A Study on the Pharmacological Aspects and Possible Side Effects of Krishna Chaturmukha Rasa

DEPARTMRNT OF PHARMACY

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Submitted by: Tanzina Mollick
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CERTIFICATION

This is to certify that, the thesis "A Study on the Pharmacological Aspects and Possible Side Effects of Krishna Chaturmukha Rasa" submitted to the Department of Pharmacy, East West University, Mohakhali, Dhaka, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm) was carried out by Tanzina Mollick (ID# 2006-1-70-024) under our guidance and supervision and that no part of the thesis has been submitted for any other degree. We further certify that all the sources of information and other facilities availed of in this connection is duly acknowledged.

Apurba 27.12.09.

Mr. Apurba Sarker Apu
Lecturer (Supervisor)
Department of Pharmacy
East West University
43, Mohakhali C/A,
Dhaka-1212

Prof. Dr. Chowdhury Faiz Hossain
Chairperson
Department of Pharmacy
East West University
43, Mohakhali C/A
Dhaka-1212

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ABSTRACT

Purpose: The research was carried out in order to study the pharmacological aspects and possible side effects of the Ayurvedic formulation, Krishna Chaturmukha Rasa mainly indicated for the treatment of epilepsy; based on neuropharmacological, psychopharmacological and analgesic and anti-inflammatory studies on animal models (*Swiss albino*).

Method: The research project was conducted using five previously established pharmacological models proposed by various scientists. The experiments were executed at three dose levels of 100 mg/kg, 200 mg/kg and 400 mg/kg on animal models in order to determine the various degrees of significance on activity resulted by Krishna Chaturmukha Rasa.

Result: In the open field test there was a significant decrease in ambulation at the dose of 400mg/kg ($p=0.034$) followed by a significant decrease in emotional defecation ($p=0.044$) at a dose of 200mg/kg. Another statistically significant result ($p=0.049$) was obtained in the forced induced swimming test in which case the mice showed prolonged time periods in the immobile phase 24 hours after treating with the drug. In the formalin induced paw licking test a highly significant result was obtained ($p=0.002$) in the analgesic phase of the experiment. Apart from this, another significant result ($p=0.016$), which represented a decrease in writhing response, was obtained in the acetic acid induced writhing test at a dose of 100 mg/kg.

Conclusion: All these results signify that the drug may cause depression as a side effect of its treatment and that it may also possess analgesic activity. However, further studies should be performed using more sophisticated models to confirm these results.

Keywords: Ayurvedic formulation, Krishna Chaturmukha Rasa, epilepsy, pharmacological aspects, side effects.

1. INTRODUCTION

1.1 Ayurveda

Ayurveda or Ayurvedic medicine is one of the world's oldest medical systems which originated in India and has evolved there over thousands of years. The uniqueness of this system is that it provides an integrated approach to prevent and treat illness through lifestyle interventions and natural therapies based on the view that the elements, forces, and principles that comprise all of nature - and that holds it together and make it function - are also seen in human beings. This system of using natural resources for betterment of health was developed through the experimentation and experiences of day-to-day life style of Indian people and supported by the diverse biodiversity in flora and fauna due to variations in geographical landscaping (Mukherjee & Wahile, 2006).

1.2 Historical Perspective of Ayurveda

Ayurveda is a Sanskrit word derived from two roots, "Ayus" and "vid," meaning life and science respectively. Ayus, or life, represents a combination of the body, the sense organs, the mind, and the soul. The Vedas are ancient Hindu books of knowledge that contain within them the science, the rhythm, and the structure of the universe and the secrets of sickness and health. There are four Vedas: Rig Veda, Sama Veda, Yajur Veda, and Atharva Veda. Ayurveda is part of this fourth Veda, which includes detailed dissertations upon the treatment of the sick using mantras, herbs, and potions (Ayurveda, 2009). Thus, Ayurveda is a combination of science and philosophy which details the many physical, mental, emotional, and spiritual components necessary for holistic health.

At its essence, Ayurveda is based on the theory that everything in the universe is interconnected. Human beings are connected with one another as well as their physical environment, thus the balance within each person and their relationship to their environment as a whole must be carefully maintained. Any imbalance, whether it's physical, emotional, mental or spiritual causes unhealthy reactions by the body. Illness then, is the result of not being in harmony with the universe (Ayurvedic Medicine: India's Science of Life, 2009). Ayurveda believes that each person's general health, often referred to as their constitution or prakriti, is unique. To evaluate each person's *prakriti* or constitution, it uses three regulatory categories called *doshas* (life forces). Each dosha aligns with the qualities of the five basic elements that are believed to govern the universe: earth, fire, air, water and ether (the upper regions of space). Each dosha also relates to specific bodily functions and the basic state of health is determined by the balance or imbalance of the doshas. Good health reigns when all three doshas: *vata* (to move), *pitta* (to heat or burn) and *kapha* (to keep together) work in balance. Each one has its role to play in the body. For example, vata, which combines ether and air, produces movement and relates mainly to the nervous system and the body's energy. Pitta, which combines fire and water, relates to the metabolism, digestion, enzymes, acid, and bile. Kapha, which combines earth and water, relates to the mucous membranes, phlegm, moisture, fat, and lymphatics. The balance of the three doshas depends on a variety of factors, in particular correct diet and exercise, good digestion, healthy elimination of body wastes, and balanced emotional and spiritual health.

1.3 Ayurveda as a Healthcare System

Ayurveda is India's traditional system of medicine that has been practiced for more than 5,000 years and takes an integrated approach toward treating and preventing illness. It is also practiced as a primary medical system in Bangladesh, Sri Lanka, Nepal and Pakistan. Ayurveda calls upon a variety of natural therapies and lifestyle changes to restore imbalance or stress in the body and spirit believed to cause illness. It elaborately deals with measures for healthful living during the entire span of life and its various phases. Besides considering the principles for maintenance of health, it has also developed a wide range of therapeutic measures to combat illness. In this manner, Ayurveda becomes one of the oldest systems of health care dealing with both the preventive and curative aspects of life in a most comprehensive way and presents a close similarity to the World Health Organization (WHO) report's concept of health propounded in the modern era.

According to a WHO report, over 80% of the world population relies on plant-based traditional medicine for their primary healthcare needs and remedies (Ayurveda with no side effect, 2007), and the use of traditional medicines is rising in the developed economies. The United States of America now professionally practices Ayurveda and considers it as a complementary alternative medicine (CAM). More than 200,000 adults in the U.S. used Ayurveda in 2006, according to the National Center for Complementary and Alternative Medicine (Korenchan, 2009).

1.4 Ayurveda in Bangladesh

Bangladesh is rich in biodiversity and has an abundant resource of herbs, plants, and trees. Based on its geographical and seasonal benefits, the country is a potential

practitioner of Ayurveda. Indeed, Bangladesh is considered as the home of medicinal plants which have occupied an important position in the socio cultural, spiritual and medicinal arena of rural and tribal lives of Bangladesh. This is of tremendous contemporary relevance because it can on one hand ensure health security to millions of people and on the other hand it can provide new and safe herbal drugs to the entire world. Relative to allopathic treatment, Ayurvedic treatment is easy to access at affordable prices and sometimes is the only source of health care available to the poor. A majority of the population is below the poverty line and for most people the only way to seek medication at an economical rate is by seeking Ayurvedic treatment. However, in light of the successful benefits of Ayurvedic medicine the demand for such preparations is increasing in both developing and developed countries. In fact, given the success and extensive presence of Ayurvedic medicine in Bangladesh, the government is considering incorporating it as one of the mainstream primary health care services. Such action is considered a cost-effective and comparatively expedient manner of providing health coverage to large segments of the rural population (Islam, 1991). Bangladesh also has high prospect in making footsteps on the global market for medicinal plants and products as nearly 650 medicinal plant species have been identified to be in use in Bangladesh with around 25 plants having high value (Parves, 2009). In view of this, Ayurvedic preparations were brought under a drug control system in 1982 to provide oversight of manufacturing and marketing (Islam, 1991). In line with increased national and international demand of Ayurvedic medicines it has become very essential that clinical examination in the extent of safety and efficacy of these formulations be carefully evaluated and their pharmacological profiles established.

Thus, taking into consideration the widespread use of Ayurvedic medicine as the popular form of drugs can not be overlooked. Keeping the present scenario in mind, this research work on Krishna Chaturmukha Rasa (CTM), an Ayurvedic formulation, explores various pharmacological aspects utilizing animal models. The objective of this research work is to have a keen insight of the possible side effect profile of this drug and to some extent, decide how reasonable the use of this drug is under the stated circumstances.

1.5 Krishna Chaturmukha Rasa

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

Krishna Chaturmukha Rasa is included (page 321) in the Bangladesh National Formulary of Ayurvedic Medicine 1992 (Approved by the Government of Bangladesh vide Ministry of Health and Family Welfare Memo No. Health-1/Unani-2/89/(Part-1) 116 dated 3-6-1991). Bangladesh National Formulary of Ayurvedic Medicine is compiled by the National Unani and Ayurvedic Formulary Committee and published by the Bangladesh Board of Unani and Ayurvedic Systems of Medicine, 38, Bangabandhu Avenue, Dhaka-1000 under the authority vested in the Board vide section 13(j) of the Bangladesh Unani and Ayurvedic practitioners Ordinance, 1983 in collaboration with the World Health Organization. Directorate of Drug Administration has issued Notification DA/Admin/1-10/96/6212 dated 19th October 1996 has issued license under Drug Act, 1940 and Rules there under and Drug (Control) Ordinance 1982 for local manufacture and sale in Bangladesh. (Published

Bangladesh Gazette #24 Part VI dated Thursday, June 11th 1998.) At present a good number of Ayurvedic manufacturers are manufacturing and marketing the Classical Ayurvedic Medicinal Preparation.

1.5.1 Drug Composition, Administration and Indication

In Ayurveda, the formulation of Krishna Chaturmukha Rasa (CTM) is given in Sanskrit as:

Rasa (parada)	1 part
Gandhaka	1 part
Loha bhasma	1 part
Abhra (abhraka) bhasma	1 part
Hema (svarna) bhasma	¼ part
Kanya (kumari) svarasa	Q.S. (for mardana)
Eranda patra (Lf.)	Q.S. (for avestana.)

The translated version of the formulation of CTM is:

Purified Murcury	1 part
Purified Sulphur	1 part
Purified Iron	1 part
Mica (unglazed)	1 part
Purified Gold	¼ part
Aloe vera	Q.S. (for manual trituration)
Ricinus communis leaves	Q.S. (for covering mixed semisolid preparation.)

At the final stage of preparation, i.e. after covering with eranda leaves, it should be left within a heap of dhanya grains (Oryza sativa) for 3 days.

CTM is available in the form of powder or pills and it must be administered orally at a dose of 125 mg. The *Anupana* (fluid vehicle taken with or after medicine) is honey or triphala kvatha. Triphala kvatha is a herbal decoction of haritaki (Terminalia chebula), bhibitaki (Terminalia belerica) and amalaki (Emblica officinalis).

The main indication for CTM is Epilepsy or *Apasmara* in Sanskrit.

1.6 Epilepsy – as seen in Ayurveda and Modern Medicine

1.6.1 Definition

In most Ayurvedic texts, epilepsy has been mentioned as *Apasmara* or *Apasmrti*, and has been described as one of the earliest eight diseases known (diagnosed) that can be controlled only with medical therapies and can sometimes be incurable and remain uncontrolled (Jain & Tandon, 2004). Epilepsy as per Ayurveda is defined as the transient derangement of memory, intelligence, and the mind, resulting in a temporary blackout of vision, loathsome activities, and unconsciousness. *Vagbhata* defines it as loss or destruction (*apaya*) of memory (*smriti*) (Shrikanthamurthy, 2002). Modern medicine defines a seizure as a transient alteration of behavior due to the disordered, synchronous, and rhythmic firing of populations of neurons. Epilepsy per se is defined as a disorder of brain function characterized by the periodic and unpredictable occurrence of seizures (McNamara, 2001). Hence, whereas the Ayurvedic definition describes the effect, the modern definition describes the cause of epilepsy.

1.6.2 Clinical Description

The clinical description as mentioned in Ayurvedic texts is classified as prodromal signs and symptoms (*purvarupa*) and clinical manifestations (*rupa*). The prodromal signs and symptoms include raising of eyebrows, frequent abnormal movements of eyes, hearing of sounds not perceived by others, excessive oozing of saliva and nasal mucus, aversion to food, anorexia, indigestion, distension of abdomen, debility, body ache, transient blackout, giddiness, profuse sweating, increased thirst, fainting, hallucinations, delusions, falling, aura, and insomnia (Sharma, 1999). This description is very similar to the description in modern medicine of generalized tonic clonic (GTC) seizures. These seizures are characterized by an abrupt onset, sometimes with premonitory symptoms, loud cry, excessive salivation, postictal unresponsiveness, headache, muscle ache, fatigue, and an increase in heart rate and pupillary size (Lowenstein, 2001).

The loss of memory as described by *Vagbhata* is similar to the description of absence seizures, which are characterized by loss of consciousness for a longer duration and are less abrupt in onset and cessation.

In Ayurveda, epilepsy is divided into four types according to the dominant *dosa* involved in the disease pathogenesis: *vataja, pittaja, kaphaja,* and *sannipataja*; signs and symptoms manifest accordingly. The *vataja* type involves *vata* as the dominating *dosa* and is characterized by having frequent fits; regaining consciousness in the shortest time interval, bulging eyes, excessive crying, frothing at the mouth, irregularly contracted fingers, reddish, rough, and blackish nails, eyes, face, and skin, hallucinations, trembling, and visions of unstable, coarse, and rough objects. *Pitta* is

the leading *dosa* in *pittaja* type with distinctive features of regaining consciousness in shorter periods, scratching the ground, greenish-yellowish and coppery nails, eyes, face, and skin, and visions of bloody, agitated, irritated, frightful, and burning objects. The *kaphaja* type denotes *kapha dosa* as the principal *dosa* including features such as delayed fits, delayed recovery, increased frothing at mouth, white nails, eyes, face, and skin, and visions of white, heavy, unctuous, smooth objects. *Sannipataja* means all the three *dosas* are in conjunction with each other. It is their simultaneous vitiation that gives rise to the combination of signs and symptoms.

Vataja and *kaphaja* types match the description of GTC seizures, *pittaja* matches complex partial seizures, and *sannipataja* matches the characteristics of a mixed-seizure profile.

1.6.3 Etiology (*Vyaadhi hetu*)

As per the classical texts, the basic etiology is threefold for epilepsy. Endogenous factors (*nija*) include genetic, congenital, constitutional (*prakriti*), enzymatic disturbance (*agni vikruti*), and idiopathic. Exogenous factors (*agantuka*) include habitual intake of unwholesome and unhygienic foods and drinks that have mutually contradictory properties (*apathya aahar*), especially those with properties similar to *vata dosa* causing its aggravation and trauma; this aggravation is due to worms (*krimijanya*) and environmental and idiopathic factors (Lowenstein, 2001). Psychological trigger factors (*manasika*) include excessive worry, grief, fear, passion, anger, anxiety, attachment, and excitement.

