TRANQUILLIZING PROPERTY OF *Clitoria ternatea* Linn. (Shankupushpam), *Acorus calamus* Linn. (Vayampu) AND *Vitex leucoxylon* Linn. (Atta nocchi) IN RATS

By
SURESH N. NAIR

THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

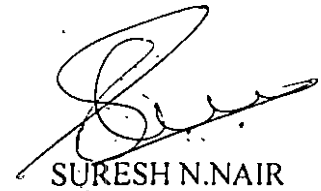
Department of Pharmacology and Toxicology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680651
KERALA

2001

DECLARATION

I hereby declare that the thesis entitled **"Tranquillizing property of *Clitoria ternatea* Linn. (Shankupushpam), *Acorus calamus* Linn. (Vayampu) and *Vitex leucoxylon* Linn. (Atta nochi) in rats"** is a bonafide record of research work done by me during course of research and that this thesis has not previously formed the basis for award to me any degree, diploma, assosiateship, fellowship or other similar title, of any other university or society

Mannuthy
23.8.2001,



SURESH N.NAIR
98 – 13 - 22

CERTIFICATE

Certified that the thesis entitled **"Tranquillizing property of *Clitoria ternatea* Linn. (Shankupushpam), *Acorus calamus* Linn. (Vayampu) and *Vitex leucoxylon* Linn. (Atta nochi) in rats"** is a record of research work done independently by **Suresh N. Nair** (98–13–22) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him

Mannuthy,
23 .8.2001

Dr.K.Venugopalan
(Chairman ,Advisory committee)
Associate Professor
Dept.Of Pharmacology &Toxicology
College of Veterinary &Animal Sciences,
Mannuthy

CERETIFICATE

We, the undersigned members of the Advisory Committee of Sri.**Suresh N.Nair**,(98-13-22) a candidate for the degree of Master of Veterinary Science in Pharmacology and Toxicology agree that the thesis entitled **"Tranquillizing property of *Clitoria ternatea* Linn. (Shankupushpam), *Acorus calamus* Linn. (Vayampu) and *Vitex leucoxylon* Linn.(Atta nochi) in rats"** may be submitted by Sri. Suresh N. Nair in partial fulfillment of the requirement of the degree.

Dr.K.Venugopalan
(Chairman, Advisory committee)
Associate Professor
Dept.of Pharmacology &Toxicology
College of Veterinary &Animal Sciences,

Dr.P.Marykutty
Professor & Head
Dept.of Pharmacology &Toxicology
College of Veterinary &Animal Sciences
(Member)

Dr.N.Gopakumar
Associate Professor
Dept.of Pharmacology &Toxicology
College of Veterinary &Animal Sciences
(Member)

Dr.V.Ramnath
Assistant Professor
Dept.of Physiology
College of Veterinary &Animal Sciences
(Member)

26/9/2001
External Examiner
Dr M.K. Rajagopalan
Prof & Head pharmacology (Rtd)
Kavitha
Indira Nagar
Mannuthy

ACKNOWLEDGEMENTS

First and foremost, I wish to express my profound sense of gratitude and indebtedness to my research guide, Dr. K. Venugopalan, Associate Professor, Department of Pharmacology & Toxicology and Chairman of my advisory committee for his gentle and inspiring guidance. I am deeply indebted to him or his invaluable suggestions, constant encouragement and support.

I would like to extend my deep sense of gratitude to the Head of the Dept. of Pharmacology & Toxicology, Dr. P. Marykutty for the valuable help, suggestions and guidance.

I express my sincere gratitude to Dr. N. Gopakumar, Professor, Department of Pharmacology & Toxicology and Dr. V. Ramnath, Assistant Professor, Dept. of Physiology and Biochemistry for their perpetual interest and creative suggestions at every stage of the research.

I am highly obliged by the help and cooperation of all the staff of our department. Thanks are due to. Dr. A. D. Joy, Dr. A. M. Chandrasekharan Nair, and Dr. C. M. Aravindakshan, Associate Professors, Dr. P. T. A. Usha, Assistant Professor and Dr. Jacob V. Cheeran and Dr. Zacharias Cherian, Professors (Retd.) are gratefully acknowledged for their help.

My sincere thanks are due to Dr. M. K. Rajagopalan, Former Professor and Head, Department of Pharmacology and Toxicology for the valuable time and effort he spent in contributing to this research.

The help rendered by Mrs. Narayanikutty, Associate Professor and Mrs. Santha Bai, Senior Programmer of Department of Statistics are specially acknowledged.

No words can implicitly express the deep gratitude to my family members, my mother, Surekha, Raju chettan and Preethy chechi for their love affection, moral support and encouragement during the course of study.

I express my heartfelt thanks to all my friends – Dr. Mini Bharatan, Dr. P. Senthilkumar, Dr. A. Thirunavukkarasu, Dr. Nisha A. R., Dr. Chandra Rajeswary, Dr P. K. Padmaraj and Dr. Jyotsana Menon for all the timely help they extended when needed. I owe a special sense of gratitude to my colleagues Dr. Binu T.V., Dr. Jayakrishnan, Dr. Jaison George, Dr. Shibu K. Jacob, and all my friends and well-wishers of P. G. Hostel.

Finally I convey my sincere thanks to my entire well - wishers and friends who have directly or indirectly helped me.

SURESH N. NAIR

Dedicated to my mother, sister and memories of my father

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

1 INTRODUCTION

Tranquillizers are agents, which exerts quietening and calming effect on animals. They decrease anxiety and sometimes reduce fear and aggression in animal species with naturally vicious or nervous temperaments (Lees, 1993) or they are the drugs that produce calmness without inducing sleep or depressing mental facilities. Tranquillizers are classified as major tranquillizers or neuroleptics and minor tranquillizers or anxiolytic sedatives or anti-anxiety drugs. The term neuroleptic is used to describe those antipsychotic agents that produce an effect on involuntary movement disorders involving extra pyramidal system (Reynolds, 1993).

The effects produced by neuroleptic agents are depression of motor activity and prolongation of sleeping time induced by hypnotics. It also impairs conditioned avoidance response to a learned sensory cue, impairment of continuous rotor pursuits and tapping speed test, lower of seizure threshold, exhibit of extrapyramidal side effects and depress limbic system. Increased prolactin and corticotropin releasing hormone and depression of chemoreceptor trigger zone are another properties but spinal reflexes and unconditioned nociceptive-avoidance reflex remain intact (Baldessarini, 1995).

There are various classes of tranquillizers based on their chemical structure. They are phenothiazine derivatives which include chlorpromazine, trifluprомazine and its congeners, butyrophenone derivatives which encompass droperidol and its congeners, dibenzepine derivatives, thioxanthene derivatives and Rauwolfia alkaloids. All these drugs have antidopaminergic activity. Now other group of drugs such as α_2 adrenergic agonists exemplified by xylazine, meditomidin and detomidine and GABA

agonists as benzodiazepine derivatives are also considered as tranquillizers (Gross and Booth, 1995).

The mechanisms of action of various agents are varied. Antidopaminergic agents block D_2 dopaminergic receptors in brain and thereby inactivate dopaminergic neurotransmission, one of the motor excitatory pathways in brain. α_2 adrenergic agonists stimulate α_2 adrenergic pathways and thus inhibit α_1 adrenergic pathway, another excitatory motor pathway in brain. Neuroleptics are highly lipophilic agents, metabolized mainly by hepatic oxidative mechanism and have complex elimination kinetics (Baldessarini 1995).

Major use of tranquillizers in veterinary practice is quietening the apprehensive animals. Tranquillizers can reduce the spontaneous motor activity in animals and thus quieten the excited animals, which help to conduct a diagnostic or minor surgical procedure on them and also prevent self-mutilation by them. Also tranquillizers can be used to quieten unfriendly, aggressive or vicious animal, which can cause damage to themselves, other animals or human beings. This effect is due to depression of motor centres as well as limbic system in brain.

Another use of tranquillizers is in anaesthetic premedication. Most of the anaesthetic agents have very narrow margin of safety. Tranquillizers when used as premedicant can considerably reduce the dose of anaesthetic agent and thus reduce the anaesthetic accidents.

Pharmacological approaches for management of behavioral problem are another area where tranquillizers are useful. Neuroleptics are useful in treating aggression, anxiety, fear, phobia, stereotypical behavior,

hyperactivity, depression and eating disorders in dogs and cats (Dodman and Shuster 1994). Thus the pharmacologic therapy can be successfully employed to complement behavioral modification therapy in a variety of animal behavior problems. These agents act by its antidopaminergic, antiserotoninergic and β-antiadrenergic activity in brain.

Another arena of usage of tranquillizers in stress conditions of domestic and wild animals in captivity. There are various kinds of stress in animals such as immobilization stress, production stress, reproduction stress, environmental stress, translocation stress, disease stress and other stresses involving human interactions. Abraham and Gogate (1989) reported the effect of immobilization stress that would produce decreased body weight and increased water intake in rats and Sahakari *et al* (1989) reported decreased rate of pup retrieval due to foot shock and immobilization stress in female rats. Monteiro *et al* (1989) reported reduced feed intake, reduced body weight gain even if fed near normal and increase in weight of various organs like cerebrum, cerebellum, pituitary, adrenals and thyroid due to immobilization stress. Saxena and Madan(1997)reported that herbals are useful in reducing various stresses in animals and act as adaptogenic agents.

Herbal medicines were in existence in India before thousands of years. Herbal drugs constitute those traditional medicines that primarily use medicinal plant preparations for the therapy. Their earliest recorded evidence of use in Indian, Chinese, Egyptian, Greek, Roman and Syrian text dates back to about 5000 years. The herbal drugs stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. At present herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects.

They also provide remedy for diseases and disorders for which there is no effective treatment in modern medicine. Moreover these drugs are made from renewable resources of raw materials by ecofriendly processes and will bring economic prosperity to the masses cultivating these raw materials. India is one of the twelve mega biodiversity centres in world having over 45,000 plant species, many of which have medicinal properties (Kamboj, 2000).

In earlier days drugs that depress central nervous system in maniac disorders were mostly derived from plant parts. Morphine was isolated from *Papaver somniferum* and reserpine isolated from *Rauwolfia serpentina,* which were the earliest tranquillizers, were all derived from plants. Isolation of active ingredient from the plant is a laborious process. But once isolated and chemical structure is elucidated, it can be synthetically manufactured in the laboratory on a large scale. Thus the plants provide a much appreciable help in the development of new and safer drugs.

Central nervous system depressant activity of *Clitoria ternatea* was reported in rats by Kulkarni *et al* (1988). Tranquillizing property of *Acorus calamus* was reported in rats by Panchal *et al* (1989). Similar property of *Vitex leucoxylon* was reported by Makwana *et al* (1994) in rats.

In the present study an attempt has been made to evaluate tranquillizing properties of alcoholic extracts of whole plant of *Clitoria ternatea*, roots and rhizomes of *Acorus calamus*, and leaves of *Vitex leucoxylon* and compare their effects to that of a standard tranquillizer, chlorpromazine.

REVIEW OF LITERATURE

2.REVIEW OF LITERATURE

2.1. *Clitoria ternatea* Linn.

It is a pretty perennial climber plant with conspicuous blue or white flowers. The seeds contain oil, resinous principle and tannins. Roots are bitter and cathartic. Root bark is diuretic and laxative (CSIR 1950).

Nadkarni (1976) stated that fresh roots of *Clitoria ternatea* has a bitter taste and is laxative and diuretic. Seeds have powerful cathartic action and root bark is demulcent, laxative and diuretic.Juices of leaves mixed with green ginger was given in cases of colliquative sweating in hectic fever.

Sathiavathy *et al.* (1976) could extract two chemical substances taraxerol and taraxerone from the roots of *Clitoria ternatea.*

Ripperger (1978) first isolated stigmast-4-one-3,6-dion successfully from *Clitoria ternatea.*

Khory and Katrak (1984) reported that the root of *Clitoria ternatea* was having a demulcent, diuretic and laxative action and was given in fever, croup, chronic bronchitis, ascites, dropsy and enlargement of abdominal viscera, and was used to remove irritation of bladder and urethra.

Kulkarni *et al.* (1988) did experiments with alcoholic extract of *Clitoria ternatea* at the dose rate of 230 mg/kg and 460 mg/kg intraperitonially and found that it produced increased sedation, diminished alertness, inhibited conditioned avoidance response and induced hypothermia equivalent to chlorpromazine at the dose of 10mg/kg intraperitonially in rats and mice. No significant anticonvulsant activity was detected against electro or chemo shock induced seizures. Analgesic and local anaesthetic properties were observed to a moderate extend

Terahara *et al.* (1998) isolated eight new anthocyanines namely ternatins C_1, C_2, C_3, C_4, C_5 and D_3 and preternatin A_3 and C_4 from *Clitoria ternatea* and they further elucidated the structure of ternatin C_1 and C_3-C_5 and preternatin A_3 by nuclear magnetic resonance method.

