PHARMACOLOGICAL STUDIES

OF

DHATRI LAUHA

A project report submitted in partial fulfilment of the requirements for the degree of Bachelor of Pharmacy

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LIST OF ABBREVIATIONS

The following abbreviations were used throughout this research work.

Acetic acid	**AA**
Control	**Con**
Control	**Ctrl**
Dilution	**Dil**
Female	**F**
Gastric Emptying	**GE**
Gastrointestinal	**GI**
Gram	**g**
Hour	**Hr**
Intra-peritoneal	**i.p**
Kilogram	**kg**
Male	**M**
Minute	**Min**
Milliliter	**ml**
Per-oral	**p.o.**
Second	**s**
Weight	**wt**
DHATRI LAUHA	**DTR**
Bangladesh Council Of Scientific And Industrial Research	**BCSIR,**
Quantity Sufficient	**Q.S./q.s.**

ABSTRACT

DHATRI LAUHA is a widely used formulation in folk and ayurvedic systems of medicine and it is commonly used for Sularoga *(colic)*, pandu *(anaemia)*, kamala *(jaundice)*, amlapitta *(hyperacidity non ulcer dyspepsia)*. This ayurvedic drug mainly formulated of Dhatri (amalaki) curna, Lauha curna (bhasma), Yastimadhuraja (curna), Amrta kvatha and these compounds are already well tested for their various types of therapeutic action and in certain diseases. In addition to mixture of these compound taken with ghee and honey for their optimum therapeutic activity. Total 9 experiments were carried out at different doses (100, 200 and 400 mg/kg, p.o.) of DHATRI LAUHA which studied in different animal model to find out the various degrees of significance of action of the drug on experimental animals. Highly significant ($p=0.009$) increase in ambulation behavior was observed in Hole board test. In Hole cross test highly significant ($p=0.005$) increase in motor activity was observed. Open field test showed significant ($p=0.036$) increase in Standing up behavior. Climbing out test produced a significant ($p=0.039$) decrease in activity. None of the other experiments resulted in significant observations but there have been overall decreases or increases in activities in different experiments in comparison to respective control groups.

All these experiment were executed in an attempt to confirm the safety of the general patients or users of the society and country as a whole. After completion of this research work now we can suggest that this DTR can be prescribed for treating different diseases without or with a minimal central nervous system side-effect. It also found that the drug has no effect on the gut motility and gastric emptying time.

1.1 Ayurveda

The term Ayurveda combines two Sanskrit words: *ayur*, which means life, and *veda*, which means science or knowledge. It is a medical system which integrates and balances the body, mind, and spirit (thus, it is considered "holistic"). This balance is necessary for contentment and good health. A primary aim of Ayurvedic medicine is to cleanse the body of substances that can cause disease. This helps re-establish the harmony and balance necessary for optimal health. Ayurveda is a major traditional medicinal system of Bangladesh and the Indian subcontinent originated several thousands year ago and it is still being successfully used in many countries. Ayurveda and variations of it have also been practiced for centuries in Pakistan, Nepal, Bangladesh, Sri Lanka, and Tibet. Ayurveda uses the concept of purification as a means to eradicate disease rather than to cure as perceived by modern medicine. When treating acute and chronic infections, Ayurveda does not aim to kill the microbes; restoration of dosa balance and host immunity (rasayna) ensures elimination of the infectious agent. Numerous Ayurvedic medicinal plants have shown strong chemotherapeutic and immunomodifying effect in experimentally induced infections.

1.2 Present scenario of Ayurveda

According to World Health Organization 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 economies such as Europe and America. 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. The global market for herbal and ayurvedic medicine is estimated to be more than $60 billion a year and many people in the West are showing growing interests. (Islam, 1991)

1.3 Ayurveda in Bangladesh

According to its geographical and seasonal benefits, this country has a lot of green trees of different class and family and people are used to heal from their diseases by using different types of plants, herbs, and their various preparation along with some rules and practice. 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 coming 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). Allopathic medicinal market in Bangladesh is worth around Tk 4,000 crore, while the market size for herbal medicines including Ayurvedic and Unani stands at more than Tk 1,000 crore. Bangladesh government termed herbs and herbal medicine as one of the five priority sectors to diversify the country's export basket. Bangladesh has prospect in making footsteps on the global market for medicinal plant and products as nearly 650 medicinal plant species have been identified to be in use in Bangladesh with around 25 plants having high value. Now-a-days many Bangladeshi companies going for herbal medicine making with an aim to take over the export potential. Though time-tested evidences show immense therapeutic benefits of theses ayurvedic drug but there is no pharmacologically established data to support these drugs for using against different diseases and for other benefits. By using Ayurvedic medicine expensive and extensive procedures of clinical investigations can be avoided in many cases and people in these selected areas have the choice to get treatment at a cheaper price depending on their choice. 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. Regarding of these clinical

examination in the extent of efficacy, safety and drug interactions of newly developed ayurvedic drugs and formulations are required to be carefully evaluated and their pharmacological profiles are also should be established. A variety of botanical products and their ayurvedic preparation have been reported to possess various activity (especially from ethnopharmacological studies), but the documented literature has centered primarily on pharmacological action in experimental animals. Except for a few phytogenic compounds (i.e., liquorice and chilli), limited clinical data are available to support the use of herbs as recommended and, thus, the data on efficacy and safety are limited.

1.4 Category of Ayurvedic medicines

Ayurvedic medicines are categorized according to whether they promote general health and longevity, enhance sexual vigor, or fight disease. The first category is known as rasayana, the second is called vajikarana, and the third is aushadhis. These categories are not mutually exclusive because some of the aushadhis may act as rasayanas and vice versa. On the basis of their origin, Ayurvedic medicines are also classified into three groups: (1)kastha ausadhis (herbal preparations), (2) rasa ausadhis (metallic preparations [e.g., bhasmas, sindoora]), and (3) jangama ausadhis (animal preparation — prepared from animal products). Depending on their form, method of preparation, ingredients, and pharmacological properties, Ayurvedic medicines are grouped as Swaras, Kalka, Hima, Phanita, Kashaya, Asavas, Aristas, Awalehas, Churnas, Vati, Gutika, Ghrita, Taila Guggulu, Bhasmas, Pishti, Parpati, Rasayoga, Sindoora, Lepa, and Anjana.

1.5 Formulation of DTR

The Formulation of Dhatri lauha (BTR) is included in the Bangladesh National Formulary of Ayurvedic Medicine 1992.

Table 01.1: The formulation of DTR given below:

	21:2 DHATRI LAUHA (Bhaisajyaratnavali, Sularogadhikara, 142 - 143½.)	
1	Dhatri (amalaki) curna (Fr. P.)	384 g
2	Lauha curna (bhasma)	192 g
3	Yastimadhuraja (curna) (Rt.)	96 g
4	Amrta kvatha (St.)	Q.S. (for bhavana)

DOSE

0.5 – 1.0 g.

1.5.1 Dhatri (Amalaki)

Common name- *Indian gooseberry* (E), Amla (H)

Sanskrit - Amalaki, Dhatri

Latin - Emblica officinalis – Fructus (Euphorbiaceae)

'Amla' literally means 'sour' another name for Amalaki is Dhatri; Dhatri means 'mother' or 'nurse', indicating that Amalaki is the ultimate carer and healer. It is the major ingredient in DTR.

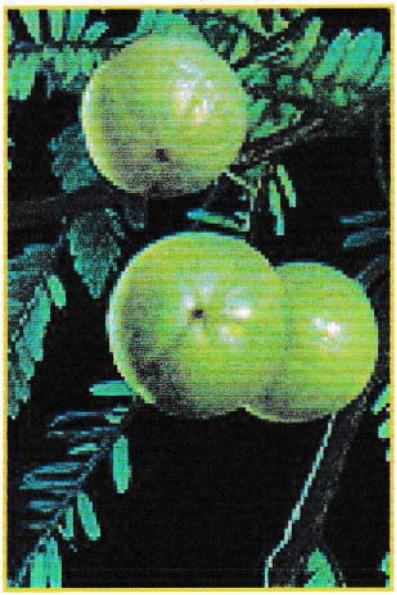


Fig: 1.1: DHATRI (AMALAKI)

Indications

GIT - Specifically indicated for digestive sensitivity; constipation, ulcers, acidity, gastritis, colitis, hepatitis, haemorrhoids. It is especially useful in inflammatory and bleeding conditions of the intestines. A small dose constipates while a larger dose is a laxative. It is a very effective liver cleanser, its sour flavour 'squeezes' the liver and its anti-oxidant properties protect it.

Heart - Its affinity for the blood helps to nourish and protect the heart. It protects by reducing elevated cholesterol and healing arterial damage. It is a super anti-oxidant and a tonic for general debility and weakness; use for palpitations and for recovery post-illness. It helps to nourish rakta dhatu and enkindles raktadhatuagni to function efficiently hence alleviating deficiency conditions such as anemia that can effect heart function. It specifically pacifies an aggravated sadhaka pitta and this influences the clarity and calmness of the mind (mdhya rasayana).

Metabolic disorders - Diabetes (pittaja prameha type) is treated by its microcirculatory stimulating and ojas enhancing properties, anaemia due to excess bile vitiating the blood, and hair loss from excess pitta burning the roots of the hair. The oil is especially good at alleviating hair loss and early greyness. It is a renowned rejuvenative and adaptogen famed for slowing age (vayahsthapan), increasing virility, promoting immunity and inducing balanced health (satmikaran). Consider using Amalaki as an immune restorative and hepatoprotective during radiotherapy and chemotherapy treatments. (Sebastian, 2006)

1.5.2 Lauha Curna (Bhasma)

Accumulated toxicity data on the hazardous effects of heavy metals have made health scientists afraid of heavy metals. As a result, renewed interest in the beneficial effects of metals and minerals is often viewed with skepticism. However, available literature from all the ancient civilizations indicates that man has used metals in disease treatment since time immemorial. Ayurvedic literature is full of the use of metals. Not only have Ayurveda and other Indian systems of medicine used metals, but their use is also amply described in Chinese and Egyptian civilizations in 2500 B.C. Metals that are extensively described in Indian and other ancient systems of medicine include silver, arsenic, copper, iron, lead, mercury, and zinc. As far as Ayurveda is concerned, metals have been used mainly as bhasma (ash). Bhasma literally means anything inorganic or organic burnt into its ash.

Bhasmas have been classified on the basis of color and appearance. A more scientific way of classification is on the basis of dominant metal and mineral group. According to this classification, bhasmas have been grouped as rajata group (silver), tamra group (copper), loha group (iron), pravala group (shells), etc. Often two metals and a metal with mineral are the ingredients of bhasmas. For example, Trivanga Bhasma contains lead, tin, and zinc. The metals yield three different types of bhasma corresponding to the nature of the ingredient used. They appear as best, medium, and inferior quality. Mercury is always used as a basic substance in the process of marana.

Iron bhasma preparation uses three basic processes: shodhana, dravana, and marana. Iron is purified by sinking the red-hot leaflet into the fresh trifala decoction (nishechan) repeatedly nine times. A freshly prepared decoction is used every time. Coarse pieces of sulfur are taken in khalva yantra and some amount of dewadali

swaras are added for bhavana (i.e., the sulfur pieces remain in good contact within this medicinal fluid). It is rubbed thoroughly and the process is repeated for at least days. When sulfur powder obtained at the end is sprinkled (pravap) over the fused iron, it is kept in liquidity. Iron bhasma always prepared with mercury; otherwise, it is not absorbed properly in the intestine. Additional processes are used to obtain the best quality iron bhasma. This includes loha maraka gana, amritkarana, and nirutthikarana. In the loha maraka, fresh lemon juice is prepared and a specific amount of hingula powder is added. Then these ingredients are mixed thoroughly. Afterward, the process of repeated dipping (nirvapana) red-hot iron leaflets in the medicinal fluid is applied to obtain loha bhasma. In the amritkarana process, equal amounts of loha bhasma and ghrita are placed in an iron pan and mixed properly under mild heat until the fat disappears. This compound now bears a yagavahi character. Finally, the properly prepared bhasma and trifala decoction (bhavana drava) is exposed to heat by the sun (suryaputa) or by the process of burning the herbs in closed freshly made mud containers (putapaka). At the end of this procedure, loha bhasma becomes the end product (niruttha and varttara) of filtration and separation procedures (nirutthikarana). The organoleptic characteristics of loha bhasma are that it is dark brown, has a faint smell, fine touch, and no taste. Iron as Fe2O3 is no more than 96.575% w/w, and iron as Fe is no more than 75% w/w present in this bhasma. Loha bhasma contains an ash value not less than 96.8% w/w and not more than 99.7% w/w. Its acid-insoluble ash is between 0.101 and 2.803% w/w. Its dose is 100 to 250 mg. (Prakash B. 1997)

Indications

Loha bhasma is a powerful hematinic Ayurvedic drug indicated in anemia. It stimulates the appetite and has a general vitalizing effect. It is readily assimilated in the body. General indications of Loha bhasma are as follows:

- Hyperacidity
- Abdominal Diseases
- Jaundice
- Anaemia
- Spleenic Disorders
- Asthma
- Leprosy

types of constipation.3 At low dose it is anti-emetic (if nausea is caused by heat) and in high doses it is an emetic.

Liver - Hepatoprotective action in hepatitis and chronic liver disease. Licorice works on ranjakapitta and soothes the heat that travels via the liver to the blood. Used for skin conditions, such as acne, with heat and inflammation.4 Its affinity for the blood and pitta help to soothe alochakapitta and any eye irritations.

Kidneys and nerves - As part of a formula for nervous exhaustion Licorice is a strong adrenal tonic giving enduring energy. It is a rasayana for the shukra dhatu and the whole reproductive system. Its cortisol-like action is useful in Addison's disease. As it is used to tonify majja dhatu, it can nourish an exhausted and hyperactive vata and pitta in such conditions as ME and CFS. The sattvic nature of Licorice calms the mind.

Urine - Its cooling action and unctuous nature are beneficial in inflammations of the urinary tract and it should be used to treat cystitis and painful, burning urination. Skin It is a useful emolliating herb for preventing itching with dry skin. Its anti-inflammatory pitta reducing effects are commonly employed to treat red, hot, inflamed skin disorders.

1.5.4 Amrta Kvatha

Common name - Guduchi, Giloy (H)

Sanskrit - Guduchi, Amrita, Chakralakshana

Latin - *Tinospora cordifolia* – Caulis (Menispermaceae) (Singh *et al.*, 2002)

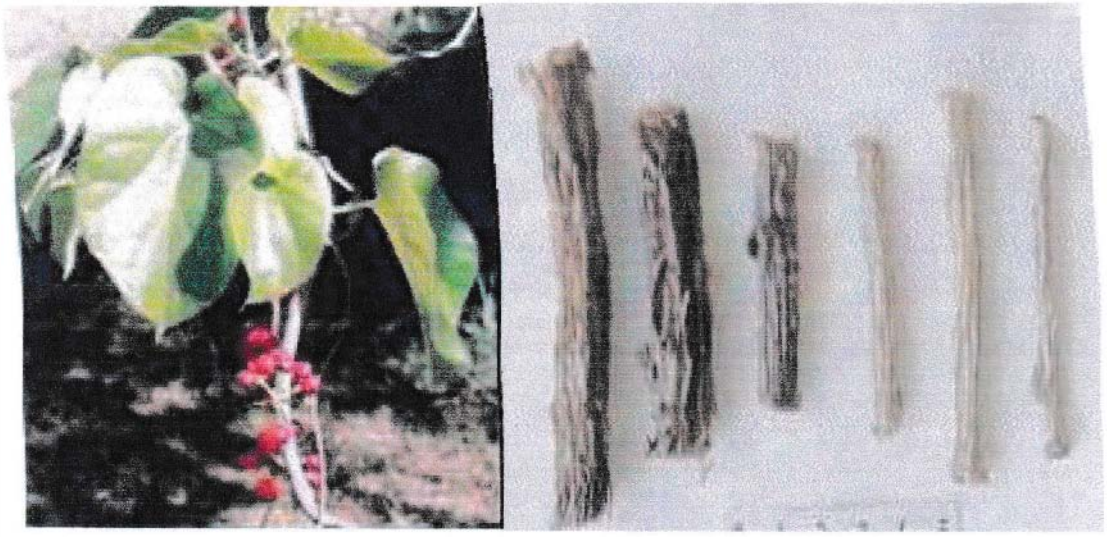


Fig: 1.3: AMRTA KVATHA

Indications

Liver - Liver damage, viral hepatitis or poisoning from alcohol, chemicals or recreational and medicinal drugs. Useful in repairing fibrosis and regenerating liver tissue.

Joints - Gout, arthritis and other inflammatory joint conditions.

Immunity - All auto-immune diseases causing inflammation. Applicable in degenerative diseases such as cancer, AIDS and arthritis as it boosts the immune system. Use to offset the ulcerative and toxic effects of chemo-radiotherapy.

Skin - Inflammatory skin conditions such as eczema, psoriasis, Systemic Lupus Erythmatosus. Specific for burning sensations on the skin.

GIT - Guduchi heals a bowel affected with constipation, intestinal bleeding, haemorrhoids or dysentery. Useful at redressing intestinal floral imbalance with candida-like symptoms such as bloating, flatulence and mal-absorption.

Metabolic - Regulates blood sugar levels via its direct effect on rakta and medas-dhatu thus benefiting diabetes and hypoglycemia. Guduchi is very calming to vata and the nervous system via its unctuous nature soothing nervous irritation.

Reproductive - Its ability to clear heat is applied when sexual dysfunction is caused by a hyper-heat condition. It is often used in formulas for male sexual dysfunction caused by pitta imbalance as its sweet post-digestive effect nourishes shukradhatu.

1.6 Therapeutic indications of DTR

1.6.1 Colic

Severe abdominal pain is often referred to as colic, which lasts with fluctuating intensity, with waves of pain lasting for a few seconds/minutes. Colic pain refers to a paroxysmal abdominal pain and it can be rather acute. It is also known as Shula in Ayurvedic terminology. Colic pain is caused due to the predominance of vayu, i.e. the aggravation of wind, and this pain can spread to other portions of the body including vulnerable parts such as the genital organs and the vascular region. Other causes include excessive food intake, flatulence, constipation, exposure to cold/damp, hernia, liver or ovary inflammation, gastric and duodenal ulcer, intestinal spasm, kidney stone, gall-stone and appendicitis whose presence confuses the matter but, since symptoms are quite different in each case, there is no difficulty in earmarking actual cause and location of pain.

There are many types of colic pain depending on the cause of the pain. These include the following: renal colic which starts from the small of the back and radiates from loin to groin/genitals; abdominal colic which occurs due to accumulation of wind in the intestines consequent upon feeding problems; infantile colic occurring due to accumulation of wind in the intestine consequent upon feeding problems; intestinal colic which occurs due to partial or complete obstruction of intestines mainly due to obstinate constipation; colic during pregnancy which has multiple causes such as wind in the abdomen, movement of foetus, displacement of uterus etc.; colic occurring due to presence of stone in the gall bladder; and menstrual colic which occurs due to interrupted menstrual flow causing pain in ovaries.

The symptoms vary according to body or organ affected. However, here are the common symptoms: the patient will suffer from constipation which may assume a chronic form, there may be nausea and vomiting as well. Sometimes the pain is so severe that the patient writhes with pain, squeezes the painful part with his hands, rolls on the floor, moans and cries due to severe impact of paroxysms of pain, bends double. If wind is the cause, he will feel relieved after the wind gets expelled.

The complications associated with this disease include excruciating pain, excessive thirst, fainting, obstruction to the passage of stool and urine, heaviness, anorexia, cough, dyspnoea and hic cup. If the patient of colic is afflicted with ten complications obstruction to the passage of stool and urine, heaviness, vomiting, fever, morbid thirst, giddiness, anorexia, emaciation, weakness and excruciating pain then he is surely to succumb to the disease.
It is mandatory and absolutely necessary that the patient is not allowed to go through any form of physical exercise, alcoholic beverages, sexual intercourse, anxiety, angers, sours, irritants, pungents, salty items, pulses and suppression of urges.

Colic pain which is associated with several complications is of serious nature and is incurable. In cases of curable colic, formulations such as DTR should be administered to pacify the patient. (Ayurvedic-medicines, 2009)

1.6.2 Anaemia

In Ayurvedic system Anaemia known as Pandu Roga which means the lack of red blood corpuscles and haemoglobin in the blood. Anaemia is the most common

disorder of the blood usually means `without blood`. This is defined as a qualitative or quantitative deficiency of haemoglobin. Haemoglobin is an important constituent of the human blood which is a molecule inside red blood cells or RBCs. It is hemoglobin which mixes with the inhaled oxygen and forms the complex oxy-haemoglobin, which gives the blood its characteristic red color. Haemoglobin is made up of two parts as haeme, an iron complex, and globin, a protein. Thus, iron is needed to make up the haeme of hemoglobin. This iron is supplemented by the diet that we consume. Anaemia leads to hypoxia i.e. lack of oxygen in organs. Since all human cells depend on oxygen for survival, varying degrees of anaemia can have a wide range of clinical consequences.

Types of Anaemia are as follows according to their causative agent as:

1. Iron Deficiency Anaemia: This is a type of Anaemia caused due to a gross deficiency of iron in the body.
2. Megaloblastic Anaemia: Megaloblastic anaemia is caused due to the deficiency of water-soluble vitamins in the blood of the person, especially vitamins B12 and folic acid.
3. Anaemia due to Underlying Diseases: Some diseases can lower the count of the red blood corpuscles in the blood. Diseases such as kidney disorders and hormonal imbalances can lose their erythrocytes.
4. Aplastic Anaemia: This is a metabolic disorder in the body when the blood is unable to make an adequate amount of red blood corpuscles.
5. Genetic Anaemia: Genetic anaemia is a deficiency of red blood corpuscles in the blood caused due to inherited factors.

