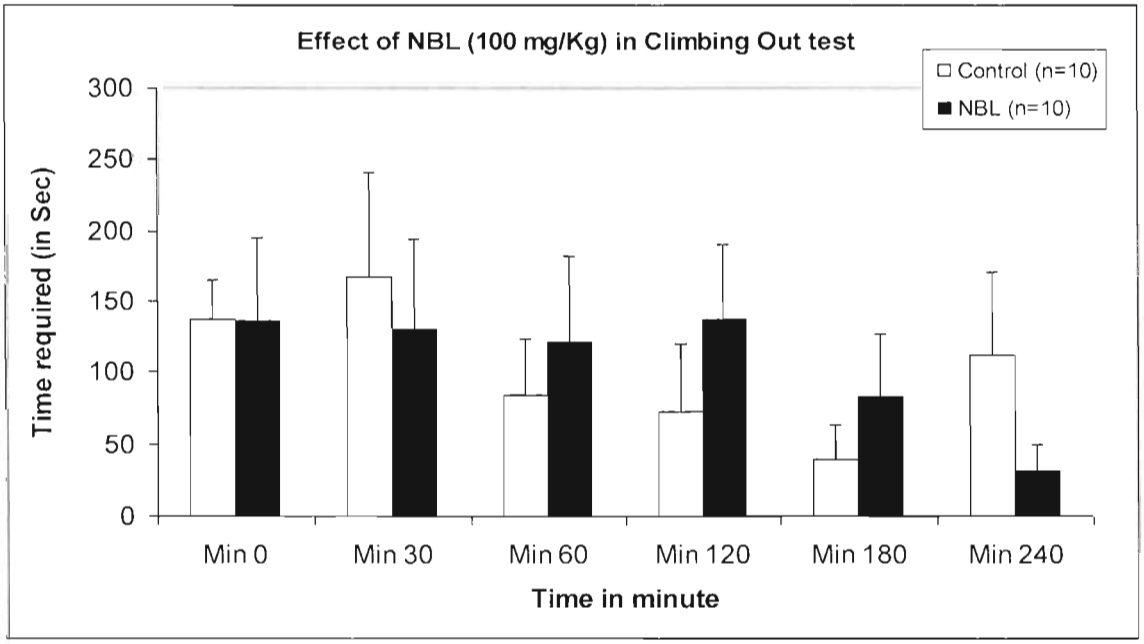
But no results are statically significant.

Tabular and Graphical presentation of the effect of NBL (100mg/kg) on the Climbing out Test utilizing Male mice

Table 4.11.1: The effect of NBL (100mg/kg) in the Climbing out Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Control (n=10)		137.40± 27.741	167.50± 74.074	84.20± 39.197	72.20± 47.549	39.24± 23.658	111.60± 59.046
NBL(n=10)		135.60± 59.476	130.10± 63.959	120.70± 61.357	137.40± 53.278	83.00± 43.789	31.20± 18.772
t/p		0.027 /0.978	0.382 /0.707	-0.501 /0.622	-0.913 /0.373	-0.854/ 0.405	1.298/ 0.211
95% confidence interval	Lower	-136.078	-168.208	-189.465	-215.228	-151.466	-56.276
	Upper	139.678	243.008	116.465	84.828	63.942	217.076

Figure 4.11.1: Graphical presentation of the effect of NBL (100 mg/Kg) in Climbing out