2.2. *Acorus calamus*

Acorus calamus is a semi aquatic perennial herb with a creeping and much branched aromatic rhizome. The dried rhizome is a common bazaar medicine and is generally used in the form of infusion. It is an aromatic bitter tonic, carminative, anti spasmodic emetic, and antidiarrhoeal (CSIR, 1950).

Nadkarni (1976) reported that the volatile essential oils in *Acorus calamus* contain Acorin, a bitter principle - acoretin, calamine, starch mucilage and a little of tannin. He also reported about its antispasmodic, nervous sedative and various other uses in Ayurveda, Siddha and Unani medicine systems.

Sathiavathi *et al.* (1976) quoted that fractionation of active principles from volatile oil by gas phase chromatography revealed presence of two components α - asarone and β - asarone. The α - asarone and β - asarone prolonged hypnosis caused by pentobarbital, hexobarbital and ethanol in mice. β-asarone facilitated the electro shock seizures while α-asarone had a protective effect. The α-asarone caused ataxia, hypnosis and loss of righting reflex in rats. The α - asarone in small doses potentiated the effect of reserpine and chlorpromazine. They also found that sedative effect of asarone was dependant on the depression of ergotropic division of hypothalamus.

Khory and Katrak (1984) studied the chemical constituents of dried rhizomes of *Acorus calamus* and found to contain Acorin – a glucoside which was very bitter and aromatic and Acoretin – a resin like substance and a crystalline alkaloid called calamine. They also reported that it could be used in habitual constipation, atonic dyspepsia, colic, flatulence, and paralytic and nervous affections.

Belova *et al.* (1985) conducted a study of biological properties of α-asarone (1 – propenyl-2, 4, 5, methoxybenzol- ethophanol), isolated from the roots of *Acorus calamus* and *Asarum europium* and found that LD_{50} of asarone for mice is 417.6 mg/kg for enteral administration. They also determined the tranquillizing, sedative, antiulcer, spasmolytic and antisclerosing activities.

Panchal *et al.* (1989) reported that water soluble dry powder of alcoholic extract of rhizomes of *Acorus calamus* at the dose rate 10, 25 and 50 mg/kg intraperitonial can antagonize spontaneous motor activity and amphetamine induced hyperactivity in mice. The sedative and tranquillizing action was less potent than chlorpromazine and local anesthetic property found to be absent.

Vohora *et al.* (1990) studied the ethanol extract of *Acorus calamus* rhizome for central nervous system effects and exhibited a large number of actions similar to α - asarone, including the apomorphine and isolation induced aggressive behaviour, amphetamine toxicity in aggregated mice and behavioral despair syndrome in forced swimming.

Thripathy and Singh (1995) conducted a clinical trial of Vaca (*Acorus calamus*) in fifty cases of depression over a period of six weeks treatment and one and a half year follow up and found that there was significant

improvement in these patients in terms of degree of severity of depression and reported better rehabilitation.

Wu *et al.* (1994) isolated a new tricyclic sesquiterpene, named calamenone and two known sesquetespines calamendiol and isocalamendiol from rhizomes of *Acorus calamus* and structure of new compound was elucidated on the basis of spectral and X - ray diffraction data.

2.3. *Vitex leucoxylon*

Vitex leucoxylon is a small to large tree with a short thick trunk and a spreading crown found throughout the Deccan Peninsula up to an altitude of 900 m and met with chiefly along the banks of streams. Leaves three to five, foliate, basal pair of leaflets much smaller than other leaflets. The leaves are smoked for relieving headache and catarrh (CSIR 1950).

Nadkarni (1976) reported that the bark and roots of *Vitex leucoxylon* are astringent and roots are vermifuge and used in intermitted fever. Leaves are smoked in catarrh and headache.

Makwana *et al.* (1994) found that cold aqueous infusion and ethanol extract of *Vitex leucoxylon*, depressed spontaneous motor activity antagonized d-amphetamine stereotypy and oxotremorine tremors at the dose rate of 100 and 200 mg/kg administered intraperitonially. It also possessed anti-inflammatory as well as analgesic properties.

2.4. Other plants having central nervous system depressant activity

Vohora *et al.* (1980) reported that biflavinoids isolated from leaves of *Taxus baccata* reduced spontaneous motor activity, pentobarbitone narcosis prolongation, reduced conditioned avoid response and induced hypothermia.

Lutterodt and Maleque (1988) reported that non-polar fractions from a methanol extract of dried leaves of *Psidium guajava* at the does rate of 3.3–6.6 mg/kg depressed spontaneous locomotor activity and tunnel running activity and abolished spontaneous locomotor reflex action at higher dose levels. They also suggested that a flavonoid compound present in it might be responsible for the action.

Jaiswal *et al.* (1994) reported that Leaf extract of *Azadirachta indica* at lower doses of 10, 20, 50, 100 mg/kg body weight dose rates produced significant antianxiety effect both in plus-maze and open field test. But at higher doses of 400 and 800 mg/kg did not show this effect. The effect at lower doses were comparable to that of diazepam 1 mg/kg.

N'gouemo *et al.* (1994) reported that ethanolic extract of leaves of *Maprounea africana* induced hypothermia, reduced latency to loss of the righting reflex and prolonged the sleeping time induced by pentobarbital in mice.

Nalini *et al.* (1995) reported that celastrus oil extracted from *Celastrus paniculatus* decreased the dopamine level in brain along with reduction of nor-epinephrine and 5-Hydroxy tryptamine.

Zia *et al.* (1995) reported that methanolic extract and bioassy directed fraction of fresh undried, uncrushed leaves of *Nerium oleander* could reduce

locomotor activity, rota-rod performance, potentiate hexobarbital sleeping time and possess analgesic activity.

Henriques *et al.* (1996) reported that alcoholic extract of *Eravatamia coronaria* at the dose rate of 150 mg/kg intraperitonially can increase pentobarbital induced sleeping time and possess analgesic activity.

Water fractions of *Hemerocallis flava* reduced the increase in locomotor activity produced by L-dopa plus benserazide and p-chlorophenylalanine and water fraction significantly decreased the concentration of dopamine and serotonine in brain stem (Hsieh *et al.*, 1996). They also suggested that reduction in locomotor activity might be related to decrease in concentration of nor-epinephrine in the cortex and concentration of dopamine and serotonine in the brainstem of rodents.

Mukherjee *et al.* (1996) reported that methanolic extract of rhizomes of *Nelumbo nucifera* reduced spontaneous activity, decreased exploratory behaviour, reduction in muscle relaxant activity by rota-rod, 30^0 inclined screen and traction test and potentiated pentobarbitone induced sleeping time when administered at the dose rates of 200, 300 and 400 mg/kg intraperitonially.

Tortoriello and Anguilar-Santamaria (1996) reported that methanolic extract of *Baccharis serraefolia* when given intraperitonially at various dose levels produced potentiation of hypnotic effect of pentobarbital, delayed onset of tonic seizures induced by strychnine and pentylene tetrazole and spasmolytic and antidiarrhoeal properties.

Soulimani *et al.* (1997) reported that lyophilized hydroalcoholic extract of aerial parts of *Passiflora incarnata* L. produced psychotropic and

anxiolytic properties at 400 mg/kg body weight dose rate and aqueous extract at 400 mg/kg body weight dose rate produced sedative effect and prolonged pentobarbitone sleeping time.

Asuzu *et al.* (1998) reported that 50 per cent methanolic extract of *Olax viridis* root when administered at the dose rate of 30 mg/kg as intraperitonially, prolonged pentobarbitone induced sleeping time without producing any evidence of motor inco-ordination.

Liao *et al* (1998) reported the central inhibitory effect of a water extract of rhizome of *Acori gramineus* wherein it reduced the locomotor activity and increased pentobarbitone-sleeping time. It also had anticonvulsant effect at higher doses. They also reported that the active ingredients of the extract compete for striatal dopamine D_1, D_2 receptors, and $GABA_A$ receptors.

Lu (1998) reported that the crude extract and water fraction of *Cistanche deserticola* could reduce spontaneous locomotor activity and prolong pentobarbitone induced sleeping time, water fraction being more active than crude extract.

Morais *et al.* (1998) reported that reticuline, a benzyl isoquinoline alkaloid isolated from *Ocotea duckei* at the rate of 50-100 mg/kg up intraperitonially produced prolongation of pentobarbital induced sleep, reduction in motor co-ordination, D-amphetamine induced hyper motility and suppression of conditioned avoidance response.

Perez.G *et al.* (1998) reported that ethanolic extract of the fruits of *Solanum nigrum* at various dose levels from 50-255 mg/kg administered intraperitonially significantly prolonged pentobarbitone induced sleeping

time, produced alteration in general behavioural pattern, reduced exploratory behavioural pattern, suppressed aggressive behaviour, affected locomotor activity by reducing spontaneous motility.

Aqueous extract of *Bambusa bambos* potentiated pentobarbitone induced sleeping time in mouse and converted sub-hypnotic dose of pentobarbitone to hypnotic dose. Aqueous extract at the rate of 200 mg/kg body weight produced marked sedation and hypothermia after 30 minutes of intraperitonial administration is rats, mice and guinea pig (Singh *et al.* 1998).

Crude alkaloidal fraction from the stem of *Tabernaemontana pandacaqui* could cause reduction in spontaneous motility, prolongation of pentobarbital sleeping time, prolongation of latency of pentylene tetrazole convulsions and antinociception but not oxotremorine induced tremors (Taesotikul *et al.*1998).

Gupta *et al.* (1999) reported that methanolic extract of leaves of *Vitex negundo* significantly potentiated the sleeping time induced by pentobarbitone sodium, diazepam and chlorpromazine and possess analgesic and anticonvulsant properties.

Hellion–Ibarrola *et al.* (1999) reported that crude hydro alcoholic extract of rhizomes of *Kyllinga brevifolia* at the rate of 100 mg/kg orally produced a decrease in spontaneous motor activity, passivity, palpebral ptosis, catatonia & stereotyped behaviour. Doses of 1, 10 and 100 mg/kg of extract produced a significant increase in the pentobarbital sleeping time in a dose dependant manner.

The essential oils isolated from *Lippia alba* (Mill) at the dose rate of 200 mg/kg given intraperitonially reduced the time of permanence on rota rod, number of rearings in open field test and decreased the body temperature (Vale *et al.* 1999).

Garin-Anguilar *et al.* (2000) reported that three alkaloidal fractions from the seeds of *Erythrina americana* at the dose rate of 3 mg/kg administered intraperitonially diminished the aggressive behaviour and they suggested that the action may be due to interaction between the cholinergic and GABA-minergic system.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Experimental animals

Study was conducted in sixty-four adult albino rats weighing 75-150g of either sex. Rats were maintained on identical feeding and managemental practices in the laboratory for one week before the commencement of study. The rats were trained for one week in actaphotometer and rota-rod. Food was withheld for 12 hours to ensure complete absorption of drugs and to prevent any possible interactions between food and the extract. Water was given *ad libitum*. The rats were grouped into eight groups, each group comprising eight animals, out of which four were males and four were females.

3.2 Drugs

3.2.1 Experimental drugs

3.2.1.1 *Clitoria ternatea*

The whole plants of *Clitoria ternatea* Linn. (Shankupushpam) were procured locally and identified (Fig: 1). The plants were cleaned, dried under shade and pulverized to a coarse powder in an electrical pulverizer. The powder was extracted in soxhelet extraction apparatus using ethanol. The extract thus obtained was kept open to facilitate complete evaporation of solvent and then kept under refrigeration. When 120g of dried powder were extracted, it yielded 11.8g of extract.

3.2.1.2 *Acorus calamus*

Dried roots and rhizomes of *Acorus calamus* Linn. (Vayampu) was purchased locally (Fig: 2). It was cleaned, chopped into pieces, dried in shade and pulverized to a coarse powder using electrical pulverizer. It was

then extracted using ethanol in a soxhelet extraction apparatus. The solvent was allowed to escape, to get a semisolid extract, with a characteristics smell. It was kept under refrigeration till use. When 120g of powder was extracted it yielded 8.5g of extract.

3.2.1.3 *Vitex leucoxylon*

The leaves of *Vitex leucoxylon* Linn. (Atta nocchi) were collected from Chinnar forests, Idukki district and identified (Fig: 3 and 4). The green leaves were cleaned, dried in shade, crushed and pulverized to a coarse powder using electrical pulverizer. Then it was extracted using ethanol in a soxhelet extraction apparatus. The solvent was removed completely by evaporation to a thick semisolid extract. 120g of *Vitex leucoxylon* powder yielded 21.5g of extract. The extract was kept under refrigeration till use.