Among the general causes of anaemia are malnutrition, faulty nutrition, wrong dietary habits, alcohol consumption and drug addiction. Anaemia may result from problems such as traumatic injury with profuse internal or external bleeding , bleeding piles, fistula , fissures , febrile diseases, diseases damaging the quality or quantity of blood, diseases of the liver (for example, jaundice and/or spleen disorders), tuberculosis and cancer. In the case of women, pregnancy related anaemia and anaemia related to menstrual problems as loss of blood through excessive menstruation are additional causes. Certain diseases like purpura and haemophilia, which are characterized by bleeding, can also be the cause. Defective blood formation because of infections, toxins, and drugs and also inadequate intake of iron and defective absorption of

substances in the diet, which enrich the blood, are the causes. Some anaemias are due to a combination of more than one of the causes enumerated above. Women are more prone to anaemia than men. This is because of the monthly loss of blood due to periods. During pregnancy or lactation, if women do not take additional supplementary diet, there are chances of becoming anemic. Children may suffer from anemia due to worms and old people may suffer from the same due to low intake of food.

The most prominent symptom of anaemia is the pale yellowish appearance of the skin, hence the Ayurvedic name of the disease is Pandu Roga. The best guide, however, is the colour of the internal lining of the eyelid. There is weakness and giddiness, the breathing is shallow, the pulse rapid, and the blood pressure is often becomes low. Others are as low grade fever at times, burning of palms and soles, tingling, numbness, yellowish and/or scanty urination, anorexia and/or indigestion, vertigo, fainting, dyspnoea at rest/ on exertion, etc. can be observed in anaemia. Amongst women, additional symptoms such as no periods or short or scanty periods, with or without leucorrhoea (white discharge) may occur. In severe cases, the tongue is often sore and the nails of the fingers brittle and concave instead of being convex. If the disease is ignored, it may turn into pernicious anaemia, which is more difficult to cure. In some severe cases, the patient may have to be given a blood transfusion to make up the loss of blood. Usually blood transfusion happens in traumas like severe haemorrhage due to injury or bursting of an ulcer in the abdominal region.

In the era of *ayurved,* no pathological techniques to measure the exact blood haemoglobin level were available but *ayurved* suggests examination of nails, skin, eyes, lips, etc., to determine the level of *pandu* (pallor) and accordingly decide upon treatment.

Anaemia can be prevented if it caused due to vitamin deficiency or iron deficiency. This can be done by including the deficient nutrients in the diet. However, anaemia caused due to inherent conditions and genetic factors cannot be prevented. Such anemic conditions must be treated with proper medical supervision. In general, *ayurved* suggests a nutritive diet and possible supplementary foods and metal ashes like *loh bhasma* for anemic conditions of the body. Non-vegetarian products such as meat, fish and milk are also recommended. Pomegranate and black grapes are some of

legumes, beet, carrots etc., add to the iron content in the blood. Honey and molasses are dietary supplements which help in building blood. Dates, raisins and prunes are good supplementary dry fruits. The exact cause of the malady should be ascertained before starting the treatment. If it is of a mild nature and has been caused by insufficient nutrition, massive doses of the substances lacking could cure it. Standard Ayurvedic preparations as DHATRI LAUHA can be used with ghee, honey for treating anaemia. (Anemia, 2009)

1.6.3 Jaundice

In Ayurveda 'Jaundice' is known as kamla. Jaundice is not a disease in itself but a symptom of other liver disorders such as gallstones, hepatitis and cirrhosis. It is a condition characterized by yellowness of skin, sclera (whites of eyes), mucous membranes and body fluids due to deposition of bile pigment resulting from excess billirubinin the blood (hyperbilirubinemia). However Jaundice is a disease for which there exists no known medicine or remedy in the allopathic medical system. It is commonly occur in newly born babies, usually within the first three days of life. An infants' liver is not yet mature enough to metabolize a molecule called billirubin, which affects the livers ability to make red cells. In the Ayurvedic teachings Jaundice is a complaint of the *pitta* region of the body, which denotes a build up of toxins in an intestinal area that is causing an in-balance in the overall *dosha.*

In modern jargon, jaundice is of three types — haemolytic, hepatic and obstructive. When the liver is laid low by an infection it can not produce enough bile to keep the body cells and tissue healthy. At this point jaundice develops and the skin and the whites of the eyes take on a yellow tint. It is not fatal in itself but its presence can greatly delay recovery from the original complaint so it is best to treat it as soon as possible to allow the body to fight back. Jaundice is caused due to following causes as obstruction of the bile ducts (by infection, tumor or gallstones), viral hepatitis (hepatitis A, hepatitis B, hepatitis C, hepatitis D, and hepatitis E), drug-induced cholestasis (bile pools in the gallbladder because of the effects of drugs), drug-induced hepatitis (hepatitis triggered by medications, including erythromycin sulfa drugs, antidepressants, anti-cancer drugs, rifampin, steroids, chlorpropamide, tolbutamide, oral contraceptives, testosterone, propylthiouracil), biliary stricture, alcoholic liver disease (alcoholic cirrhosis), pancreatic carcinoma (cancer of the pancreas), primary biliary cirrhosis, ischemic hepatocellular jaundice (jaundice caused by inadequate oxygen or inadequate blood flow to the liver), intrahepatic cholestasis

of pregnancy (bile pools in the gallbladder because of the pressure in the abdomen with pregnancy), hemolytic anemia, congenital disorders of bilirubin metabolism (Gilbert's syndrome, Dubin-Johnson syndrome, Rotor's syndrome), chronic active hepatitis, autoimmune hepatitis, malaria, deficiency of iron in the blood is usually the main cause of jaundice. In a majority of the cases, it happens due to alcoholism. Hepatic jaundice due to liver problem and obstructive condition may be possible due to bile stone and roundworm. Epidemic of jaundice may be possible due to bacteria like spirochaetosis ictero-haemonrhagica, pneumonia, typhoid and syphilis.

It usually affects children and young adults more than other groups. The disease normally spreads due to dirty water and infected needles of syringes. A jaundice bacterium is located in the patients stool. Jaundice lasts for one to six weeks.

As symptoms of Jaundice yellow colour first appears in the whites of eyes and then spreads to the whole of the skin. Excess of bile pigments circulating in the blood give the skin a yellow colour. Since the bile does not go into the intestines as it normally does, the stools of the jaundice patient lose their typical brownish colour and in severe cases, these are almost whitish. If the liver is inflamed, it is painful and tender to touch. Jaundice patients may also feel fever (100-102°C). General symptoms of Jaundice include as follows: Yellow skin, yellow eyes, dark or reddish urine, orange urine, red urine, bronze skin, loss of appetite, bitter taste in mouth, furry tongue, pale faeces, foul-smelling faeces, nausea, itching skin, lethargy, slow pulse, confusion, indigestion, headache. Jaundice is detectable clinically by liver functioning test.

As a complication of Jaundice, it is advised to avoid alcohol, which is toxic to the liver, avoid fried food and large meals, avoid eating out during rainy days, drink water after boiling it, avoid uses infected syringes, avoid processed food products that contain preservatives, artificial flavours and other additives, Living rooms should be properly ventilated. Bilirubin test is must. The patient should take complete rest. According to the patient's condition, protein diet like soyabin, eggs, dal and milk can be given. The patient can take carbohydrate diet like chappatis, bread, boiled potatoes, etc. Take fresh fruit or lemon juice and coconut water. Fresh vegetables having a bitter taste are useful in this condition. Take Vitamin C (500 mg) twice a day. This can reduce the duration of jaundice. Drugs to induce more urination are helpful in expelling excess bile from the blood.

Amlapitta is classified into two types as- regurgitation through the mouth (urdhvaga amlapitta) and a similar type of movement in the lower GI tract (adhoga amlapitta).

In Ayurveda, hyperacidity may be an expression of excessive digestive enzymes (pachaka pitta) due to stimulation from vata. This excessive enzyme secretion will dry the mucous (kaphadhara kala) lining of the stomach, leading to irritation, hypersecretion, acidity, and eventually ulceration. Pitta is at the root of such a disorder. Pitta which increases by the etiological factors (especially with its acid state), results in hyperacidity. This state of pitta is burnt out to cause acid eructation. Thus hyperacidity is a hypersecretory disorder of the stomach and duodenum. It involves the pathological state of pitta locally and blood systemically. According to Ayurvedic medicine, hyperacidity is mainly caused by eating foods that are excessively sour, hot, and spicy; increasing the intake of alcohol; and eating oily, fried, or irritant foods. Violation of rules for eating and lifestyle changes, including overeating, having an incompatible diet, eating irritant and contaminated food, eating when suffering from indigestion, living in temperate climate, and experiencing factors that increase pitta (e.g., negative emotions and control of natural urges) are causes this disease. Dietary and lifestyle choices may contribute to GERD. Certain foods and beverages, including chocolate, peppermint, fried or fatty foods, coffee, or alcoholic beverages, may weaken the LES, causing reflux and heartburn. Studies show that cigarette smoking relaxes the LES. Obesity and pregnancy can also cause GERD. Doctors believe that some people suffer from GERD due to a condition called hiatal hernia. Patients with connective tissue diseases, such as scleroderma, and chronic respiratory disease, such as asthma and cystic fibrosis, institutionalized and intellectually handicapped patients. Patients nursed in a supine position for prolonged periods are at increased risk of reflux disease as hyperacidity. (Mitra, *et al.,* 2004)

The most prevalent symptom is a burning pain in the area of the lower chest or upper abdomen. The pain of an ulcer can last anywhere from 30 min to a few hours. Additional symptoms that may be experienced are weight loss, a decrease in appetite, anaemia, headache, nausea, and vomiting. There may be times when the pain of an ulcer seems to be gone and then it suddenly returns. Pain associated with ulcers affect different people in different ways. Some people experience pain immediately after eating, whereas others may not be bothered for several hours. It is beneficial to figure

out what may be responsible for producing excess stomach acid so these symptoms can be avoided.

The diagnosis of this condition is made by identifying a combination of signs and symptoms such as indigestion , bitter and acid regurgitation , substernal burning , hypersalivation , fatigue, and increased stress .

Approximately 15 to 20% of adults experience amlapitta at least once a week. Obesity (body mass index [BMI] >30 kg/m), alcohol consumption (>7 standard drinks/week), and a first-degree heartburn increase the risk of having hyperacidity symptoms.

In Ayurveda, the following procedures are used in treating hyperacidity as:

1. Emesis therapy with the water extracts of snakeguard (*Trichosanthes dioica*) and neem (*Azadirachta indica*) followed by purgation with powders of trivrit (*Operculina turpethum*) or any one of the following: avipattikara curna, amalakyadi curna, abhayarishta, or triphala curna.
2. If the burning pain is intensive, the patient is advised to apply whole body massage with candana taila or lakshadi taila.
3. In conditions of regurgitation through the mouth, purgation is advised. In case of lower GI symptoms due to hyperacidity, emesis is the choice.

In allopathy with the introduction of acid-suppressive drugs like histamine-2 blockers in the 1970s, the treatment of PUD was revolutionized. By the 1980s, the advent of Helicobacter pylori brought about a dramatic idea about of curing this disease.

Dhatri lauha is frequently used in amlapitta with ghee & honey.

AIMS OF THE STUDY

This research was carried out in order to characterize the pharmacological profile of the marketed Ayurvedic medicinal preparation specially DHATRI LAUHA (DTR) on the following aspects:

1. To see whether DTR has any effect on Gastro-intestinal motility or emptying.
2. Analgesic and Anti-inflammatory properties of DTR are also possible site of interest.
3. To monitor whether DTR has any effect on oxygen consumption.
4. To evaluate neuropharmacological effects of DTR.
5. To find out whether DTR has any Psychopharmacological effects or not.

All these studies were performed in an effort to ensure the safety of the general patients or users of the country as a whole.

Chapter Two

Materials and Methods

2.1 Collection of the Ayurvedic Formulation

For the pharmacological study DHATRI LAUHA (DTR) was collected from Sree Kundeswari Aushadhalaya Ltd, Chittagong, Bangladesh.

2.2 Dose

For the pharmacological experiment, the powdered tablets 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 experiments the drugs were administered per oral route.

Table 02.1 Doses Used In Different Experiments

SL. No.	Experiment Name	Dose
01	Hole Cross Test	100 mg/kg, 200 mg/kg, 400 mg/kg Body Wt
02	Hole Board Test	100 mg/kg, 200 mg/kg, 400 mg/kg Body Wt
03	Open Field Test	100 mg/kg, 200 mg/kg, 400 mg/kg Body Wt
04	Climbing Out Test	100 mg/kg, 200 mg/kg, 400 mg/kg Body Wt
05	Acetic Acid Induced Writhing Test	100 mg/kg, 200 mg/kg, 400 mg/kg Body Wt
06	Formalin Induced Paw Licking Test	100 mg/kg, 200 mg/kg, 400 mg/kg Body Wt
07	Gastrointestinal motility test	100mg/kg body wt
08	Gastric Emptying Test	100mg/kg body wt
09	Hypoxia Test	100mg/kg body wt

2.3 Experimental Animals

Male and Female mice (Swiss-Webster strain, 20-40 gm body weight) bred in the Animal House of the Department of Pharmacy, Jahangirnagar University, were used for the pharmacological experiments. They were kept in cages having dimensions of 30 x 20 x 13 cm^3 and soft wood shavings were employed as bedding in the cages.

The animals were provided with standard laboratory food and tap water '*ad libitum*' and maintained at natural day night cycle. They were fed with "mouse chow" (prepared according to the formula developed at BCSIR). 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. For both control and drug group female mice were used in all experiments.

2.4 Controls

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 per 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.

2.5 Pharmacological Study with Animal Models

2.5.1 Hole Cross Test

Principle: To test the effect of drug on motor activity by counting the number of holes the mice crossed.

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

Reagent: Not applicable

2.5.2 Hole Board Test

Principle: 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 (1964), Boissier, Simon and Lwoff (1964) and Boissier and Simon (1967). The test enables the initial exploratory activity of the animal and its variations brought about by psychotropic elements of a drug to be unmistakably assessed. The hole board test was carried out to investigate the effect of the drug on the exploratory behavior of the animals. Exploration can be defined as a broad category of behavior, the consequences of which are to provide the organism with information about the exteroceptive environment. The principle of the test is that a novel situation of open field evokes in the animals a pattern of behavior characterized by exploration (head dipping through the holes), locomotion

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).

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

Reagent: Not applicable

2.5.3 The Open Field Test

Principle: To test whether the drug has any effect on the parameters as follows- ambulation, center ambulation, standing behaviour and emotional defecation. These actually denote whether the drug is stimulant or depressant.

Procedure: In this experiment, the method of Gupta (1971) was employed. The floor of an open field of half square meter was divided in to a series of squares, each alternatively colored black and white. The apparatus had a wall of 40 cm. The number of squares, traveled by the animal, was recorded for a period of two minutes.

Reagents: Not applicable

2.5.4 Climbing Out Test

Principle: To analyse the stimulant or depressant property of the drug by calculating the time when mice is climbing out from the box.

Procedure: This experiment was carried out by the method of Sandberg (1957). The animals were put in a cage with dimension of 60 X 50 X 30 cm and having dark walls. 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.

Reagents: Not applicable

2.5.5 Acetic Acid Induced Abdominal Writhing Assay

Principle: To analyse whether the drug has any analgesic property or not by counting the writhing.

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

Reagent: 0.06% acetic acid

Percent protection was calculated as follows: -

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

2.5.6 Formalin Test

Principle: To monitor the analgesic and anti-inflammatory activity by counting the number of paw licking.

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

Reagent: 1% formalin

2.5.7 Hypoxia Test

Principle: To analyse whether the drug has any effect on the dissolved oxygen in blood in accordance with oxygen consumption level also and it is done by calculating the survival time.

Procedure: In this experiment the method of Caillard *et al.*, (1975) was employed. Three set of ten mice per groups were used. 2 hr after the treatment, the hypoxia time was recorded individually for all the animals. The animals were placed in an empty glass jar of 300 ml capacity attached with an electronic watch; the jars were made air tight with greased glass stoppers and the time until the onset of convulsion was recorded.

2.5.8 Gastric Emptying Measurements

Principle: To monitor the effect of drug on gastric emptying by weighing the food material given and food material found in stomach.

Procedure: Gastric emptying of the solid nutrient meal measured in experimental mice by minor modifications of the two techniques previously described (Martinez *et al.*, 2002).

Sixteen Swiss-Webster male mice were fasted for 18 hours prior to the experiment. Out of the 16, 8 were randomly chosen as the CR (drug) group and the remaining 8 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 hr. feeding period, CR was orally administered to the mice of CR group at 200mg/kg (2 x 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 CR. 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 (Shimadzu) 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 follows:

$$\% \text{ GE} = 1 - \frac{\text{Gastric contents}}{\text{Total food intake}} \times 100$$

Reagent: Not applicable

2.5.9 Gastro-Intestinal Motility Test

Principle: Gastrointestinal (GI) 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. GI motility test was carried out to find the effect of the drugs on the peristaltic movement of the GI tract.

Procedure: The experiment was carried out by the method previously described by Chatterjee, 1993. $BaSO_4$ 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 test drug. 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 ileocecal junction). The test drug was compared with the control group administered with distilled water.

Reagents: Barium sulphate ($BaSO_4$); Carboxy methyl cellulose (CMC)

2.5.10 Statistical Analysis

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

3.1 Hole Cross Test

As spontaneous movements of the animals include, by definition, both the propulsive and non-propulsive movements of the animal, and as the fluctuating and multifarious nature of many overt movements patterns impossible, to accurately measure the effects of a drug on the spontaneous motor activity of animals by using a single experimental procedure, the hole cross test was performed (Robbing, 1977). The weight range of female mice for this experiment was 20- 25 g.

STATISTICAL FINDINGS

At dose of 100mg /Kg response of the mice at min 30 and min 60 overall decreased but the increase result was found in min 120, min 180 and min 180. But no results were statistically significant. (Table 3.1, Fig. 3.1).

At dose of 200mg /Kg response of the mice at min 30 ($p=0.005$) the increasing result was found to be statistically highly significant. Although there was overall increases the response none of the other results were statistically significantly different from the corresponding control animals. . (Table 3.2, Fig. 3.2).

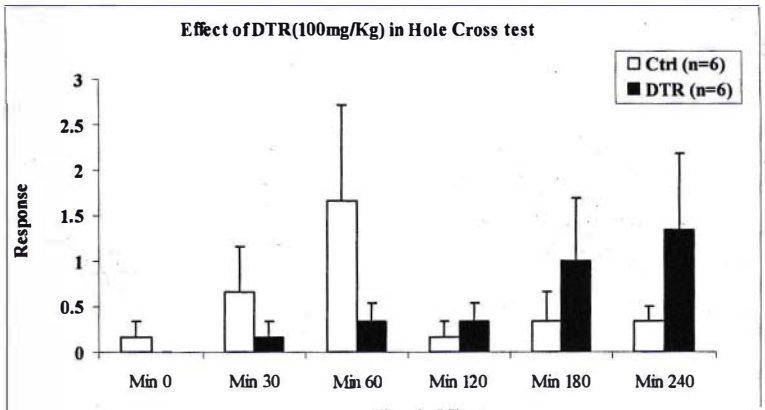
At dose of 400mg /Kg the response of the mice overall decreased compared to the control animals. . (Table 3.3, Fig. 3.3).