Modern medicine describes the etiology of epilepsy in a similar manner while giving an age-wise classification of the causes. In neonates, genetic disorders, metabolic disturbances, and acute central nervous system (CNS) infection are the common causes. Infants and children have genetic disorders, febrile seizures, traumas, and CNS infections as common causes. Etiology for adults includes trauma, infection, tumor, and metabolic disorders in the case of older adults (Lowenstein, 2001).

1.6.4 Pathogenesis

In epilepsy, *vata* is predominately vitiated and is the fundamental cause in the pathology of the disease. Neurological disorders, as understood in Ayurveda, are considered to be due to imbalance of *vata.* All the three etiological factors contribute in the accumulation and vitiation of *vata*, affecting consciousness and all the sense organs. After it is aggravated, the *dosa* spreads throughout the body and through the nerves (*dhamanis*), leading to a manifestation of the epileptic episode. The agitated *vata dosa* abruptly proceeds through the nerves of the body, shaking it in a quick succession (shaking jerks) called "*akshepaka,*" which means convulsion. Another term for this condition is *apatanaka* where the patient falls on the ground without spasm at intervals that could be correlated with absence-seizure type of epilepsy.

According to modern medicine, epilepsy results from a focus of hyperexcitable neurons in the cortex. During seizures, the permeability of the cytoplasmic membrane of the neurons changes. This results in increased levels of intracellular calcium and extracellular potassium, which contributes to overall excitability of the epileptic neuronal aggregate. The neurons become susceptible to hypoglycemia, hyponatremia,

hypocalcemia, sleep deprivation, and photic stimulation (McNamara and Lowenstein, 2001).

An epileptic seizure has two phases: initiation phase and seizure propagation phase. The initiation phase is characterized by two concurrent events: high frequency bursts of action potentials and hypersynchronization. In epileptogenesis, a normal neuronal network transforms into one that is chronically hyperexcitable. Ayurveda also has the concept of seizure propagation where the *vata dosa* spreads rapidly throughout the body.

1.6.5 Rational behind the use of Ayurvedic Medicine in Epilepsy

The incidence of epilepsy in the elderly is rising and an ideal anticonvulsant for use in an elderly patient in modern medicine is yet available (Arroyo & Kramer, 2001). Another fact that must be considered is that the elderly may have many disorders, take multiple concomitant medications, and have different metabolic features; they are also more sensitive to the adverse effects of drugs. Furthermore, febrile seizures are the most common convulsive events in childhood, occurring in 2 to 5% of children. Approximately 20 to 30% of these children may have recurrence during a subsequent febrile infection. These could be nonepileptic. Because the side effects of the antiepileptic drugs outweigh their benefits, their continuous use is no longer recommended (Rantala *et al.*, 2000). In both these circumstances, Ayurveda as an individualized therapy could be of great value.

Ayurvedic preparations in epilepsy treatment will result in milder and fewer adverse effects and toxicities than modern medicine; and the ultimate expenses would be

certainly less than the conventional therapy. In many countries the vast majority of sufferers remain untreated (WHO, 2002). The use of Ayurvedic therapies could help solve this problem worldwide.

In case of conventional drug therapy, such as Carbamazepine, treatment has potential risks and these must be weighed against benefits before initiating therapy (Ramadasan *et al.*, 2000). Weight gain in patients on antiepileptic drugs disturbs the general health. It causes cosmetic adverse effects and can have serious psychological effects that may lead to withdrawal of the drug (Jallon *et al.*, 2001). Hepatotoxicity and teratogenecity of antiepileptic drugs like valproate are rare, but severe adverse effects have been reported (Bialer, 1999). Hepatoprotective Ayurvedic therapies may be used as an adjuvant to protect from hepatotoxicity of conventional drugs. All conventional antiepileptic drugs may provoke positive or negative psychiatric reactions in individual patients, and these reactions depend on the strength of the drugs and genetic and biographic psychiatric predisposition of the patient (Schmitz, 1999).

These adverse side effects can be prevented using Ayurvedic therapies where the concept of *prakriti* plays a vital role in the treatment.

1.7 Prevalence of Epilepsy in Bangladesh

Bangladesh is one of the densely populated countries in the world where infectious diseases, malnutrition and many chronic neurological disorders are quite common. Although there is no national statistics yet in our country but there are some hospital based studies that reflect to some extent the situation of epilepsy in Bangladesh (Mannan, 2004). Studies in developed countries shows prevalence rate of about 5 per

1,000 populations whereas in developing countries it is higher. Men are more affected than female and rural populations are affected more than the urban populations. Based on the prevalence rate of 10 per 1,000 populations, the number of epilepsy patients in Bangladesh is about 1.3 million (Mannan, 2004). The common ages of epileptic patients in Bangladesh are between 16 to 31 years. The etiology varies with age. Birth trauma, birth asphyxia, central nervous system infections are common in neonate and infancy whereas head trauma, brain tumor, stroke, infections are common causes in middle aged and elderly.

Vast majority of the people in Bangladesh have superstitious beliefs about epilepsy. This belief usually is a strong barrier for total care of patients with epilepsy. Misunderstanding and negative attitude of the parents, family members and society towards epilepsy are still prevalent. Thus, many patients with epilepsy are still neglected in diagnosis, treatment, education, rehabilitation and other social needs. The epilepsy patients are often reluctant to seek advice from physicians (Mannan, 2004).

Only 29% perceive epilepsy as a disease, 50% dropped out from school (58% of whom due to epilepsy), and 52% of patients had to change job because of epilepsy. Appropriate antiepileptic drugs are sometimes unavailable in Bangladesh (Mannan, 2004). The BSMMU study showed 23% of patients found it difficult to continue treatment due to financial problem. Financial factor is likely to partly account for the treatment gap.

Based on the facts and figures that have been stated, it is even more evident that the use of Ayurvedic medicines, in this case CTM, can be effectively used to treat epileptic patients in Bangladesh in an affordable and beneficial approach.

1.8 Purpose of the Present Study

In the past, research on Ayurveda was sparse and often poorly designed. Since Ayurveda has been practiced in India for thousands of years, the efficacy of the system is accepted there without question. However, during the last several decades, a great deal of effort has been made to study the scientific basis of Ayurvedic medicine. Moreover, the extensive use of Ayurvedic medicine as a means of treatment from disease on both a national and international basis has made clinical examination essential in the extent of safety and efficacy. Keeping such state of affairs in mind, this research work on Krishna Chaturmukha Rasa (CTM), an Ayurvedic formulation, encompasses the pharmacological and toxicological aspects of the preparation and to some extent decides how reasonable the use of this drug is under the stated circumstances. The methodologies utilized will be based on animal models. The ultimate result of this project will be in supplementing and complementing the existing health care facilities of our country and in the long run will ensure total coverage of the population in terms of public health.

1.9 Accomplishment of the Purpose

In order to accomplish this purpose, the research project is conducted using previously established models proposed by various scientists based on animal studies. These proposed methodologies are used to analyze the neuropharmacological, psychopharmacological and analgesic and anti-inflammatory effects of CTM at three

dose levels of 100 mg/kg, 200 mg/kg and 400 mg/kg; a brief discussion of which is discussed in the following segment.

The neuropharmacological effects are studied by conducting the hole cross test and the open field test. The hole cross test is performed based on the established procedure proposed by Robbins in 1977, in order to determine the stimulatory or depressive effect of the test drug by accurately measuring the effects of the drug on the spontaneous motor activity of animals. The open field test is carried out, based on the model proposed by Gupta in 1971, to verify the same purpose but in this case the animal is provided with a different and more complex environment to explore. The theory behind this test is that when the animal is placed in the unfamiliar region of an open field it results in a pattern of behavior characterized by exploration (movement towards center region and standing up), locomotion (ambulation past squares) and emotional defecation. It has been considered that the exploration evoked under an unfamiliar environment is modified with physiological factors such as curiosity, fear and anxiety and the modulation of these factors after the administration of a drug.

Psychopharmacological effects are investigated by performing the forced induced swimming test. The traditional version of this test was developed by Roger Porsolt and colleagues in 1977, in order to study any anti-depressant activity of the test drug.

Finally, the analgesic and anti-inflammatory effects are determined by the procedures of the formalin induced paw licking test and the acetic acid induced writhing test. The formalin pain test is performed according to the model proposed by Tjolsen and colleagues in 1992; it is very useful for evaluating the mechanism of pain and

analgesia and thus finds a means of finding out whether the test drug has any analgesic and anti-inflammatory effects. 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. The acetic acid induced writhing test is conducted to assess the existence of non-narcotic analgesic property of the test drug based on the methodology proposed by Tang *et al* in 1984. The pain sensation is initiated by using acetic acid. The acetic acid induced writhing is inversely proportionate to the non-narcotic analgesic property

2. MATERIALS & METHODS

The entire research project was conducted on five pharmacological models in order to study the neuropharmacological, psychopharmacological and analgesic and anti-inflammatory effects of CTM. A detailed discussion is given on the materials required and the methods followed for each of the experiments.

2.1 Source of the Ayurvedic formulation, CTM

The Ayurvedic formulation, CTM (Batch number 001), was collected from "Sri Kundeswari Aushadhalaya Ltd." located in Chittagong, Bangladesh. The formulation was available in the form of tablets packed in suitable containers.

2.2 Dose and Route of Administration

The tablets were powdered, by grinding with the aid of a mortar and pestle, and made into a solution with distilled water. The solution was then administered at a volume such that it would permit optimal dosage accuracy without contributing much to the total increase in the body fluid. For all the pharmacological studies the drugs were administered by the oral route [per oral, (p.o.)] at the doses of 100mg/kg, 200mg/kg and 400mg/kg body weight.

2.3 Experimental Animal

Only Female mice of the Swiss-Webster strain having a weight range between 20-40 gm were experimented on for pharmacological effects. The mice were bred in the animal house of the Department of Pharmacy, Jahangirnagar University, where they were kept in cages having dimensions of 30 x 20 x 13 cm and soft wood shavings employed as bedding in the cages.

The animals were provided with standard laboratory food and tap water *'ad libitum'* and maintained at the natural day and night cycle. They were fed with "mouse chow" (prepared according to the formula developed at BCSIR, Dhaka).

At the beginning of each experiment the animals were carefully divided into two groups consisting of an equal number of mice. One group was assigned as the drug group, in which case the mice were administered with drug; and the other group was assigned as the control group which was administered with distilled water as par the same volume as the drug treated group. Each mouse was then marked on the base of the tail in order to serve as an identification mark for that particular animal. This way the response of a particular mouse prior to and after drug administration could be noted distinctly. Six to ten mice were taken for both the control and the drug group and the experiments were simultaneously employed for both.

2.4 Experiments conducted and Doses employed

Experiment	Dose
Hole cross test	100 mg/kg, 200 mg/kg and 400 mg/kg
Open field test	100 mg/kg, 200 mg/kg and 400 mg/kg
Forced induced swimming test	100 mg/kg
Formalin induced paw licking test	100 mg/kg
Acetic acid induced writhing test	100 mg/kg, 200 mg/kg and 400 mg/kg

2.5 Pharmacological studies with Animal models

2.5.1 Hole Cross Test

Materials required:

12 female mice	Methanol (VWR, England)
Hole cross apparatus	Feeding needle
Stopwatch	Counter
Cotton	

Method employed:

The 12 mice were divided into two groups; 6 for the drug group and 6 for the control group. Each mouse was placed on one side of the hole cross apparatus (a box having dimensions of 30 X 20 X 14 cm, constructed with a dividing wall with a hole of 3 cm in diameter at a height of 4.5 cm from the floor) and the 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 minutes prior to and 30, 60, 120 and 240 minutes after oral administration of CTM and was compared with the control group.

2.5.2 Open Field Test

Materials required:

12 female mice	Methanol (VWR, England)
Open field apparatus	Feeding needle
Stopwatch	Counter
Cotton	

Method employed:

The 12 mice were divided into two groups; 6 for the drug group and 6 for the control group. Each mouse was placed on one corner of the open field apparatus (a box consisting of a half square meter floor divided into a series of squares alternatively coloured black and white and enclosed by walls 40 cm in height) and the number of ambulation (expressed as the number of squares traveled), the number of times it entered the center region, the number of stand ups and number of fecal boluses expelled by the mouse were 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 group.

2.5.3 Forced Induced Swimming Test

Materials required:

20 female mice	Cotton
Glass case	Feeding needle
Stopwatch	

Method employed:

The 20 mice were divided into two groups; 10 for the drug group and 10 for the control group. Each mouse was exposed to a 15-min pre-swim 24 h before a 5-min test exposure in 15–18 cm of 25°C water. Following an initial period in which the mouse produces escape-directed behaviors, it will adopt an immobile posture, which is believed to reflect either a failure to persist with escape-directed behavior or a passive behavior to cease active forms of coping to the stressful stimuli. The period for which the mice attained an immobile posture was recorded and compared with the control group.

2.5.4 Formalin Induced Paw Licking Test

Materials required:

20 female mice	Methanol (VWR, England)
10 glass jars	Feeding needle
Stopwatch	Micro liter syringe (Hamilton, Switzerland)
Electronic balance (Shimadzu, Japan)	Formalin (BDH, England)
Measuring cylinder	

Method employed:

The 20 mice were divided into two groups; 10 for the drug group and 10 for the control group. Two hours after drug administration, Formalin (1%) was administered to each mouse by intraplantar route (IP), and immediately the licking time was registered for 5 minutes (first phase, neurogenic). Twenty minutes after the beginning of the experiment (second phase, inflammatory) the licking time was registered for another 5 min. The same method was employed for the control group and results were compared.

2.5.5 Acetic Acid (AA) Induced Writhing Test

Materials required:

20 female mice	Methanol (VWR, England)
10 separate mice cases for observation	Feeding needle
Counter	Sterile syringe
Acetic acid (COO, Germany)	Injection needle

Method employed:

The 20 mice were divided into two groups; 10 for the drug group and 10 for the control group. In case of each mouse muscular contraction was induced by the intraperitonial injection of Acetic Acid (0.6%, 0.25ml/animal). Then CTM was administered orally 45 minutes before the intraperitonial injection of 0.6% AA. The mice were then individually cased to count the 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 drug group and control group.

2.6 Statistical Analysis

The various degrees of significance on activity were determined by analyzing all the data obtained using SPSS (Statistical Package for Social Science) for WINDOWS™ (Version 12). Data were presented as Mean ± SEM (standard error of the mean) and unpaired "t" tests were done for statistical significance tests.