3.2.2 Standard Drug

Chlorpromazine tablets were purchased locally and was pulverized into a fine power and suspended in 250ml five percent gum acacia.

3.2.3 Control Drug

The vehicle used for administration of the drug itself is used as negative control. Vehicle used for administration of plant extracts was five per cent gum acacia prepared by dissolving five gram of gum acacia in 100ml distilled water by constant stirring.

3.3 Equipments

Two equipments described by Turner (1965) were used to assess the degree of tranquillization and reduction in motor activity.

3.3.1 Actaphotometer

It consists of a cubical box with photoelectric cells in perpendicular directions to the light sources. (Fig: 5) When the ray of light was cut then the automatic counter counts once. So the count will be proportional to spontaneous motor activity. Readings were done at 0.5 hrs, 1hrs, 1.5 hrs, 2 hrs, 2.5 hrs, 3 hrs, 3.5 hrs and 4 hrs respectively after administration of test drug (Panchal *et al* 1989).

3.3.2 Rotarod

It consists of a rough metallic rod rotating at various fixed speed with a counter. (Fig: 6) The time permanence on rota rod in second was taken as a measure of forced locomotor activity, which analyzed statistically and compared with control (Bansinath *et al.*, 1982; quoted by Makwana *et al.*, 1994). Readings were taken at 0.5 hrs, 1hr, 1.5 hrs, 2 hrs, 2.5 hrs, 3 hrs, 3.5 hrs and 4 hours respectively after administration of test drug.

3.3.3 Rating scale for aggressive behavior

This arbitrary scale was formulated by Brady and Nauta (1957) quoted by Garin-Angular *et al.* (2000). The scale is as follows:

1	To come up to	0	Nothing
		1	Scrape
		2	Posture aggressive
		3	Attack
2	To capture in home cage	0	Nothing
		1	Scrape
		2	Posture aggressive
		3	Attack
3	Resistance to leave home cage	0	Nothing
		1	Scrape
		2	Posture aggressive
		3	Attack
4	Squealing and vocalization to capture and handling	0	Nothing
		1	When touch it
		2	Every one when touch it
		3	All the time

5	Urination /or defecation to capture and handling	0	Nothing
		1	Urination or defecation
		2	Urination and defecation
		3	Abundant urination and defecation
6	To the presentation of forceps in close proximity of snout.	0	Nothing
		1	Scrape
		2	Posture aggressive
		3	Attack
7	To the prodding with forceps	0	Nothing
		1	Scrape
		2	Posture aggressive
		3	Attack

Aggressive behaviour rating was done using rating scale during the peak phase of tranquillization

3.3.4 Haematological study

Blood samples were collected from orbital plexus of rats and hematological parameter like total erythrocytic count, total leueocytic count and hemoglobin concentration were assessed at the peak of tranquillization by the methods described by Schalm (1975). It was compared with the standard values given by Hrapkiewics (1998) and assessed whether they are falling within the normal range of the species.

3.4 Preparation of drugs for administration

1. Control:	Five percent gum acacia alone at the rate of 1ml/kg body weight
2. Standard drug:	Chlorpromazine 25mg dissolved in 250 ml five per cent gum acacia so that 1 ml of standard contain 0.1mg of chlorpromazine
3.Plant extracts:	For 250mg/kg dose rates 500mg of extract in 10ml of five percent gum acacia so that 1 ml contain is equivalent to 50mg of extract. For 500 mg/kg dose rate 1000mg of extract dissolved in 10ml of five percent gum acacia so that 1 ml contain 100mg extract.

3.5 Experimental design

Healthy animals without any defects and those which were capable of remaining on the rota rod were only selected. Eight animals four each male and female are selected for each group.

All the drugs were given by oral route using a metallic stomach tube into the oesophagus. The treatment schedule undertaken was as follows

First group (G.1) *Clitoria ternatea* 250 mg/kg	Alcoholic extract of *Clitoria ternatea* at the dose rate of 250 mg/kg body weight. Spontaneous motor activity, forced motor activity and suppression of aggressive behaviour were measured using actaphotometer, rota rod and aggressive behaviour test score respectively at 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 hours. Blood for haematology was collected during peak of tranquillization.
Second group (G2) *Clitoria ternatea* 500 mg/kg	Alcoholic extract of *Clitoria ternatea* extract orally at the dose rate of 500mg/kg as 1g of dried extract dissolved in 10ml of five percent gum acacia and spontaneous motor activity, forced motor activity and suppression of aggressive behaviour were measured using actaphotometer,

	rota rod and aggressive behaviour test score respectively at 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 hours. Blood for haematology was collected during peak of tranquillization.
Third group (G3) *Acorus calamus* 250 mg/kg	Alcoholic extract of *Acorus calamus* orally at the dose rate of 250 mg/kg as 500 mg dried extract dissolved in 10ml of 5per cent gum acacia and spontaneous motor activity, forced motor activity and suppression of aggressive behaviour were measured using actaphotometer rota rod and aggressive behaviour test score respectively at 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 hours. Blood for haematology was collected during peak of tranquillization.
Fourth group (G4) *Acorus calamus* 500 mg/kg	Alcoholic extract of *Acorus calamus* orally at the rate of 500mg/kg as 1g of dried extract dissolved in 10ml of 5per cent gum acacia and spontaneous motor activity, forced motor activity and suppression of aggressive behaviour were measured using actaphotometer, rota rod and aggressive behaviour test score respectively at 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 hours. Blood for

	haematology was collected during peak of tranquillization.
Fifth group (G5) *Vitex leucoxylon* 250 mg/kg	Alcoholic extract of *Vitex leucoxylon* orally at the dose rate of 250 mg/kg as 500 mg of dried extract dissolved in 10ml of 5per cent gum acacia and spontaneous motor activity, forced motor activity and suppression of aggressive behaviour were measured using actaphotometer, rota rod and aggressive behaviour test score respectively at 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 hours. Blood for haematology was collected during peak of tranquillization.
Sixth group (G6) *Vitex leucoxylon* 500 mg/kg	Alcoholic extract of *Vitex leucoxylon* orally at the dose rate of 500mg/kg as 500mg of dried extract dissolved in 10ml of 5per cent gum acacia and spontaneous motor activity, forced motor activity and suppression of aggressive behaviour were measured using actaphotometer, rota rod and aggressive behaviour test score respectively at 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 hours. Blood for haematology was collected during peak of tranquillization.

Seventh group (G7) standard drug chlorpromazine	The standard drug chlorpromazine at the dose rate of 7 mg/kg orally (Barns and Eltherington, 1973) and spontaneous motor activity, forced motor activity and suppression of aggressive behaviour were measured using actaphotometer, rota rod and aggressive behaviour test score respectively at 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 hours. Blood for haematology was collected during peak of tranquillization.
Eight group (G8) control drug	The vehicle alone at the dose rate of 7 ml/kg body weight and spontaneous motor activity, forced motor activity and suppression of aggressive behaviour were measured using actaphotometer, rota rod and aggressive behaviour test score respectively at 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 hours. Blood for haematology was collected during peak of tranquillization.

Table 1. Treatment groups and their dose regimen.

Plants	Dose of extract (mg/kg)	
Clitoria ternatea	250(G1)	500(G2)
Acorus calamus	250(G3)	500(G4)
Vitex leucoxylon	250(G5)	500(G6)
Chlorpromazine	7(G7)	
Control (gum acacia)	7ml/kg (G8)	

The data obtained were analyzed statistically and values of plant extracts were compared to control and standard drug using analysis of variance.

RESULTS

4. RESULTS

Results obtained were tabulated and presented in tables 2 to 33 and the statistical analysis were done and is given in tables 34-37.

All the plants produced significant tranquillization compared to the standard drug but the effect was much less than the standard group.

4.1. *Clitoria ternatea* @ 250mg/kg.

At all the time intervals it showed significant difference from control and standard drug both on actaphotometer and rota-rod at 1% level. Maximum decrease in spontaneous motor activity was observed at 2 hrs after administration of drug with counts of 79 ± 6.523 when compared to the counts of 132.25 ± 10.868 of the control animals. The decrease of counts started at 0.5 hrs with 114.5 ± 8.036 of the extract compared to 216.625 ± 12.198 of control, kept on decreasing up to 94.75 ± 6.972, which was still significantly higher when compared to the standard drug group (Table 36).

Maximum decrease in rota- rod performance time was observed at 1.5 hrs with average of 243.625 ± 11.052 seconds when compared to control with 641.375 ± 15.793 seconds at the same time. Here also tranquillization was started at 0.5 hrs evidenced by 280.875 ± 13.195 seconds of test group compared to 622.625 ± 12.159 seconds of control. The time then decreased gradually to a minimum at 1.5 hours then remained at that level up to 2.5 hrs and thereafter started increasing. But even at 4 hrs, it differed significantly from control with 277.25 ± 9.468 seconds of treated compared to 655.875 ± 9.235 seconds of control (table 37).

At all the time the test drug showed significantly lower effect than the standard drug.

Aggressive behaviour was also reduced as evidenced by total score of 3 when compared to 8-11 by control group of animals (Table 12 and 4) .

Hematological values were all in normal range and were comparable to control group as seen in the table no 13.

Observation of spontaneous motor activity and forced motor activity, are given in table nos.10 and 11 respectively and graphical representation in comparison to control and standard are given in Fig.7 and 8. Statistical analysis table are given in table 34 to 37.

4.2. *Clitoria ternatea* @ 500mg/kg

This drug showed significant difference from control group and comparable values with standard drug at 1hrs, 1.5hrs, 3.5hrs and 4hrs on actaphotometer and at 1.5hrs, 2hrs, 3hrs, 3.5hrs, 4hrs in the rota-rod significance (at 1% level).

On actaphotometer, the drug showed maximum effect at 1.5 hours with counts of 42.25 ± 2.043 when compared to 140.875 ± 11.148 of control group. The effect started at 0.5 hrs with counts of 97.750 ± 3.437 compared to control of 216.625 ± 12.98, proceeded to minimum counts at 1.5 hrs and then gradually increased to 54.25 ± 3.76 compared to 125.625 ± 11.063 of control at 4 hrs(Table 36).

Maximum decrease in rota-rod performance was noted at 1.5 hrs itself with average time of 148.25 ± 11.621 seconds of treated group compared to 641.375 ± 15.793 seconds of control group. The effect started at 0.5 hrs itself with time of 200.25 ± 8.33 seconds of treated compared to 622.625 ± 12.159 seconds of control, decreased minimum at 1.5 hrs and then gradually

increased to 168.75 ± 11.632 seconds at 4 hrs. Time of control group at 4 hrs was 655.75 ± 9.235 seconds (Table 37).

This drug showed effect much closer to standard drug on actaphotometer and rota-rod. On actaphotometer, the average count was statistically insignificant even though it was higher than standard group at 1, 1.5, 3.5 and 4 hours. On rota-rod it showed time of permanence statistically insignificant compared to standard drug at 1.5, 2,3, 3.5 and 4 hours.

Aggressive behaviour was also reduced, as evidenced by on behavioral test score to 3 of test group when compared to 8-11 of control group. (Table 16).

Hematological values were all on normal range and were comparable to control group. (Table 17)

Observation of spontaneous motor activity and forced motor activity are given in table nos.15 and 16 respectively and graphical representation in comparison to control and standard are given in Fig 7 and 8. Statistical analysis table are given in table 34 to 37.

4.3. *Acorus calamus* @ 250mg/kg

At all the time intervals it showed significant difference from control as well as standard group on actaphotometer and rota-rod at 1% level.

The extract showed its maximum effect on actaphotometer at 1 hrs, with count of 91.125 ± 5.226 compared to 172.625 ± 5.105 of control. The effect started at 0.5 hr with 119.00 ± 4.115 and decreased to a minimum at 1 hour, then gradually increased to 120.25 ± 4.858 at 4 hours. Corresponding

values for control were 216.625 ± 12.198 and 125.625 ± 11.063 respectively (Table 36).

On rota-rod, the extract showed its peak effect at 1hr itself, with average time of 271.0 ± 13.612 seconds. Here also the effect started at 0.5 hrs as indicated by the time of permanence of 338.625 ± 14.973 seconds of drug compared to 622.625 ± 12.159 seconds of control. The effect reached maximum at 1 hr, then increased gradually to 324.75 ± 15.78 at 4 hrs, which was far less than 655.875 ± 9.235 seconds of control (Table 37).