Table 3.1: The effect of DTR (100mg /kg) in the Hole Cross Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		0.167± 0.167	0.667± 0.494	1.667± 1.054	0.167± 0.167	0.333± 0.333	0.167± 0.167
DTR (n=6)		0.000± 0.000	0.167± 0.167	0.333± 0.211	0.333± 0.211	1.000± 0.683	1.333± 0.843
t/p		1.000/ 0.363	0.958/ 0.360	1.240/ 0.266	-0.620/ 0.549	-0.877/ 0.401	-1.357/ 0.229
95% confidence interval	Lower	-0.262	-0.663	-1.370	-0.765	-2.360	-3.329
	Upper	0.595	1.663	4.036	0.432	1.027	0.996

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

Figure 3.1: The effect of DTR (100mg/Kg) in Hole Cross Test



Chapter Three

Results and Discussion

Table 3.2: The effect of DTR (200mg /kg) in the Hole Cross Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		2.333± 1.054	0.000± 0.000	0.333± 0.211	0.500± 0.224	0.000± 0.000	0.167± 0.167
DTR (n=6)		2.000± 0.856	2.333± 0.494	2.333± 1.054	1.667± 0.919	1.500± 0.671	1.667± 0.615
t/p		0.245/ 0.811	-4.719/ **0.005****	-1.861/ 0.092	-1.234/ 0.267	-2.236/ 0.076	-2.355/ 0.059
95% confidence interval	Lower	-2.693	-3.604	-4.395	-3.523	-3.224	-3.076
	Upper	3.359	-1.062	0.395	1.189	0.224	0.076

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

Figure 3.2: The effect of DTR (200mg/Kg) in Hole Cross Test

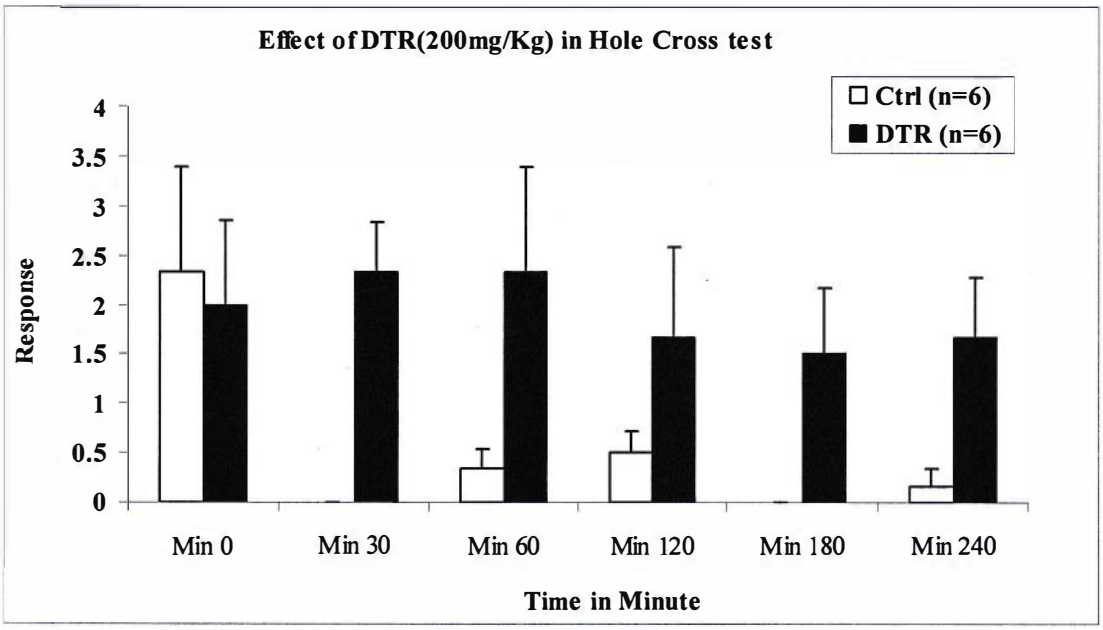
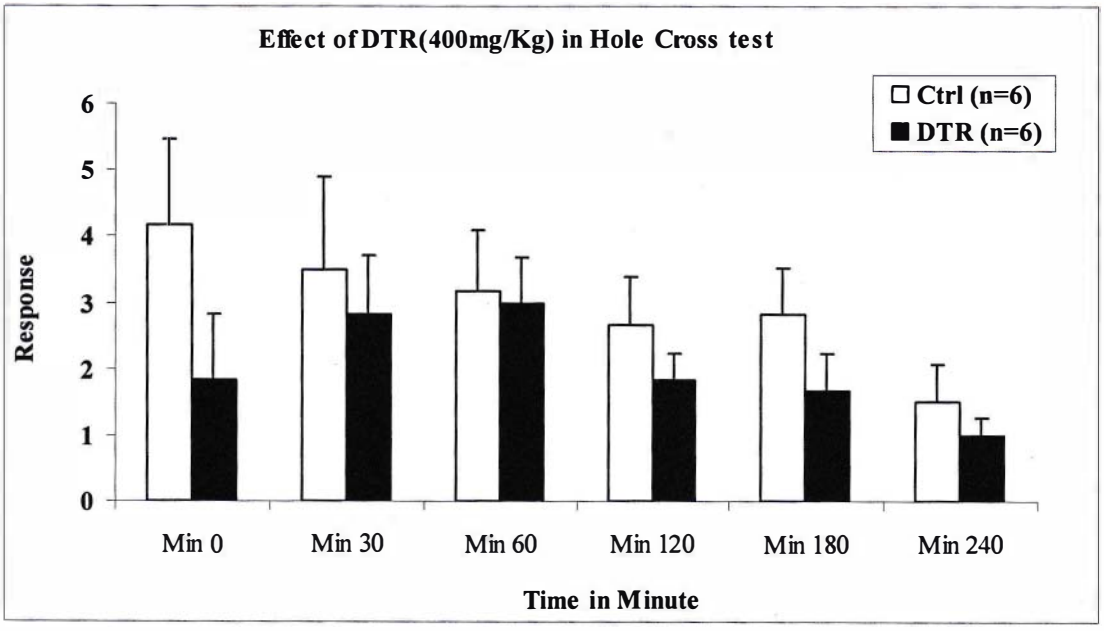


Table 3.3: The effect of DTR (400mg /kg) in the Hole Cross Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		4.167± 1.302	3.500± 1.408	3.167± 0.910	2.667± 0.715	2.833± 0.703	1.500± 0.563
DTR (n=6)		1.833± 0.980	2.833± 0.872	3.000± 0.683	1.833± 0.401	1.667± 0.558	1.000± 0.258
t/p		1.432/ 0.183	0.402/ 0.696	0.146/ 0.886	1.016/ 0.333	1.300/ 0.223	0.808/ 0.438
95% confidence interval	Lower	-1.298	-3.025	-2.368	-0.994	-0.833	-0.880
	Upper	5.964	4.358	2.702	2.660	3.166	1.880

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

Figure 3.3: The effect of DTR (400mg/Kg) in Hole Cross Test



3.2 Hole Board Test

The experiment was carried out to get a clear picture of the effect of the drugs under consideration on the pattern of behavior characterized by spontaneous ambulatory activity, exploratory activity and emotional defecation of the animals. This experiment presents with a different and more complex environment to explore. The weight range of mice for this experiment was 25- 30 g.

STATISTICAL FINDINGS

3.2.1 Ambulation

At dose of 100mg /kg the response of the mice overall increased, whereas at min 180 (p=0.010) the result of increase was found to be statistically significant and in min 240 it was found as statistically highly significant (p=0.009). (Table 3.4, Fig. 3.4).

At dose of 200mg /kg the response of the mice overall increased in min 60, min 120 and min 240. The exceptions were in min 30 and min 180 whereas the responses were decreased compared to the respective control animals. But no results found as statically significant. (Table 3.5, Fig. 3.5).

At dose of 400mg /Kg the response of the mice were overall increased except in min 30 the response were found as decreased. (Table 3.6, Fig. 3.6).

Table 3.4: The effect of DTR (100 mg/kg) on Ambulation in Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		17.000± 4.066	14.500± 7.032	23.167± 7.534	14.000± 4.397	14.833± 3.646	7.167± 2.587
DTR (n=6)		28.833± 4.771	27.500± 6.908	29.000± 3.256	24.833± 4.665	28.000± 2.000	25.000± 4.830
t/p		-1.888/ 0.088	-1.319/ 0.217	-0.711/ 0.501	-1.690/ 0.122	-3.166/ **0.010***	-3.254/ **0.009****
95% confidence interval	Lower	-25.800	-34.963	-25.354	-25.117	-22.433	-30.043
	Upper	2.134	8.963	13.688	3.450	-3.901	-5.624

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

Figure 3.4: The effect of DTR (100mg/Kg) on Ambulation in the Hole Board Test.

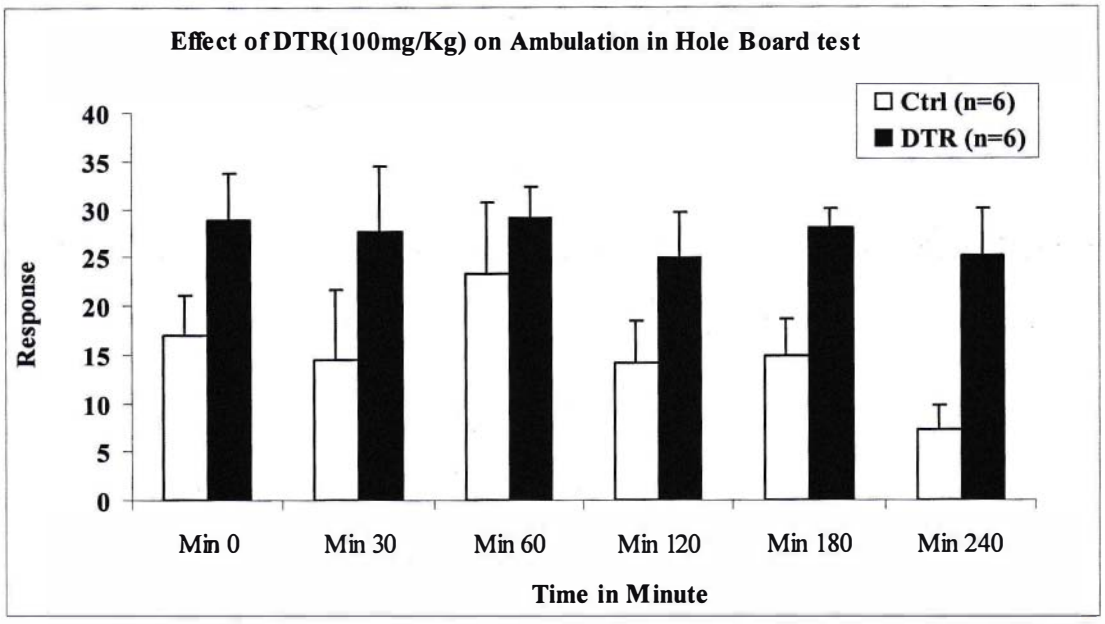


Table 3.5: The effect of DTR (200mg /kg) on Ambulation in the Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		16.833± 7.821	20.333± 9.028	11.833± 5.003	16.833± 7.989	14.167± 8.553	13.167± 9.354
DTR (n=6)		11.667± 3.818	14.833± 4.199	16.333± 3.703	17.500± 1.522	12.833± 1.701	16.167± 3.478
t/p		0.594/ 0.566	0.552/ 0.598	-0.723/ 0.486	-0.082/ 0.938	0.153/ 0.884	-0.301/ 0.770
95% confidence interval	Lower	-14.224	-18.000	-18.368	-21.155	-20.600	-25.236
	Upper	24.558	29.000	9.368	19.822	23.267	19.236

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

Figure 3.5: The effect of DTR (200mg/Kg) on Ambulation in the Hole Board Test

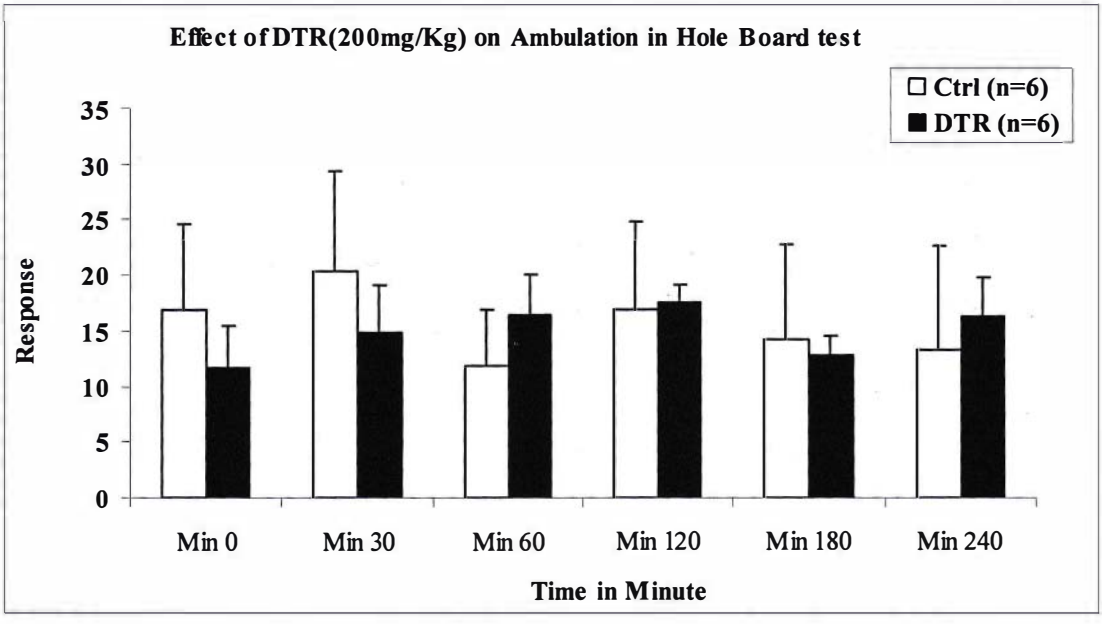
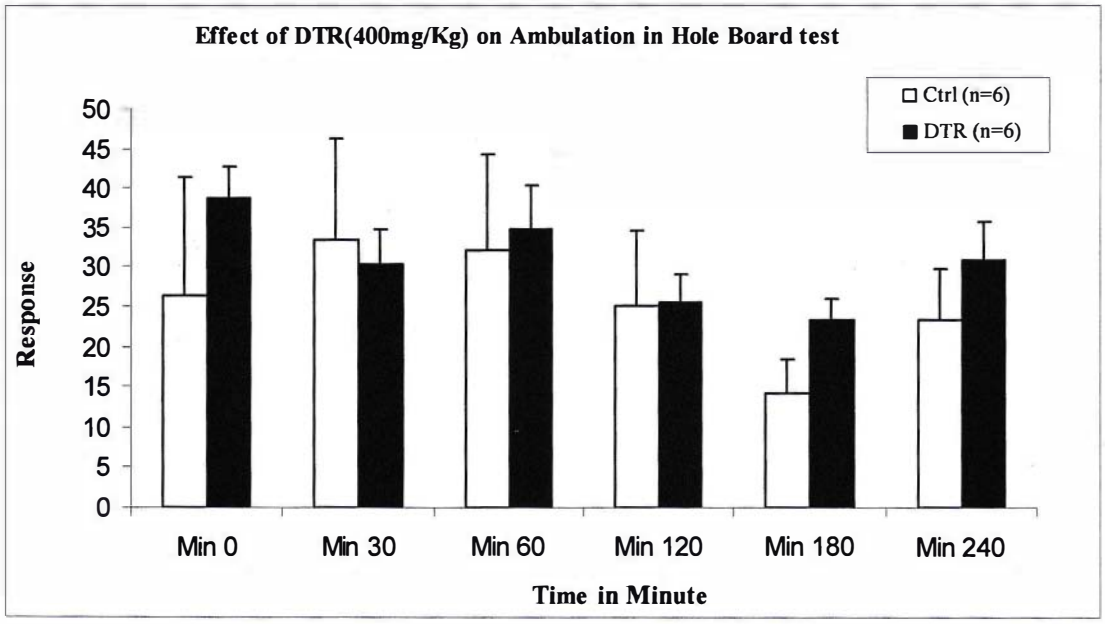


Table 3.6: The effect of DTR (400mg /kg) on Ambulation in the Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		26.333± 15.136	33.333± 12.808	32.000± 12.332	25.000± 9.623	14.167± 4.159	23.167± 6.462
DTR (n=6)		38.667± 3.947	30.333± 4.432	34.833± 5.412	25.500± 3.510	23.333± 2.654	30.667± 4.924
t/p		-0.788/ 0.449	0.221/ 0.829	-0.210/ 0.838	-0.049/ 0.962	-1.858/ 0.093	-0.923/ 0.378
95% confidence interval	Lower	-47.187	-27.198	-32.840	--23.323	-20.159	-25.602
	Upper	22.520	33.198	27.173	22.323	1.826	10.602

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

Figure 3.6: The effect of DTR (400mg/Kg) on Ambulation in the Hole Board Test



3.2.2 Head dipping

At dose of 100mg /Kg the response of the mice overall increased but none of the result found as statistically significant. (Table 3.7, Fig. 3.7).

At dose of 200mg /Kg the response of the mice found as increased min30, min120, and min240. The exception was found in min 60 and min 180 as decreased. (Table 3.8, Fig. 3.8).

At dose of 400mg /Kg the response of the mice overall increased but none of the result was found as statistically significant and the exception was found in min 30 as decreased. (Table 3.9, Fig. 3.9).

Table 3.7: The effect of DTR (100mg /kg) on Head Dipping in the Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		0.500± 0.342	1.333± 0.615	0.500± 0.500	0.167± 0.167	0.333± 0.333	0.500± 0.342
DTR (n=6)		2.833± 1.515	1.833± 1.222	1.333± 1.333	1.167± 1.167	2.500± 1.628	1.833± 1.327
t/p		-1.503/ 0.188	-0.365/ 0.722	-0.585/ 0.571	-0.849/ 0.416	-1.304/ 0.245	-0.973/ 0.370
95% confidence interval	Lower	-6.217	-3.549	-4.006	-3.626	-6.341	-4.736
	Upper	1.550	2.549	2.340	1.626	2.007	2.069

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

Figure 3.7: The effect of DTR (100mg/Kg) on Head Dipping in the Hole Board Test

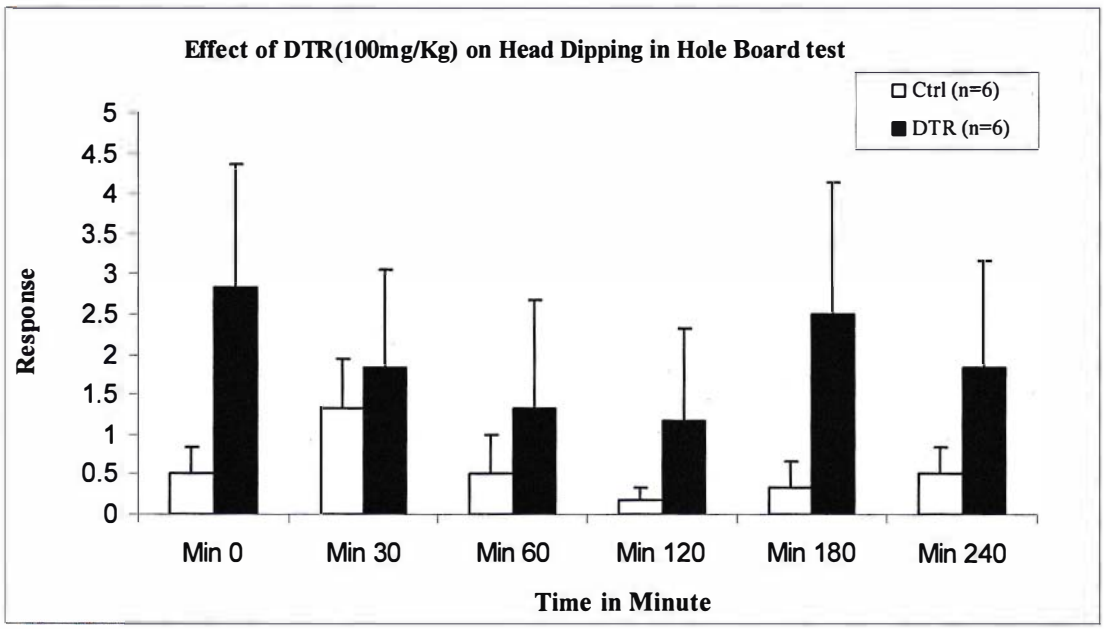


Table 3.8: The effect of DTR (200mg /kg) on Head Dipping in the Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		5.667± 2.836	4.500± 1.765	6.000± 2.129	2.167± 0.833	3.333± 1.085	1.667± 0.919
DTR (n=6)		4.333± 2.076	5.167± 2.548	5.000± 1.713	4.000± 2.251	1.833± 0.872	2.000± 0.966
t/p		0.379/ 0.712	-0.215/ 0.834	0.366/ 0.722	-0.764/ 0.463	1.077/ 0.307	-0.250/ 0.808
95% confidence interval	Lower	-6.499	-7.574	-5.088	-7.181	-1.603	-3.304
	Upper	9.165	6.241	7.088	3.515	4.603	2.638