Upon analysis, a "p" value was obtained that determines the appropriateness of rejecting the null hypothesis. The "p" values range between 0 to 1; and the smaller it is the smaller the probability of rejecting the null hypothesis.

For all the data analyzed, $p = 0.05$ was assigned as the level of significance; $p = 0.01$ was assigned to represent a high level of significance; and $p = 0.001$ was assigned to represent a very high level of significance.

3. RESULTS & DISCUSSION

3.1 HOLE CROSS TEST

Statistical findings:

At the dose of 100mg/kg, an overall increase in activity was observed throughout the allotted time intervals of the experiment by the CTM treated female mice in comparison to the control group.

At the dose of 200mg/kg, there was an overall decrease in response by the drug group throughout the course of the experiment relative to the corresponding control group. However, a variation was observed at min 180 where increased hole cross activity was exhibited by the drug group relative to the control group.

At the dose of 400mg/kg, the CTM treated female mice were observed to show increased response throughout the course of the experiment in comparison to the corresponding control group. But, a variation was observed at min 240 where the drug group showed a decrease in response relative to the control group.

None of the results were found to be statistically significant ($p > 0.05$).

Tabular and Graphical presentation of the effect of CTM (100 mg/kg, 200 mg/kg, and 400 mg/kg) in the Hole Cross Test using female mice:

Table 3.1.01: The effect of CTM (100mg /kg) in the Hole Cross Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		0.167± 0.167	0.333± 0.333	0.333± 0.211	0.333± 0.211	0.333± 0.211	0.333± 0.211
CTM(n=6)		0.667± 0.333	1.667± 0.667	1.667± 0.803	1.667± 0.803	1.000± 0.365	1.500± 0.671
t/p		-1.342/ 0.209	-1.789/ 0.104	-1.606/ 0.162	-1.606/ 0.162	-1.581/ 0.145	-1.659/ 0.148
95% confidence interval	Lower	-1.330	-2.994	-3.392	-3.392	-1.606	-2.88878
	Upper	0.330	0.327	0.725	0.725	0.273	0.555

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

Figure 3.1.01: Graphical Presentation of the Effect of CTM (100mg/kg) in the Hole Cross Test

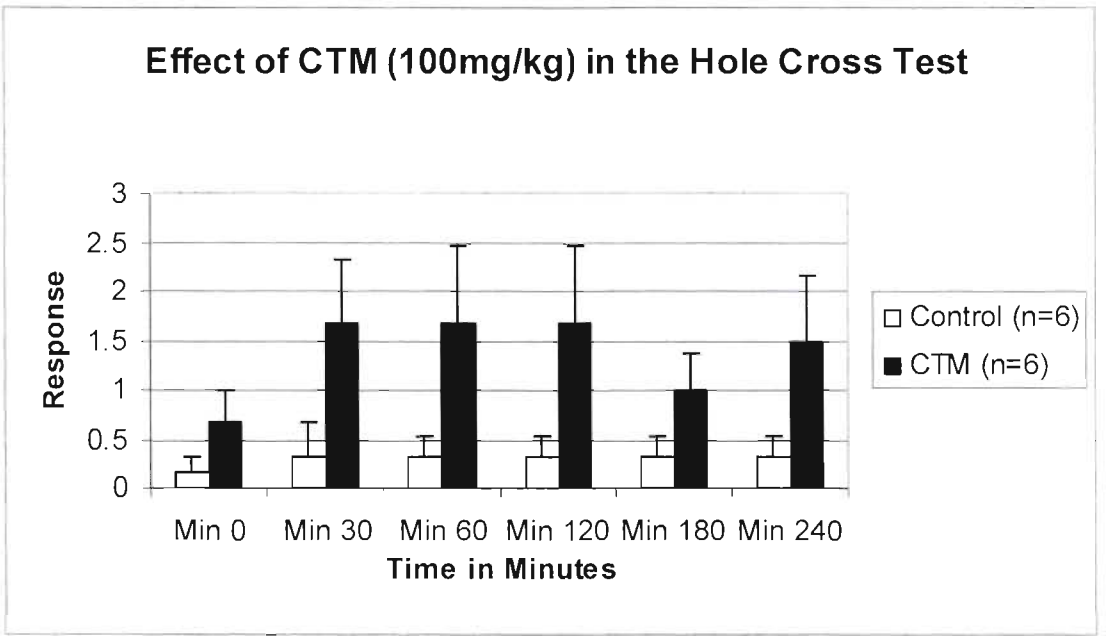


Table 3.1.02: The effect of CTM (200mg /kg) in the Hole Cross Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		4.167± 1.740	4.333± 1.706	4.500± 1.057	3.333± 1.116	2.167± 0.749	4.167± 1.138
CTM(n=6)		3.667± 1.406	3.333± 1.585	3.667± 1.687	2.000± 0.931	2.667± 1.308	2.500± 0.991
t/p		0.223/ 0.828	0.429/ 0.677	0.419/ 0.686	0.918/ 0.380	-0.332/ 0.747	1.104/ 0.295
95% confidence interval	Lower	-4.485	-4.188	-3.718	-1.904	-3.859	-1.696
	Upper	5.485	6.188	5.385	4.571	2.859	5.029

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

Figure 3.1.02: Graphical Presentation of the Effect of CTM (200mg/kg) in the Hole Cross Test

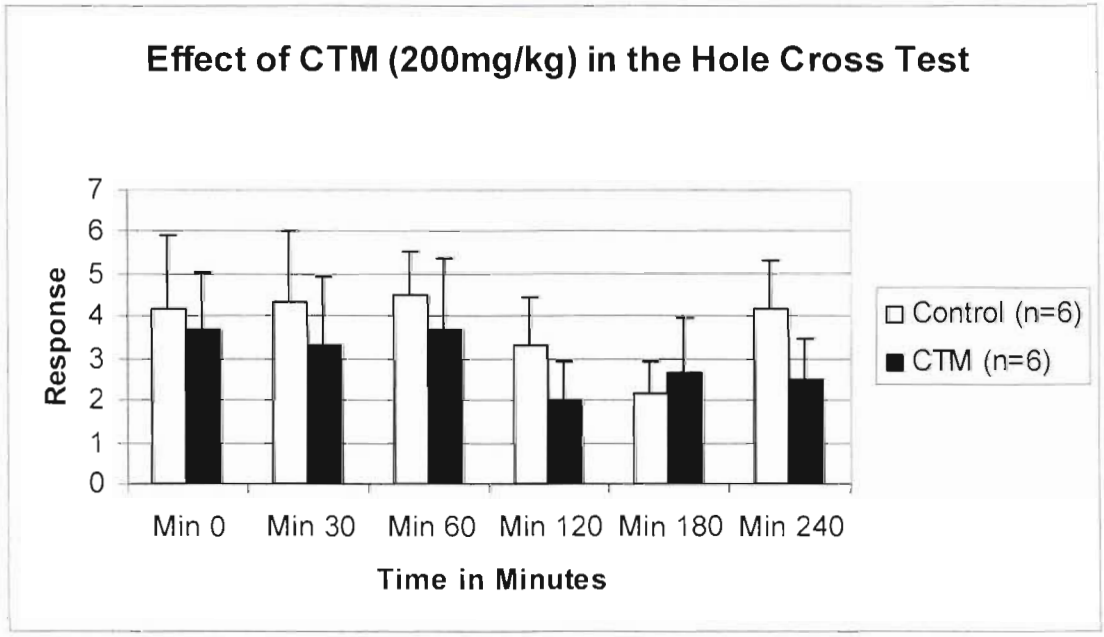
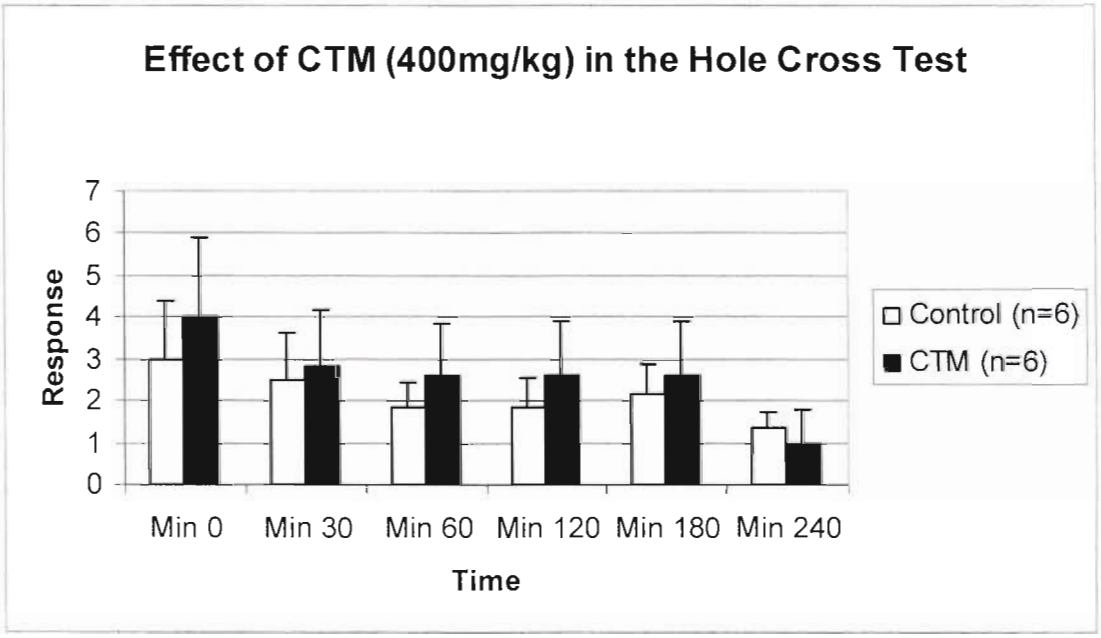


Table 3.1.03: The effect of CTM (400mg /kg) in the Hole Cross Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		3.000± 1.414	2.500± 1.147	1.833± 0.601	1.833± 0.703	2.167± 0.703	1.333± 0.421
CTM(n=6)		4.000± 1.924	2.800± 1.356	2.600± 1.249	2.600± 1.288	2.600± 1.288	1.000± 0.775
t/p		-0.428/ 0.679	-0.170/ 0.869	-0.586/ 0.572	-0.548/ 0.597	-0.310/ 0.764	0.397/ 0.701
95% confidence interval	Lower	-6.286	-4.288	-3.727	-3.931	-3.598	-1.568
	Upper	4.286	3.688	2.194	2.398	2.731	2.234

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

Figure 3.1.03: Graphical Presentation of the Effect of CTM (400mg/kg) in the Hole Cross Test



3.2 OPEN FIELD TEST

Statistical findings:

Total Ambulation

At the dose of 100mg/kg, CTM treated female mice exhibited an overall increase in ambulation throughout all the time intervals relative to the control group. However, an exception was observed at Min 30 where a decrease ambulatory activity occurred in comparison to the control group.

At the dose of 200mg/kg, the female mice of the drug group (CTM) were observed to show an overall decrease an ambulatory activity, relative to the control group, throughout the course of the experiment; except at Min 120 where ambulation of both the drug and corresponding control groups was somewhat similar.

At the dose of 400mg/kg, a significant decrease in ambulation was exhibited at Min 240 by the drug group (**p=0.034***) compared to the control. Otherwise, an overall decrease in ambulation was observed in case of the drug group in comparison to the control at all time intervals. An exceptional result was demonstrated at Min 60 where the female mice treated with CTM showed an increase in ambulation relative to the control group.

The results obtained for the doses of 100mg/kg and 200mg/kg were all statistically insignificant ($p > 0.05$).

Total Ambulation in Center Region

At the dose of 100mg/kg, the total ambulation in the center region was found to be 0 for both the drug group and the corresponding control group at Min 60, Min 120 and Min 240. Variation occurred at two instances; at Min 30 CTM treated female mice showed a decrease in response

relative to the control group, and at Min 180 center ambulation was observed for the control group while no response was shown by the drug group.

At the dose of 200mg/kg, an overall decrease in response was observed for the drug group in comparison to the corresponding control group, and at Min 240 the response showed by both the drug and control groups was equal.

At the dose of 400mg/kg, compared to the control group the CTM treated female mice showed decreased response at Min 30 and no response at Min 240. Equal responses were seen for both groups at Min 60, however a variation was observed at Min 120 and Min 180 where the drug group showed increased response compared to the control group which showed no response.

None of the results were found to be statistically significant ($p > 0.05$).

Total Standing Up Behaviour

At the dose of 100mg/kg, an overall increase in standing up behaviour was exerted by CTM treated female mice throughout the course of the experiment in comparison to the control group. Conversely, decreased activity was observed for the drug group relative to the control group at Min 30.

At the dose of 200mg/kg, the drug group showed overall decreased activity compared to the control group. Equal responses were observed for both groups at Min 180; however, a variation was observed at Min 120 where the drug group showed increased activity compared to the corresponding control group.

At the dose of 400mg/kg, at Min 30, Min 120 and Min 240 decreased standing behaviour was exerted by the drug group compared to the control group. Contrary to this result, the drug group showed increased activity relative to the control group at Min 60 and Min 180.

None of the results however were found to be statistically significant ($p > 0.05$).

Emotional Defecation

At the dose of 100mg/kg, increase in defecation was exhibited by the drug group compared to the control group at Min 30 and Min 60. Similar defecation patterns were observed at Min 120 and Min 240. No defecation was seen for the drug group at Min 180 relative to the corresponding control group.

At the dose of 200mg/kg, there was a decrease in the defecation pattern for the CTM treated female mice compared to the corresponding control group for Min 30 and Min 60. The result obtained at Min 60 was statistically significant (**$p=0.044$***). In contrast to this, increased defecation was observed for the drug group compared to control in the time intervals that followed.

At the dose of 400mg/kg, an overall increased defecation pattern was observed for the drug group in comparison to the control group; similar responses were seen for both groups at Min 120. However, there was a decrease in defecation for the drug group at Min 180 compared to the control group.

No result obtained at 100mg/kg and 400mg/kg were statistically significant ($p > 0.05$).