At all the time the test drug showed significantly lower effect than the standard drug.

Aggressive behaviour was also reduced as indicated by total score of 3, when compared to 8-11 by control group of animals. (Table 20).

Hematological values were all in normal range and were comparable to control group. (Table 21).

Observation of spontaneous motor activity and forced motor activity are given in table nos. 18 and 19 respectively and graphical representation in comparison to control and standard are given in Fig 9 and 10. Statistical analysis table are given in table 34 to 37.

4.4. *Acorus calamus* @ 500mg/kg

The test group showed significant difference from control group and standard group on actaphotometer and rota-rod, lowered aggressive behavioral test score at all the time intervals at 1% level.

Maximum decrease in actaphotometer counts were observed at 1 hour with a count of 87.375 ± 4.674 compared to 172.625 ± 5.105 of control group. The effect started at 0.5 hours with counts of 126.375 ± 2.758 compared to 216.625 ± 12.198 of control, showed maximum effect at 1 hrs and then increased to 115.625 ± 5.844 compared to control with 125.625 ± 11.063. But statistical analysis by least significant difference test indicated comparable values for 250mg/kg and 500mg/kg dose rates at 0.5, 1, 2, 2.5, 3, 3.5 and 4 hours (Table 36).

On rota-rod, peak effect was observed at 1 hours itself with average time of permanence of 209.375 ± 6.753 seconds compared to 640.25 ± 12.219 seconds control group. Here also the effect started at 0.5 hours with time of 239 ± 4.451 seconds of test group compared to control with 622.625 ± 12.159 seconds, showed maximum effect at 1 hour and then increased to 249.375 ± 5.155 seconds at 4 hours (Table 37).

At all the time the test drug showed significantly lower effect than the standard drug.

Aggressive behaviour was also reduced in animals as indicated by the average score of 3 when compared to the range of 8-11 of the control group of animals (Table 24).

All the hematological parameters fell in normal range (Table 25).

Observation of spontaneous motor activity and forced motor activity are given in table nos.22 and 23 respectively and graphical representation in comparison to control and standard are given in Fig 9 and 10 respectively. Statistical analysis table are given in table 34 to 37.

4.5.*Vitex leucoxylon* @ 250mg/kg.

This drug was found to be the least effective among tested group. It showed significant difference from control as well as standard in rota-rod performance, but on actaphotometer it showed significant difference from control only up to 1.5 hours. Behavioural test score was also slightly higher than other groups though were much less than the control group.

On actaphotometer maximum reduction in spontaneous movements were noted at 1 hour, with an average count of 109.00 ± 3.468 when compared to 172.625 ± 5.105 of control. Effect started at 0.5 hrs with a count of 134.5±3.256 compared to 216.625±12.198. But from 2 hrs the counts did not differ statistically from that of control group up to 4 hours (Table 36).

On rota-rod at all the time intervals it showed statistically significance difference from standard drug and control drug. Maximum effect was noted at 1 hour with an average time of 386.125 ± 7.474 seconds compared to 640.25 ± 12.219 seconds of control group. The effect started at 0.5 hrs with average time of permanence of 421.0 ± 4.377 seconds compared to control of 622.625 ± 12.159 seconds and persisted up to 4 hours, 427. 625 ± 4.854 seconds compared to 655.875 ± 9.235 seconds of control (Table 37).

The test drug showed significantly lower effect than the standard drug at all the time interval.

Aggressive behaviour was reduced as indicated by the score of 4 when compared to 8-11 of control group, but slightly elevated when compared to 3 of all other groups (Table 28).

All the hematological parameters were in normal range (Table 29).

Observation of spontaneous motor activity and forced motor activity are given in table nos. 26 and 27 respectively and graphical representation in comparison to control and standard are given in Fig. 11 and 12 respectively. Statistical analysis table are given in table 34-37.

4.6. *Vitex leucoxylon* @ 500mg/kg

At all the time intervals it showed significant difference from control group and standard group at 1% level.

The drug showed maximum effect at 1 hour on actaphotometer with an average count of 90.875 ± 3.906 compared to 172.625 ± 5.105 of control. The effect started at 0.5 hours with an average count of 111.500 ± 2.397 compared to 216.625 ± 12.198 of control group. Maximum effect noted at 1 hour and then increased up to 4 hours but much less than control even at 4 hours, as shown by 114.750 ± 4.373 compared to 125.625 ± 11.063 of control group (Table 36).

On rota-rod, this drug showed maximum effect at one hour with average time of permanence of 293.25 ± 8.927 seconds compared to 640.25 ± 12.219 seconds of control. Effect started at 0.5 hours with a time of 328.125 ± 9.235 compared to 622.625 ± 12.159 seconds of control and maintained even at 4 hours as shown by 327.625 ± 10.030 seconds of test compared to 655.875 ± 9.235 seconds of control group (Table 37).

The test drug showed significantly lower effect than the standard drug at all the time interval.

Aggressive behaviour was reduced as indicated by the score of 4 when compared to 8-11 of control group, but slightly elevated when compared to 3 of all other groups (Table 32).

All the hematological parameters were in normal range (Table 33).

Observation of spontaneous motor activity and forced motor activity are given in table nos. 30 and 31 respectively and graphical representation in comparison to control and standard are given in Fig. 11and 12 respectively. Statistical analysis table are given in table 34 to 37.

Table 2. Control group (G8) - Gum acacia: Actaphotometer counts/10 mts.

Animal No	Body wt	Qnty of extract (ml)	0.5 hours	1 hours	1.5 hours	2 hours	2.5 hours	3 hours	3.5 hours	4 hours
1	104	0.7	224	186	108	98	123	64	88	94
2	112	0.8	180	151	110	82	89	83	115	97
3	93	0.65	152	156	203	168	192	196	181	179
4	96	0.7	244	170	135	127	131	123	147	116
5	102	0.7	258	179	141	137	134	148	136	110
6	98	0.7	231	181	118	126	119	131	122	120
7	109	0.8	218	166	161	162	143	167	135	121
8	84	0.6	226	192	151	158	163	169	179	168

Table 3. Control group (G8) - Gum acacia: Rota-rod performance.

Animal No.	Body wt	Qnty of extract (ml)	0.5hours	1 hour	1.5 hours	2 hours	2.5 hours	3hours	3.5 hours	4 hours
1	104	0.1	610	682	698	612	631	631	605	661
2	112	0.11	610	620	642	682	693	638	698	613
3	93	0.09	694	641	642	655	631	682	672	698
4	96	0.1	631	628	676	651	662	631	676	661
5	102	0.1	635	646	596	621	671	541	610	652
6	98	0.1	621	689	676	691	705	711	694	673
7	109	0.11	574	636	639	651	668	543	637	661
8	84	0.08	606	580	562	604	613	574	583	628

Table 4. Control group (G8) - Aggressive behaviour test score.

Animal No.	Aggressive Behaviour Rating Score							Total Score
	B1	B2	B3	B4	B5	B6	B7	
1	2	2	1	1	2	1	2	11
2	1	1	1	1	2	1	2	9
3	2	2	1	1	2	1	2	11
4	1	1	1	1	2	1	1	8
5	2	2	1	1	2	1	2	11
6	2	2	1	1	2	1	2	11
7	1	2	1	1	1	1	1	8
8	2	2	1	1	2	1	2	11

B1 - To come up to
B2 - To capture in home cage
B3 - Resistance to leave home cage
B4 - Squealing and vocalization during handling
B5 - Defaecation /and urination during capture
B6 – To presentation of forceps to close proximity of snout
B7 - To prodding with forceps

Table 5. Control group (G8) - Haematology.

Animal No:	TEC ($X10^6$/microL)	TLC (/microL)	Hb (g/dL)	Neutrophill %	Lymphocyte %	Eosinophill %	Monocyte %
1	9.31	8710	14.2	21	76	3	0
2	8.79	6940	14.6	27	72	1	0
3	9.03	7310	15.2	30	69	1	0
4	7.93	8460	14.6	23	74	3	0
5	8.31	9120	15.8	21	77	2	0
6	8.38	6980	15.2	26	74	0	0
7	8.95	7140	15.6	25	72	3	0
8	8.93	7460	15.4	24	72	3	1
Normal Value	7.0-10.0	6000 -17000	10.0 - 18.0	9.0 - 34	65 - 85	0 - 5	0 - 6

Table 6. Standard drug group (G7) - Chlorpromazine 7 mg/kg : Actaphotometer counts/10 mts.

Animal No	Body wt	Qnty of extract (ml)	0.5 hours	1 hours	1.5 hours	2 hours	2.5 hours	3 hours	3.5 hours	4 hours
1	122	0.85	82	27	24	28	23	32	38	39
2	130	0.9	61	41	26	29	22	31	42	49
3	114	0.8	83	59	25	23	19	27	41	40
4	116	0.8	45	40	24	17	23	19	31	35
5	112	0.8	52	37	28	30	26	36	39	41
6	123	0.85	63	29	28	31	23	22	35	34
7	132	0.9	56	24	27	25	28	24	38	36
8	114	0.8	68	28	33	24	29	25	40	47

Table 7. Standard drug group (G7) - Chlorpromazine 7 mg/kg : Rota rod performance .

Animal No	Body wt	Qnty of extract (ml)	0.5 hours	1 hours	1.5 hours	2 hours	2.5 hours	3 hours	3.5 hours	4 hours
1	122	0.85	147	135	142	210	135	123	141	158
2	130	0.9	170	162	140	143	120	110	131	129
3	114	0.8	181	92	82	65	68	74	85	83
4	116	0.8	175	150	142	138	140	103	148	163
5	112	0.8	162	135	98	112	102	146	141	156
6	123	0.85	149	112	119	117	121	115	131	147
7	132	0.9	179	136	149	121	136	130	141	152
8	114	0.8	206	180	136	120	131	170	165	183

Table 8. Standard drug group (G7) - Chlorpromazine 7 mg/kg : Aggressive behaviour test score.

Animal No.	Aggressive Behaviour Rating Score							Total Score
	B1	B2	B3	B4	B5	B6	B7	
1	0	1	0	0	1	0	1	3
2	0	1	0	0	1	0	1	3
3	0	1	0	0	1	0	1	3
4	0	1	0	0	1	0	1	3
5	0	1	0	0	1	0	1	3
6	0	1	0	0	1	0	1	3
7	0	1	0	0	1	0	1	3
8	0	1	0	0	1	0	1	3

B1 - To come up to
B2 - To capture in home cage
B3 - Resistance to leave home cage
B4 - Squealing and vocalization during handling
B5 - Defaecation /and urination during capture
B6 – To presentation of forceps to close proximity of snout
B7 - To prodding with forceps

Table 9. Standard drug group (G7) - Chlorpromazine 7 mg/kg : Haematology .

Animal No:	TEC ($X10^6$/microL)	TLC (/microL)	Hb (g/dL)	Neutrophill %	Lymphocyte %	Eosinophill %	Monocyte %
1	6.23	8460	15.6	21	77	2	0
2	6.13	7230	14.2	26	73	1	0
3	5.16	10130	14.6	30	68	2	0
4	5.43	9370	14.6	25	72	3	0
5	5.89	9260	15.2	26	72	1	1
6	6.43	8430	15.6	27	73	0	0
7	6.23	9460	15.2	23	74	3	0
8	5.19	9010	14.8	26	71	3	0
Normal Value	7.0-10.0	6000 -17000	10.0 - 18.0	9.0 - 34	65 - 85	0 - 5	0 - 6

Table 10. Test drug group (G1) - *Clitoria ternatea* 250 mg/kg : Actaphotometer counts/10 mts.

Animal No	Body wt	Qnty of extract (ml)	0.5 hours	1 hours	1.5 hours	2 hours	2.5 hours	3 hours	3.5 hours	4 hours
1	150	0.75	117	88	75	78	71	77	84	75
2	132	0.65	121	94	76	73	70	81	90	92
3	146	0.7	158	135	119	110	114	125	114	129
4	132	0.65	112	94	85	81	88	89	96	94
5	128	0.6	83	64	59	61	65	69	74	88
6	131	0.65	112	87	81	83	79	86	91	101
7	142	0.7	90	61	60	51	65	59	54	67
8	134	0.65	123	104	108	95	104	99	103	112

Table 11. Test drug group (G1) - *Clitoria ternatea* 250 mg/kg : Rota rod performance.