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

Figure 3.8: The effect of DTR (200mg/Kg) on Head Dipping in the Hole Board Test

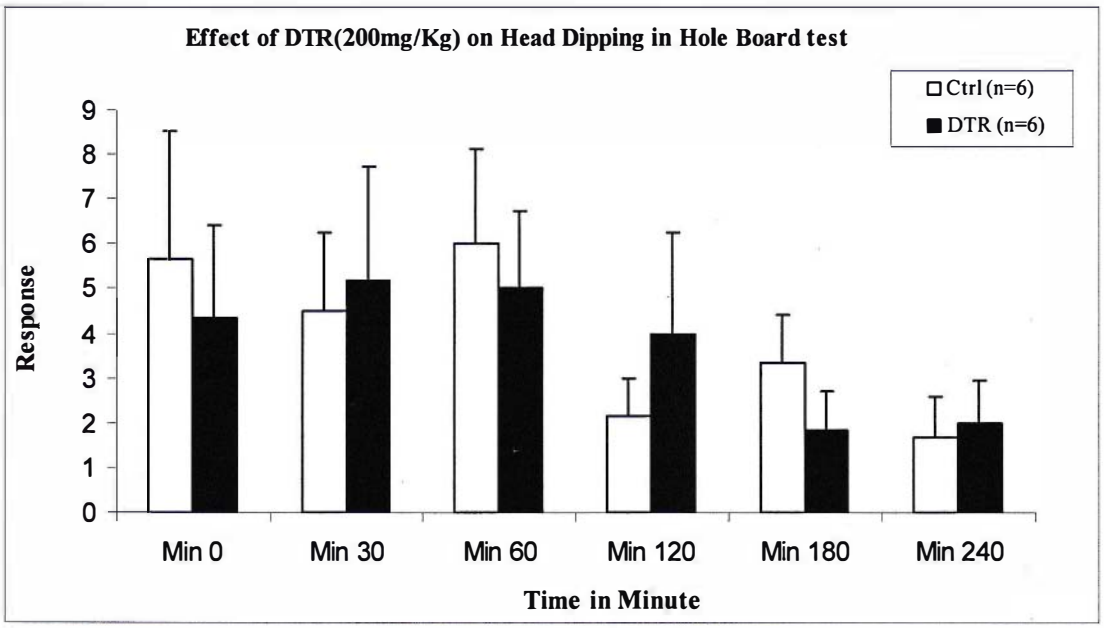
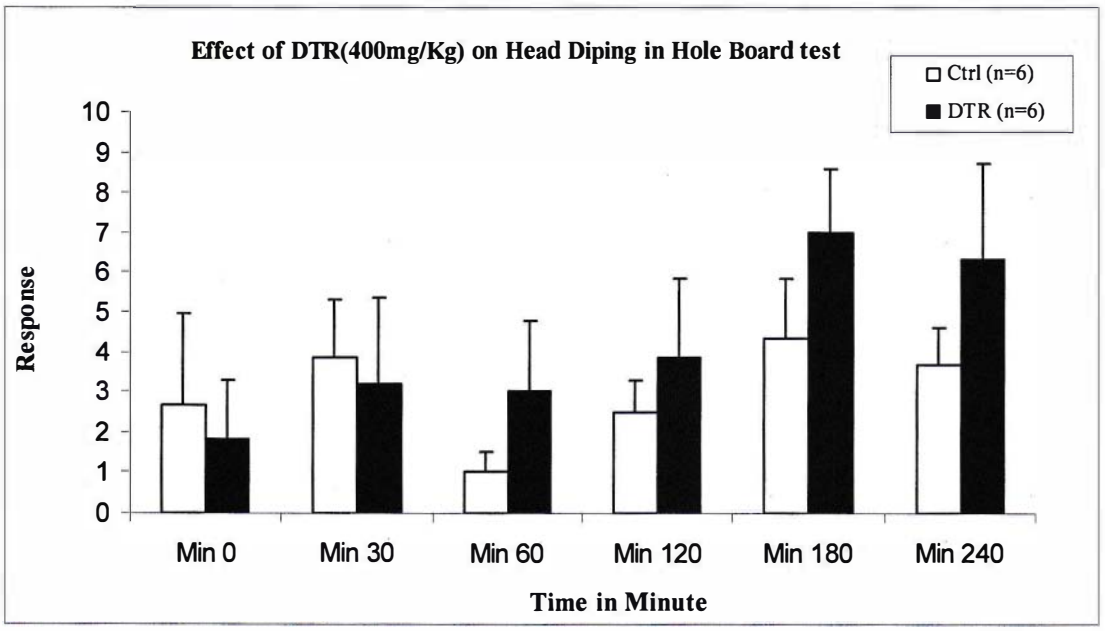


Table 3.9: The effect of DTR (400mg /kg) on Head Dipping in the Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		2.667± 2.275	3.833± 1.493	1.000± 0.516	2.500± 0.764	4.333± 1.520	3.667± 0.919
DTR (n=6)		1.833± 1.447	3.167± 2.197	3.000± 1.770	3.833± 1.990	7.000± 1.571	6.333± 2.404
t/p		0.309/ 0.764	0.251/ 0.807	-1.085/ 0.321	-0.625/ 0.553	-1.220/ 0.250	-1.036/ 0.337
95% confidence interval	Lower	-5.175	-5.252	-6.541	-6.464	-7.537	-8.863
	Upper	6.842	6.585	2.541	3.797	2.204	3.529

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

Figure 3.9: The effect of DTR (400mg/Kg) on Head Dipping in the Hole Board Test



3.2.3 Emotional defecation

At dose of 100mg /Kg the response of the mice found as increased in min60, min240 and the exception was found in min 30, min 120 as decreased and in min 180 the response was found as same. (Table 3.10, Fig. 3.10).

At dose of 200mg /Kg the response of the mice overall increased but none of the result was found as statistically significant. (Table 3.11, Fig. 3.11).

At dose of 400mg /Kg the response of the mice overall increased in min 30, min 60, min 120 but none of the result was found as statistically significant and the exception was found in min 180 and min 240 as decreased. (Table 3.12, Fig. 3.12).

Table 3.10: The effect of DTR (100mg /kg) on Emotional Defecation in the Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		1.167± 0.401	1.333± 0.615	0.333± 0.211	0.333± 0.333	0.333± 0.333	0.333± 0.211
DTR (n=6)		1.000± 0.365	0.667± 0.333	0.500± 0.224	0.167± 0.167	0.333± 0.333	0.667± 0.333
t/p		0.307/ 0.765	0.953/ 0.363	-0.542/ 0.599	0.447/ 0.664	0.000/ 1.000	-0.845/ 0.418
95% confidence interval	Lower	-1.042	-0.891	-0.851	-0.664	-1.050	-1.212
	Upper	1.376	2.225	0.518	0.997	1.050	0.545

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

Figure 3.10: The effect of DTR (100mg/Kg) on Emotional Defecation in the Hole Board Test

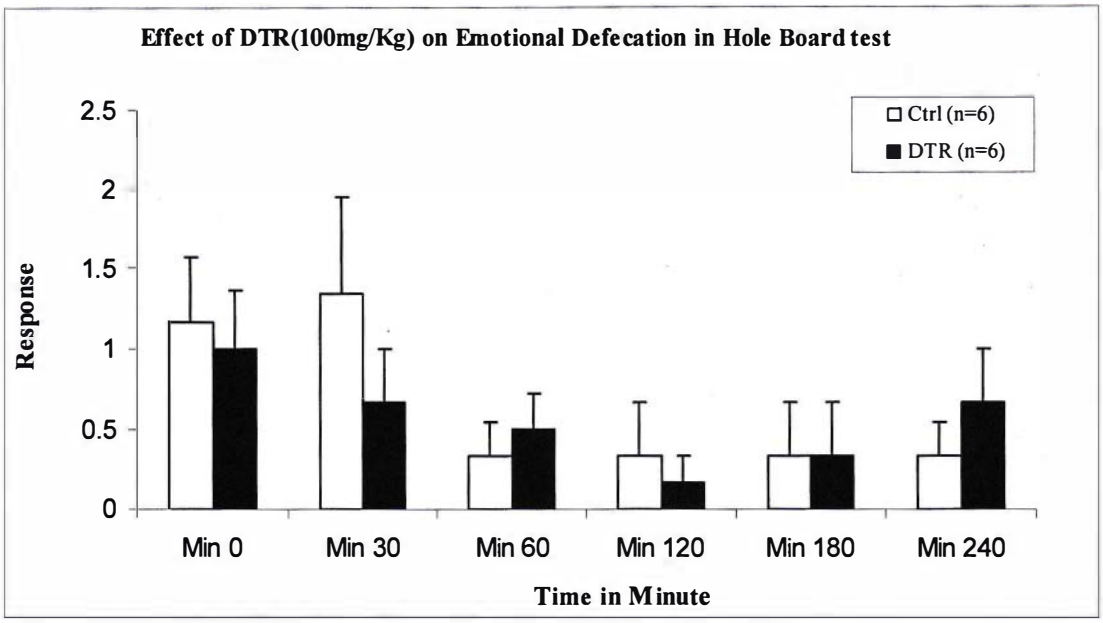


Table 3.11: The effect of DTR (200mg /kg) on Defecation in the Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		1.333± 0.803	0.500± 0.224	0.500± 0.342	0.167± 0.167	0.000± 0.000	0.000± 0.000
DTR (n=6)		2.000± 0.447	1.000± 0.516	1.167± 0.401	1.167± 0.543	0.167± 0.167	0.167± 0.167
t/p		-0.725/ 0.485	-0.889/ 0.395	-1.265/ 0.235	-1.762/ 0.129	-1.000/ 0.363	-1.000/ 0.363
95% confidence interval	Lower	-2.714	-1.754	-1.841	-2.393	-0.595	-0.595
	Upper	1.381	0.754	0.508	0.393	0.262	0.262

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

Figure 3.11: The effect of DTR (200mg/Kg) on Emotional Defecation in the Hole Board Test

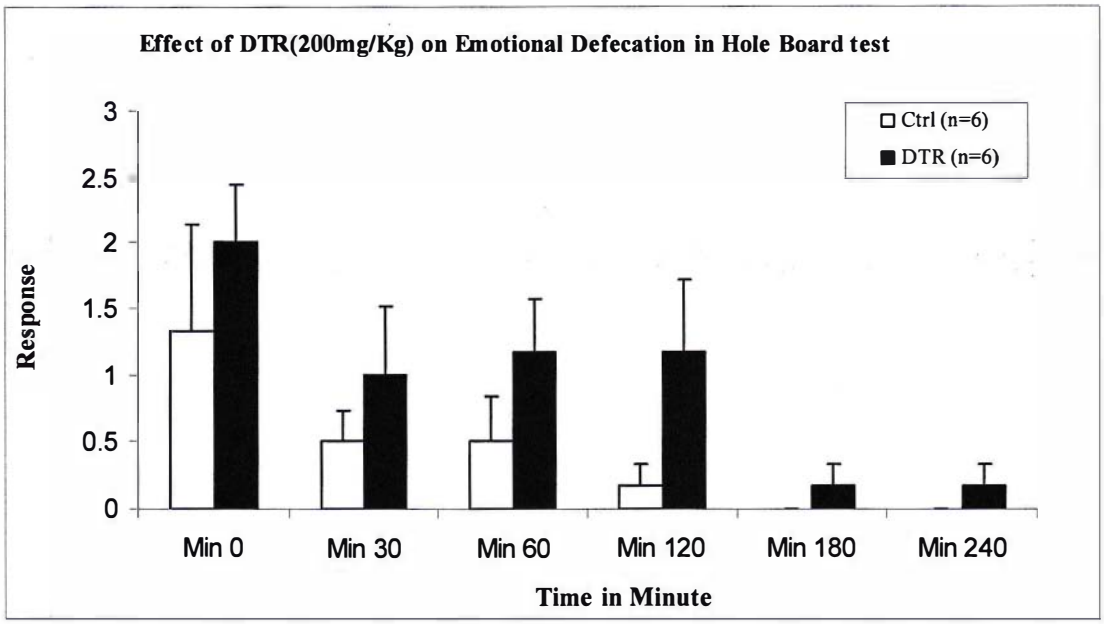
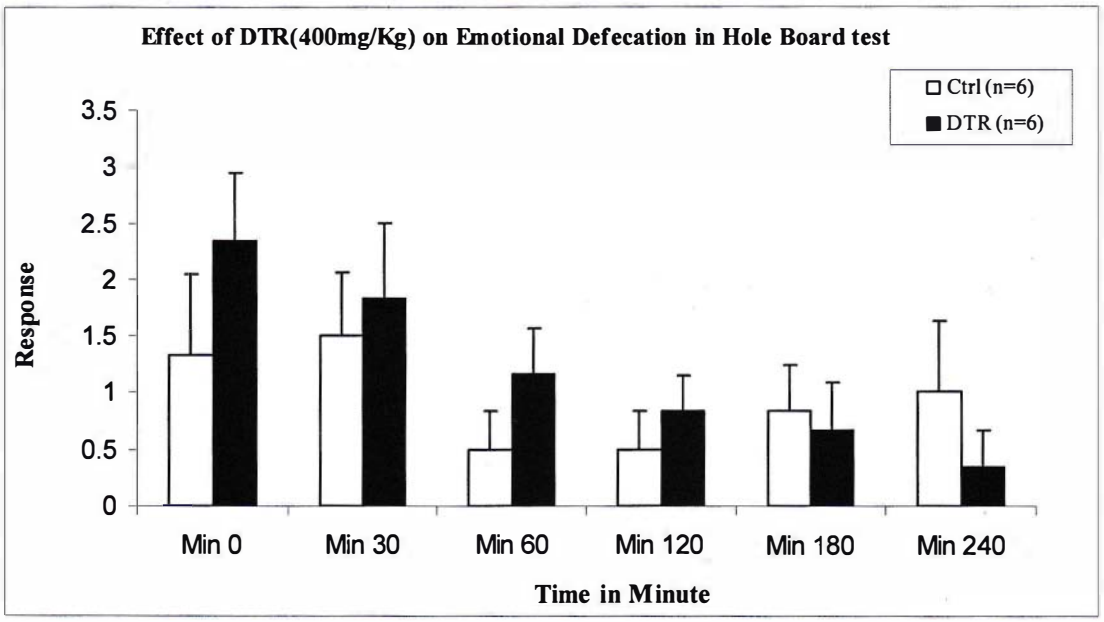


Table 3.12: The effect of DTR (400mg /kg) on Defecation in the Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		1.333± 0.715	1.500± 0.563	0.500± 0.342	0.500± 0.342	0.833± 0.401	1.000± 0.632
DTR (n=6)		2.333± 0.615	1.833± 0.654	1.167± 0.401	0.833± 0.307	0.667± 0.422	0.333± 0.333
t/p		-1.061/ 0.314	-0.386/ 0.707	-1.265/ 0.235	-0.725/ 0.485	0.286/ 0.780	0.933/ 0.373
95% confidence interval	Lower	-3.101	-2.256	-1.841	-1.357	-1.130	-0.926
	Upper	1.101	1.589	0.508	0.690	1.464	2.260

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

Figure 3.12: The effect of DTR (400mg/Kg) on Emotional Defecation in the Hole Board Test



3.3 Open field

The experiment was carried out to get a clear picture of the effect of the drugs under consideration on the pattern of behavior. This experiment presents with a different and more complex environment to explore. The weight range of female mice for this experiment was 25- 30 g.

STATISTICAL FINDINGS

3.3.1 Ambulation

DTR treated mice at dose 100 mg/Kg levels exerted overall increase in ambulation compared to the control animals except in min 30 it was found as decreased. (Table 3.13, Fig. 3.13)

At dose 200 mg/Kg, exerted overall increase in ambulation compare with the control group but none of them were found as significant. (Table 3.14, Fig. 3.14)

At dose 400 mg/Kg levels exerted overall decrease in ambulation compared to the control animals except in min 240 as it was found as almost similar to the control animals. (Table 3.14, Fig. 3.14)

Table 3.13: The effect of DTR (100mg /kg) on Ambulation in the Open Field Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		97.500± 11.445	89.167± 16.993	64.833± 8.738	25.000± 13.209	23.000± 8.622	27.167± 9.379
DTR (n=6)		112.333± 10.382	82.667± 20.381	73.500± 9.062	31.833± 14.791	35.833± 5.724	32.167± 9.432
t/p		-0.960/ 0.360	0.245/ 0.811	-0.688/ 0.507	-0.345/ 0.738	-1.240/ 0.243	-0.376/ 0.715
95% confidence interval	Lower	-49.262	-52.625	-36.716	-51.017	-35.892	-34.637
	Upper	19.596	65.625	19.383	37.351	10.225	24.637

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

Figure 3.13: The effect of DTR (100 mg/Kg) on Ambulation in the open field test.

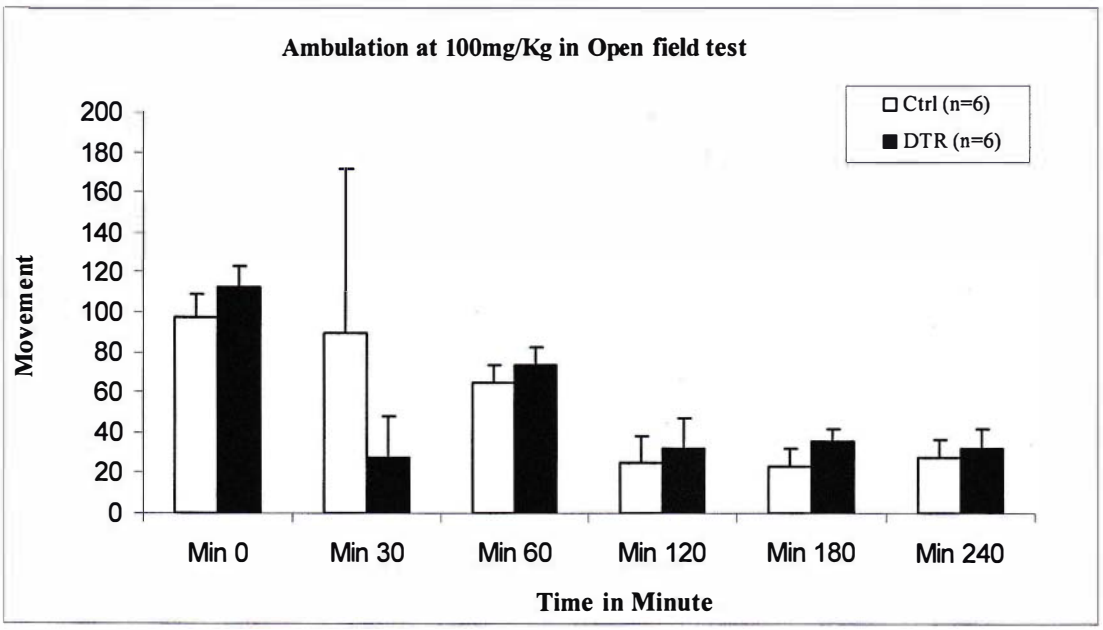


Table 3.14: The effect of DTR (200mg /kg) on Ambulation in the Open Field Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		76.667± 22.543	33.000± 16.348	21.167± 8.224	22.833± 11.984	8.833± 3.146	6.833± 3.487
DTR (n=6)		134.333± 21.773	74.833± 28.669	38.333± 13.994	40.500± 13.488	40.833± 21.706	16.333± 8.728
t/p		-1.840/ 0.096	-1.268/ 0.234	-1.058/ 0.315	-0.979/ 0.351	-1.459/ 0.202	-1.011/ 0.336
95% confidence interval	Lower	-127.497	-115.367	-53.333	-57.869	-87.704	-30.442
	Upper	12.164	31.701	19.000	22.535	23.704	11.442

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

Figure 3.14: The effect of DTR (200 mg/Kg) on Ambulation in the open field test.

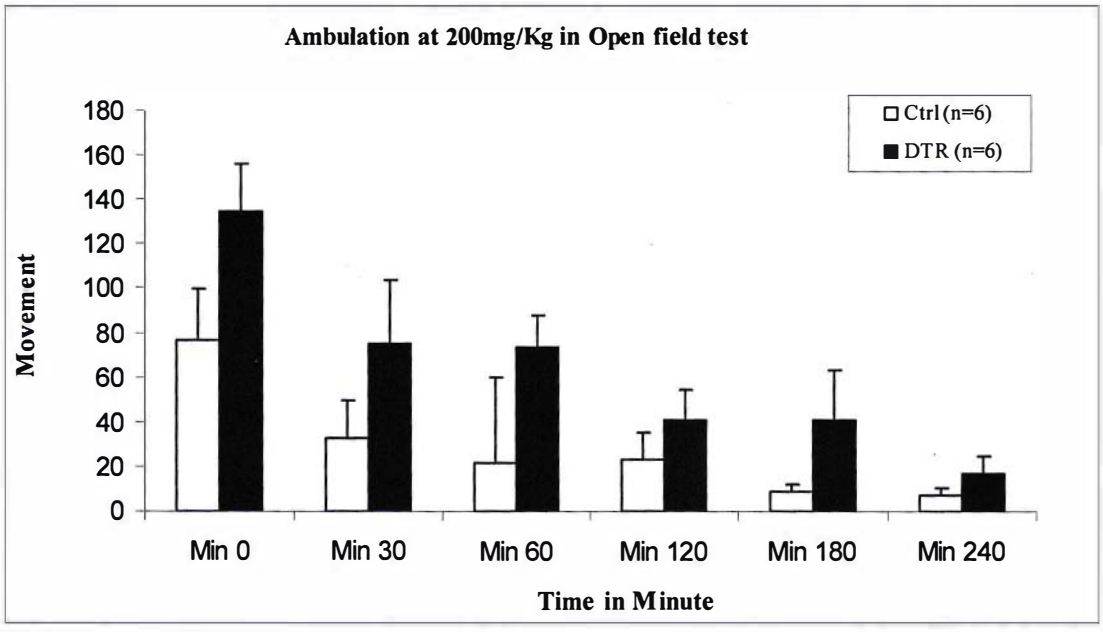
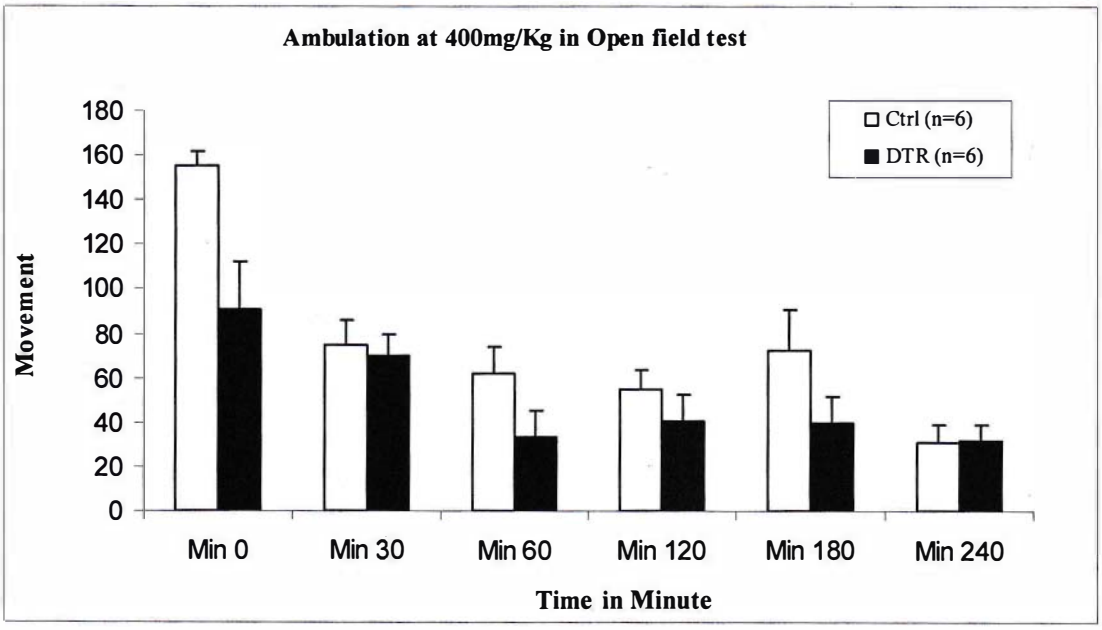


Table 3.15: The effect of DTR (400mg /kg) on Ambulation in the Open Field Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		155.167± 6.274	74.667± 11.307	61.833± 12.068	55.167± 8.845	72.333± 18.645	31.167± 8.163
DTR (n=6)		90.667± 21.263	70.333± 9.570	33.667± 11.687	40.833± 11.473	39.500± 12.622	32.000± 6.938
t/p		2.909/ 0.028	0.293/ 0.776	1.677/ 0.125	0.989/ 0.346	1.458/ 0.175	-0.078/ 0.940
95% confidence interval	Lower	9.948	-28.672	-9.264	-17.944	-17.335	-24.703
	Upper	119.052	37.339	65.597	46.611	83.002	23.036

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

Figure 3.15: The effect of DTR (400 mg/Kg) on Ambulation in the open field test.