Tabular and Graphical presentation of the effect of CTM (100 mg/kg, 200 mg/kg, and 400 mg/kg) in the Open Field Test using female mice:

Table 3.2.01: The effect of CTM (100mg /kg) on Ambulation in the Open Field Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		91.500± 22.235	58.500± 15.524	22.000± 10.733	28.000± 7.633	25.000± 10.680	7.667± 2.765
CTM(n=6)		62.667± 16.992	50.000± 20.456	57.833± 23.878	36.167± 18.227	29.667± 3.809	50.167± 28.307
t/p		1.030/ 0.327	0.331/ 0.747	-1.369/ 0.201	-0.413/ 0.688	-0.412/ 0.689	-1.494/ 0.166
95% confidence interval	Lower	-33.519	-48.718	-94.165	-52.197	-29.932	-105.872
	Upper	91.185	65.718	22.498	35.863	20.599	20.872

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

Figure 3.2.01: Graphical Presentation of the effect of CTM (100 mg/Kg) on Ambulation in the Open Field Test

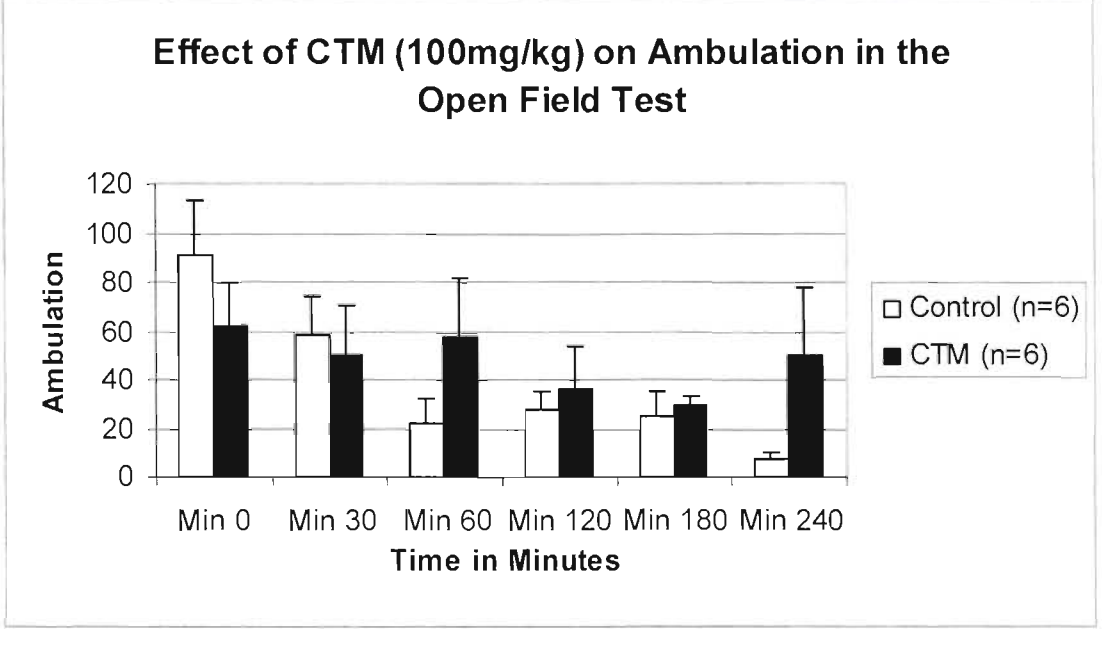


Table 3.2.02: The effect of CTM (200mg /kg) on Ambulation in the Open Field Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		125.667± 9.945	79.000± 4.050	46.333± 5.004	34.500± 9.828	22.167± 11.412	32.000± 7.234
CTM(n=6)		102.833± 5.250	72.167± 3.609	33.167± 5.199	35.167± 7.683	19.167± 7.002	22.167± 10.216
t/p		2.030/ 0.070	1.260/ 0.236	1.825/ 0.098	-0.053/ 0.958	0.224/ 0.827	0.786/ 0.450
95% confidence interval	Lower	-2.224	-5.254	-2.912	-28.461	-26.832	-18.058
	Upper	47.891	18.920	29.245	27.128	32.832	37.725

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

Figure 3.2.02: Graphical Presentation of the effect of CTM (200 mg/Kg) on Ambulation in the Open Field Test

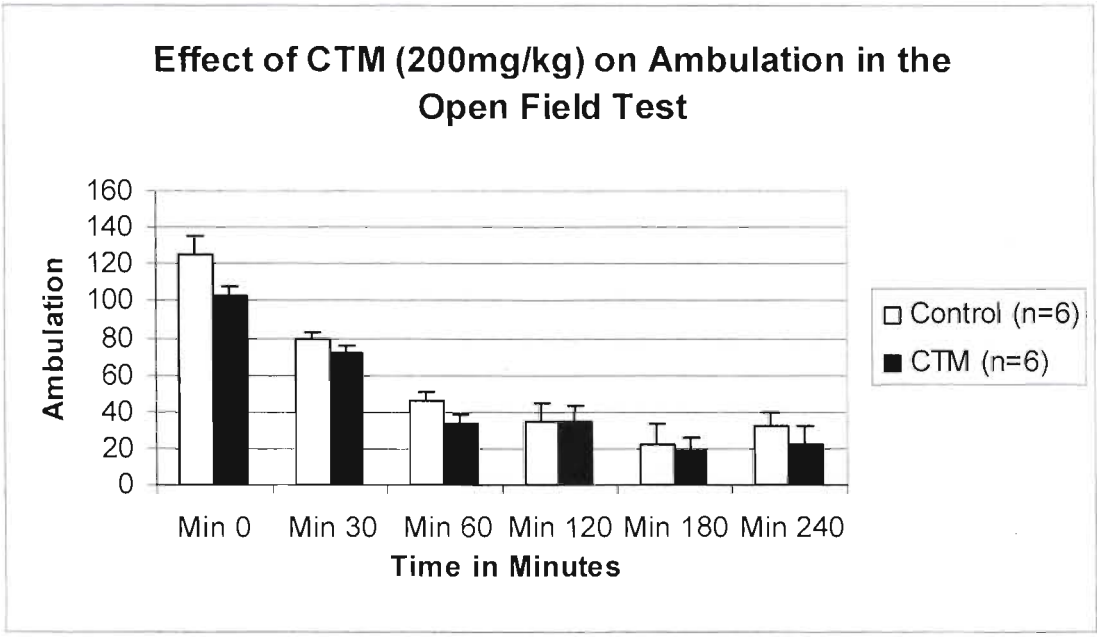


Table 3.2.03: The effect of CTM (400mg /kg) on Ambulation in the Open Field Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		104.667± 10.648	55.000± 14.114	38.833± 7.106	40.167± 7.808	45.833± 7.427	33.667± 4.145
CTM(n=6)		108.500± 9.276	53.833± 3.763	59.500± 9.814	32.500± 10.797	37.167± 9.188	18.833± 4.370
t/p		-0.271/ 0.792	0.080/ 0.939	-1.706/ 0.119	0.575/ 0.578	0.734/ 0.480	2.463/ **0.034***
95% confidence interval	Lower	-35.299	-35.024	-47.664	-22.022	-17.658	1.414
	Upper	27.632	37.357	6.331	37.356	34.992	28.253

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

Figure 3.2.03: Graphical Presentation of the effect of CTM (400 mg/Kg) on Ambulation in the Open Field Test

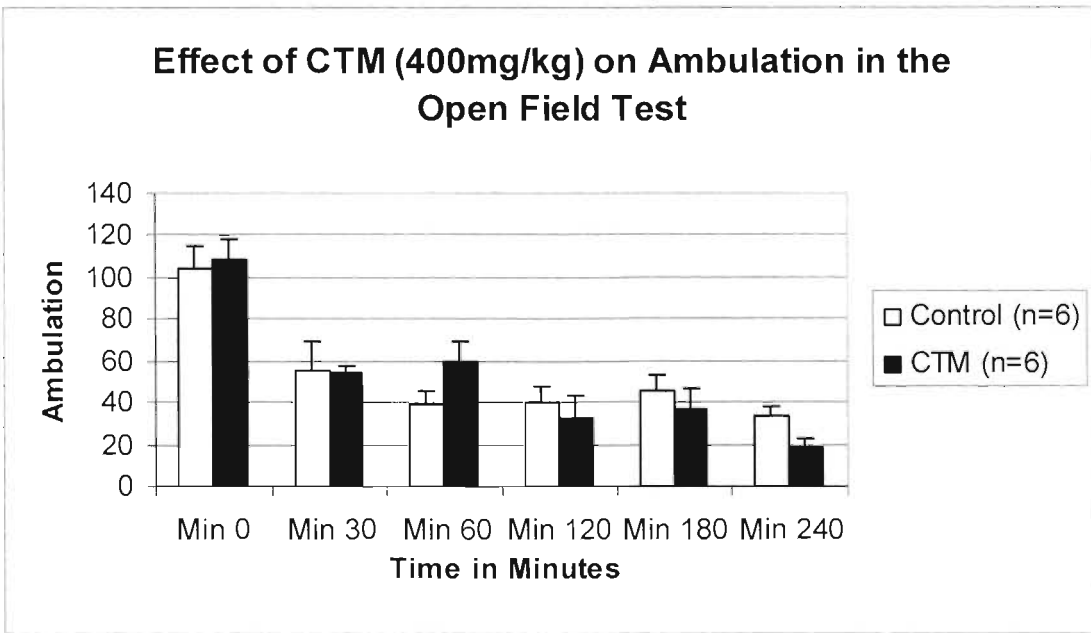


Table 3.2.04: The effect of CTM (100mg /kg) on Center Ambulation in the Open Field Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		0.500± 0.500	0.333± 0.211	0.000± 0.000	0.000± 0.000	0.333± 0.333	0.000± 0.000
CTM(n=6)		0.500± 0.500	0.167± 0.167	0.000± 0.000	0.000± 0.000	0.000± 0.000	0.000± 0.000
t/p		0.000/ 1.000	0.620/ 0.549	0.000/ 0.000	0.000/ 0.000	1.000/ 0.363	0.000/ 0.000
95% confidence interval	Lower	-1.576	-0.432	0.000	0.000	-0.524	0.000
	Upper	1.576	0.765	0.000	0.000	1.190	0.000

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

Figure 3.2.04: Graphical Presentation of the effect of CTM (100 mg/Kg) on Center Ambulation in the Open Field Test

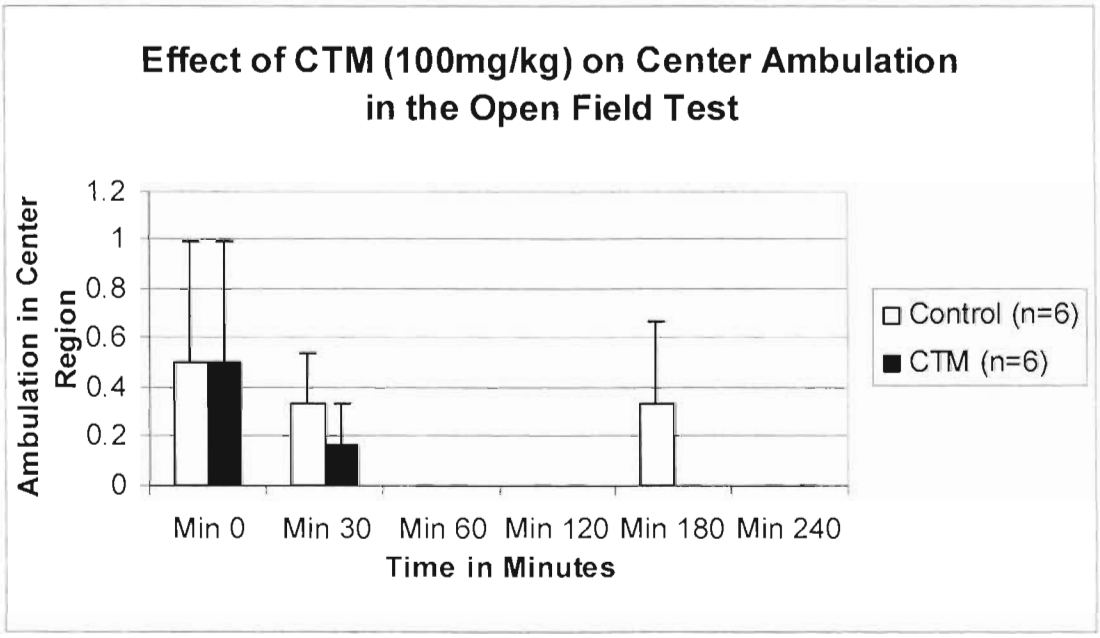


Table 3.2.05: The effect of CTM (200mg /kg) on Center Ambulation in the Open Field Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		4.167± 1.470	1.833± 0.749	0.333± 0.211	0.667± 0.422	0.333± 0.333	0.167± 0.167
CTM(n=6)		1.333± 0.494	0.500± 0.224	0.167± 0.167	0.167± 0.167	0.167± 0.167	0.167± 0.167
t/p		1.827/ 0.098	1.706/ 0.140	0.620/ 0.549	1.103/ 0.309	0.447/ 0.664	0.000/ 1.000
95% confidence interval	Lower	-0.622	-0.589	-0.432	-0.588	-0.664	-0.525
	Upper	6.289	3.255	0.765	1.588	0.997	0.525

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

Figure 3.2.05: Graphical Presentation of the effect of CTM (200 mg/Kg) on Center Ambulation in the Open Field Test

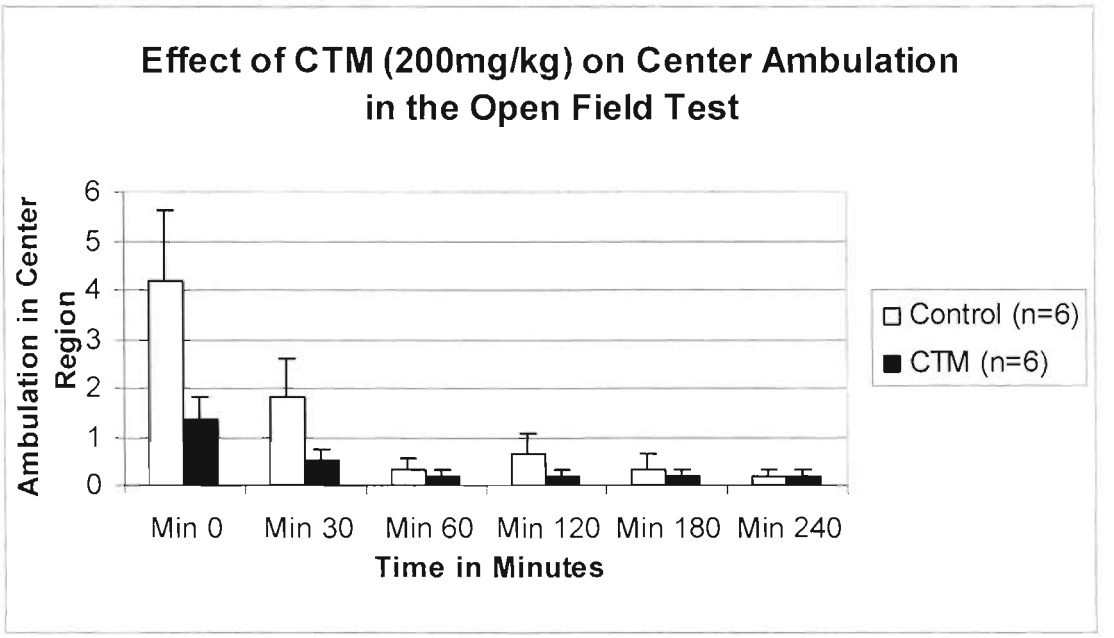


Table 3.2.06: The effect of CTM (400mg /kg) on Center Ambulation in the Open Field Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		2.000± 1.033	0.667± 0.333	0.167± 0.167	0.000± 0.000	0.000± 0.000	0.167± 0.167
CTM(n=6)		1.333± 0.494	0.500± 0.342	0.167± 0.167	0.333± 0.333	0.167± 0.167	0.000± 0.000
t/p		0.582/ 0.573	0.349/ 0.734	0.000/ 1.000	-1.000/ 0.363	-1.000/ 0.363	1.000/ 0.363
95% confidence interval	Lower	-1.885	-0.897	-0.525	-1.190	-0.595	-0.262
	Upper	3.218	1.230	0.525	0.524	0.262	0.595