Animal No	Body wt	Qnty of extract (ml)	0.5 hours	1 hours	1.5 hours	2 hours	2.5 hours	3 hours	3.5 hours	4 hours
1	150	0.75	319	242	262	260	268	275	291	323
2	132	0.65	235	226	209	215	211	235	286	294
3	146	0.7	265	248	236	240	229	244	265	291
4	132	0.65	236	219	204	211	215	231	263	248
5	128	0.6	258	231	225	227	239	229	256	240
6	131	0.65	306	276	267	253	241	261	270	268
7	142	0.7	297	261	250	258	271	265	251	269
8	134	0.65	331	305	296	291	279	284	281	285

Table 12. Test drug group (G1) - *Clitoria ternatea* 250 mg/kg : Aggressive behaviour test score.

Animal No.	Aggressive Behaviour Rating Score							Total Score
	B1	B2	B3	B4	B5	B6	B7	
1	0	1	0	0	1	0	1	3
2	0	1	0	0	1	0	1	3
3	0	1	0	0	1	0	1	3
4	0	1	0	0	1	0	1	3
5	0	1	0	0	1	0	1	3
6	0	1	0	0	1	0	1	3
7	0	1	0	0	1	0	1	3
8	0	1	0	0	1	0	1	3

B1 - To come up to
B2 - To capture in home cage
B3 - Resistance to leave home cage
B4 - Squealing and vocalization during handling
B5 - Defaecation /and urination during capture
B6 – To presentation of forceps to close proximity of snout
B7 - To prodding with forceps

Table 13. Test drug group (G1). *Clitoria ternatea* 250 mg/kg: Haematology.

Animal No:	TEC ($X10^6$/microL)	TLC (/microL)	Hb (g/dL)	Neutrophill %	Lymphocyte %	Eosinophill %	Monocyte %
1	8.79	8970	14.8	21	76	3	0
2	9.23	9120	14.8	21	78	1	0
3	8.53	10120	15.4	25	73	2	0
4	7.82	9780	15.2	26	73	1	0
5	7.26	9460	15.6	32	66	2	0
6	7.43	7120	15.2	30	68	2	0
7	8.21	8430	14.6	28	72	0	0
8	8.01	8320	14.8	23	77	0	0
Normal Value	7.0-10.0	6000 -17000	10.0 - 18.0	9.0 - 34	65 - 85	0 - 5	0 - 6

Table 14. Test drug group (G2) - *Clitoria ternatea* 500 mg/kg : Actaphotometer counts/10 minutes.

Animal No	Body wt	Qnty of extract (ml)	0.5 hours	1 hours	1.5 hours	2 hours	2.5 hours	3 hours	3.5 hours	4 hours
1	143	0.7	102	51	52	58	61	59	65	67
2	134	0.65	94	44	42	44	48	56	54	61
3	145	0.7	90	40	38	39	45	32	39	48
4	129	0.6	108	41	34	43	33	38	39	41
5	136	0.65	111	52	49	44	47	43	49	51
6	126	0.6	82	41	41	44	49	45	50	50
7	137	0.65	102	48	40	41	48	49	46	53
8	144	0.7	93	45	42	44	51	53	52	63

Table 15. Test drug group (G2) - *Clitoria ternatea* 500 mg/kg : Rota rod performance.

Animal No	Body wt	Qnty of extract (ml)	0.5 hours	1 hours	1.5 hours	2 hours	2.5 hours	3 hours	3.5 hours	4 hours
1	143	0.7	226	206	204	198	193	204	226	232
2	134	0.65	198	182	181	161	173	162	183	195
3	145	0.7	214	192	142	153	150	162	159	166
4	129	0.6	205	181	165	161	151	153	162	181
5	136	0.65	184	152	123	138	129	132	151	156
6	126	0.6	231	196	143	152	161	142	148	146
7	137	0.65	162	136	119	112	118	126	124	133
8	144	0.7	182	122	109	118	109	136	129	141

Table 16. Test drug group (G2) - *Clitoria ternatea* 500 mg/kg : Aggressive behaviour test score

Animal No.	Aggressive Behaviour Rating Score							Total Score
	B1	B2	B3	B4	B5	B6	B7	
1	0	1	0	0	1	0	1	3
2	0	1	0	0	1	0	1	3
3	0	1	0	0	1	0	1	3
4	0	1	0	0	1	0	1	3
5	0	1	0	0	1	0	1	3
6	0	1	0	0	1	0	1	3
7	0	1	0	0	1	0	1	3
8	0	1	0	0	1	0	1	3

B1 - To come up to
B2 - To capture in home cage
B3 - Resistance to leave home cage
B4 - Squealing and vocalization during handling
B5 - Defaecation /and urination during capture
B6 – To presentation of forceps to close proximity of snout
B7 - To prodding with forceps

Table 17. Test drug group (G2). *Clitoria ternatea* 500 mg/kg : Haematology.

Animal No:	TEC ($X10^6$/microL)	TLC (/microL)	Hb (g/dL)	Neutrophill %	Lymphocyte %	Eosinophill %	Monocyte %
1	8.13	8760	14.8	33	66	1	0
2	7.86	9240	14.8	21	74	5	0
3	7.46	8120	14.8	26	73	1	0
4	7.78	7150	14.6	23	76	1	0
5	8.41	7960	15.6	24	75	1	0
6	8.43	8130	15.8	26	72	1	1
7	8.23	8830	15.2	27	71	2	0
8	8.81	9120	15.4	22	76	2	0
Normal Value	7.0-10.0	6000 -17000	10.0 - 18.0	9.0 - 34	65 - 85	0 - 5	0 - 6

Table 18. Test drug group (G3) - *Acorus calamus* 250 mg/kg : Actaphotometer counts/10 minutes.

Animal No	Body wt	Qnty of extract (ml)	0.5 hours	1 hours	1.5 hours	2 hours	2.5 hours	3 hours	3.5 hours	4 hours
1	132	0.65	123	81	85	93	99	103	105	121
2	128	0.6	98	73	78	85	84	102	89	92
3	148	0.7	114	88	89	93	99	105	119	123
4	136	0.65	132	115	123	121	126	114	129	134
5	133	0.65	133	107	117	114	129	134	131	135
6	142	0.7	115	99	102	101	107	105	112	117
7	145	0.7	125	89	95	91	96	109	115	127
8	138	0.65	112	77	79	85	89	99	119	113

Table 19. Test drug group (G3) - *Acorus calamus* 250 mg/kg : Rota rod performance.

Animal No	Body wt	Qnty of extract (ml)	0.5 hours	1 hours	1.5 hours	2 hours	2.5 hours	3 hours	3.5 hours	4 hours
1	132	0.65	389	293	311	304	328	343	340	361
2	128	0.6	293	233	283	269	254	272	269	289
3	148	0.7	356	301	312	336	329	343	346	372
4	136	0.65	403	326	334	343	356	367	391	383
5	133	0.65	323	236	232	247	271	269	291	298
6	142	0.7	343	303	329	318	332	315	324	340
7	145	0.7	315	245	251	267	271	279	301	290
8	138	0.65	287	231	227	251	246	253	249	265

Table 20. Test drug group (G3) - *Acorus calamus* 250 mg/kg : Aggressive behaviour test score.

Animal No.	Aggressive Behaviour Rating Score							Total Score
	B1	B2	B3	B4	B5	B6	B7	
1	0	1	0	0	1	0	1	3
2	0	1	0	0	1	0	1	3
3	0	1	0	0	1	0	1	3
4	0	1	0	0	1	0	1	3
5	0	1	0	0	1	0	1	3
6	0	1	0	0	1	0	1	3
7	0	1	0	0	1	0	1	3
8	0	1	0	0	1	0	1	3

B1 - To come up to
B2 - To capture in home cage
B3 - Resistance to leave home cage
B4 - Squealing and vocalization during handling
B5 - Defaecation /and urination during capture
B6 – To presentation of forceps to close proximity of snout
B7 - To prodding with forceps

Table 21. Test drug group (G3) - *Acorus calamus* 250 mg/kg : Haematology.

Animal No:	TEC ($X10^6$/microL)	TLC (/microL)	Hb (g/dL)	Neutrophill %	Lymphocyte %	Eosinophill %	Monocyte %
1	8.04	8760	15.2	21	79	0	0
2	8.28	8920	15.4	25	74	1	0
3	7.93	9140	14.8	31	67	2	0
4	7.31	9320	14.8	22	75	3	0
5	8.93	8120	14.6	21	78	1	0
6	8.83	8340	15.6	32	67	1	1
7	8.46	7460	14.2	21	77	2	0
8	8.49	7930	14.8	30	69	1	0
Normal Value	7.0-10.0	6000 -17000	10.0 - 18.0	9.0 - 34	65 - 85	0 - 5	0 - 6

Table 22. Test drug group (G4) - *Acorus calamus* 500 mg/kg : Actaphotometer counts/10 minutes.

Animal No	Body wt	Qnty of extract (ml)	0.5 hours	1 hours	1.5 hours	2 hours	2.5 hours	3 hours	3.5 hours	4 hours
1	135	0.65	116	65	63	71	79	88	84	94
2	128	0.6	131	83	89	95	93	109	108	113
3	142	0.7	126	85	78	81	82	81	93	95
4	139	0.65	124	73	84	88	89	96	93	106
5	146	0.75	118	101	105	104	112	110	116	121
6	136	0.65	123	98	103	110	118	122	120	126
7	141	0.7	135	95	96	108	114	119	123	131
8	138	0.65	138	99	97	117	116	123	130	139

Table 23. Test drug group (G4) - *Acorus calamus* 500 mg/kg : Rota rod performance.

Animal No	Body wt	Qnty of extract (ml)	0.5 hours	1 hours	1.5 hours	2 hours	2.5 hours	3 hours	3.5 hours	4 hours
1	135	0.65	253	243	232	244	256	261	258	272
2	128	0.6	231	214	222	241	238	242	246	261
3	142	0.7	242	203	219	212	236	241	232	246
4	139	0.65	233	216	214	223	241	246	241	247
5	146	0.75	231	201	212	219	223	226	234	242
6	136	0.65	238	191	202	205	216	226	229	237
7	141	0.7	223	183	193	195	208	215	221	228
8	138	0.65	261	224	221	231	237	246	257	262

Table 24. Test drug group (G4) - *Acorus calamus* 500 mg/kg : Aggressive behaviour test score.

Animal No.	Aggressive Behaviour Rating Score							Total Score
	B1	B2	B3	B4	B5	B6	B7	
1	0	1	0	0	1	0	1	3
2	0	1	0	0	1	0	1	3
3	0	1	0	0	1	0	1	3
4	0	1	0	0	1	0	1	3
5	0	1	0	0	1	0	1	3
6	0	1	0	0	1	0	1	3
7	0	1	0	0	1	0	1	3
8	0	1	0	0	1	0	1	3

B1 - To come up to
B2 - To capture in home cage
B3 - Resistance to leave home cage
B4 - Squealing and vocalization during handling
B5 - Defaecation /and urination during capture
B6 – To presentation of forceps to close proximity of snout
B7 - To prodding with forceps

Table 25. Test drug group (G4) - *Acorus calamus* 500 mg/kg : Haematology.

Animal No:	TEC ($X10^6$/microL)	TLC (/microL)	Hb (g/dL)	Neutrophill %	Lymphocyte %	Eosinophill %	Monocyte %
1	8.01	8420	14.8	19	79	2	0
2	7.93	8820	14.2	27	71	2	0
3	7.91	8730	15.6	28	71	1	0
4	7.76	8110	15.6	29	70	1	0
5	7.16	8910	14.6	28	70	1	1
6	7.27	8820	14.8	24	73	2	1
7	7.14	8430	14.8	25	74	1	0
8	7.12	8110	14.6	24	72	4	0
Normal Value	7.0-10.0	6000 -17000	10.0 - 18.0	9.0 - 34	65 - 85	0 - 5	0 - 6

Table 26. Test drug group (G5) - *Vitex leucoxylon* 250 mg/kg : Actaphotometer counts/10 minutes.

Animal No	Body wt	Qnty of extract (ml)	0.5 hours	1 hours	1.5 hours	2 hours	2.5 hours	3 hours	3.5 hours	4 hours
1	153	0.8	142	112	111	121	128	131	139	146
2	147	0.75	131	101	106	118	123	129	128	134
3	142	0.7	134	98	104	114	118	123	129	133
4	148	0.75	138	114	112	118	123	128	137	132
5	142	0.7	114	94	98	96	108	114	123	119
6	150	0.8	143	116	123	132	138	146	144	151
7	145	0.75	139	118	119	128	126	138	143	149
8	148	0.75	135	119	114	128	123	129	133	139

Table 27. Test drug group (G5) - *Vitex leucoxylon* 250 mg/kg : Rota rod performance.