3.3.2 Center ambulation

DTR treated mice at dose levels 100 mg/Kg exerted overall increase in total movement in the center region except in min 120 and min 240 and at min 30 it was found as similar to the compared control animals. (Table 3.16, Fig. 3.16).

At dose level 200 mg/kg except in min 0 and min 30 the total ambulation in the center region had decreased but none of them were found a statistically significant. (Table 3.17, Fig. 3.17)

At dose 400 mg/Kg overall decrease in total movement in the center region except in min 30 and min 120 compared to the control animals and none of them were found as statistically significant. (Table 3.18, Fig. 3.18)

Table 3.16: The effect of DTR (100mg /kg) on Center Ambulation in the Open Field Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		0.333± 0.211	0.167± 0.167	0.333± 0.211	0.000± 0.000	0.000± 0.000	0.000± 0.000
DTR (n=6)		0.833± 0.401	0.167± 0.167	0.833± 0.307	0.000± 0.000	0.000± 0.000	0.333± 0.333
t/p		-1.103/ 0.304	0.000/ 1.000	-1.342/ 0.209	0.000/ 0.000	0.000/ 0.000	-1.000/ 0.363
95% confidence interval	Lower	-1.556	-0.525	-1.330	0.000	0.000	-1.190
	Upper	0.556	0.525	0.330	0.000	0.000	0.524

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

Figure 3.16: The effect of DTR (100 mg/Kg) on Center Ambulation in the open field test.

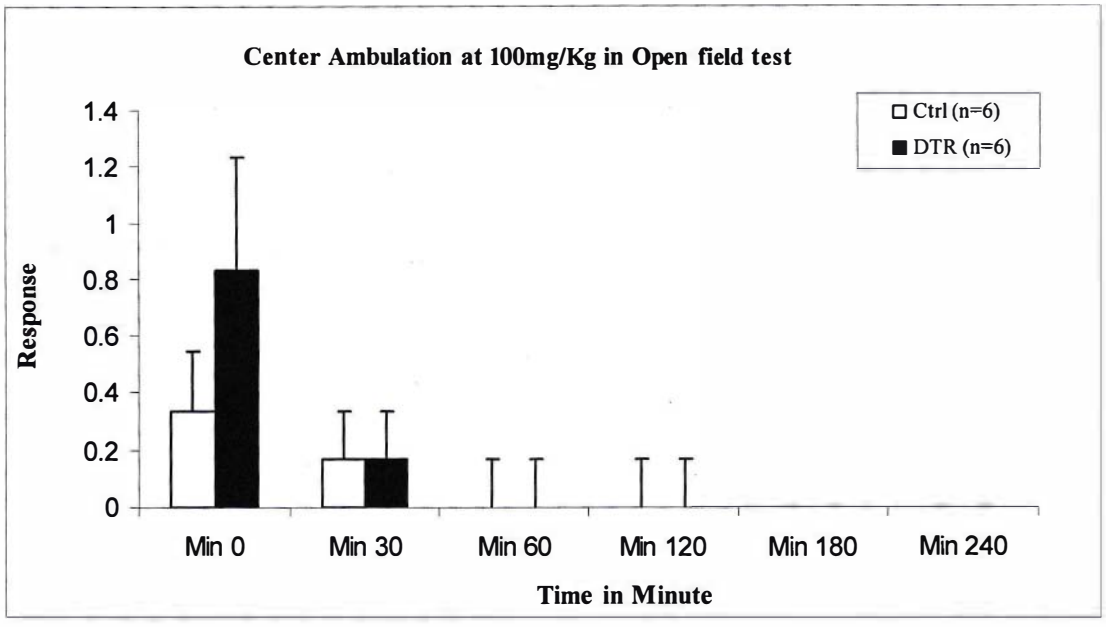


Table 3.17: The effect of DTR (200mg /kg) on Center Ambulation in the Open Field Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		0.333± 0.333	0.167± 0.167	0.000± 0.000	0.000± 0.000	0.000± 0.000	0.000± 0.000
DTR (n=6)		0.667± 0.333	0.333± 0.333	0.167± 0.167	0.167± 0.167	0.000± 0.000	0.000± 0.000
t/p		-0.707/ 0.496	-0.447/ 0.664	-1.000/ 0.363	-1.000/ 0.363	0.000/ 0.000	0.000/ 0.000
95% confidence interval	Lower	-1.384	-0.997	-0.595	-0.595	0.000	0.000
	Upper	0.717	0.664	0.262	0.262	0.000	0.000

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

Figure 3.17: The effect of DTR (200 mg/Kg) on Center Ambulation in the open field test.

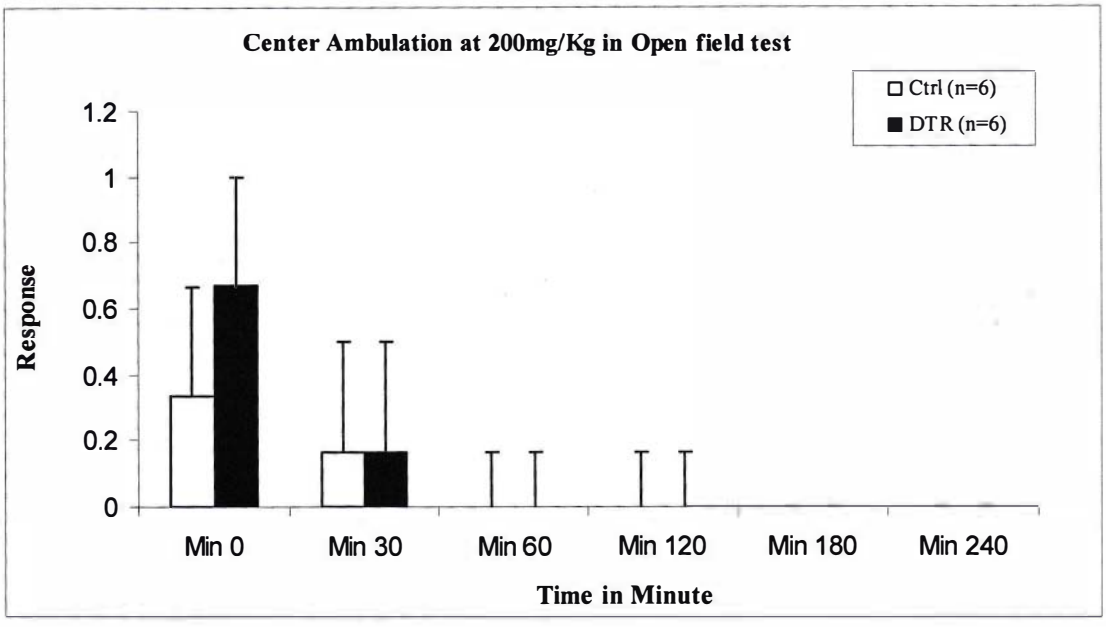
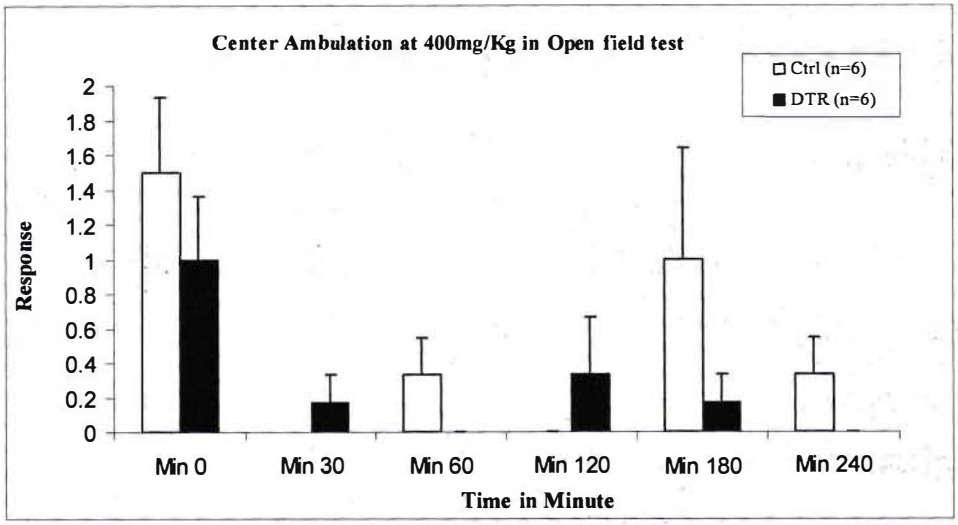


Table 3.18: The effect of DTR (400mg /kg) on Center Ambulation in the Open Field Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		1.500± 0.428	0333± 0.333	0.333± 0.211	0.000± 0.000	1.000± 0.632	0.333± 0.211
DTR (n=6)		1.000± 0.365	0.167± 0.167	0.000± 0.000	0.333± 0.333	0.167± 0.167	0.000± 0.000
t/p		0.889/ 0.395	0.447/ 0.664	1.581/ 0.175	-1.000/ 0.363	1.274/ 0.231	1.581/ 0.175
95% confidence interval	Lower	-0.754	-0.664	-0.209	-1.190	-0.624	-0.209
	Upper	1.754	0.997	0.875	0.524	2.291	0.875

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

Figure 3.18: The effect of DTR (400 mg/Kg) on Center Ambulation in the open field test.



3.3.3 Standing up behaviour

At dose 100 mg/Kg except at min 120 and min 240 the number of standing was decreased comparing with the control group. (Table 3.19, Fig. 3.19)

Similarly at dose 200 mg/kg except at min 180 and min 240 the number of standing was increased comparing with the control group. (Table 3.20, Fig. 3.20)

The exceptions were at the highest dose of 400 mg/Kg where DTR treated mice exerted a decrease in the standing up behavior in comparison to that of control group and in min 30 it was found as statistically significant (p=0.036*). (Table 3.21, Fig. 3.21)

Table 3.19: The effect of DTR (100mg /kg) on Standing Up Behavior in the Open Field Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		8.167± 1.851	11.833± 3.400	8.000± 2.017	4.167± 2.272	2.833± 1.701	2.167± 0.703
DTR (n=6)		7.000± 0.966	8.167± 2.104	6.833± 1.833	4.500± 1.821	2.500± 0.847	2.333± 1.229
t/p		0.559/ 0.589	0.917/ 0.381	0.428/ 0.678	-0.114/ 0.911	0.175/ 0.864	-0.118/ 0.909
95% confidence interval	Lower	-3.486	-5.243	-4.906	-6.821	-3.901	-3.322
	Upper	5.820	12.576	7.239	6.154	4.567	2.989

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

Figure 3.19: The effect of DTR (100 mg/Kg) on Standing up Behavior in the open field test.

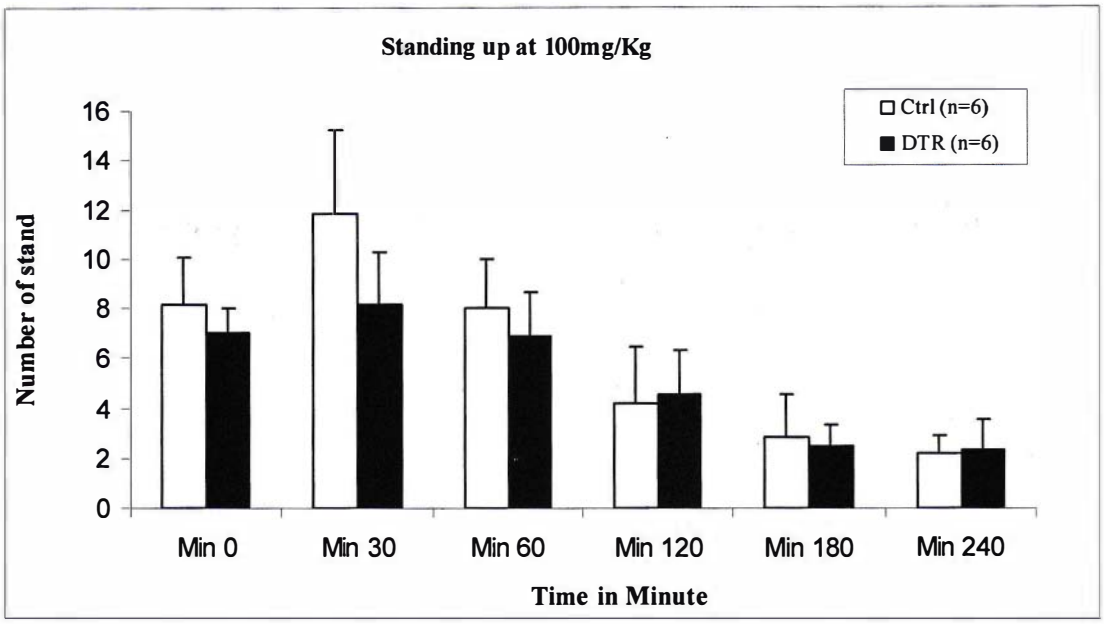


Table 3.20: The effect of DTR (200mg /kg) on Standing Up Behavior in the Open Field Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		3.667± 1.022	1.667± 1.116	2.833± 1.108	1.000± 0.632	0.667± 0.422	0.333± 0.333
DTR (n=6)		4.833± 1.447	3.833± 0.980	3.500± 1.432	2.000± 0.683	2.500± 1.176	0.333± 0.211
t/p		-0.659/ 0.525	-1.459/ 0.175	-0.368/ 0.720	-1.074/ 0.308	-1.467/ 0.191	0.000/ 1.000
95% confidence interval	Lower	-5.114	-5.476	-4.701	-3.074	-4.860	-0.879
	Upper	2.781	1.142	3.367	1.074	1.193	0.879

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

Figure 3.20: The effect of DTR (200 mg/Kg) on Standing Up Behavior in the open field test.

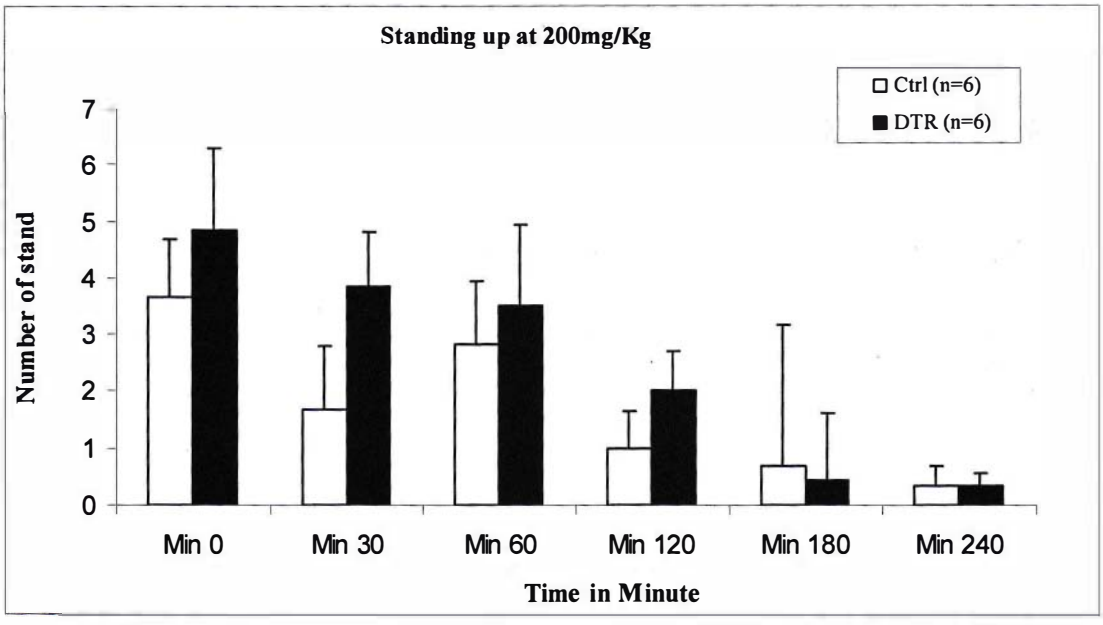
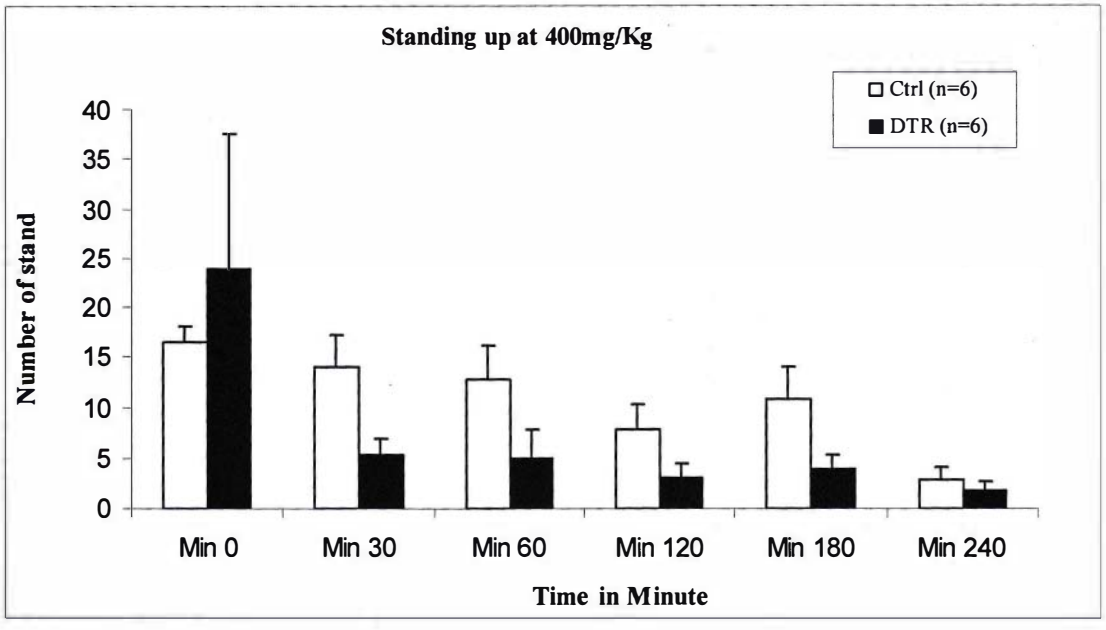


Table 3.21: The effect of DTR (400mg /kg) on Standing Up Behaviour in the Open Field Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		16.500± 1.522	14.000± 3.183	12.667± 3.393	7.833± 2.358	10.833± 3.177	2.833± 1.195
DTR (n=6)		23.833± 13.651	5.333± 1.647	5.000± 2.733	3.000± 1.461	3.833± 1.515	1.833± 0.792
t/p		-0.534/ 0.605	2.418/ **0.036***	1.760/ 0.109	1.742/ 0.112	1.989/ 0.075	0.697/ 0.501
95% confidence interval	Lower	-37.939	0.681	-2.040	-1.347	-0.843	-2.195
	Upper	23.272	16.652	17.373	11.014	14.843	4.195

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

Figure 3.21: The effect of DTR (400 mg/Kg) on Standing up Behaviour in the Open field test.



3.3.4 Emotional defecation

At dose 100mg/kg increased number of stool counted compared to the control group. (Table 3.22, Fig. 3.22)

At dose 200mg/kg the number of stool found more than control group except in min 60 and min 240 found as similar. (Table 3.23, Fig. 3.23)

At dose 400mg/kg in min 60 and min 240 the number of stool counted more whereas in min 30 and in min 120 the number of stool count decreased. In min 180, it was found as similar to the control group. (Table 3.24, Fig. 3.24)

Table 3.22: The effect of DTR (100mg /kg) on Emotional Defecation in the Open Field Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		0.500± 0.224	0.833± 0.477	0.333± 0.211	0.333± 0.211	0.167± 0.167	0.500± 0.342
DTR (n=6)		0.833± 0.654	1.167± 0.601	1.000± 0.447	0.833± 0.307	0.667± 0.333	0.833± 0.167
t/p		-0.482/ 0.640	-0.434/ 0.673	-1.348/ 0.207	-1.342/ 0.209	-1.342/ 0.209	-0.877/ 0.401
95% confidence interval	Lower	-1.873	-2.043	-1.768	-1.330	-1.330	-1.180
	Upper	1.207	1.377	0.435	0.330	0.330	0.513

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

Figure 3.22: The effect of DTR (100 mg/Kg) on Emotional Defecation in the Open field test.