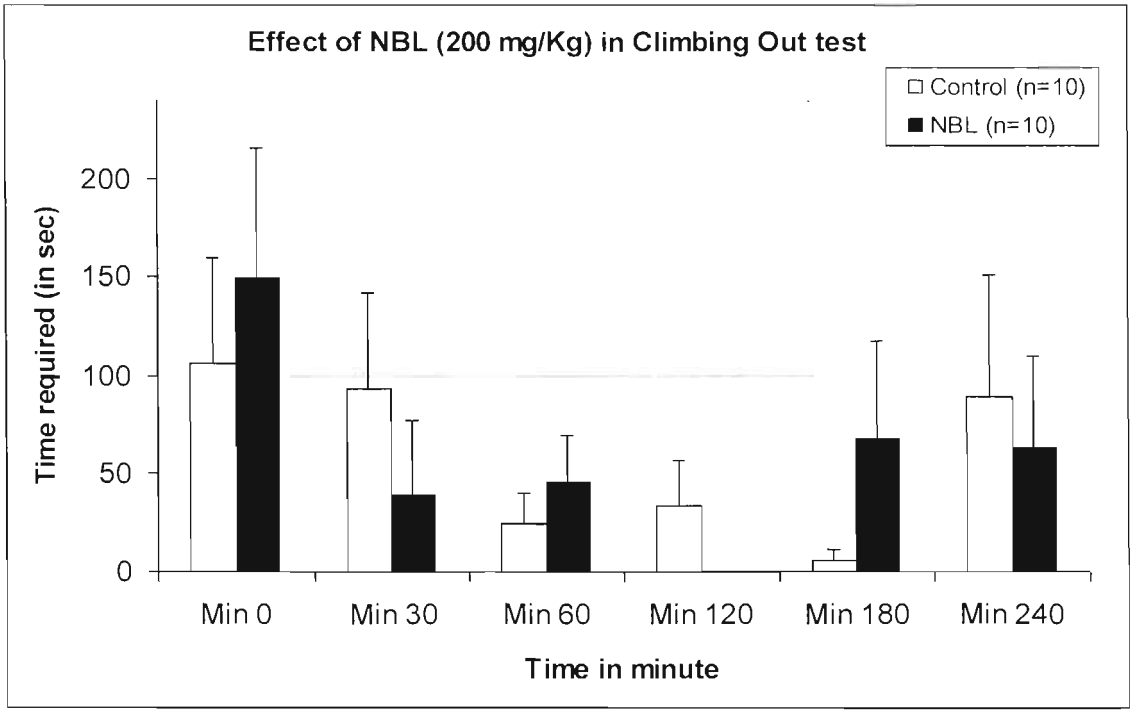


Tabular and Graphical presentation of the effect of NBL (200mg/kg) on the Climbing out Test utilizing Male mice

Table 4.11.2: The effect of NBL (200mg/kg) in the Climbing out Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Control (n=10)		106.20± 53.742	93.00± 49.232	24.00± 16.199	34.00± 23.104	5.50± 5.500	89.30± 61.652
NBL(n=10)		149.10± 66.305	38.80± 38.800	45.40± 24.769	0.00± 0.00	68.20± 49.904	63.80± 46.298
t/p		-0.503/ 0.621	0.865/ 0.399	-0.723/ 0.479	1.472/ 0.175	-1.249/ 0.243	0.331/ 0.745
95% confidence interval	Lower	-222.213	-77.493	-83.579	-18.264	-175.865	-136.483
	Upper	136.413	185.893	40.779	86.264	50.465	187.483

Figure 4.11.2: Graphical presentation of the effect of NBL (200 mg/Kg) in Climbing out