Animal No	Body wt	Qnty of extract (ml)	0.5 hours	1 hours	1.5 hours	2 hours	2.5 hours	3 hours	3.5 hours	4 hours
1	153	0.8	408	371	387	384	398	412	419	421
2	147	0.75	423	361	373	389	399	411	412	421
3	142	0.7	431	411	414	423	429	426	433	438
4	148	0.75	411	378	376	389	398	409	413	423
5	142	0.7	428	393	390	399	418	414	427	438
6	150	0.8	436	411	423	429	419	433	438	441
7	145	0.75	429	403	409	418	423	428	422	438
8	148	0.75	402	361	371	376	389	387	396	401

Table 28. Test drug group (G5) - *Vitex leucoxylon* 250 mg/kg : Aggressive behaviour test score.

Animal No.	Aggressive Behaviour Rating Score							Total Score
	B1	B2	B3	B4	B5	B6	B7	
1	0	1	0	1	1	0	1	4
2	0	1	0	1	1	0	1	4
3	0	1	0	1	1	0	1	4
4	0	1	0	0	1	0	1	3
5	0	1	0	1	1	0	1	4
6	0	1	0	1	1	0	1	4
7	0	1	0	0	1	0	1	3
8	0	1	0	1	1	0	1	4

B1 - To come up to
B2 - To capture in home cage
B3 - Resistance to leave home cage
B4 - Squealing and vocalization during handling
B5 - Defaecation /and urination during capture
B6 – To presentation of forceps to close proximity of snout
B7 - To prodding with forceps

Table 29. Test drug group (G5) - *Vitex leucoxylon* 250 mg/kg : Haematology.

Animal No:	TEC ($X10^6$/microL)	TLC (/microL)	Hb (g/dL)	Neutrophill %	Lymphocyte %	Eosinophill %	Monocyte %
1	9.01	8430	15.8	21	78	1	0
2	8.87	7640	15.6	27	70	3	0
3	7.54	7940	14.8	24	72	2	0
4	8.24	8120	15.2	24	71	2	1
5	8.59	8070	15.2	26	76	0	0
6	8.99	7410	15.4	20	78	1	1
7	8.12	8910	15.2	28	70	2	0
8	8.23	8810	15.4	27	72	1	0
Normal Value	7.0-10.0	6000 -17000	10.0 - 18.0	9.0 - 34	65 - 85	0 - 5	0 - 6

Table 30. Test drug group (G6 -. *Vitex leucoxylon* 500 mg/kg : Actaphotometer counts/10 minutes.

Animal No	Body wt	Qnty of extract (ml)	0.5 hours	1 hours	1.5 hours	2 hours	2.5 hours	3 hours	3.5 hours	4 hours
1	151	0.8	120	102	105	104	112	116	121	132
2	142	0.75	116	97	96	103	108	112	120	119
3	144	0.75	109	83	85	93	92	99	108	115
4	146	0.75	117	83	93	95	98	102	113	120
5	138	0.65	106	86	89	87	91	95	96	99
6	144	0.75	99	73	74	75	81	85	84	94
7	148	0.75	114	101	98	110	113	116	114	121
8	147	0.75	121	102	104	105	104	105	112	118

Table 31. Test drug group (G6) - *Vitex leucoxylon* 500 mg/kg : Rota rod performance.

Animal No	Body wt	Qnty of extract (ml)	0.5 hours	1 hours	1.5 hours	2 hours	2.5 hours	3 hours	3.5 hours	4 hours
1	151	0.8	353	308	314	319	310	331	343	359
2	142	0.75	363	331	339	332	346	359	369	361
3	144	0.75	321	291	298	311	308	331	329	333
4	146	0.75	316	293	299	311	293	310	323	319
5	138	0.65	302	283	272	285	280	291	290	301
6	144	0.75	287	246	263	253	264	273	269	279
7	148	0.75	339	282	296	294	298	297	311	323
8	147	0.75	344	312	322	318	325	338	339	346

Table 32. Test drug group (G6) - *Vitex leucoxylon* 500 mg/kg : Aggressive behaviour test score.

Animal No.	Aggressive Behaviour Rating Score							Total Score
	B1	B2	B3	B4	B5	B6	B7	
1	0	1	0	0	1	0	1	3
2	0	1	0	0	1	0	1	3
3	0	1	0	0	1	0	1	3
4	0	1	0	0	1	0	1	3
5	0	1	0	0	1	0	1	3
6	0	1	0	0	1	0	1	3
7	0	1	0	0	1	0	1	3
8	0	1	0	0	1	0	1	3

B1 - To come up to
B2 - To capture in home cage
B3 - Resistance to leave home cage
B4 - Squealing and vocalization during handling
B5 - Defaecation /and urination during capture
B6 – To presentation of forceps to close proximity of snout
B7 - To prodding with forceps

Table 33. Test drug group (G6) - *Vitex leucoxylon* 500 mg/kg : Haematology.

Animal No:	TEC ($X10^6$/microL)	TLC (/microL)	Hb (g/dL)	Neutrophill %	Lymphocyte %	Eosinophill %	Monocyte %
1	8.16	8410	15.2	21	77	2	0
2	8.94	9610	15.4	25	74	1	0
3	9.01	10110	16.2	23	74	3	0
4	8.23	8430	15.2	20	78	2	0
5	9.12	10230	16.2	25	72	2	1
6	8.45	9440	15.2	24	73	3	0
7	7.89	11130	14.8	23	75	1	1
8	8.14	9840	15.2	23	73	4	0
Normal Value	7.0-10.0	6000 -17000	10.0 - 18.0	9.0 - 34	65 - 85	0 - 5	0 - 6

Table 34. ANALYSIS OF VARIANCE TABLE – Actaphotometer.

Time intervals		Degrees of freedom	Sum of squares	Mean square	F-Value
0.5 hours	Between	7	106223.25	15174.75	52.525 **
	Within	56	16178.75	288.906	
1 hour	Between	7	97246.688	13892.38	73.548 **
	Within	56	10577.75	188.888	
1.5 hours	Between	7	73645.609	10520.8	39.338 **
	Within	56	14976.875	267.444	
2. hours	Between	7	71947.483	10278.21	40.624 **
	Within	56	14168.5	253.009	
2.5 hours	Between	7	79026.109	11289.44	43.265 **
	Within	56	14612.375	260.935	
3 hours	Between	7	81595.5	11656.5	30.052 **
	Within	56	21721.5	387.884	
3.5 hours	Between	7	76312.609	10901.8	42.259 **
	Within	56	14446.625	257.975	
4 hours	Between	7	69337.938	9905.42	35.988 **
	Within	56	15413.5	275.241	

** significant at 1 per cent level

Table 35. ANALYSIS OF VARIANCE TABLE – Rota rod.

Time intervals		Degrees of freedom	Sum of squares	Mean square	F-Value
0.5 hours	Between	7	1172598.484	167514.069	213.326 **
	Within	56	43973.875	785.248	
1 hour	Between	7	1419860.734	202837.248	244.487 **
	Within	56	46460.125	829.645	
1.5 hours	Between	7	1512955.734	216136.533	220.363 **
	Within	56	54925.875	980.819	
2. hours	Between	7	1531252.5	218750.357	255.053 **
	Within	56	48029.25	857.665	
2.5 hours	Between	7	1636340.359	233762.908	313.379 **
	Within	56	41772.875	745.944	
3 hours	Between	7	1399926.984	199989.569	182.309 **
	Within	56	61430.875	1096.98	
3.5 hours	Between	7	1477941.734	211134.533	234.741 **
	Within	56	50368.375	899.435	
4 hours	Between	7	1474197.484	210599.641	256.645 **
	Within	56	45952.875	820.587	

** significant at 1 per cent level

Table 36. Summary of observations – actaphotometer..

Time intervals	Groups	Average± Standard error LSD value (p<0.05)
0.5 hours	Control	216.625±12.198 [a]
	Standard	63.75±4.78 [e]
	Clitoria ternatea 250 mg/kg	114.5±8.036 [cd]
	Clitoria ternatea 500 mg/kg	97.75± 3.437 [d]
	Acorus calamus 250 mg/kg	119± 4.115 [bc]
	Acorus calamus 500 mg/kg	126.375± 2.758 [bc]
	Vitex leucoxylon 250 mg/kg	134.5± 3.256 [bc]
	Vitex leucoxylon 500 mg/kg	111.5± 2.397 [cd]
LSD Value = 52.525		

Time intervals	Groups	Average± Standard error LSD value (p<0.05)
1 hours	Control	152.625±5.105 [a]
	Standard	35.625±4.027 [d]
	Clitoria ternatea 250 mg/kg	90.875±8.213 [c]
	Clitoria ternatea 500 mg/kg	45.25±1.644 [d]
	Acorus calamus 250 mg/kg	91.125±5.226 [c]
	Acorus calamus 500 mg/kg	87.375±4.674 [c]
	Vitex leucoxylon 250 mg/kg	109±3.468 [bc]
	Vitex leucoxylon 500 mg/kg	90.875±3.907 [c]
LSD Value = 73.548		

Time intervals	Groups	Average± Standard error LSD value (p<0.05)
1.5 hours	Control	140.875±11.148 [a]
	Standard	26.875±1.048 [d]
	Clitoria ternatea 250 mg/kg	82.875±7.495 [c]
	Clitoria ternatea 500 mg/kg	42.25±2.043 [d]
	Acorus calamus 250 mg/kg	96±5.961 [bc]
	Acorus calamus 500 mg/kg	89.375±4.975 [c]
	Vitex leucoxylon 250 mg/kg	110.875±2.867 [bc]
	Vitex leucoxylon 500 mg/kg	93±3.635 [c]
LSD Value = 16.38		

Time intervals	Groups	Average± Standard error LSD value (p<0.05)
2 hours	Control	132.25±10.868 [a]
	Standard	25.875±1.63 [e]
	Clitoria ternatea 250 mg/kg	79±6.523 [c]
	Clitoria ternatea 500 mg/kg	44.625±2.019 [d]
	Acorus calamus 250 mg/kg	97.875±4.688 [b]
	Acorus calamus 500 mg/kg	96.75±5.6 [b]
	Vitex leucoxylon 250 mg/kg	119.375±3.985 [a]
	Vitex leucoxylon 500 mg/kg	96.5±4.052 [b]
LSD Value = 15.93		

Time intervals	Groups	Average± Standard error LSD value (p<0.05)
2.5 hours	Control	136.75±10.861 [a]
	Standard	24.125 ±1.17 [e]
	Clitoria ternatea 250 mg/kg	82±6.541 [c]
	Clitoria ternatea 500 mg/kg	47.75±2.719 [d]
	Acorus calamus 250 mg/kg	103.625±5.755 [b]
	Acorus calamus 500 mg/kg	100.375±5.752 [b]
	Vitex leucoxylon 250 mg/kg	123.375±3.012 [a]
	Vitex leucoxylon 500 mg/kg	99.875±4.016 [b]
LSD Value = 16.18		

	Groups	Average± Standard error LSD value (p<0.05)
3 hours	Control	135.125 ±15.797 [a]
	Standard	27±2.001 [e]
	Clitoria ternatea 250 mg/kg	85.625±7.099 [c]
	Clitoria ternatea 500 mg/kg	46.875±3.249 [d]
	Acorus calamus 250 mg/kg	108.875±3.935 [b]
	Acorus calamus 500 mg/kg	106±5.65 [b]
	Vitex leucoxylon 250 mg/kg	129.75±3.359 [a]
	Vitex leucoxylon 500 mg/kg	103.75±3.836 [bc]
LSD Value = 19.73		

Time intervals	Groups	Average± Standard error LSD value (p<0.05)
3.5 hours	Control	137.875±11.109 [a]
	Standard	38 ±1.255 [d]
	Clitoria ternatea 250 mg/kg	88.25±6.47 [c]
	Clitoria ternatea 500 mg/kg	49.25±2.988 [d]
	Acorus calamus 250 mg/kg	116.125±4.55 [b]
	Acorus calamus 500 mg/kg	108.375±5.89 [b]
	Vitex leucoxylon 250 mg/kg	134.5±2.659 [a]
	Vitex leucoxylon 500 mg/kg	108.5±4.448 [b]
LSD Value = 16.09		

Time intervals	Groups	Average± Standard error LSD value (p<0.05)
4 hours	Control	125.625±11.063 [ab]
	Standard	40.125±1.93 [d]
	Clitoria ternatea 250 mg/kg	94.75±6.972 [c]
	Clitoria ternatea 500 mg/kg	54.25±3.076 [d]
	Acorus calamus 250 mg/kg	120.25±4.858 [b]
	Acorus calamus 500 mg/kg	115.625±5.844 [b]
	Vitex leucoxylon 250 mg/kg	137.875±3.762 [ab]
	Vitex leucoxylon 500 mg/kg	114.7512.37±4.373 [b]
LSD Value = 16.62		

Table 36. Summary of observations – rotarod.