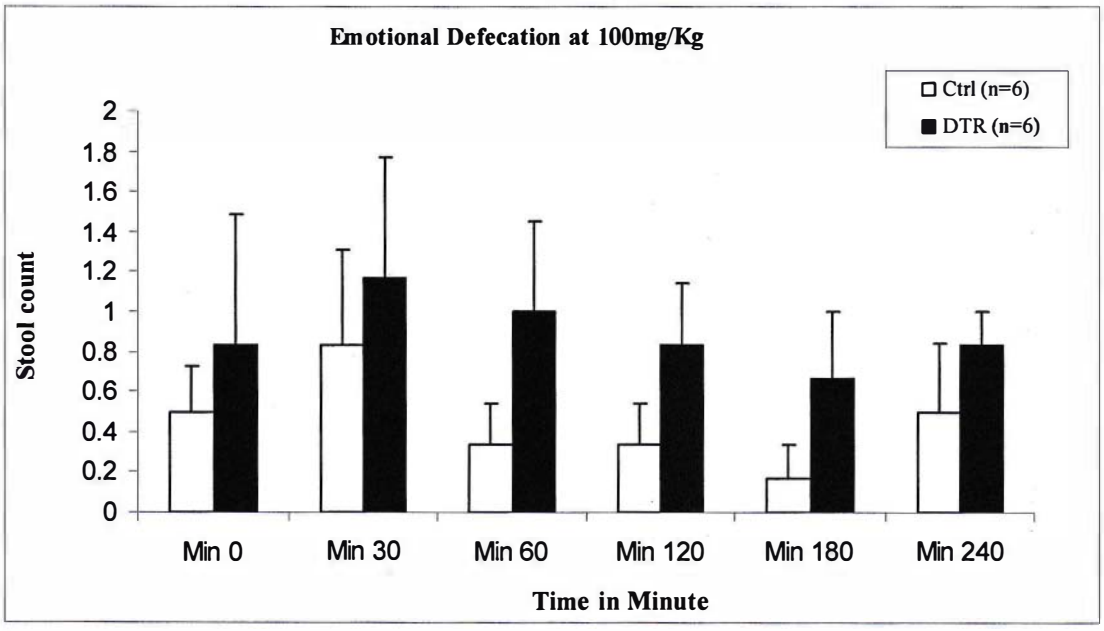


Table 3.23: The effect of DTR (200mg /kg) on Emotional Defecation in the Open field test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		0.500± 0.342	1.167± 0.477	0.833± 0.477	0.167± 0.167	1.167± 0.477	1.833± 0.703
DTR (n=6)		1.500± 0.224	1.500± 0.619	0.833± 0.543	1.8333± 0.65405	1.333± 0.422	1.833± 0.601
t/p		-2.449/ **0.034***	-0.426/ 0.679	0.000/ 1.000	-2.469/ 0.051	-0.262/ 0.799	0.000/ 1.000
95% confidence interval	Lower	-1.910	-2.075	-1.610	-3.344	-1.586	-2.061
	Upper	-0.090	1.408	1.610	0.010	1.252	2.061

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

Figure 3.23: The effect of DTR (200 mg/Kg) on Emotional Defecation in the open field test.

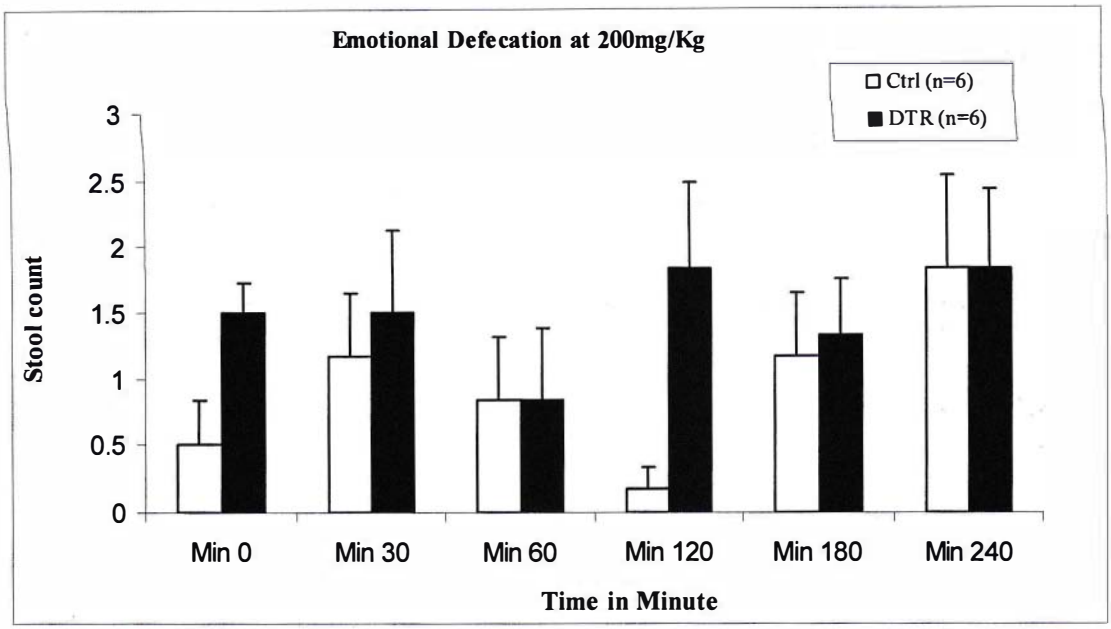
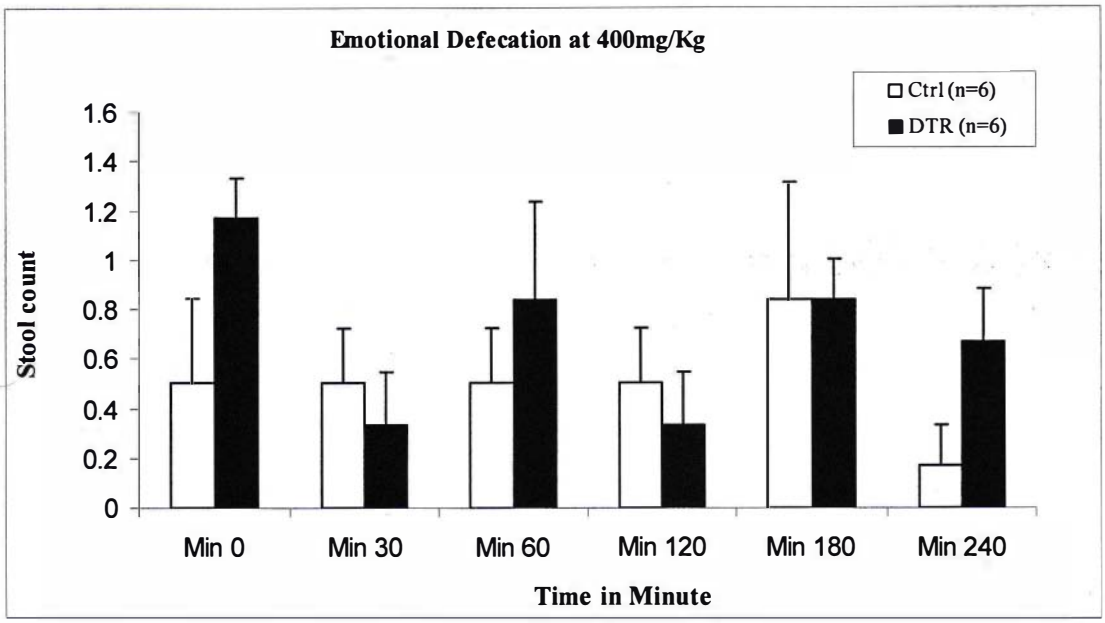


Table 3.24: The effect of DTR (400mg /kg) on Emotional Defecation in the Open field test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		0.500± 0.342	0.500± 0.224	0.500± 0.224	0.500± 0.224	0.833± 0.477	0.167± 0.167
DTR (n=6)		1.167± 0.167	0.333± 0.211	0.833± 0.401	0.333± 0.211	0.833± 0.167	0.667± 0.211
t/p		-1.754/ 0.110	0.542/ 0.599	-0.725/ 0.489	0.542/ 0.599	0.000/ 1.000	-1.861/ 0.092
95% confidence interval	Lower	-1.513	-0.518	-1.397	-0.518	-1.126	-1.099
	Upper	0.180	0.851	0.730	0.851	1.126	0.099

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

Figure 3.24: Graphical Presentation of the effect of DTR (400 mg/Kg) on Emotional Defecation in the open field test.



3.4 Climbing Out Test

The experiment was carried out by the method of Sandberg (1957).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. For this experiment female mice weight 25-30g had used.

STATISTICAL FINDINGS

DTR treated mice at dose levels (100 mg/Kg) exerted increase in time taken to come out of the cage in min 120, min180 and min 240. The exceptions were in min 30 time required for the drug treated mice to come out the cage was decreased than the control group. But no results found as statically significant. (Table 3.25, Fig. 3.25)

DTR treated mice at dose levels (200 mg/Kg) exerted decrease in time taken to come out of the cage in min60, min 180 and min240. The exceptions were in min 120 time required for the drug treated mice to come out the cage was increased than the control group. At min 30 ($p=0.039$) the result of decrease was found to be statistically significant. (Table 3.26, Fig. 3.26)

DTR treated mice at dose levels (400 mg/Kg) exerted decrease in time taken to come

min 120 time required for the drug treated mice to come out the cage was increased than the control group. But no results found as statically significant (Table 3.27, Fig. 3.27)

Table 3.25: The effect of DTR (100mg /kg) in the Climbing out Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=10)		108.100± 71.286	54.500± 43.923	12.200± 8.826	40.500± 27.542	65.200± 43.107	.000± .000
DTR (n=10)		134.600± 69.997	22.200± 11.522	12.500± 9.064	94.500± 44.226	138.700± 48.842	39.400± 28.570
t/p		-.265/.794	.711/.486	-.024/.981	-1.036/.314	-1.128/.274	-1.379/.201
95% confidence interval	Lower	-236.395	-63.102	-26.880	-163.462	-210.364	-104.031
	Upper	183.395	127.702	26.280	55.462	63.364	25.231

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

Figure 3.25: The effect of DTR (100mg/Kg) in Climbing Out Test

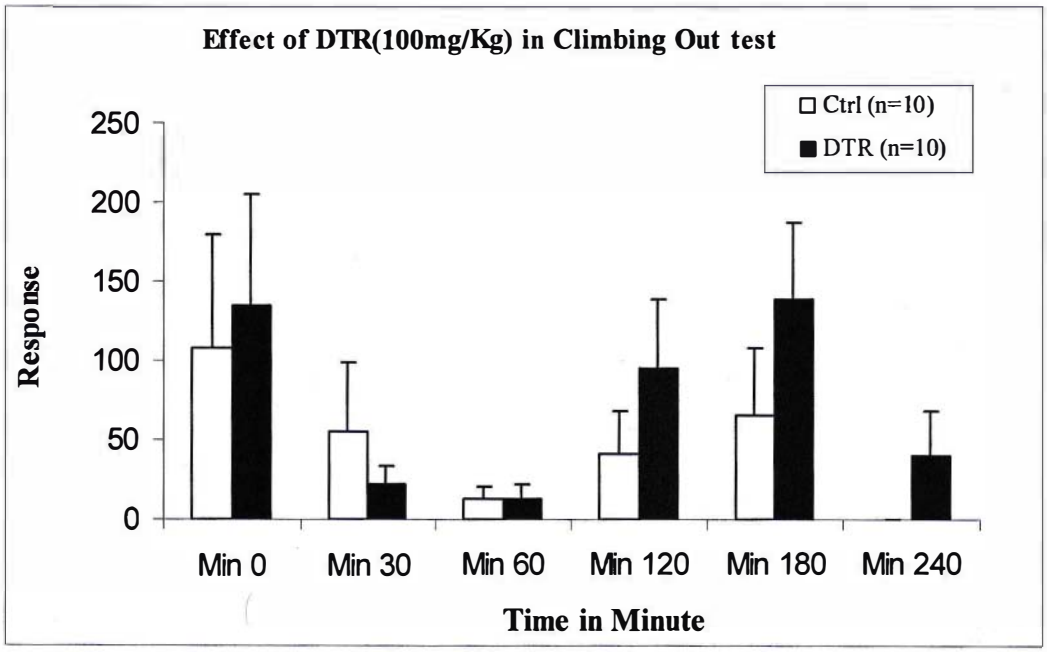


Table 3.26: The effect of DTR (200mg /kg) in the Climbing Out Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=10)		80.100± 38.548	198.200± 72.650	74.000± 44.683	14.400± 9.951	157.600± 81.134	122.500± 56.373
DTR (n=10)		50.800± 24.747	110.700± 51.494	47.300± 24.424	127.000± 59.499	125.200± 59.152	99.500± 61.173
t/p		-.682/.506	**2.393/.039***	1.349/.208	-1.767/.108	.200/.845	1.315/.211
95% confidence interval	Lower	-225.890	10.805	-40.458	-181.232	-183.230	-53.084
	Upper	116.890	341.194	163.458	21.032	221.030	219.284

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

Figure 3.26: The effect of DTR (200mg/Kg) in Climbing Out Test

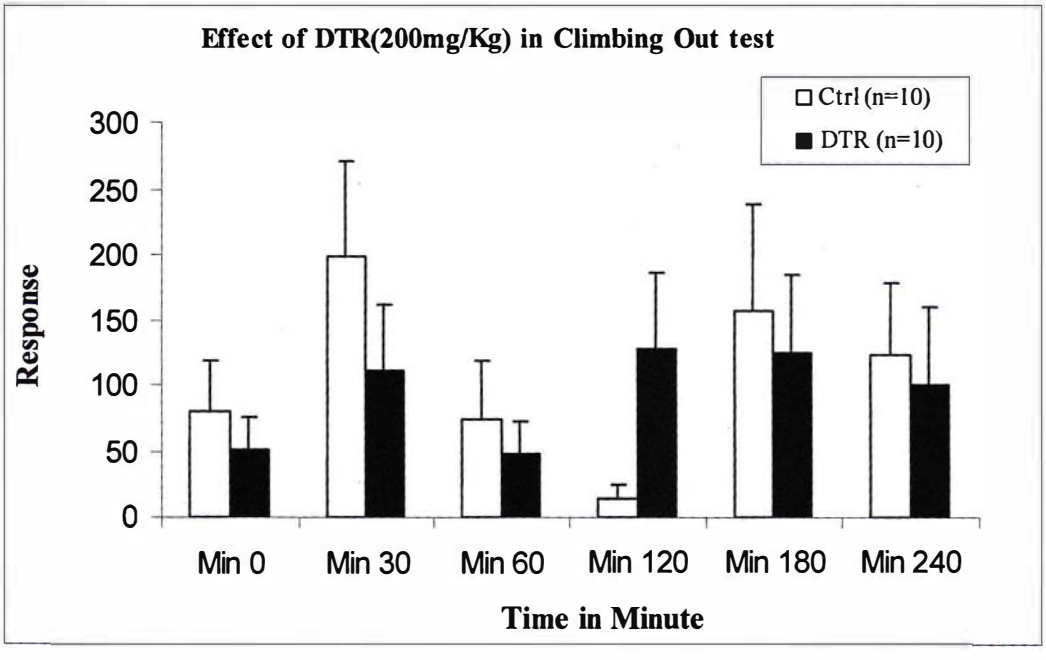
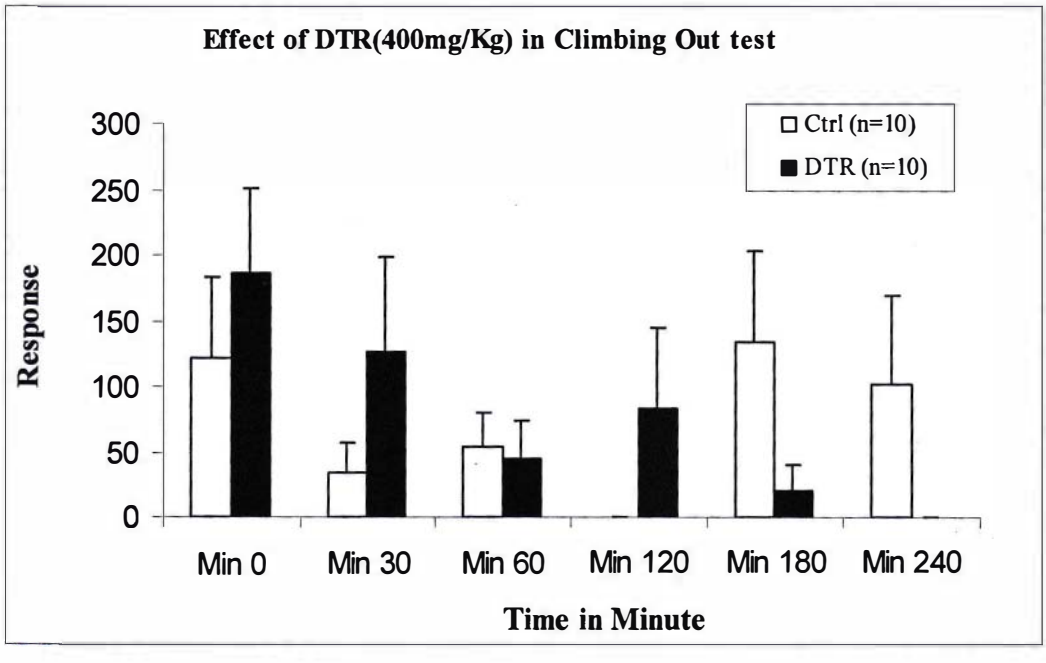


Table 3.27: The effect of DTR (400mg /kg) in the Climbing Out Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=10)		122.2±60.169	34.1±22.657	53.2±27.177	0±0	133.1±70.527	100.9±67.686
DTR (n=10)		185.4±65.153	126.1±72.982	44.1±29.727	83.4±61.671	19.7±19.7	0±0
t/p		-.713/.485	-1.204/.255	.226/.824	-1.352/.209	1.549/.151	1.491/.170
95% confidence interval	Lower	-249.523	-260.736	-75.520	-222.910	-48.921	-52.217
	Upper	123.123	76.736	93.720	56.110	275.721	254.017

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

Figure 3.27: The effect of DTR (400mg/Kg) in Climbing Out Test



3.5 Acetic acid induced writhing test

The experiment was carried out to find out the existence of non-narcotic analgesic property. The pain sensation was initiated by using Acetic acid.The Acetic acid induced writhing is inversely proportionate to the non-narcotic analgesic property. For this experiment female mice weight 25-30g had used. The results that were found in the following study are given below:

STATISTICAL FINDINGS

3.5.1 Writhing response

DTR (100mg/kg) treated mice exerted a decrease in writhing response compare to the control group from the initial 1st min to 4th min except at 5th min it was increased. But all results were statically insignificant. (Table 3.28, Fig. 3.28).

At dose, 200mg/kg, DTR treated mice showed decreasing response compare to the control group from the initial at min 1st, min 3rd and min 4th whereas in min 2nd and min 5th results of the response was found as decreased. (Table 3.29, Fig. 3.29).

DTR (400mg/kg) treated group exerted an increase in writhing response compare to the corresponding control group except min 5th where response was decreased. But no results were statistically significant. (Table 3.30, Fig. 3.30).

The percent of protection by DTR was:

- 17.83% (100 mg/kg)
- 1.52% (200 mg/kg)
- - 4.40% (400mg/kg)

Table 3.28: The effect of DTR (100mg/kg) in the Acetic Acid Induced Writhing Test.

Group		1st Min	2nd Min	3rd t Min	4th Min	5th Min
Ctrl (n=10)		4±0.988	3.8±0.891	3.1±0.721	2.5±0.582	2.3±0.578
DTR (n=10)		3±0.471	2.8±0.416	2.2±0.359	2.3±0.472	2.6±0.476
t/p		.913/.378	1.016/.329	1.116/.284	.267/.793	-.400/.694
95% confidence interval	Lower	-1.36862	-1.13085	-.83913	-1.37530	-1.87375
	Upper	3.36862	3.13085	2.63913	1.77530	1.27375

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

Figure 3.28: The effect of DTR (100mg/Kg) in the Acetic Acid Induced writhing test.