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

Figure 3.2.06: Graphical Presentation of the effect of CTM (400 mg/Kg) on Center Ambulation in the Open Field Test

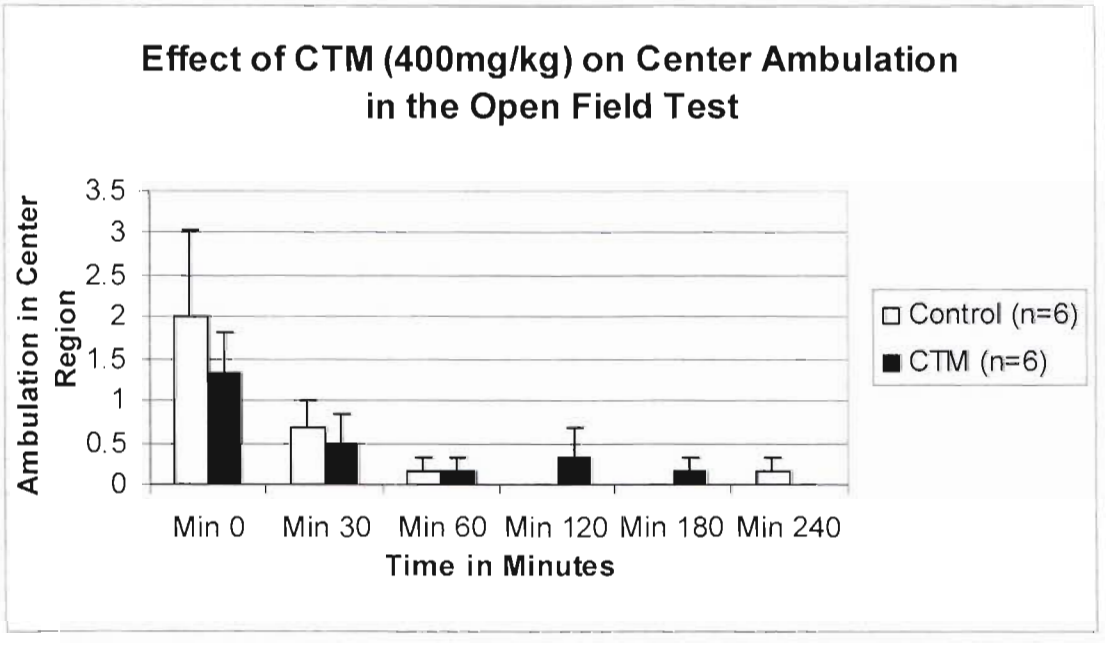


Table 3.2.07: The effect of CTM (100mg /kg) on Standing Up Behaviour in the Open Field Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		4.667± 0.882	5.167± 1.558	3.000± 1.844	2.833± 1.078	2.333± 1.764	0.333± 0.211
CTM(n=6)		4.167± 1.851	5.000± 3.055	4.500± 1.522	3.333± 1.706	3.500± 0.806	2.500± 1.727
t/p		0.244/ 0.812	0.049/ 0.962	-0.627/ 0.544	-0.248/ 0.809	-0.602/ 0.561	-1.245/ 0.241
95% confidence interval	Lower	-4.069	-7.475	-6.827	-4.996	-5.488	-6.044
	Upper	5.069	7.808	3.827	3.996	3.154	1.710

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

Figure 3.2.07: Graphical Presentation of the effect of CTM (100 mg/Kg) on Standing Up Behavior in the Open Field Test

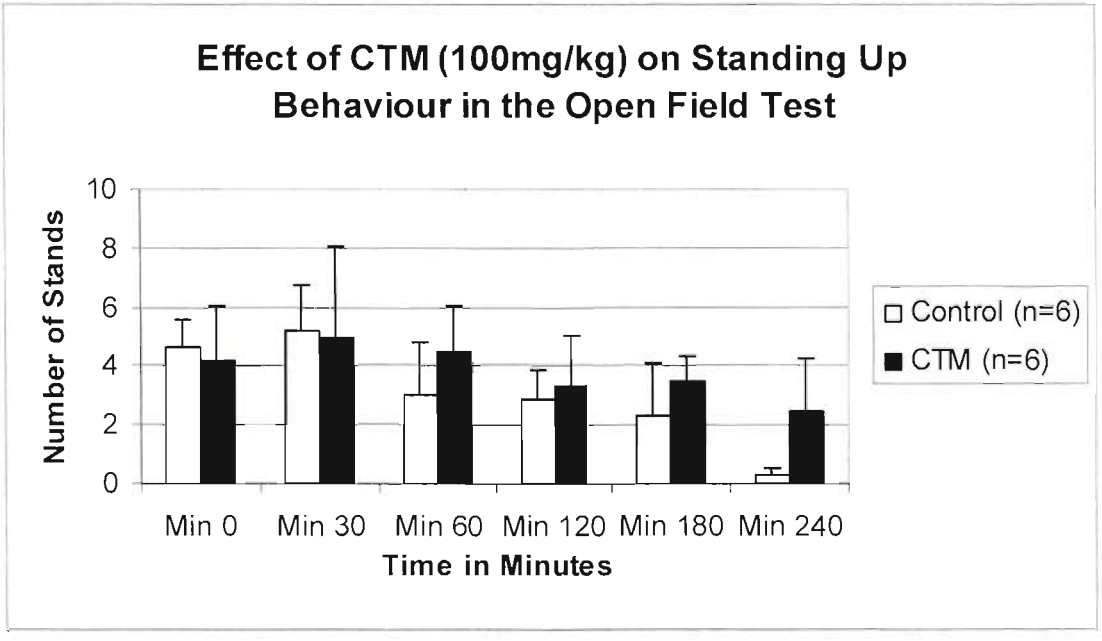


Table 3.2.08: The effect of CTM (200mg /kg) on Standing Up Behaviour in the Open Field Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		12.167± 1.167	9.833± 2.272	7.333± 2.092	4.833± 1.579	3.167± 1.493	3.667± 0.955
CTM(n=6)		7.500± 1.765	8.167± 1.400	5.333± 1.520	6.000± 1.732	3.167± 2.007	3.500± 1.607
t/p		2.205/ 0.052	0.625/ 0.546	0.773/ 0.457	-0.498/ 0.629	0.000/ 1.000	0.089/ 0.931
95% confidence interval	Lower	-0.048	-4.280	-3.763	-6.389	-5.573	-3.998
	Upper	9.382	7.613	7.763	4.056	5.573	4.332

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

Figure 3.2.08: Graphical Presentation of the effect of CTM (200 mg/Kg) on Standing Up Behavior in the Open Field Test

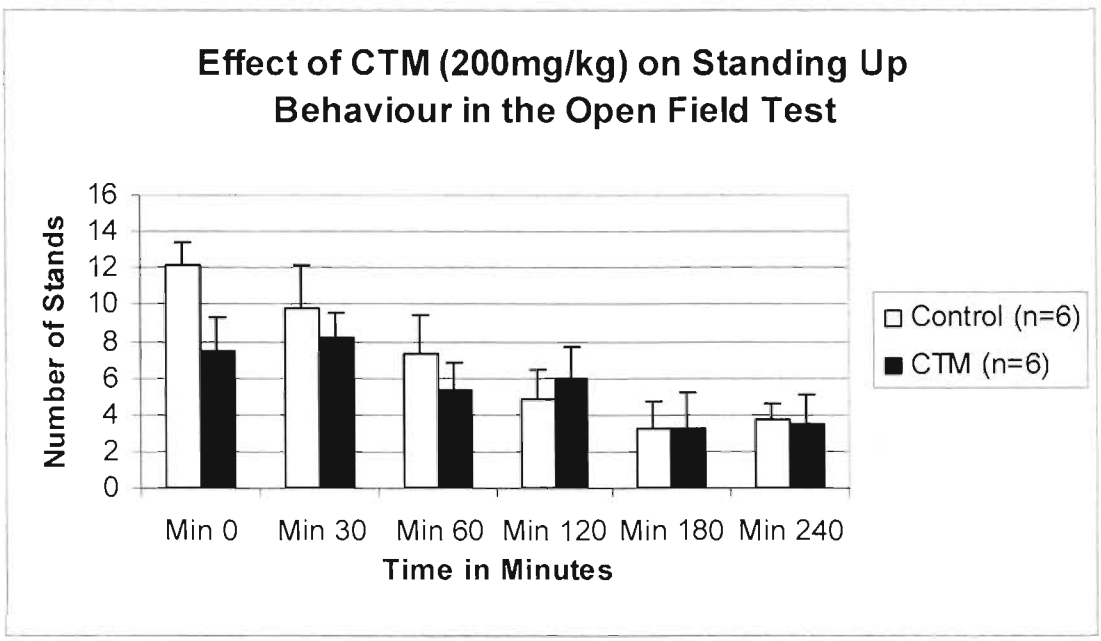


Table 3.2.09: The effect of CTM (400mg /kg) on Standing Up Behaviour in the Open Field Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		9.500± 2.592	7.667± 2.155	5.167± 1.600	4.333± 1.256	3.667± 1.229	3.333± 1.085
CTM(n=6)		8.833± 2.242	5.667± 0.989	6.833± 1.195	4.000± 2.517	4.000± 0.966	1.333± 0.558
t/p		0.195/ 0.850	0.843/ 0.419	-0.834/ 0.423	0.119/ 0.908	-0.213/ 0.835	1.639/ 0.132
95% confidence interval	Lower	-6.969	-3.283	-6.117	-5.934	-3.817	-0.719
	Upper	8.303	7.283	2.783	6.600	3.150	4.719

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

Figure 3.2.09: Graphical Presentation of the effect of CTM (400 mg/Kg) on Standing Up Behavior in the Open Field Test

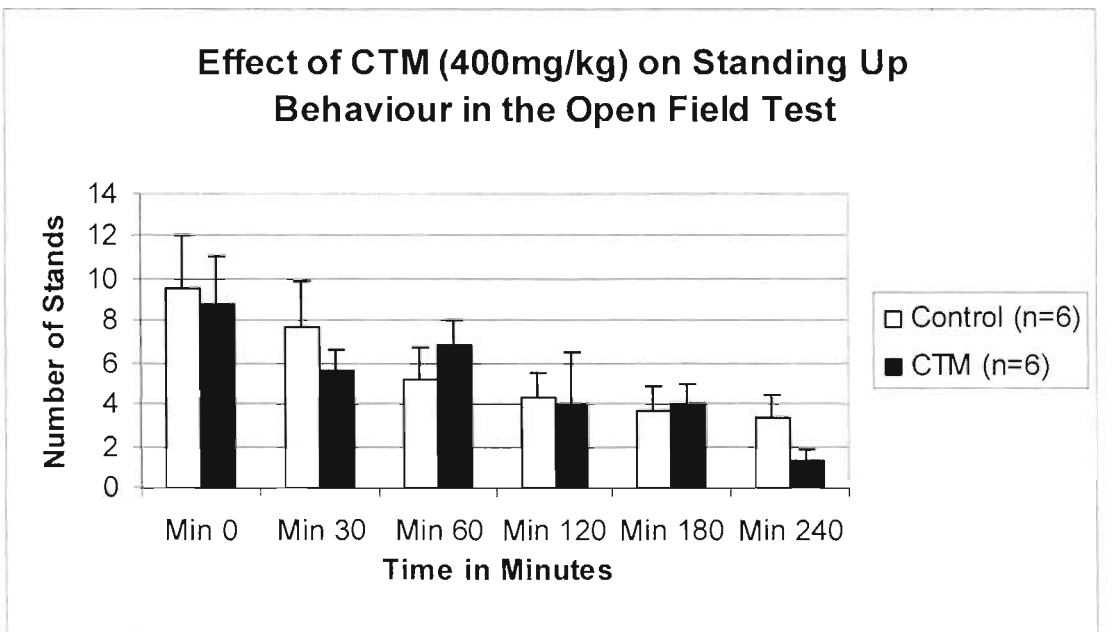


Table 3.2.10: The effect of CTM (100mg /kg) on Emotional Defecation in the Open Field Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		0.667± 0.333	0.333± 0.211	0.333± 0.211	0.500± 0.342	0.167± 0.167	0.333± 0.333
CTM(n=6)		1.667± 0.422	0.667± 0.333	0.667± 0.333	0.500± 0.224	0.000± 0.000	0.333± 0.211
t/p		-1.861/ 0.092	-0.845/ 0.418	-0.845/ 0.418	0.000/ 1.000	1.000/ 0.363	0.000/ 1.000
95% confidence interval	Lower	-2.198	-1.212	-1.212	-0.910	-0.262	-0.879
	Upper	0.198	0.545	0.545	0.910	0.595	0.879

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

Figure 3.2.10: Graphical Presentation of the effect of CTM (100 mg/Kg) on Emotional Defecation in the Open Field Test