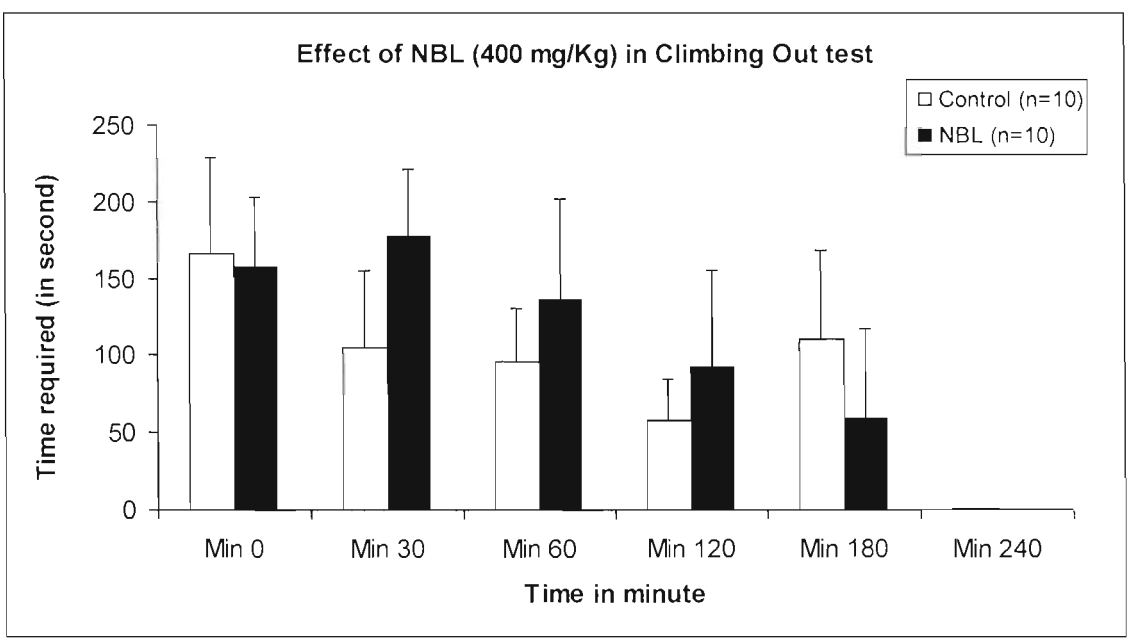


Tabular and Graphical presentation of the effect of NBL (400mg/kg) on the Climbing out Test utilizing Male mice

Table 4.11.3: The effect of NBL (400mg/kg) in the Climbing out Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Control (n=10)		166.10± 63.155	105.30± 49.888	96.20± 34.106	57.90± 27.450	110.90± 57.821	0.60± 0.60
NBL(n=10)		157.70± 45.156	177.00± 43.696	135.70± 66.324	92.50± 62.219	58.70± 58.700	0.0± 0.0
t/p		0.108/ 0.915	-1.081/ 0.294	-0.530/ 0.603	-0.509/ 0.603	0.634/ 0.534	1.0/ 0.343
95% confidence interval	Lower	-154.711	-211.030	-196.186	-177.474	-120.906	-0.757
	Upper	171.511	67.630	117.186	108.274	225.306	1.957

Figure 4.11.3: Graphical presentation of the effect of NBL (400 mg/Kg) in Climbing out



4.12 Forced Induced Swimming Test:

***Statistical findings and Discussion*:**

NBL treated male mice, initially in 2nd hour, NBL (100 mg/kg) treated group showed an increase in immobile phase in swimming test in 1st minute, 2nd minute and 3-6 Minute.

In 1st minute *(p=0.096)* and 2nd minute *(p=0.083)* the increase in immobile phase is statistically Noticeable and 3-6 minute after 2 hour the increase of immobile phase was statistically highly significant *(p=0.002**)*.

At 24hr NBL (100 mg/kg) treated group showed an increase in immobile phase in swimming test in 1st minute *(p=0.233)*, 2nd minute *(p=0.278)* and 3-6 Minute *(p=0.432)*. But no results were statistically significant

Thus NBL was may have depressant activity.

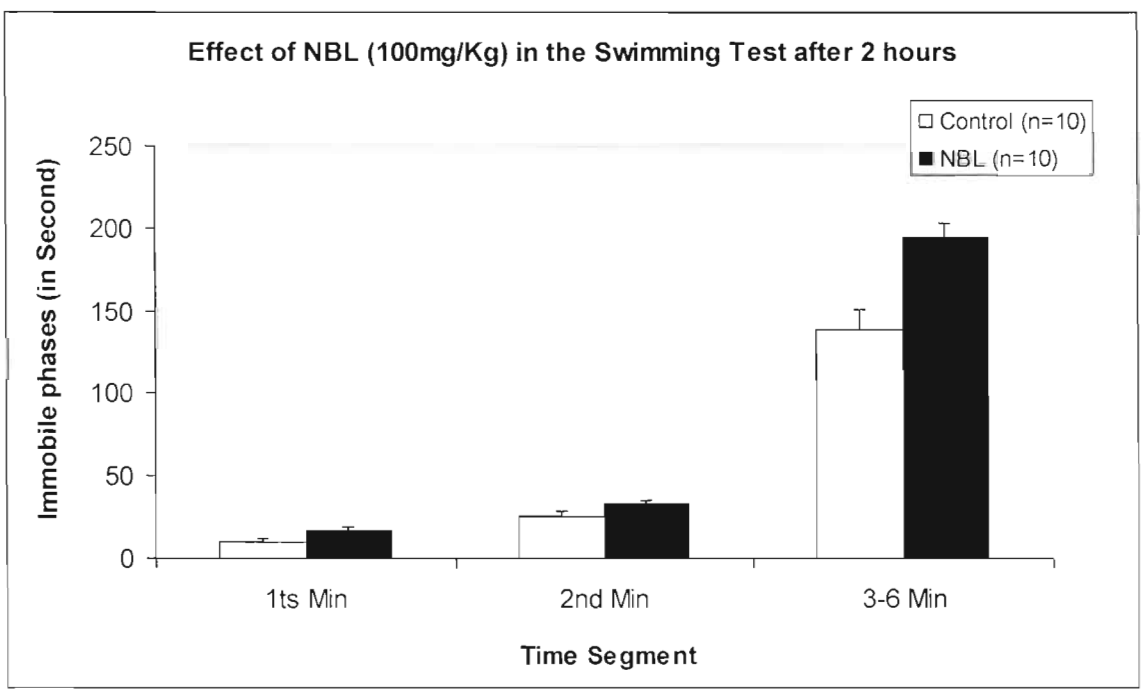
Tabular and Graphical presentation of the effect of NBL (100mg/kg) on the Swimming Test utilizing Male mice after 2 hours:

Table 4.12: The effect of NBL (100mg/kg) in the swimming test after 2 hours

Group		1st min	2nd min	3 to 6 Min
Ctrl (n=10)		10.10±2.243	25.00±3.339	138.80±12.206
NBL(n=9)		15.89±2.40	32.56±2.102	194.00±8.651
t/p		-1.763/**0.096**	-1.840/**0.083**	-3.613/**0.002****
95% confidence interval	Lower	-12.715	-16.220	-87.435
	Upper	1.137	1.109	-22.965

** (≤ 0.01) = Highly Significant,

Figure 4.12.1: Graphical presentation of the effect of NBL (100mg/Kg) in the Swimming Test after 2 hours



Tabular and Graphical presentation of the effect of NBL (100mg/kg) on the Swimming Test utilizing Male mice after 24 hours

Table 4.12.2: The effect of NBL (100mg/kg) in the Swimming Test after 24 hours

Group		1st min	2nd min	3 to 6 Min
Ctrl(n=10)		7.50±3.585	18.50±3.967	161.30±11.149
NBL(n=9)		13.78±3.570	24.0±2.693	174.33±11.736
t/p		-1.237/0.233	-1.121/0.278	-0.805/0.432
95% confidence interval	Lower	-16.985	-15.852	-47.216
	Upper	4.430	4.852	21.149

Figure 4.12.2: Graphical Presentation of the effect of NBL (100mg/Kg) in the Swimming Test after 24 hours