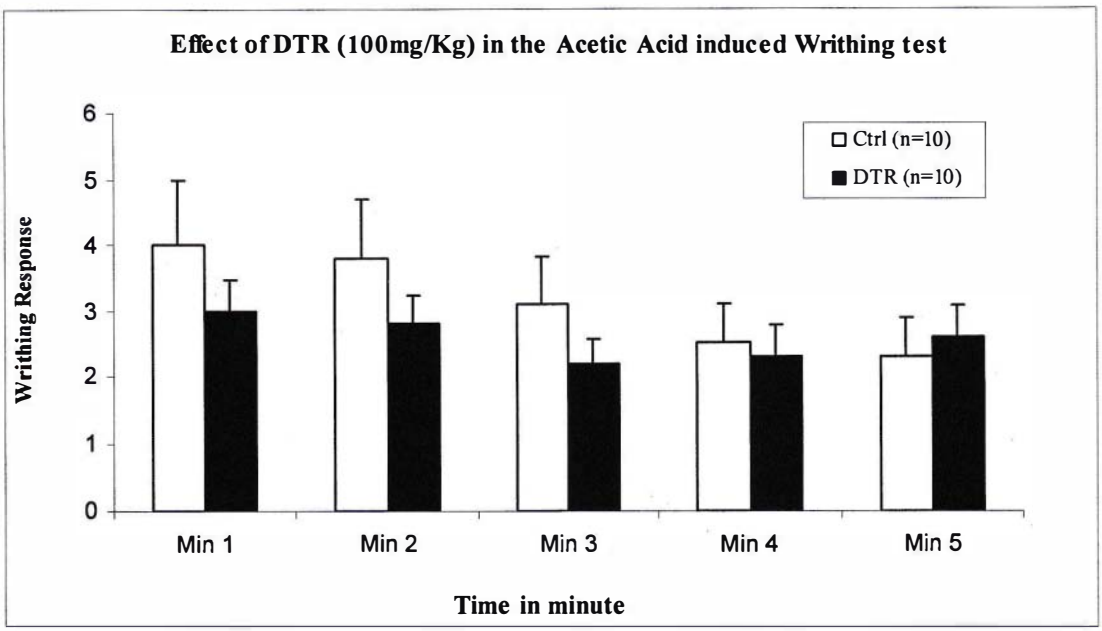


Table 3.29: The effect of DTR (200mg/kg) in the Acetic Acid Induced Writhing Test.

Group		1st Min	2nd Min	3rd t Min	4th Min	5th Min
Ctrl (n=10)		3.7±0.558	3.9±0.481	4.7±0.578	4.2±0.727	3.3±0.597
DTR (n=10)		3.6±0.653	4.4±0.635	3.9±0.458	3.9±0.737	3.7±0.26
t/p		.116/.909	-.627/.539	1.084/.293	.290/.775	-.614/.550
95% confidence interval	Lower	-1.705	-2.176	-.750	-1.875	-1.815
	Upper	1.905	1.176	2.350	2.475	1.015

Figure 3.29: The effect of DTR (200mg/Kg) in the Acetic Acid induced writhing test.

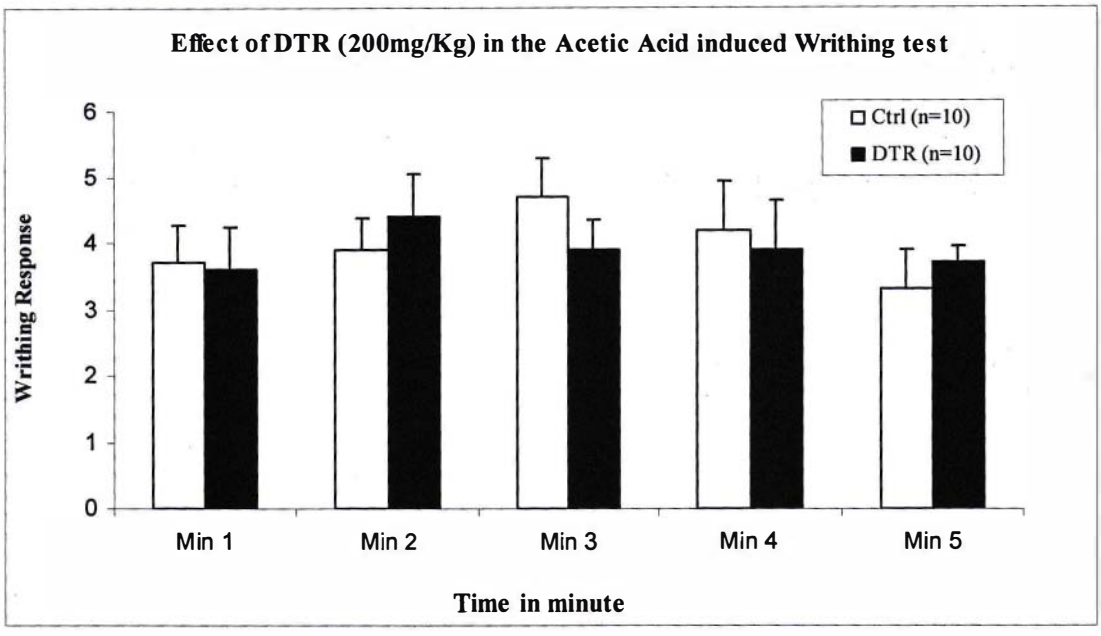
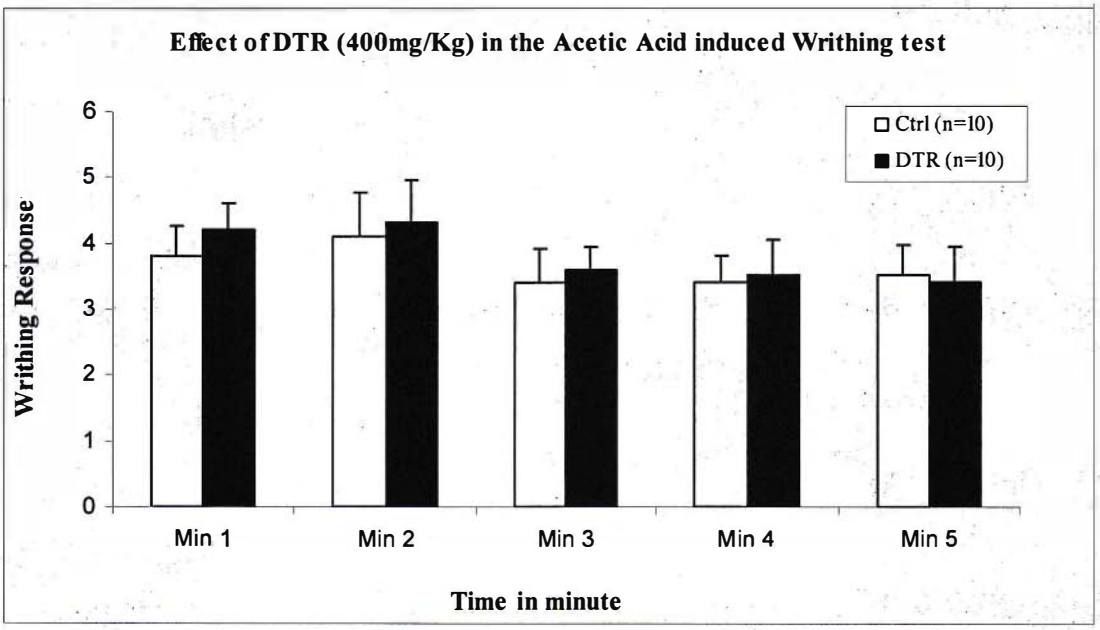


Table 3.30: The effect of DTR (400mg/kg) in the Acetic Acid Induced Writhing Test.

Group		1st Min	2nd Min	3rd t Min	4th Min	5th Min
Ctrl (n=10)		3.800±.466	4.100±.674	3.400±.498	3.400±.400	3.500±.453
DTR (n=10)		4.200±.416	4.300±.650	3.600±.339	3.500±.542	3.400±.541
t/p		-.640/.530	-.213/.833	-.331/.744	-.148/.884	.142/.889
95% confidence interval	Lower	-1.71389	-2.16835	-1.46831	-1.51628	-1.38392
	Upper	.91389	1.76835	1.06831	1.31628	1.58392

Figure 3.30: The effect of DTR (400mg/Kg) in the Acetic Acid Induced Writhing Test



3.6 Formalin Induced Paw Licking Test

The formalin pain test is very useful for evaluating the mechanism of pain and analgesia (Tjolsen et al., 1992). Drugs, which act mainly centrally, such as narcotic analgesics inhibit both phases of pain in this model while peripherally acting drugs such as aspirin and indomethacin, only inhibit the late phase (Santos et al., 1994). The weight range of female mice for this experiment was 25- 30 g.

STATISTICAL FINDINGS

DTR at dose 100mg /kg exerted increasing analgesic activity and anti-inflammatory activity in mice compared to the respective control group but none of the results were statistically significant. (Table 3.34, Fig. 3.34, 3.35).

Table: 3.34: The effect of DTR (100 mg/kg) in the Formalin Induced Paw licking (Analgesic + Inflammation) Test.

Group		Analgesic (1st Phase)	Inflammation (2nd Phase)
Ctrl (n=6)		58.800±6.209	14.800±5.293
DTR (n=6)		63.000±14.277	20.000±6.768
t/p		-.270/.790	-.605/.553
95% confidence interval	Lower	-36.909	-23.251
	Upper	28.509	12.851

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

Figure: 3.34: The effect of DTR (100mg /Kg) in the Formalin Induced Paw licking (Analgesic) Test.

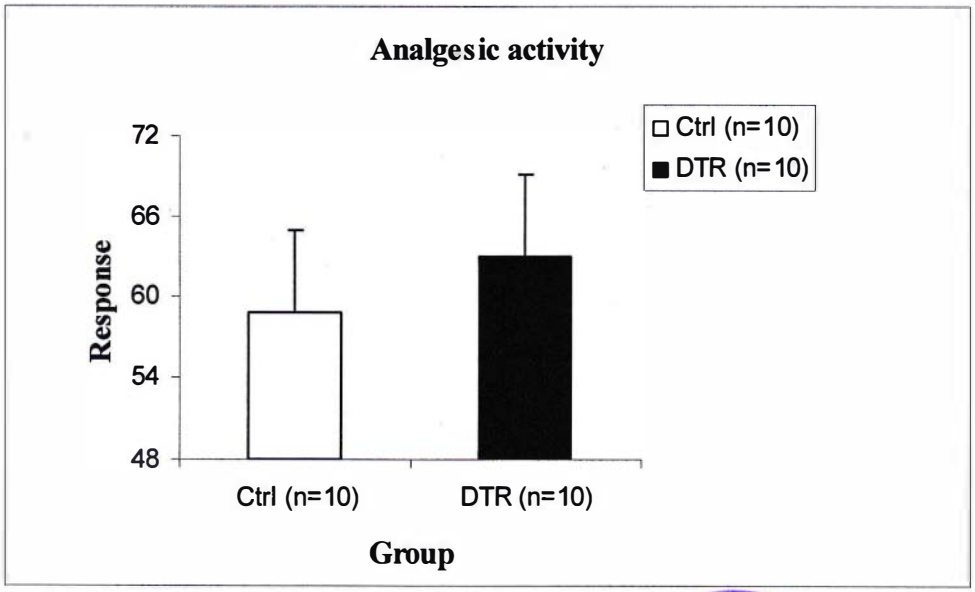
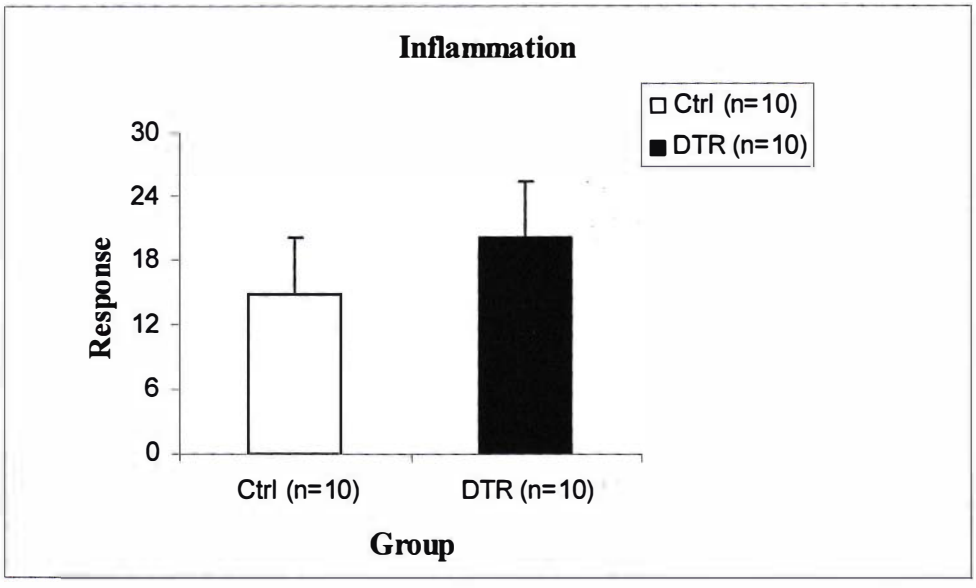


Figure: 3.35: The effect of DTR (100mg /Kg) in the Formalin Induced Paw licking (Inflammation) Test.



3.7 Gastro-Intestinal Motility

Gastro-intestinal motility test was carried out to find the effect of the drugs on the peristaltic movement of the gastro-intestinal tract. The weight range of female mice for this experiment was 30- 35 g. The results are summarized below.

STATISTICAL FINDINGS

At 1st Hour

After 15 minutes study:

As evident from the table below, DTR is found to increase the gut motility of the experimental mice in the 15 minutes study, but the increase was not statistically significant. . (Table 3.35, Fig. 3.36).

After 30 minutes study:

In the 30 minutes study, DTR is found to decrease the gastrointestinal motility of the experimental mice when compared to the corresponding control group. This decrease is statistically insignificant. . (Table 3.36, Fig. 3.37).

At 2nd Hour

After 15 minutes study:

As evident from the table below, DTR is found to decrease the gut motility of the experimental mice in the 15 minutes study and the decrease was statistically insignificant. (Table 3.37, Fig. 3.38).

After 30 minutes study:

Here, in 30 minutes study, DTR is found to increase the gastrointestinal motility of the experimental mice and the increase was statistically insignificant.. (Table 3.38, Fig. 3.39).

At 3rd Hour

After 15 minutes study:

After 15 minutes study it was observed that the gut motility of the DTR treated mice decreased. . (Table 3.39, Fig. 3.40).

After 30 minutes study:

During the 30 minutes study, the drug DTR was found to decrease (slightly) the gastrointestinal motility compared to the control group mice. . (Table 3.40, Fig. 3.41).

At 4th Hour

After 15 minutes study:

In the 15 minutes study, the drug DTR was also found to decrease the gastrointestinal motility of the experimental mice. But the result was statistically insignificant. . (Table 3.41, Fig. 3.42).

After 30 minutes study:

As evident from the table below, DTR was found to decrease the gut motility of the experimental mice in the 30 minutes study and this result was statistically insignificant. . (Table 3.42, Fig. 3.43).

Table: 3.35: The effect of DTR (100 mg/kg) on Gastrointestinal Motility Test after 1st hour 15 minutes study period.

Group		% Traversed
Ctrl (n=8)		65.829 ± 3.836
DTR (n=8)		68.470 ± 3.839
t/p value		-.487/.634
95% confidence interval	Lower	-14.281
	Upper	9.000

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

Figure 3.36: The effect of DTR (100 mg/Kg) on Gastrointestinal Motility Test after 1st hour 15 minutes study.

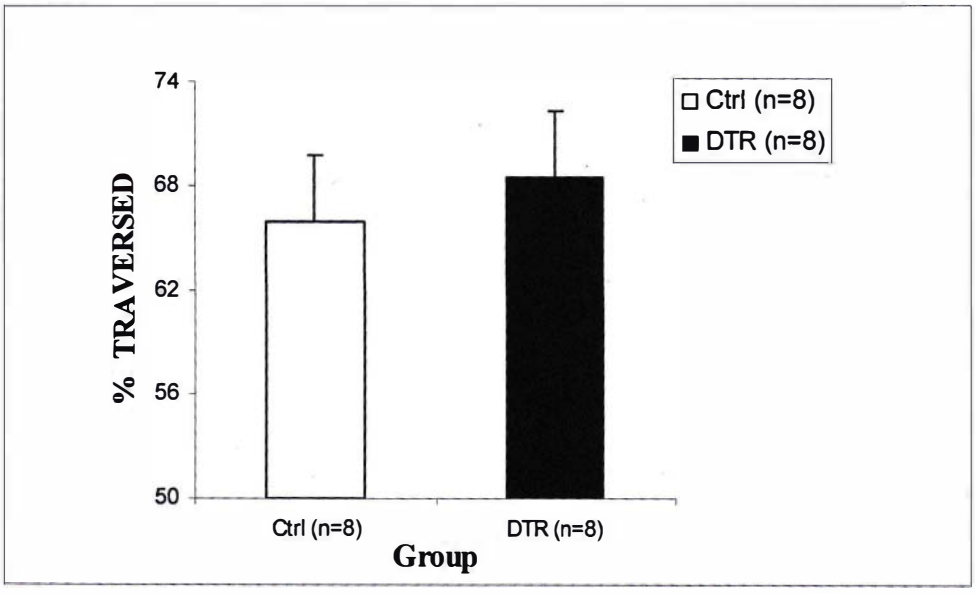


Table 3.36: The effect of DTR (100 mg/kg) on Gastrointestinal Motility Test after 1st hour 30 minutes study period.

Group		% Traversed
Ctrl (n=8)		69.820 ± 6.221
DTR (n=8)		63.706 ± 5.165
t/p value		.756/.462
95% confidence interval	Lower	-11.230
	Upper	23.457

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

Figure 3.37: The effect of DTR (100 mg/Kg) on Gastrointestinal Motility Test after 1st hour 30 minutes study.

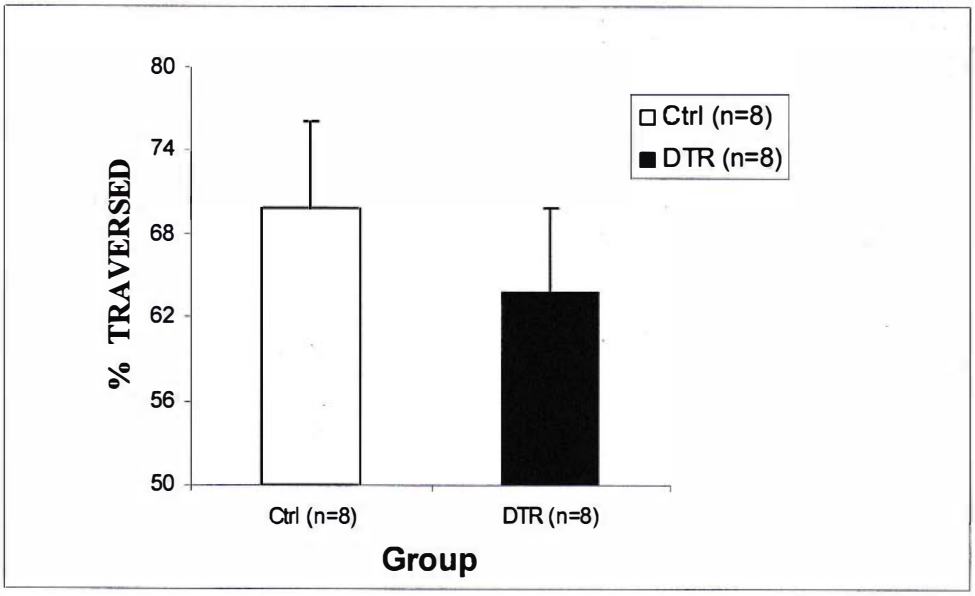


Table 3.37: The effect of DTR (100 mg/kg) on Gastrointestinal Motility Test after 2nd hour 15 minutes study period.

Group		% Traversed
Ctrl (n=8)		63.265 ± 3.726
DTR (n=8)		59.519 ± 5.935
t/p value		.534/.601
95% confidence interval	Lower	-11.285
	Upper	18.776

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

Figure 3.38: The effect of DTR (100 mg/Kg) on Gastrointestinal Motility Test after 2nd hour 15 minutes study.

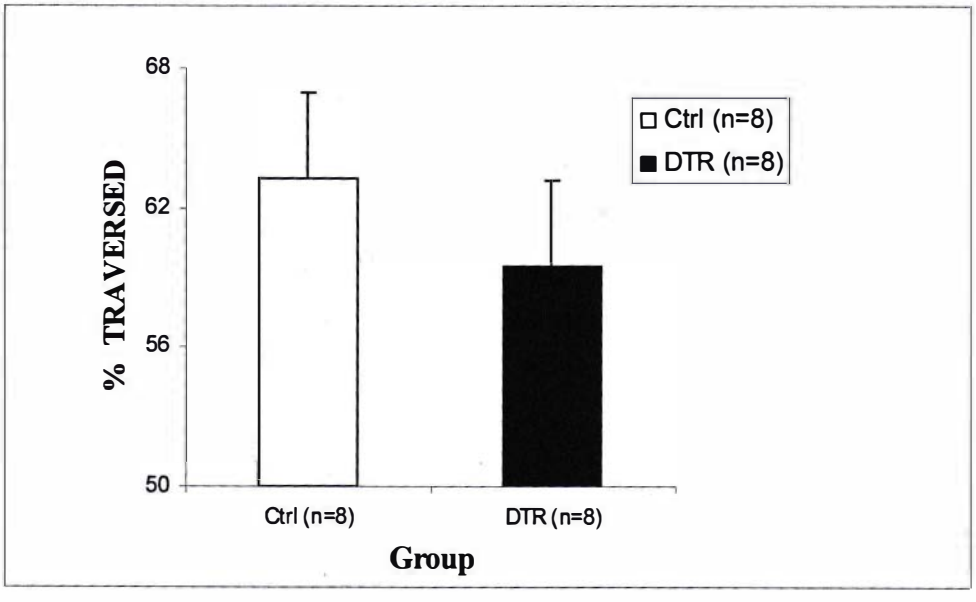


Table 3.38: The effect of DTR (100 mg/kg) on Gastrointestinal Motility Test after 2nd hour 30 minutes study period.