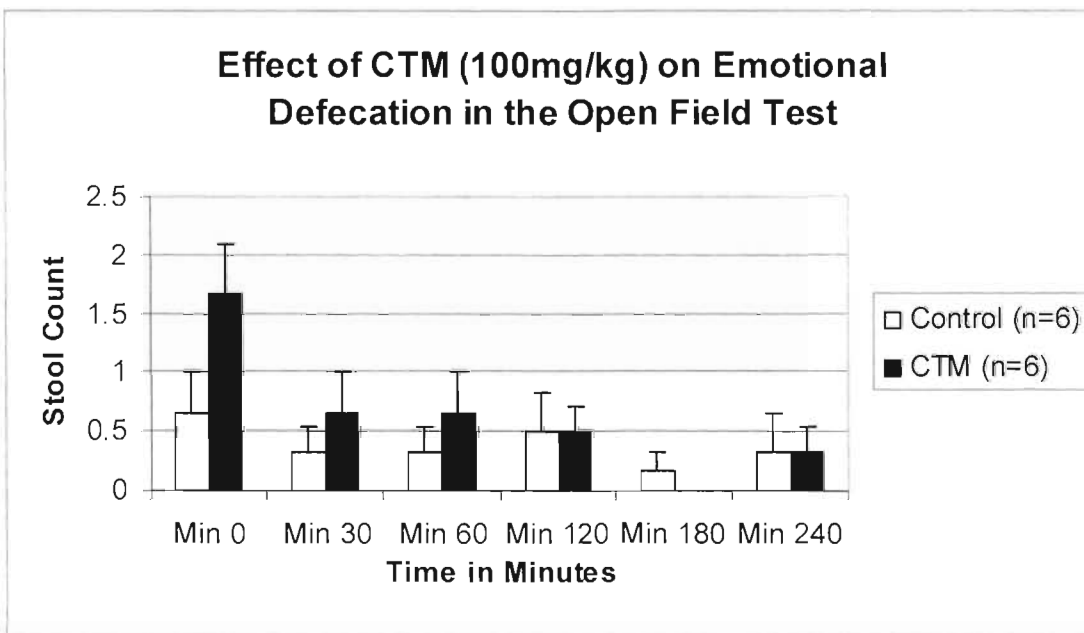


Table 3.2.11: The effect of CTM (200mg /kg) on Emotional Defecation in the Open Field Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		0.333± 0.211	1.167± 0.601	1.167± 0.401	0.000± 0.000	0.000± 0.000	0.500± 0.342
CTM(n=6)		0.333± 0.211	0.500± 0.224	0.167± 0.167	0.500± 0.500	0.667± 0.667	0.667± 0.494
t/p		0.000/ 1.000	1.040/ 0.323	2.301/ **0.044***	-1.000/ 0.363	-1.000/ 0.363	-0.277/ 0.787
95% confidence interval	Lower	-0.664	-0.762	0.032	-1.785	-2.380	-1.506
	Upper	0.664	2.095	1.968	0.785	1.047	1.172

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

Figure 3.2.11: Graphical Presentation of the effect of CTM (200 mg/Kg) on Emotional Defecation in the Open Field Test

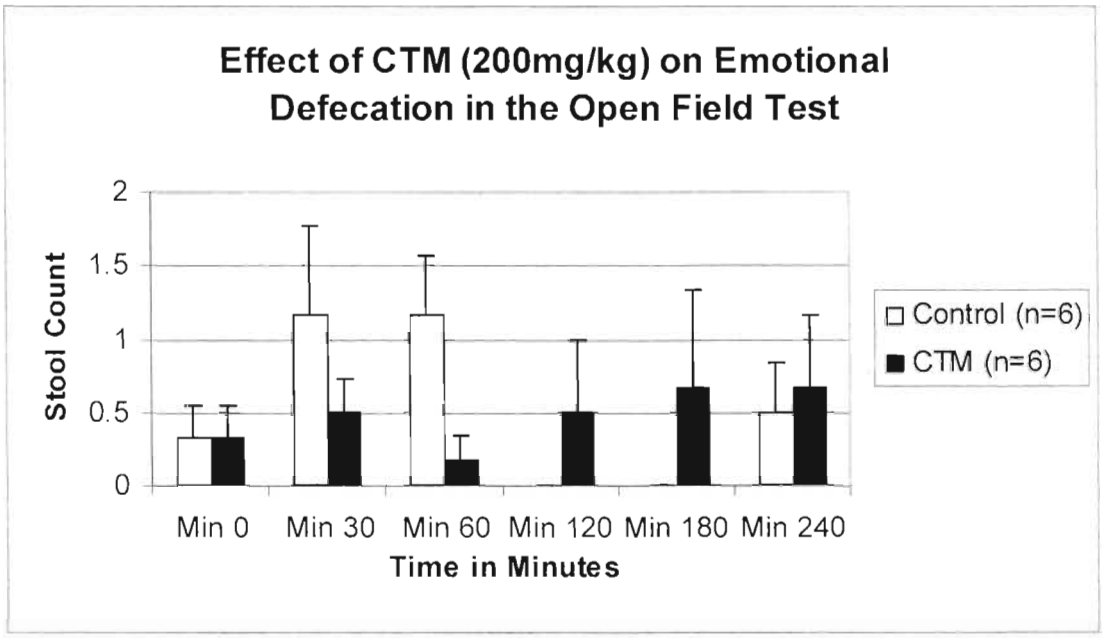
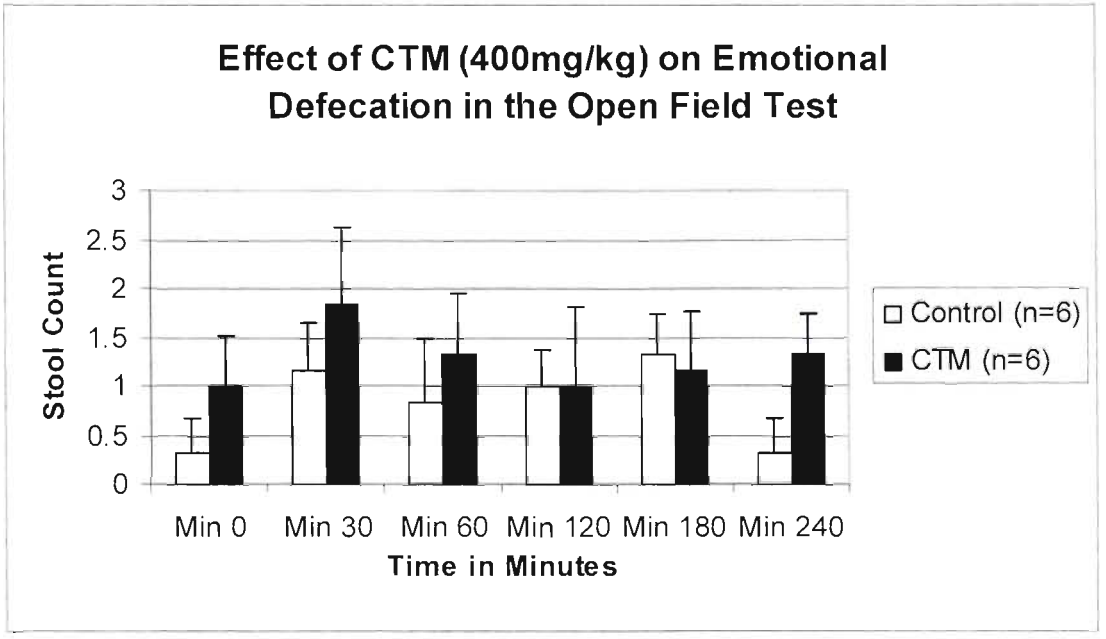


Table 3.2.12: The effect of CTM (400mg /kg) on Emotional Defecation in the Open Field Test

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		0.333± 0.333	1.167± 0.477	0.833± 0.654	1.000± 0.365	1.333± 0.422	0.333± 0.333
CTM(n=6)		1.000± 0.516	1.833± 0.792	1.333± 0.615	1.000± 0.817	1.167± 0.601	1.333± 0.422
t/p		-1.085/ 0.304	-0.721/ 0.488	-0.557/ 0.590	0.000/ 1.000	0.227/ 0.825	-1.861/ 0.092
95% confidence interval	Lower	-2.036	-2.728	-2.500	-1.993	-1.469	-2.198
	Upper	0.703	1.394	1.500	1.993	1.802	0.198

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

Figure 3.2.12: Graphical Presentation of the effect of CTM (400 mg/Kg) on Emotional Defecation in the Open Field Test



3.3 FORCED INDUCED SWIMMING TEST

tatistical findings:

'wo hours after treating female mice with CTM (100mg/kg), it was observed that the time for vhich the mice remained immobile was similar for both the drug and control groups at the 1st and 2nd Min. However, in the 3 to 6 Min study period the drug group showed increase in immobility ime relative to the corresponding control group. However, none of the results were statistically significant ($p > 0.05$).

Twenty-four hours after treating female mice with CTM (100mg/kg), the time for which the mice remained immobile for the drug group increased at the 2nd Min and the 3 to 6 Min study period relative to the corresponding control group. A statistically significant result (**p=0.049***) was obtained at the 3 to 6 Min experimental time segment indicating that the drug may have depressant activity. Apart from this, the immobility time was similar for both the drug and control groups at the 1st Min.

Tabular and Graphical presentation of the effect of CTM (100 mg/kg) in the Forced Induced Swimming Test using female mice after 2 hours and 24 hours:

Table 3.3.01: The effect of CTM (100mg/kg) in the Forced Induced Swimming Test after 2 hours

Group		1st Min	2nd Min	3rd to 6th Min
Ctrl(n=10)		12.667± 3.383	30.889± 4.976	163.444± 9.764
CTM(n=10)		11.778± 3.922	29.444± 4.035	171.667± 14.196
t/p		0.172/ 0.866	0.225/ 0.824	-0.477/ 0.640
95% confidence interval	Lower	-10.091	-12.137	-44.748
	Upper	11.869	15.026	28.303

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

Figure 3.3.01: Graphical presentation of the effect of CTM (100mg/kg) in the Forced Induced Swimming Test after 2 hours

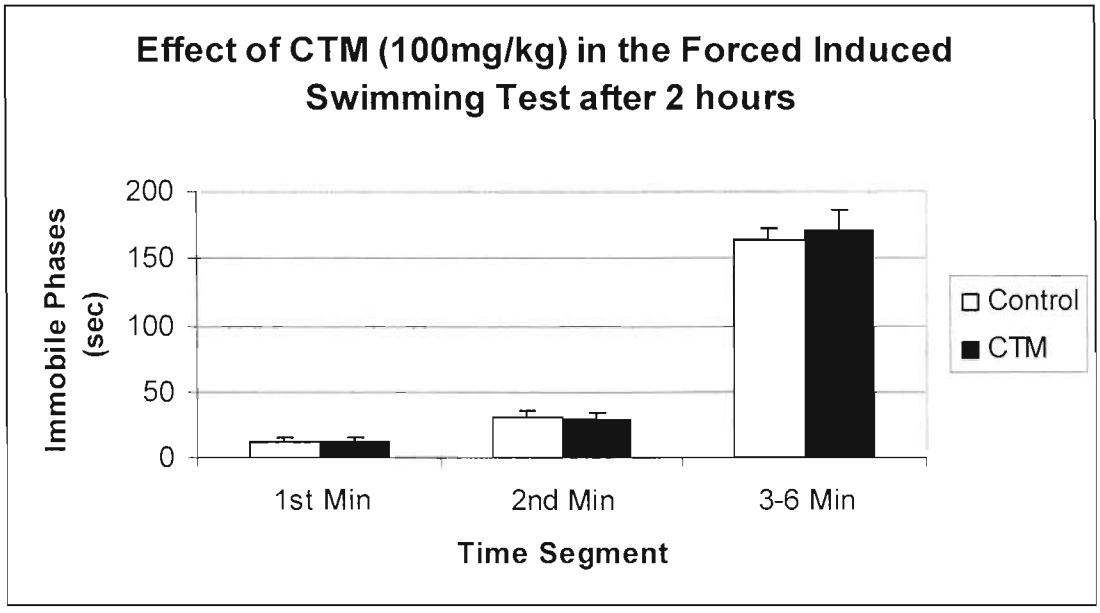
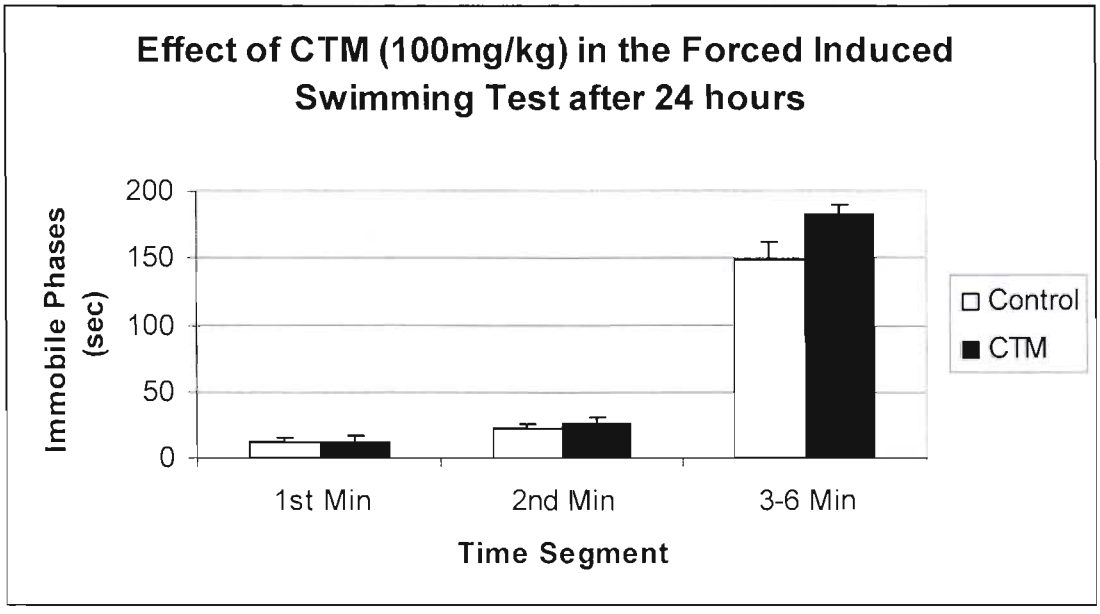


Table 3.3.02: The effect of CTM (100mg/kg) in the Forced Induced Swimming Test after 24 hours

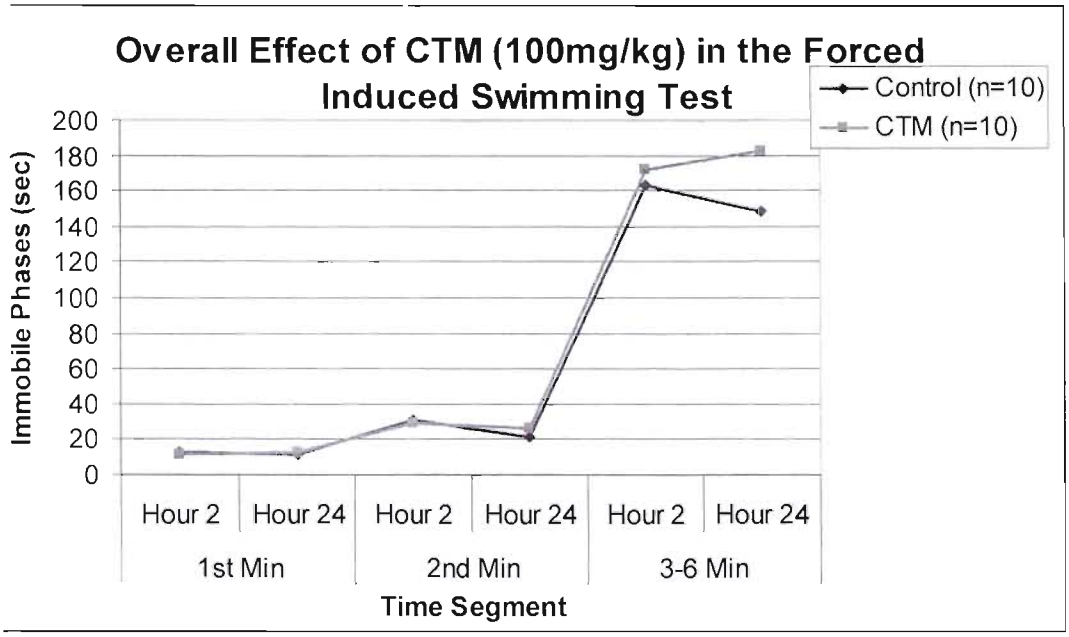
Group		1st Min	2nd Min	3rd to 6th Min
Ctrl(n=10)		11.444± 3.477	21.667± 4.460	149.000± 13.927
CTM(n=10)		12.778± 4.907	26.111± 4.866	182.778± 6.315
t/p		-0.222/0.827	-0.673/ 0.510	-2.209/ **0.049***
95% confidence interval	Lower	-14.082	-18.437	-67.378
	Upper	11.415	9.548	-0.177