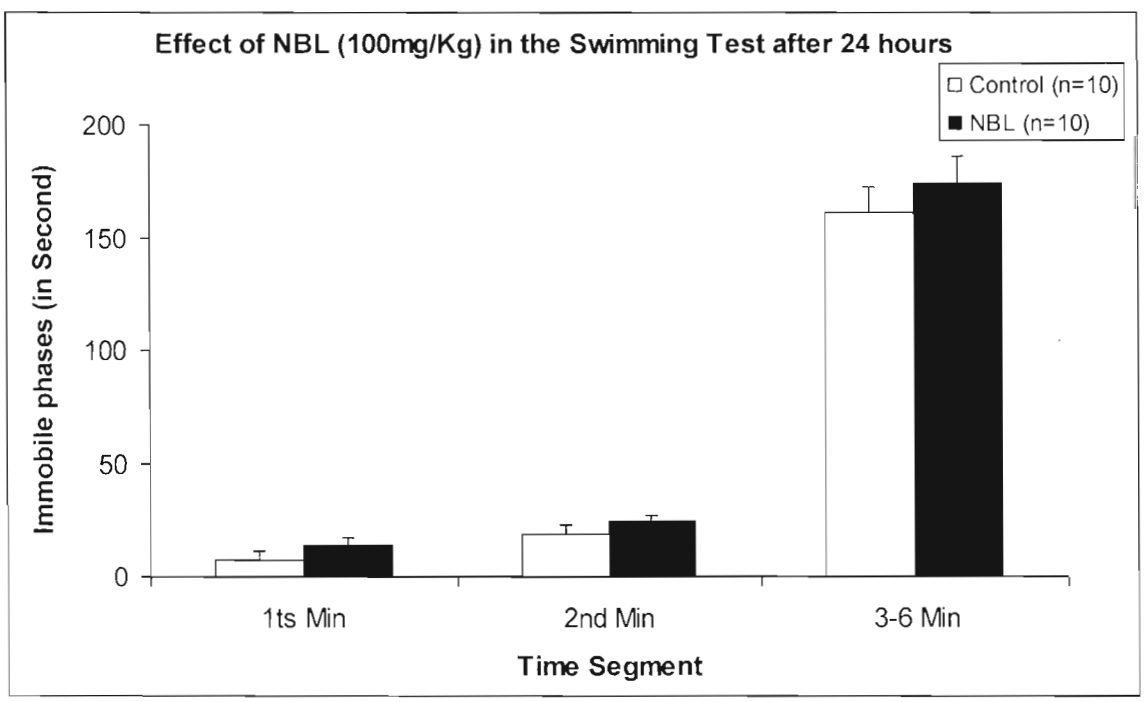


Figure 4.12.3: Line Graphical presentation of the effect of NBL (100mg/Kg) in the Swimming Test after 2nd and 24 hours

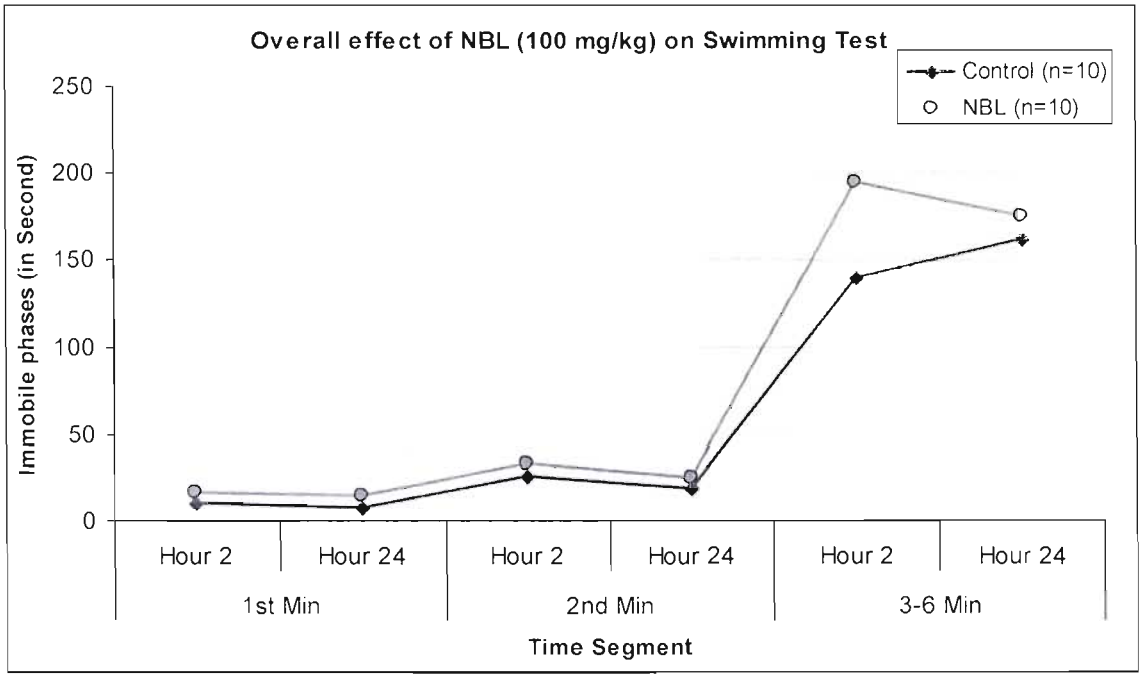
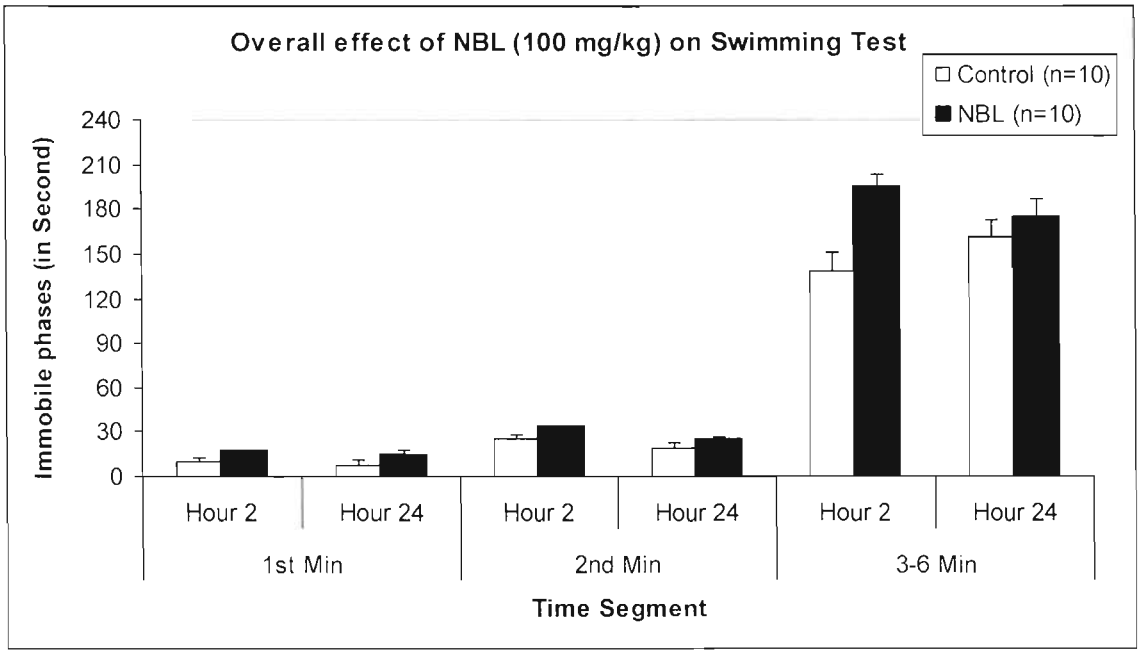