Group		% Traversed
Ctrl (n=8)		70.956 ± 3.896
DTR (n=8)		77.131 ± 2.022
t/p value		-1.407/.181
95% confidence interval	Lower	-15.589
	Upper	3.240

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

Figure 3.39: The effect of DTR (100 mg/Kg) on Gastrointestinal Motility Test after 2nd hour 30 minutes study.

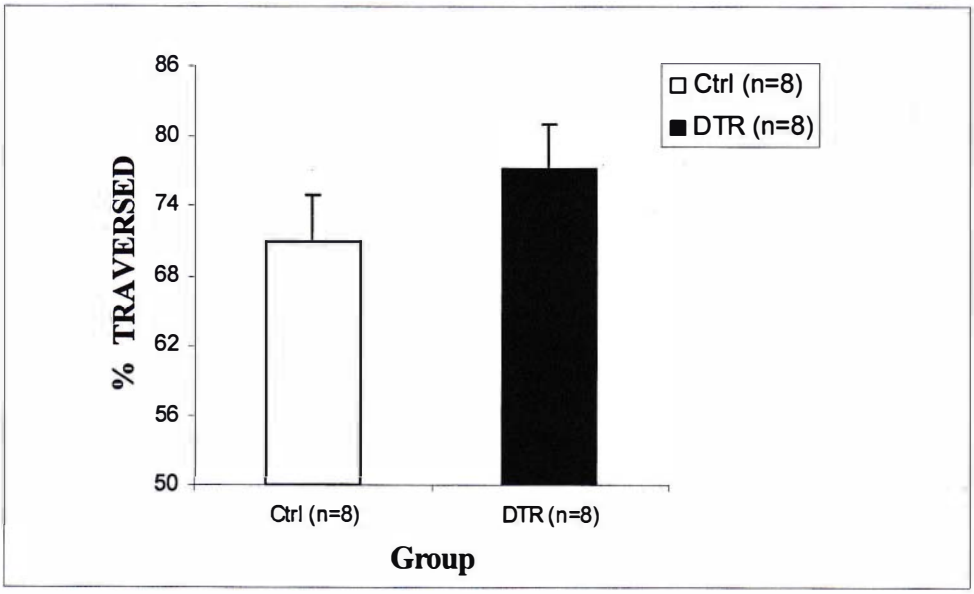


Table 3.39: The effect of DTR (100 mg/kg) on Gastrointestinal Motility Test after 3rd hour 15 minutes study period.

Group		% Traversed
Ctrl (n=8)		72.755 ± 6.048
DTR (n=8)		62.887 ± 4.569
t/p value		1.302/.214
95% confidence interval	Lower	-6.390
	Upper	26.128

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

Figure 3.40: The effect of DTR (100 mg/Kg) on Gastrointestinal Motility Test after 3rd hour 15 minutes study.

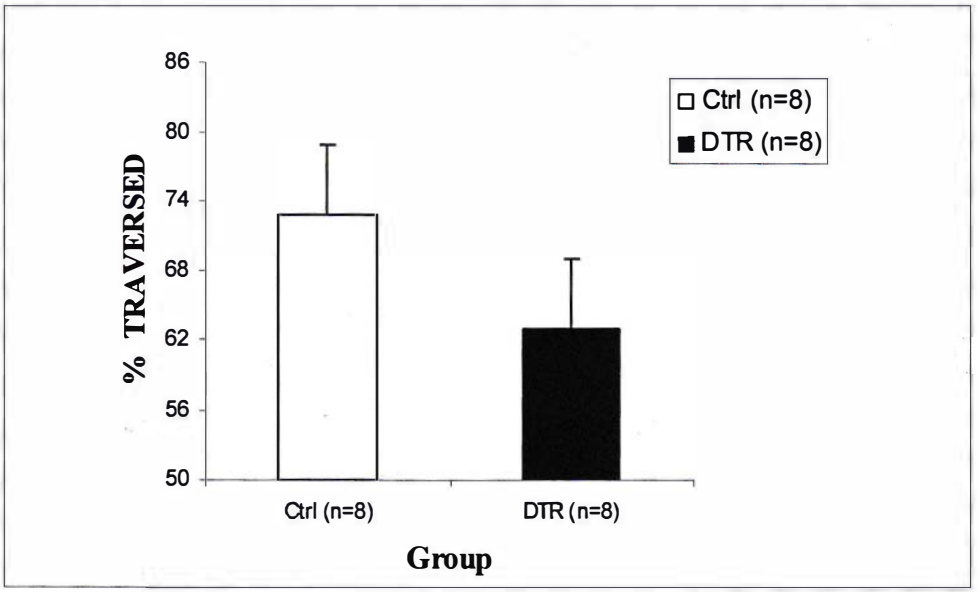


Table 3.40: The effect of DTR (100 mg/kg) on Gastrointestinal Motility Test after 3rd hour 30 minutes study period.

Group		% Traversed
Ctrl (n=7)		72.980 ± 5.802
DTR (n=7)		70.236 ± 5.159
t/p value		.353/.730
95% confidence interval	Lower	-14.173
	Upper	19.660

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

Figure 3.41: The effect of DTR (100 mg/Kg) on Gastrointestinal Motility Test after 3rd hour 30 minutes study.

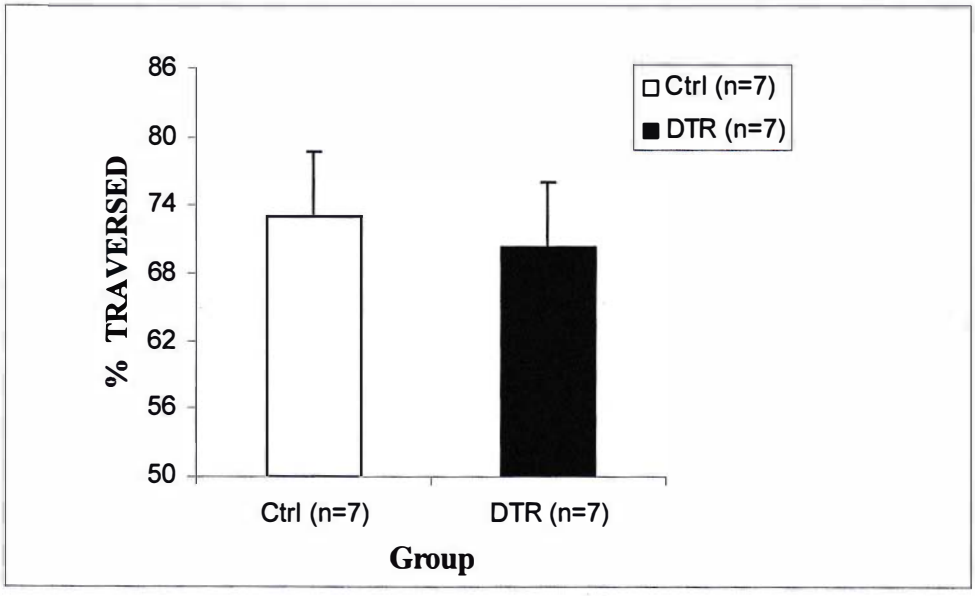


Table 3.41: The effect of DTR (100 mg/kg) on Gastrointestinal Motility Test after 4th hour 15 minutes study period.

Group		% Traversed
Ctrl (n=8)		64.046 ± 3.815
DTR (n=8)		61.578 ± 4.696
t/p value		.408/.689
95% confidence interval	Lower	-10.509
	Upper	15.446

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

Figure 3.42: The effect of DTR (100 mg/Kg) on Gastrointestinal Motility Test after 4th hour 15 minutes study.

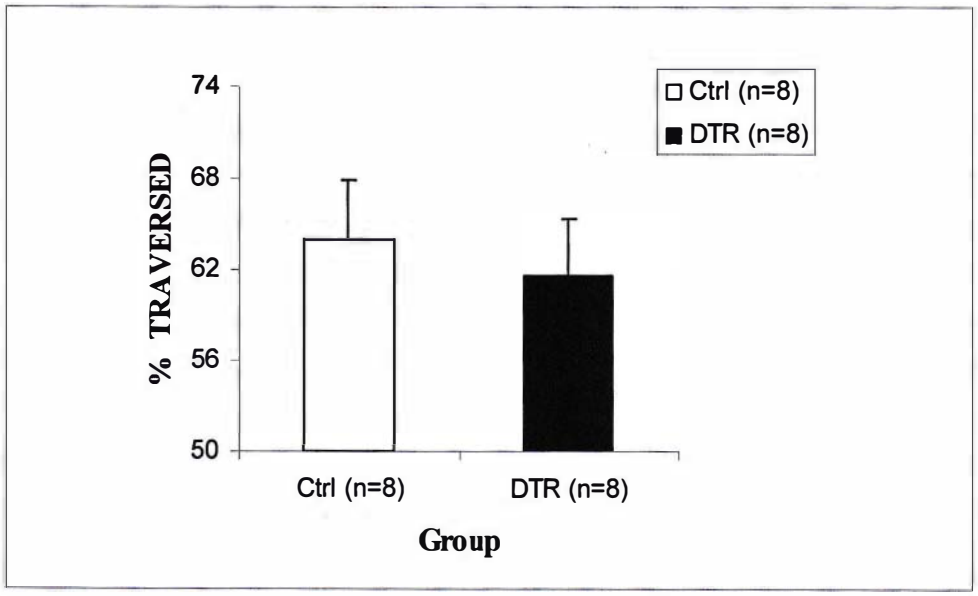


Table 3.42: The effect of DTR (100 mg/kg) on Gastrointestinal Motility Test after 4th hour 30 minutes study period.

Group		% Traversed
Ctrl (n=8)		84.920 ± 3.036
DTR (n=8)		74.612 ± 5.619
t/p value		1.614/.129
95% confidence interval	Lower	-3.391
	Upper	24.008

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

Figure 3.43: The effect of DTR (100 mg/Kg) on Gastrointestinal Motility Test after 4th hour 30 minutes study.

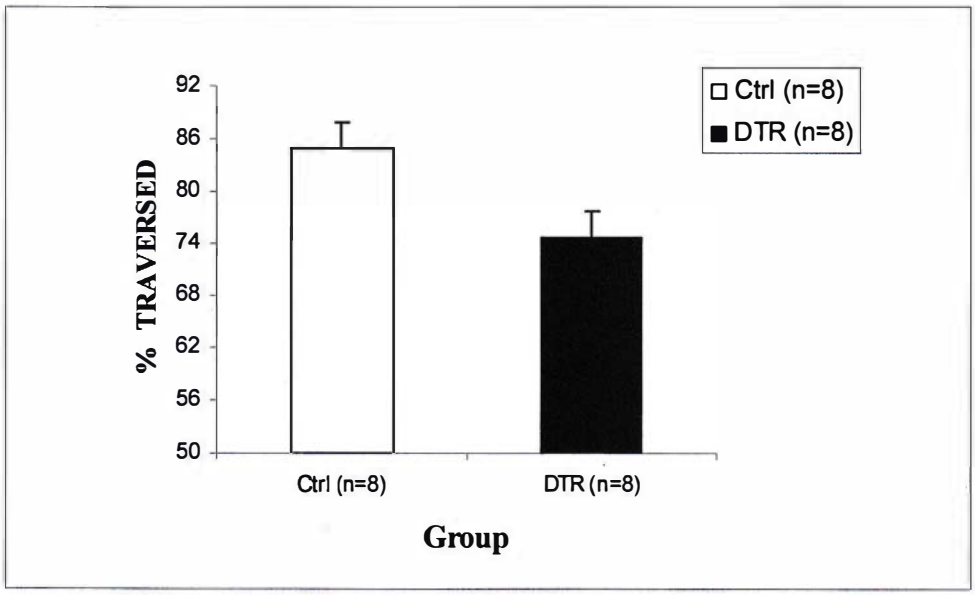
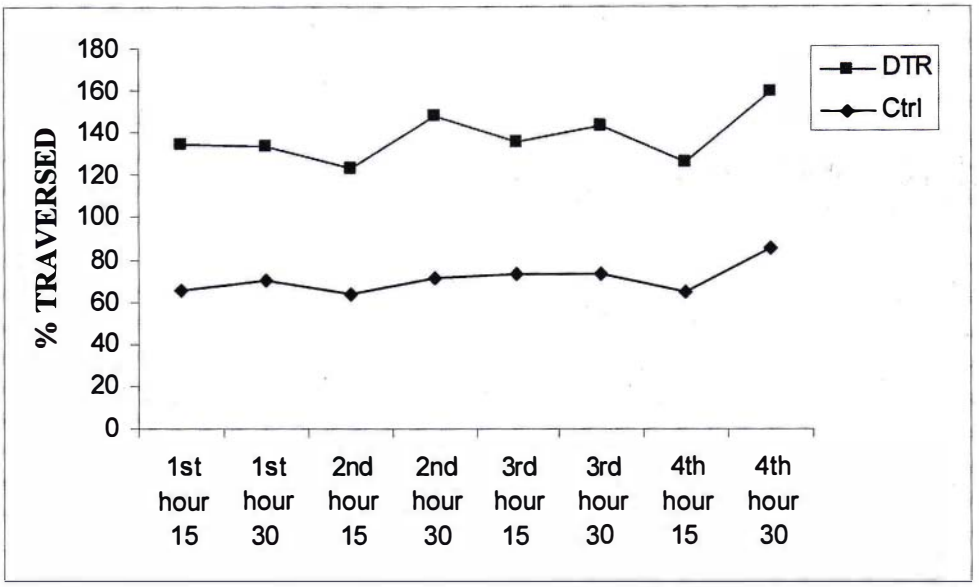


Figure 3.44: The overall effect of DTR (100 mg/kg) on Gastrointestinal Motility Test from 1st to 4th hour study period.



3.8 Gastric Emptying Test

Gastric emptying test was carried out in order to assess the effect of the DTR on the emptying of a solid meal from the gastric cavity. The weight range of female mice for this experiment was 30- 35 g. The results are summarized below.

STATISTICAL FINDINGS

At the dose 100 mg/kg DTR treated group exerted an increase in gastric emptying in the 2nd hour and a decrease in gastric emptying in the 4th hour as compared to the corresponding control group. But none of the results were found as statistically significant. . (Table 3.43, 3.44, Fig. 3.45).

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

2nd Hour (81.687- 69.799) = + 11.888 % (Increase)

4th Hour (77.497- 79.761) = - 2.264 % (Decrease)

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

Table 3.43: The effect of DTR (100mg/kg) on Gastric Emptying Test after 2nd hour study.

Group		% Gastric Emptying
Ctrl (n=10)		69.799±5.998
DTR (n=10)		81.687±2.492
t/p value		-1.830/.092
95% confidence interval	Lower	-26.038
	Upper	2.262

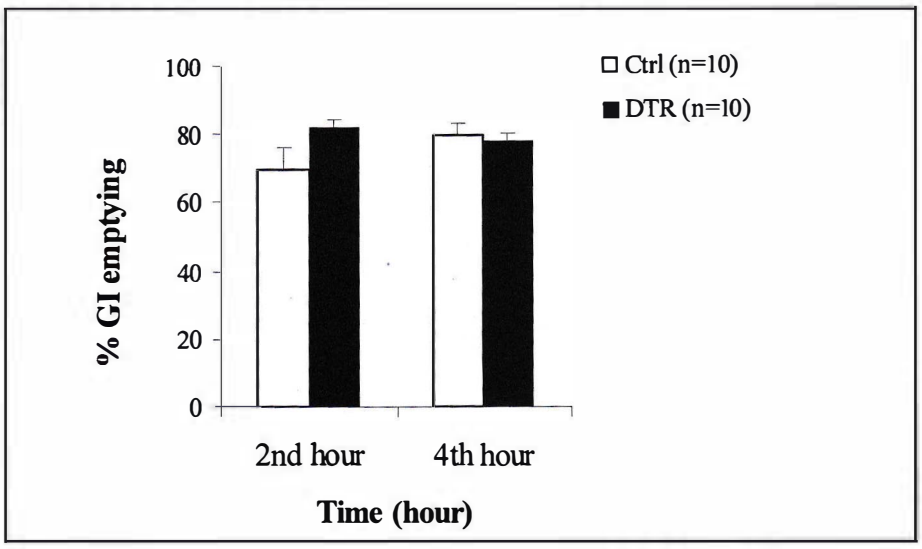
N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Table 3.44: The effect of DTR (100mg/kg) on Gastric Emptying Test after 4th hour study.

Group		% Gastric Emptying
Ctrl (n=10)		79.761±3.539
DTR (n=10)		77.497±2.367
t/p value		0.532/0.601
95% confidence interval	Lower	-6.682
	Upper	11.210

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

Figure 3.45: The effect of DTR (100mg/kg) on Gastric Emptying Test after 2nd hour and 4th hour study.



3.9 Hypoxia Test

This experiment was 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. The weight range of female mice for this experiment was 20- 26 g. The experimental results are analyzed below:

STATISTICAL FINDINGS

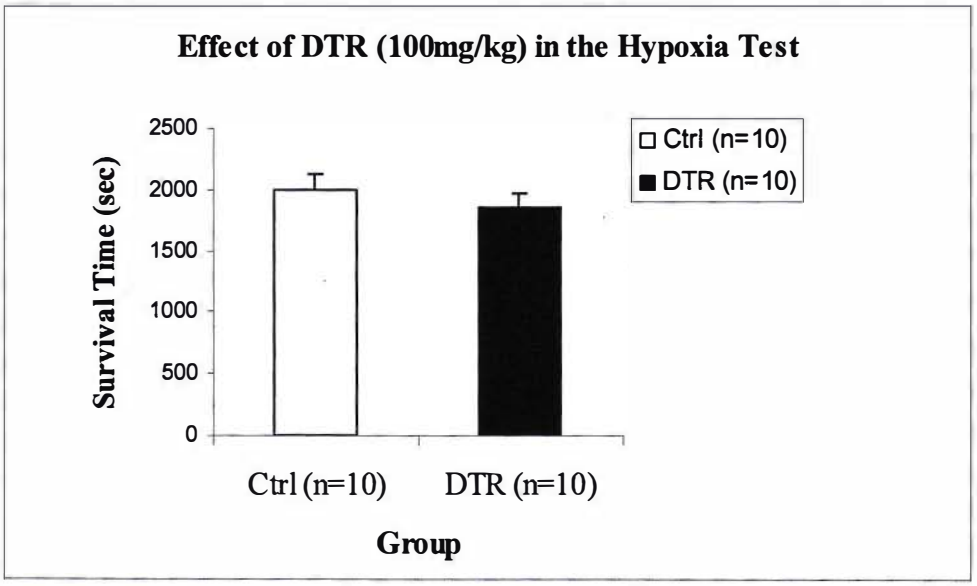
At the dose 100 mg/kg, DTR treated group showed a decrease in survival time as compared to the corresponding control group. The result was found to be statistically insignificant. (Table 3.45, Fig. 3.46).

Table 3.45: The effect of DTR (100mg/kg) in the Hypoxia Test.

Group		Survival Time (sec)
Ctrl (n=10)		1998.70± 119.950
DTR (n=10)		1850.20 ± 60.018
t/p		1.107/.288
95% confidence interval	Lower	-140.731
	Upper	437.731

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

Figure 3.46: The effect of DTR (100mg/kg) in Hypoxia Test.



Chapter Four

Conclusion & References

CONCLUSION

The Hole Cross Test produced mixed results which showed that the motor activity both increased and decreased at different doses but in 200mg /kg dose at min 30 ($p=0.005$) showed highly significant increase of motor activity.

The Open Field Test did not produce significant results throughout the experiment in all the 3 doses except for dose 400 mg/kg where significant ($p=0.036$) decrease was found in standing behavior.

According to the above results for both Hole cross test and Open field test we can assume that the drug may have a little stimulant property and no depressant property.

In Hole Board Test, at dose of 100mg /kg DTR showed significant ($p=0.010$) increase and highly significant ($p=0.009$) decrease of ambulatory activity whereas other results were insignificant which indicates that DTR may not have any effect on emotional defecation and the ambulatory activity was changed in a time dependant manner.

In Climbing Out Test at dose 200mg/kg and at min 30, a significant ($p=0.039$) decrease in activity was observed which indicates that DTR may have depressant property.

The Acetic Acid Writhing Test showed insignificant result. So DTR may not have any analgesic property.

In Formalin Test DTR exerted increasing analgesic activity and anti-inflammatory activity but none of the results were statistically significant.

In Gastro Intestinal Motility Test DTR showed both increase and decrease effect on gut motility but none of the results were statistically significant.

The effects of DTR on Gastric Emptying Test were insignificant and therefore the drug may not affect the rate of expulsion of solid meal from the gastric cavity.

In Hypoxia Test, DTR treated group showed a decrease in survival time as compared to the corresponding control group but the results were insignificant and it concludes that DTR may not have any effect on oxygen consumption.

All these experiment were executed in an attempt to confirm the safety of the general patients or users of the society and country as a whole. After completion of this research work now we can suggest that this DTR can be prescribed for treating different diseases without or with a minimal central nervous system side-effect. It also

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APPENDIX

Different Preparations and their constituents used throughout the research work

Sl. No.	Preparations	Ingredients	Amount
01	Barium sulphate	Carboxy methyl cellulose	1 g
		Barium sulphate	30 g
		Water	q.s. to 200 ml
02	0.6% Acetic Acid	Acetic acid	0.12ml
		Water	q.s. to 20 ml
03	1 % Formalin	Formalin	0.1 ml
		Water	q.s. to 10 ml