Time intervals	Groups	Average± Standard error LSD value (p<0.05)
0.5 hours	Control	622.625±12.159 [a]
	Standard	171.125±6.742 [g]
	Clitoria ternatea 250 mg/kg	280.875±13.195 [d]
	Clitoria ternatea 500 mg/kg	200.25±8.33 [f]
	Acorus calamus 250 mg/kg	338.625±14.973 [c]
	Acorus calamus 500 mg/kg	239±4.451 [e]
	Vitex leucoxylon 250 mg/kg	421±4.377 [b]
	Vitex leucoxylon 500 mg/kg	328±9.235 [c]
LSD Value = 28.07		

Time intervals	Groups	Average± Standard error LSD value (p<0.05)
1 hours	Control	640.25±12.219 [a]
	Standard	137.75±9.726 [g]
	Clitoria ternatea 250 mg/kg	251±10.161 [d]
	Clitoria ternatea 500 mg/kg	170.85±10.773 [f]
	Acorus calamus 250 mg/kg	271±13.612 [cd]
	Acorus calamus 500 mg/kg	209.375±6.753 [e]
	Vitex leucoxylon 250 mg/kg	386.125±7.474 [b]
	Vitex leucoxylon 500 mg/kg	293.25±8.927 [c]
LSD Value = 28.85		

Time intervals	Groups	Average± Standard error LSD value (p<0.05)
1.5 hours	Control	641.375±15.793 [a]
	Standard	126±8.563 [e]
	Clitoria ternatea 250 mg/kg	243.625±11.052 [d]
	Clitoria ternatea 500 mg/kg	148.25±11.621 [e]
	Acorus calamus 250 mg/kg	281.125±15.797 [c]
	Acorus calamus 500 mg/kg	214.375±4.331 [d]
	Vitex leucoxylon 250 mg/kg	393.5±7.379 [b]
	Vitex leucoxylon 500 mg/kg	300.375±8.839 [c]
LSD Value = 31.37		

Time intervals	Groups	Average± Standard error LSD value (p<0.05)
2 hours	Control	645.875±11.179 [a]
	Standard	128.25±14.337 [e]
	Clitoria ternatea 250 mg/kg	244.375±9.425 [d]
	Clitoria ternatea 500 mg/kg	149.125±9.602 [e]
	Acorus calamus 250 mg/kg	291.875±13.505 [c]
	Acorus calamus 500 mg/kg	221.25±6.049 [d]
	Vitex leucoxylon 250 mg/kg	400.875±7.025 [b]
	Vitex leucoxylon 500 mg/kg	302.875±8.818 [c]
LSD Value = 29.33		

Time intervals	Groups	Average± Standard error LSD value (p<0.05)
2.5 hours	Control	659.25 ±11.331[a]
	Standard	119.125 ±8.478[f]
	Clitoria ternatea 250 mg/kg	244.125 ±9.178[d]
	Clitoria ternatea 500 mg/kg	148 ±10.012[e]
	Acorus calamus 250 mg/kg	298.375 ±14.927[c]
	Acorus calamus 500 mg/kg	231.875 ±5.424[d]
	Vitex leucoxylon 250 mg/kg	409.125 ±5.208[b]
	Vitex leucoxylon 500 mg/kg	303 ±9.044[c]
LSD Value = 27.36		

	Groups	Average± Standard error LSD value (p<0.05)
3 hours	Control	618.875 ±21.945[a]
	Standard	121.375 ±10.175[e]
	Clitoria ternatea 250 mg/kg	253 ±7.464[d]
	Clitoria ternatea 500 mg/kg	152.125 ±8.8[e]
	Acorus calamus 250 mg/kg	305.125 ±14.998[c]
	Acorus calamus 500 mg/kg	237.875 ±5.172[d]
	Vitex leucoxylon 250 mg/kg	415 ±5.105[b]
	Vitex leucoxylon 500 mg/kg	316.25 ±10.048[c]
LSD Value = 33.17		

Time intervals	Groups	Average± Standard error LSD value (p<0.05)
3.5 hours	Control	646.875 ±15.588[a]
	Standard	135.375 ±8.139[f]
	Clitoria ternatea 250 mg/kg	270.375 ±5.084[d]
	Clitoria ternatea 500 mg/kg	160.25 ±11.473[f]
	Acorus calamus 250 mg/kg	313.875 ±16.189[c]
	Acorus calamus 500 mg/kg	239.75 ±4.688[e]
	Vitex leucoxylon 250 mg/kg	418.5 ±4.974[b]
	Vitex leucoxylon 500 mg/kg	321.625 ±11.133[c]
LSD Value = 30.04		

Time intervals	Groups	Average± Standard error LSD value (p<0.05)
4 hours	Control	655.875 ±9.235
	Standard	146.375 ±10.518[e]
	Clitoria ternatea 250 mg/kg	277.25 ±9.468[d]
	Clitoria ternatea 500 mg/kg	168.75 ±11.632[e]
	Acorus calamus 250 mg/kg	324.75 ±15.779[c]
	Acorus calamus 500 mg/kg	249.375 ±5.155[d]
	Vitex leucoxylon 250 mg/kg	427.625 ±4.854[b]
	Vitex leucoxylon 500 mg/kg	327.625 ±10.03[c]
LSD Value =28.69		

Fig. 1: *Clitoria ternatea* – whole plant.

Fig. 2: *Acorus calamus* - whole plant with rhizome.


CLITORIA TERNATEA
PAPILIONACEAE

Fig. 3: *Vitex leucoxylon* – whole plant.

Fig. 4: *Vitex leucoxylon* - leaves.

Fig. 5: Actaphotometer.

Fig. 6: Rota rod.


INCO
PHOTO-ACTOMETER
COUNTING
POWER

Fig.7: *Clitoria ternatea* - Actaphotometer.

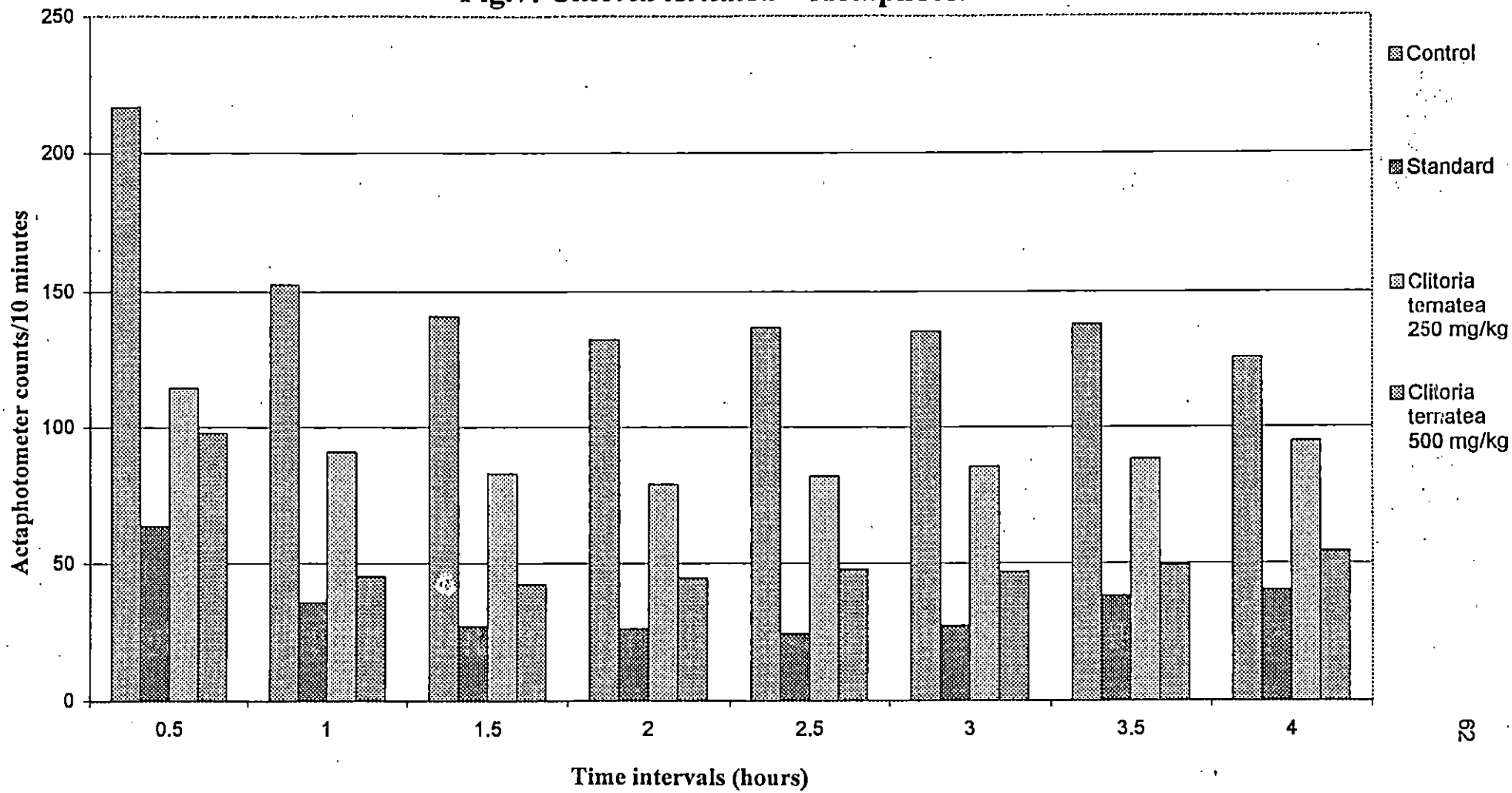


Fig. 8: *Clitoria ternatea* - Rotarod.

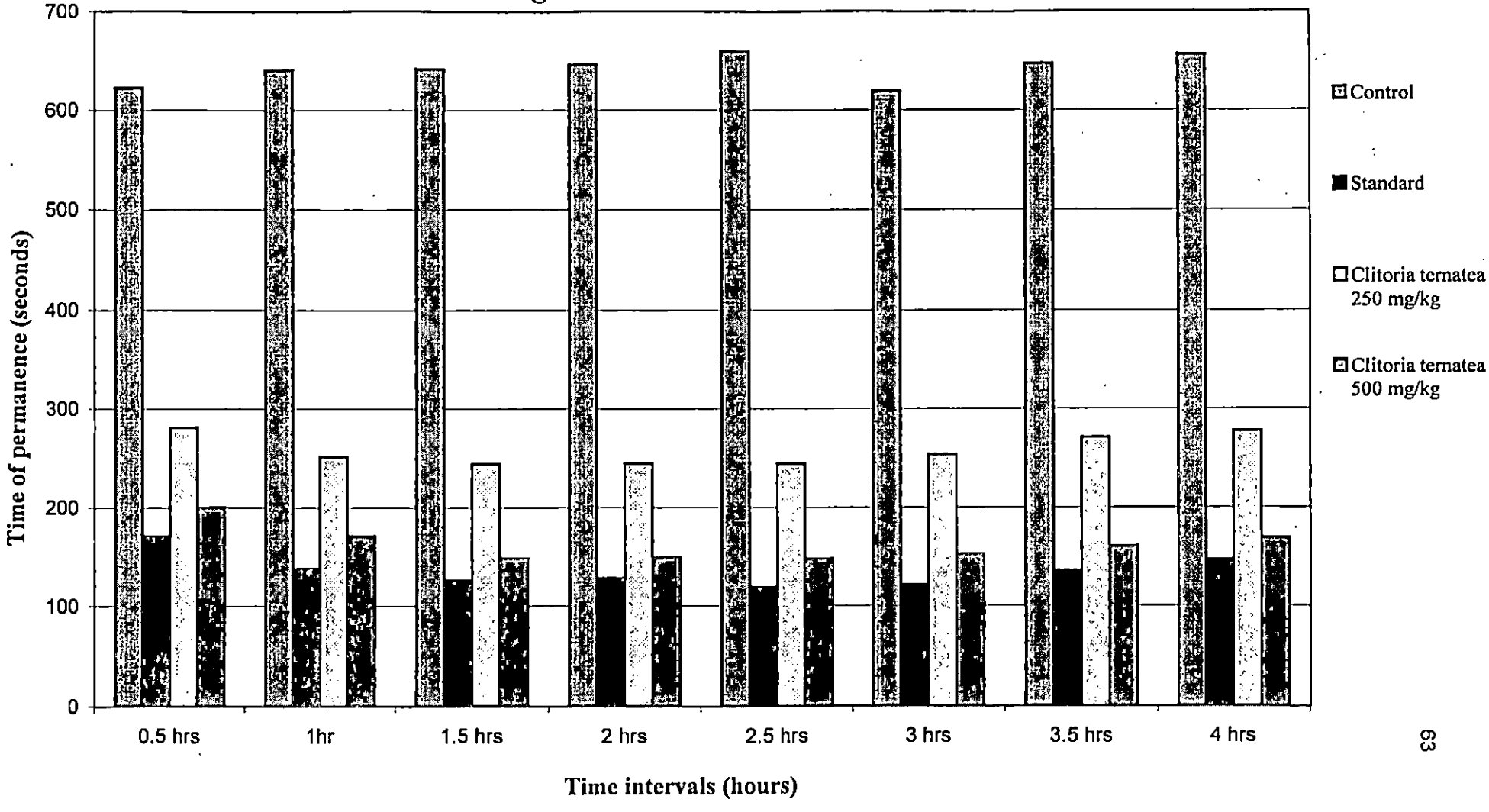


Fig.9: *Acorus calamus* - Actaphotometer.