Figure 4.12.4: Bar Graphical presentation of the effect of NBL (100mg/Kg) in the Swimming Test after 2nd and 24 hours



4.13 Rotarod test with constant speed model

***Statistical findings and Discussion*:**

Total fall

NBL (at doses 100mg/Kg) treated male mice exerted an increase in total fall all through out the 240 min study compared with the control group.

NBL treated group exerted an increase in total fall compare to the corresponding control group at minute 180 which is statically significant *(p=0.036*)*

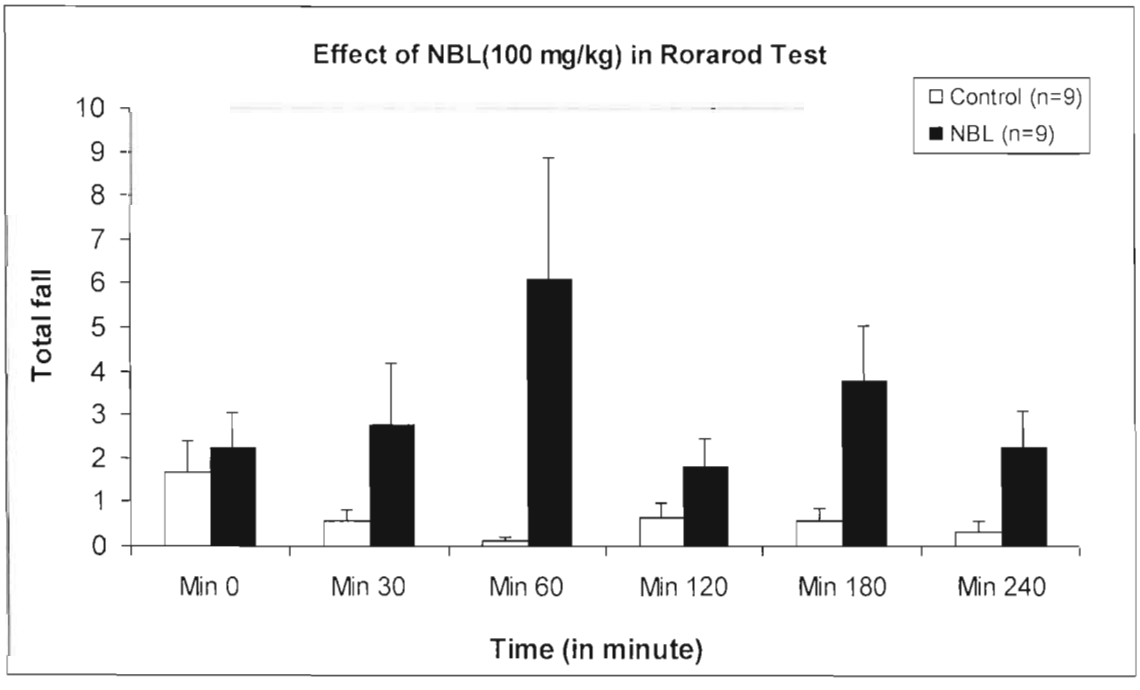
NBL treated group exerted an increase in total fall compare to the corresponding control group at min60 (p=0.060) and min240 (p=0.063) which is statically not significant but Noticeable.