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

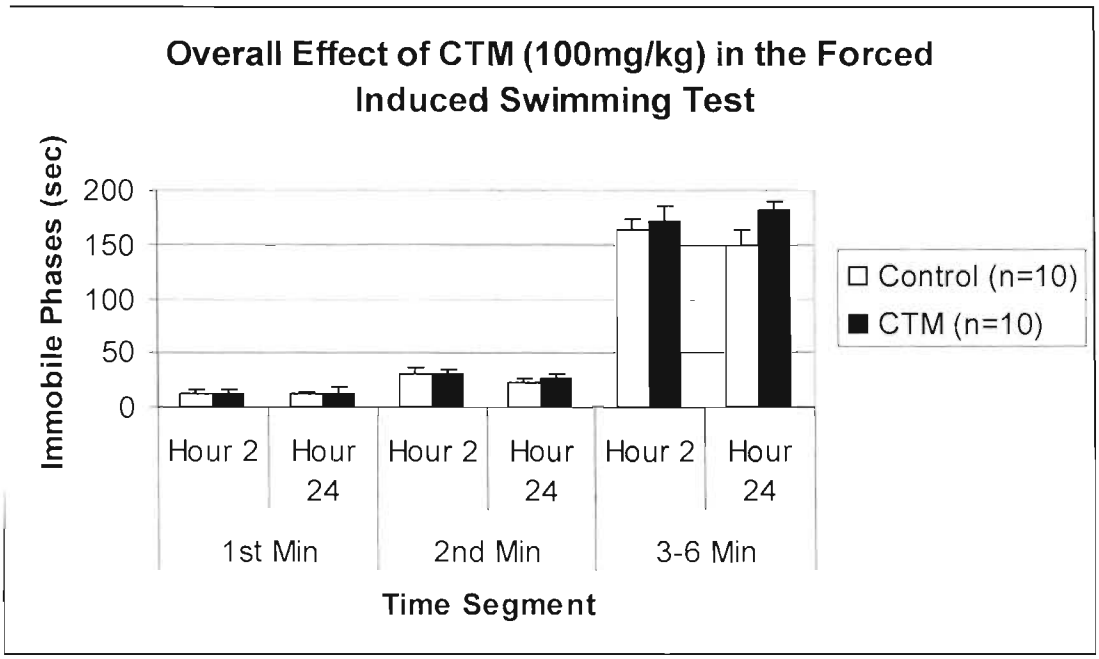
Figure 3.3.02: Graphical presentation of the effect of CTM (100mg/kg) in the Forced Induced Swimming Test after 24 hours



;ure 3.3.03: Line graphical presentation of the effect of CTM (100mg/kg) in the Forced luced Swimming Test after 2 and 24 hours



igure 3.3.04: Bar graphical presentation of the effect of CTM (100mg/kg) in the Forced ıduced Swimming Test after 2 and 24 hours



3.4 FORMALIN INDUCED PAW LICKING TEST

Statistical findings:

At the dose of 100mg/kg, CTM treated female mice showed a decrease in mean paw licking time compared to the corresponding control group in the first five minutes of the experimental study period which refers to the analgesic phase of the experiment. Within this time, a highly significant result was obtained (**p=0.002****) which means that CTM may possess analgesic activity.

In case of the last five minutes of the experimental study period, it was observed that the drug group exerted an increase in mean paw licking time in comparison to the respective control group. No result was found to be statistically significant ($p > 0.05$). Hence, CTM may not posses any anti-inflammatory activity.

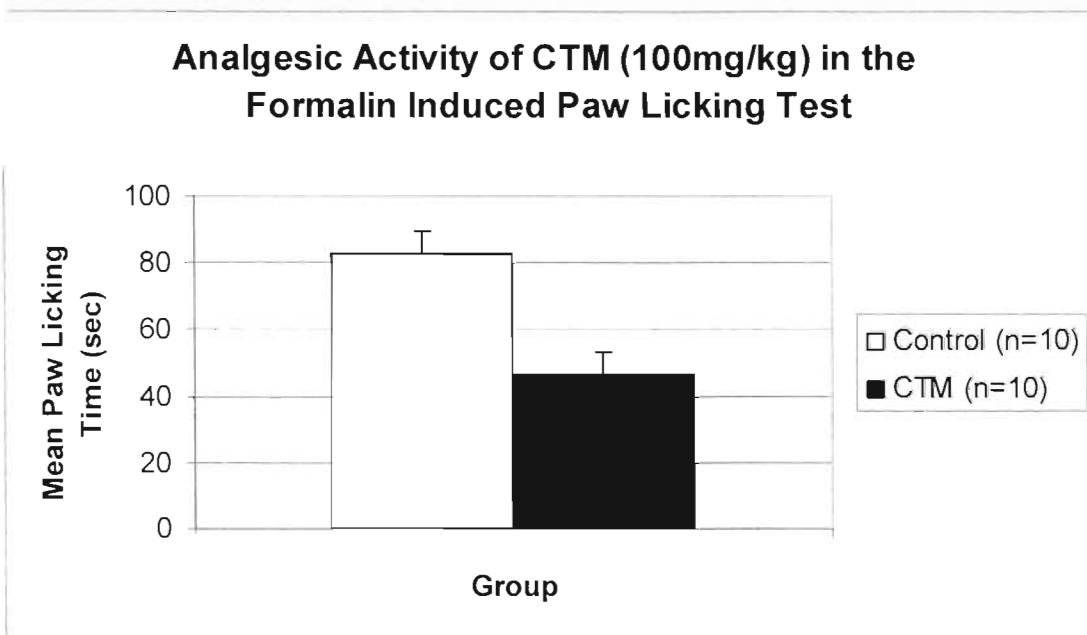
Tabular and Graphical presentation of the effect of CTM (100 mg/kg, 200 mg/kg, and 400 mg/kg) in the Formalin Induced Paw Licking (Analgesic + Inflammation) Test using female mice:

le 3.4.01: The effect of CTM (100 mg/kg) in the Formalin Induced Paw licking algesic + Inflammation) Test

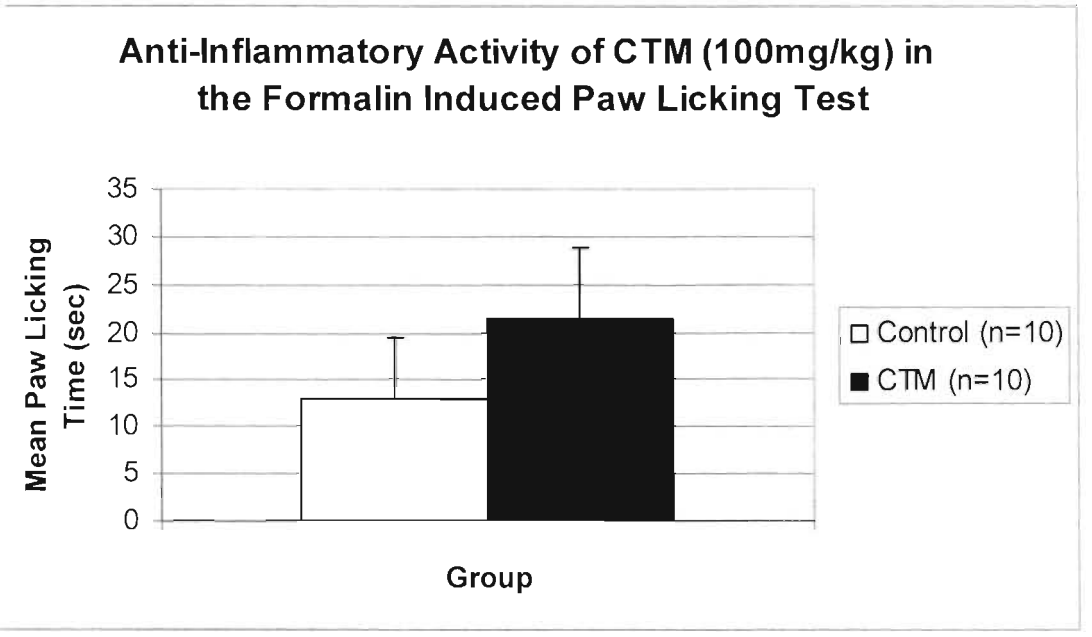
Group		Analgesic (1st Phase)	Inflammation (2nd Phase)
Ctrl(n=10)		82.600±6.964	13.000±6.347
CTM(n=10)		46.700±6.736	21.300±7.540
t/p		3.705/ **0.002****	-0.842/ 0.411
95% confidence interval	Lower	15.545	-29.006
	Upper	56.255	12.406

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

igure 3.4.01: Graphical presentation of the effect of CTM (100mg/kg) in the Formalin ıduced Paw licking (Analgesic) Test



;ure 3.4.02: Graphical presentation of the effect of CTM (100mg/kg) in the Formalin luced Paw licking (Inflammation) Test



3.5 ACETIC ACID INDUCED WRITHING TEST

ıtistical findings:

the dose of 100mg/kg, CTM treated female mice showed a decrease in the number of writhing mpared to the corresponding control group at the 1st, 2nd and 3rd minutes. At the 2nd minute ere was a significant decrease in the writhing response by the drug group (**p=0.016***). In ntrast to these results, an increase in the writhing response was observed for the drug group at e 4th and 5th minutes within the five minute study period.

t the dose of 200 mg/kg, an overall increase in writhing response was observed for the drug roup in comparison to the control group throughout the five minute study period. However, here was a variation in result at the 3rd minute in which case there was a decrease in writhing esponse by the drug group relative to the control group.

At the dose of 400 mg/kg, an overall decrease in the writhing response was exhibited by the drug group relative to the corresponding control group throughout the five minute study period.

None of the results at 200mg/kg and 400mg/kg doses were statistically significant ($p > 0.05$).

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

- 26.50 % at 100 mg/kg
- -21.54 % at 200 mg/kg
- 20.88 % at 400 mg/kg

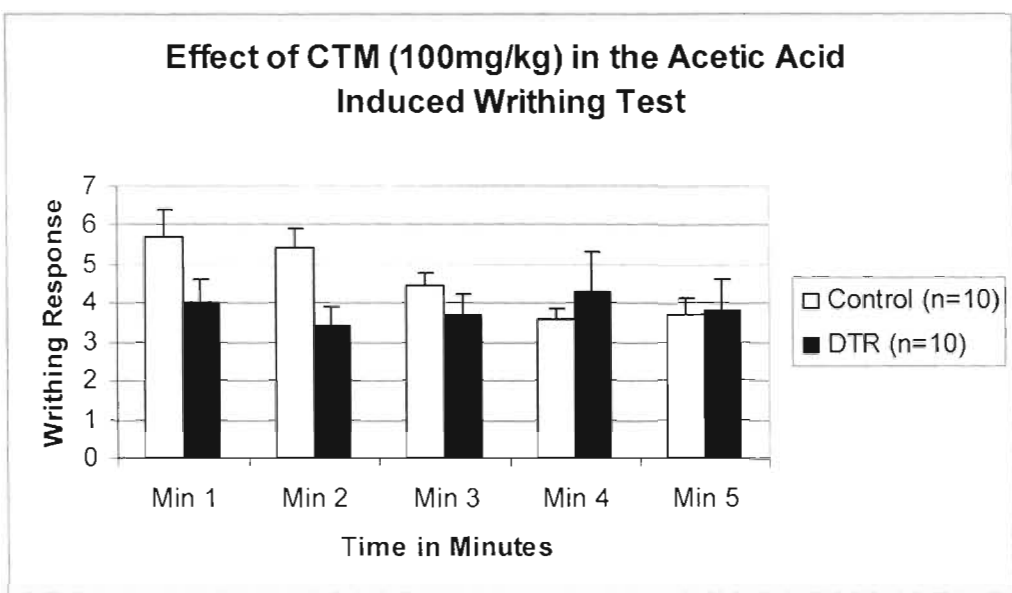
ɔular and Graphical presentation of the effect of CTM (100 mg/kg, 200 /kg, and 400 mg/kg) in the AA Induced Paw Licking Test using female ɛe:

ɔle 3.5.01: The effect of CTM (100mg/kg) in the AA Induced Writhing Test

Group		1st Min	2nd Min	3rd Min	4th Min	5th Min
Ctrl(n=10)		5.714± 0.680	5.43± 0.481	4.429± 0.369	3.571± 0.297	3.714± 0.421
CTM(n=10)		4.000± 0.596	3.400± 0.521	3.700± 0.559	4.300± 1.012	3.800± 0.786
t/p		1.879/ 0.080	2.730/ **0.016***	0.985/ 0.340	-0.585/ 0.567	-0.085/ 0.934
95% confidence interval	Lower	-0.231	0.445	-0.849	-3.383	-2.239
	Upper	3.659	3.613	2.306	1.926	2.067

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

ɪigure 3.5.01: Graphical presentation of the effect of CTM (100mg/kg) in the AA Induced Writhing Test



le 3.5.02: The effect of CTM (100mg/kg) in the AA Induced Writhing Test from Min 0-udy period.