Tabular and Graphical presentation of the effect of NBL (100mg/kg) in the Rota rod Test utilizing Male mice.

Table 4.12.1: The effect of NBL (100mg/kg) in the Total fall of the Rota rod test

Group		Time in Minute (Mean ± SEM)					
		Min0	Min30	Min60	Min120	Min 180	Min240
Ctrl(n=9)		1.67± 0.745	0.56± 0.242	0.11± .0111	0.67± 0.289	0.56± 0.294	0.33± 0.236
NBL(n=9)		2.22± 0.846	2.78± 1.412	6.11± 2.736	1.78± 0.662	3.78± 1.267	2.22± 0.862
t/p		-0.493/ 0.629	-1.551/ 0.157	-2.191/ **0.060**	-1.538/ 0.143	-2.478/ **0.036***	-2.113/ **0.063**
95% confidence interval	lower	-2.946	-5.494	-12.310	-2.702	-6.171	-3.905
	Upper	1.835	1.050	0.310	0.480	-0.273	0.127

Figure 4.13.1: Graphical presentation of the effect of NBL (100mg/kg) in the Rota rod Test utilizing Male mice.



Chapter -5

Conclusion

Conclusions:

According to a WHO report, over 80% of the world population relies on plant-based traditional medicine for their primary healthcare needs and remedies, and the use of traditional medicines is rising in the developed countries. Ayurvedic is a traditional medicine native to the Indian subcontinent and practiced in other parts of the world as a form of alternative medicine. Ayurvedic drug can be used to treat a range of disorders including acne, anemia, anxiety, arthritis, asthma, constipation, depression, diabetes, eating disorder, headache, hypertension, impotence, insomnia, menstrual difficulties, migraine, muscle cramps, obesity, osteoporosis, smoking, stress, yeast infection etc.

At present 204 companies are manufacturing Ayurvedic medicines and there are 237 allopathic, 297 Unani and 77 Homeopathic drug manufacturing companies in Bangladesh. It can easily be assumed that a considerable part of population use ayurvedic product in Bangladesh. Nabayas Louha is one of the popular ayurvedic iron preparation, currently manufacturing by "Sree Kundeswari Aushadhalaya Ltd.". In this research work it was tried to characterize the effect of ayurvedic iron preparation Nabayas Louha on different physiological systems of animal model (*Swiss albino* mice).

In this report it was found that iron preparation Nabayas Louha increase gastrointestinal motility and gastrointestinal emptying rate on mice and Nabayas Louha may have depression activity on animal trial subjected on *Swiss albino* mice without any major side effect. In addition it should rather be emphasized that to establish these findings there is a need for a comprehensive study and large scale clinical trial to ensure the safety of the general patients/users of the country.

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Abbreviation

Abbreviations

The following abbreviations were used throughout this research work

AA	Acetic acid
Con	Control
Dil	Dilution
F	Female
GE	Gastric Emptying
GI	Gastrointestinal
G	Gram
Hr	Hour
i.p.	Intra-peritoneal
kg	Kilogram
M	Male
Min	Minute
ml	milliliter
mg	milligram
NBL	Nabayas Louha
p.o.	Per oral
s	Second
SEM	Standard error mean
wt	weight