Group		Mean Writhing (Min0 to 5)	% Protection
CTRL(n=10)		22.857±0.857	26.50%
CTM(n=10)		19.200±3.228	
t/p		1.095/0.299	
95% nfidence interval	Lower	-3.76044	
	Upper	11.07472	

gure 3.5.02: Graphical presentation of the effect of CTM (100mg/kg) on Total Writhing esponse from Min 0-5 study period

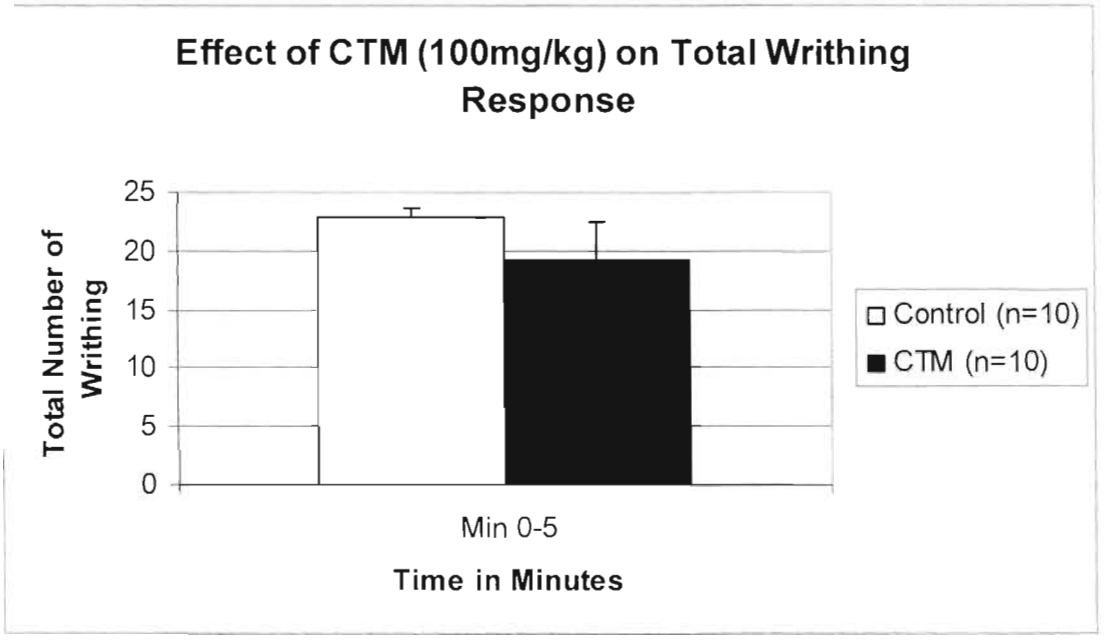
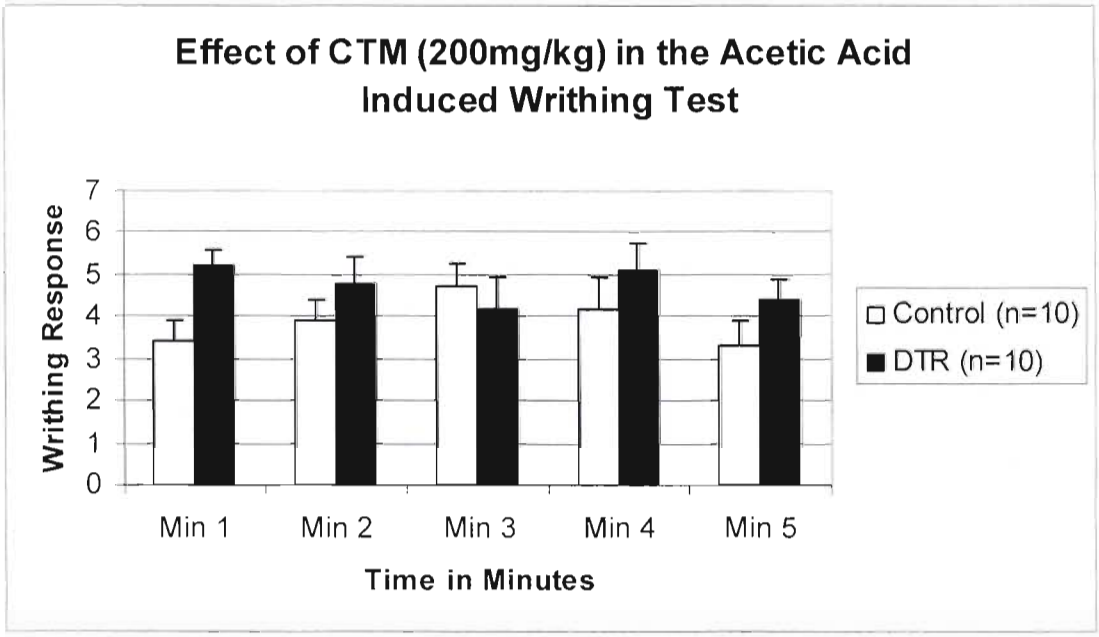


Table 3.5.03: The effect of CTM (200mg/kg) in the AA Induced Writhing Test

Group		1st Min	2nd Min	3rd Min	4th Min	5th Min
Ctrl(n=10)		3.400± 0.499	3.900± 0.482	4.700± 0.578	4.200± 0.727	3.300± 0.597
CTM(n=10)		5.200± 0.416	4.800± 0.646	4.200± 0.712	5.100± 0.674	4.400± 0.499
t/p		-2.770/ 0.013*	-1.116/ 0.279	0.545/ 0.592	-0.908/ 0.376	-1.414/ 0.175
95% confidence interval	Lower	-3.165	-2.594	-1.427	-2.983	-2.735
	Upper	-0.435	0.794	2.427	1.183	0.535

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

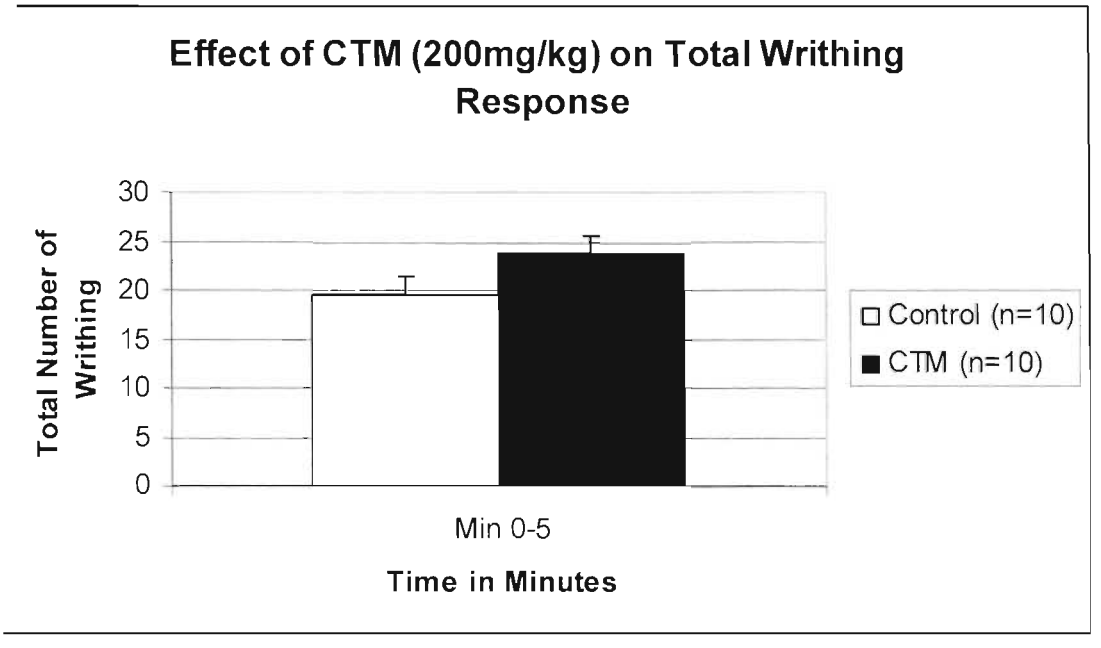
Figure 3.5.03: Graphical presentation of the effect of CTM (200mg/kg) in the AA Induced Writhing Test



.e 3.5.04: The effect of CTM (200mg/kg) in the AA Induced Writhing Test from Min 01-
:udy period.

Group		Mean Writhing (Min0 to 5)	% Protection
CTRL(n=10)		19.500±1.821	
CTM(n=10)		23.700±1.915	
t/p		-1.589/0.129	- 21.54%
95% confidence interval	Lower	-9.75234	
	Upper	1.35234	

;ure 3.5.04: Graphical presentation of the effect of CTM (200mg/kg) on Total Writhing
sponse from Min 0-5 study period

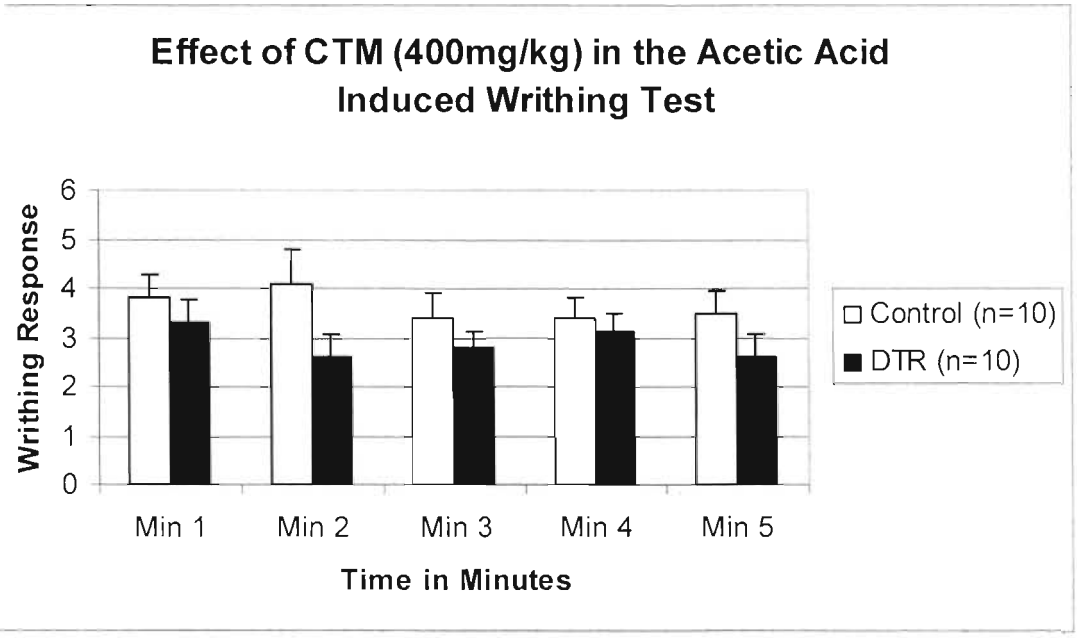


3.5.05: The effect of CTM (400mg/kg) in the AA Induced Writhing Test

Group		1st Min	2nd Min	3rd Min	4th Min	5th Min
Ctrl(n=10)		3.800± 0.467	4.100± 0.674	3.400± 0.499	3.400± 0.400	3.500± 0.453
CTM(n=10)		3.300± 0.473	2.600± 0.476	2.800± 0.327	3.100± 0.407	2.600± 0.476
t/p		0.753/ 0.461	1.818/ 0.086	1.006/ 0.328	0.526/ 0.605	1.369/ 0.188
95% dence interval	Lower	-0.895	-0.234	-0.653	-0.899	-0.481
	Upper	1.895	3.234	1.853	1.499	2.281

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

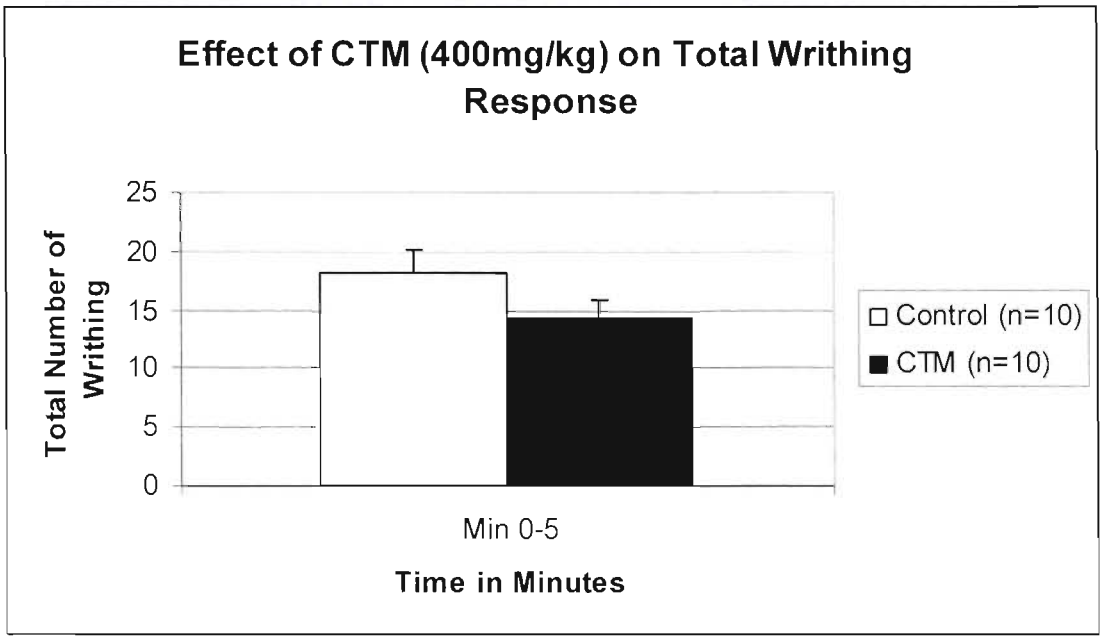
ure 3.5.05: Graphical presentation of the effect of CTM (400mg/kg) in the AA Induced ithing Test



able 3.5.06: The effect of CTM (400mg/kg) in the AA Induced Writhing Test from Min 01-5 study period.

Group		Mean Writhing (Min0 to 5)	% Protection
CTRL(n=10)		18.200 ± 1.931	20.88%
CTM(n=10)		14.400 ± 1.507	
t/p		1.551/0.138	
95% confidence interval	Lower	-1.34619	
	Upper	8.94619	

Figure 3.5.06: Graphical presentation of the effect of CTM (400mg/kg) on Total Writhing Response from Min 0-5 study period



4. CONCLUSION

basis of the results obtained after thorough analysis of Krishna Chaturmukha
. the neuropharmacological, psychopharmacological and analgesic and anti-
natory studies on animal models a conclusion may be drawn that upon long
eatment with the drug in Epilepsy, the patient may experience depression as a
fect. This determination can be confirmed based on the significant results
d in the open field test where there was a decrease in ambulation and the
induced swimming test where an increase in time spent in the immobile phase
served. All these results indicate a depressant activity related to CTM. Apart
his, it was also found that CTM may possess analgesic activity as was seen
he results of the formalin induced paw licking test and the AA induced writhing
However, in order to confirm such results it is very much necessary to conduct
experiments utilizing more sophisticated and modern pharmacological models. It
o evident that there is a need for comprehensive clinical trials in order to ensure
ıfety of the patients.

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ABBREVIATIONS

cid	AA
	Control
	Control
	Female
	g
ı	kg
itoneal	IP
	Min/min
:	ml
	p.o.
	sec
mber of mice taken	n
Chaturmukha Rasa	CTM