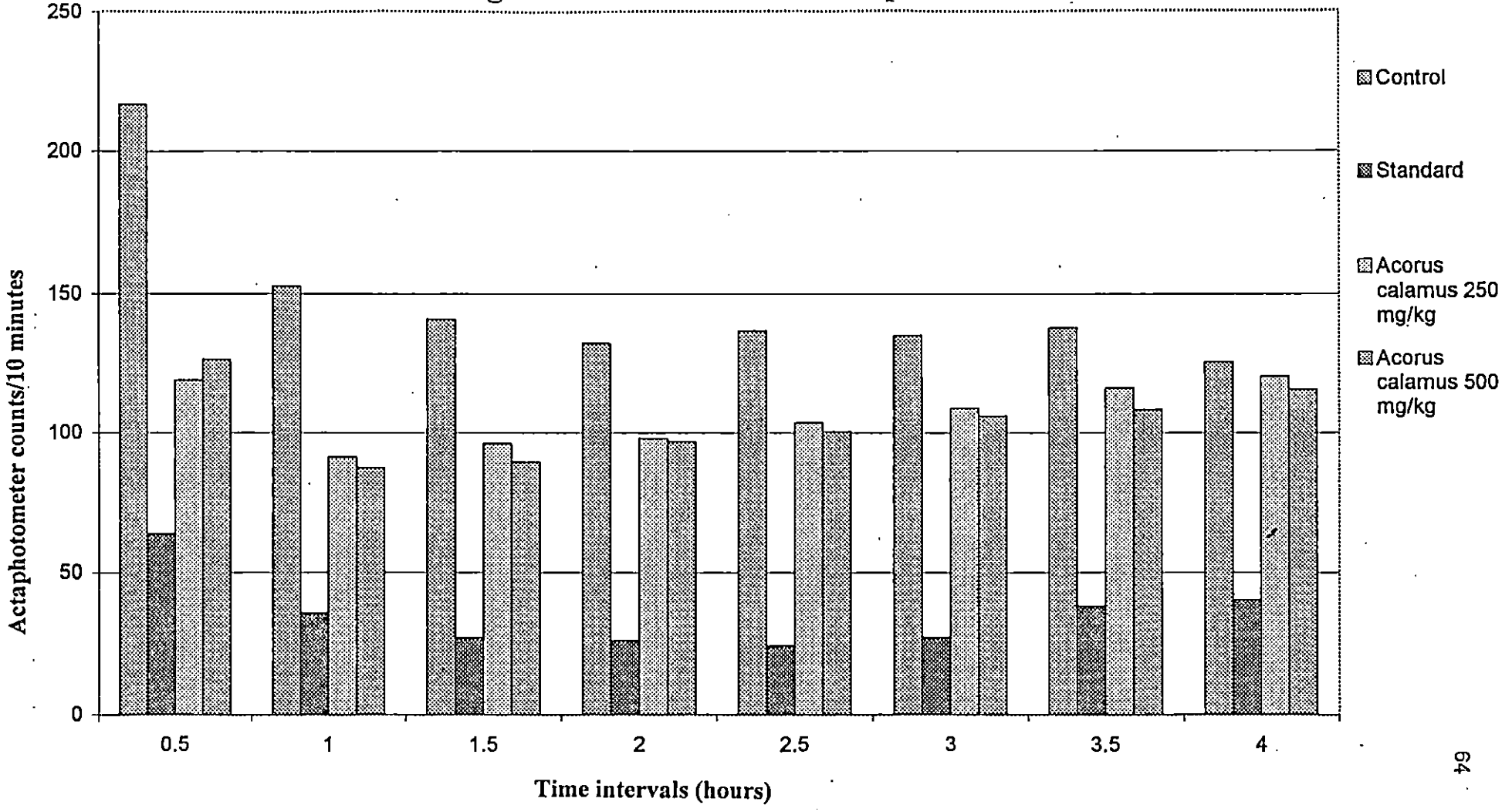


Fig.10: *Acorus calamus* - Rotarod -

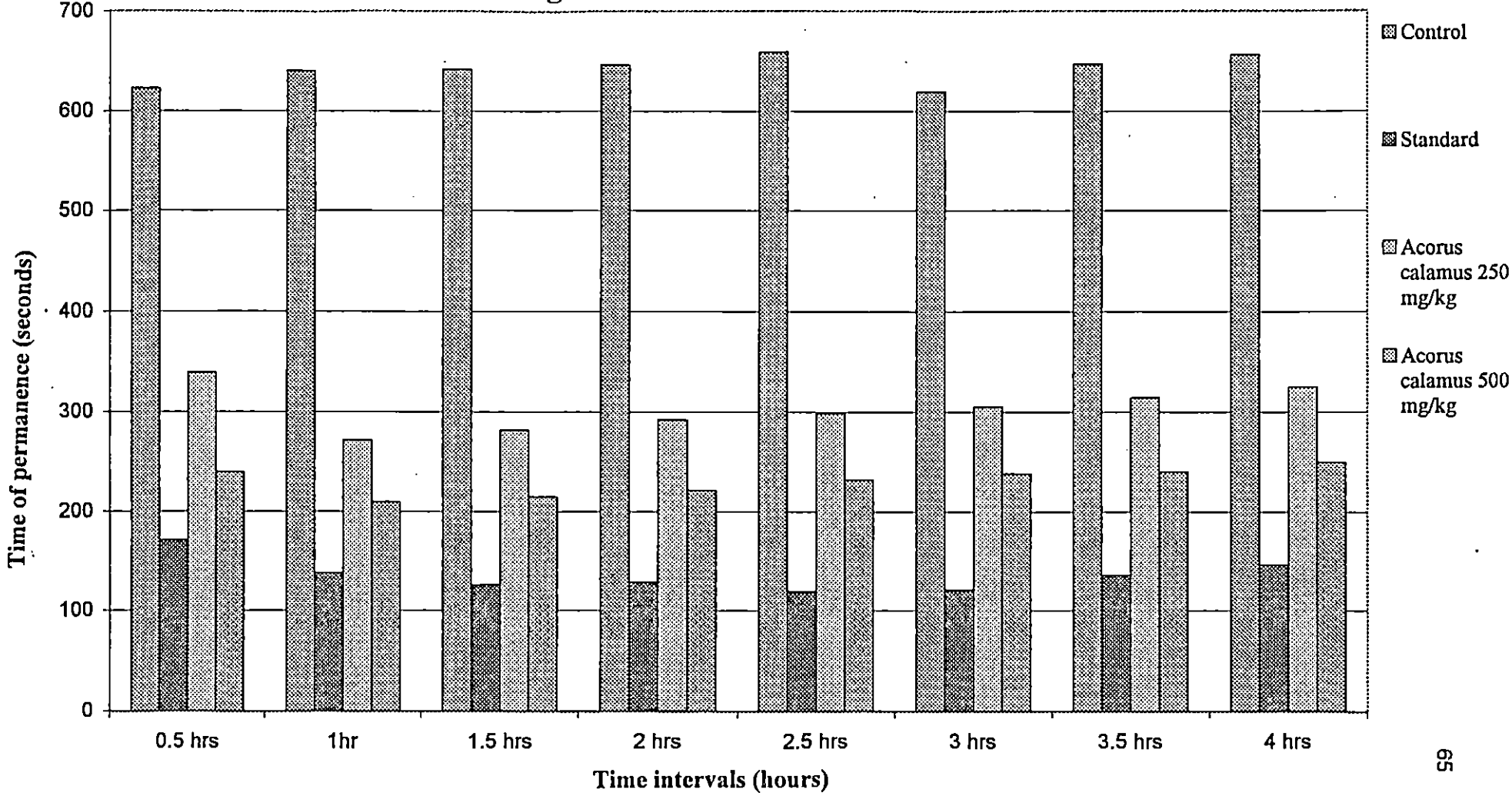


Fig.11: *Vitex leucoxylon* - Actaphotometer.

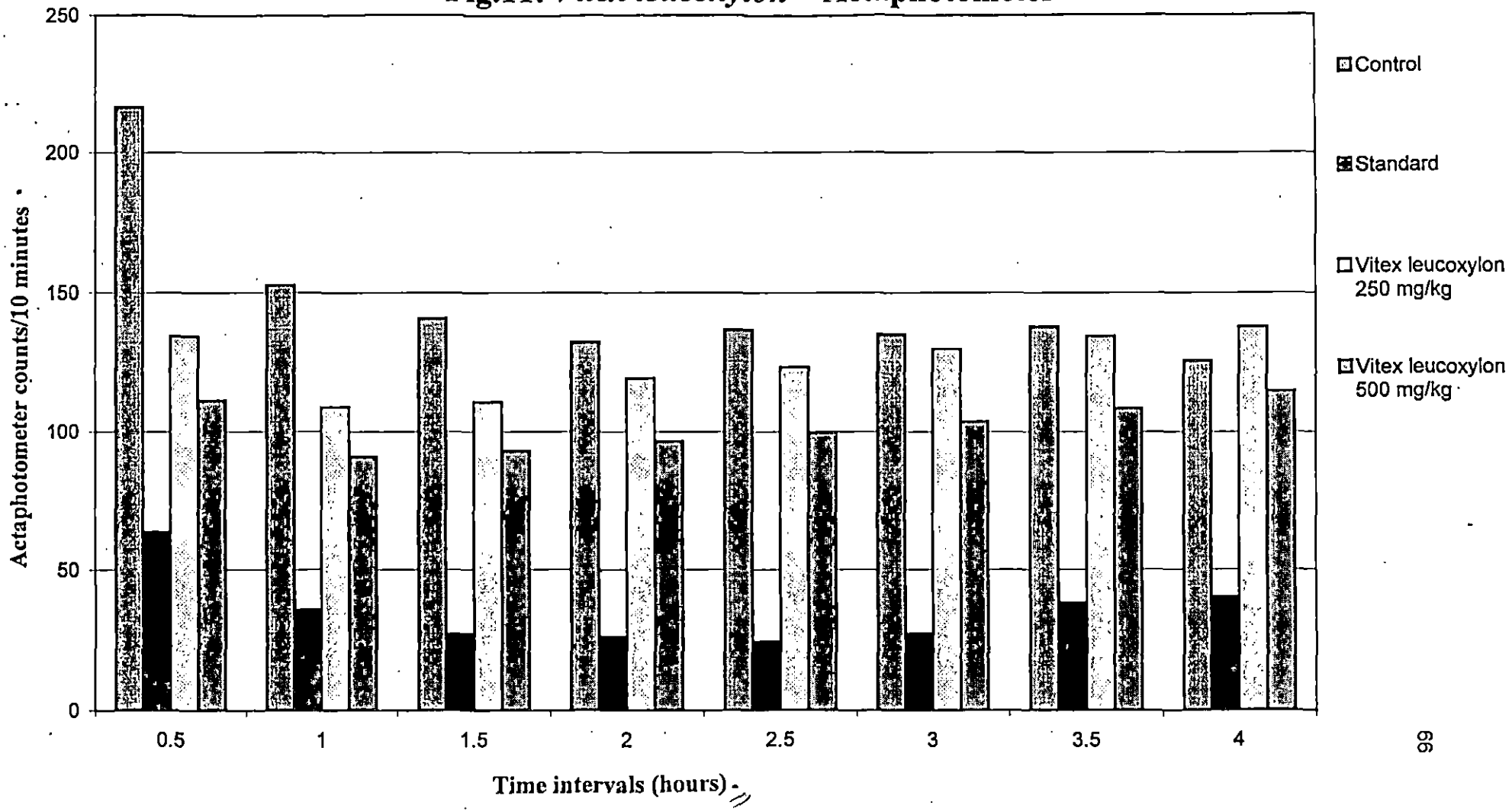
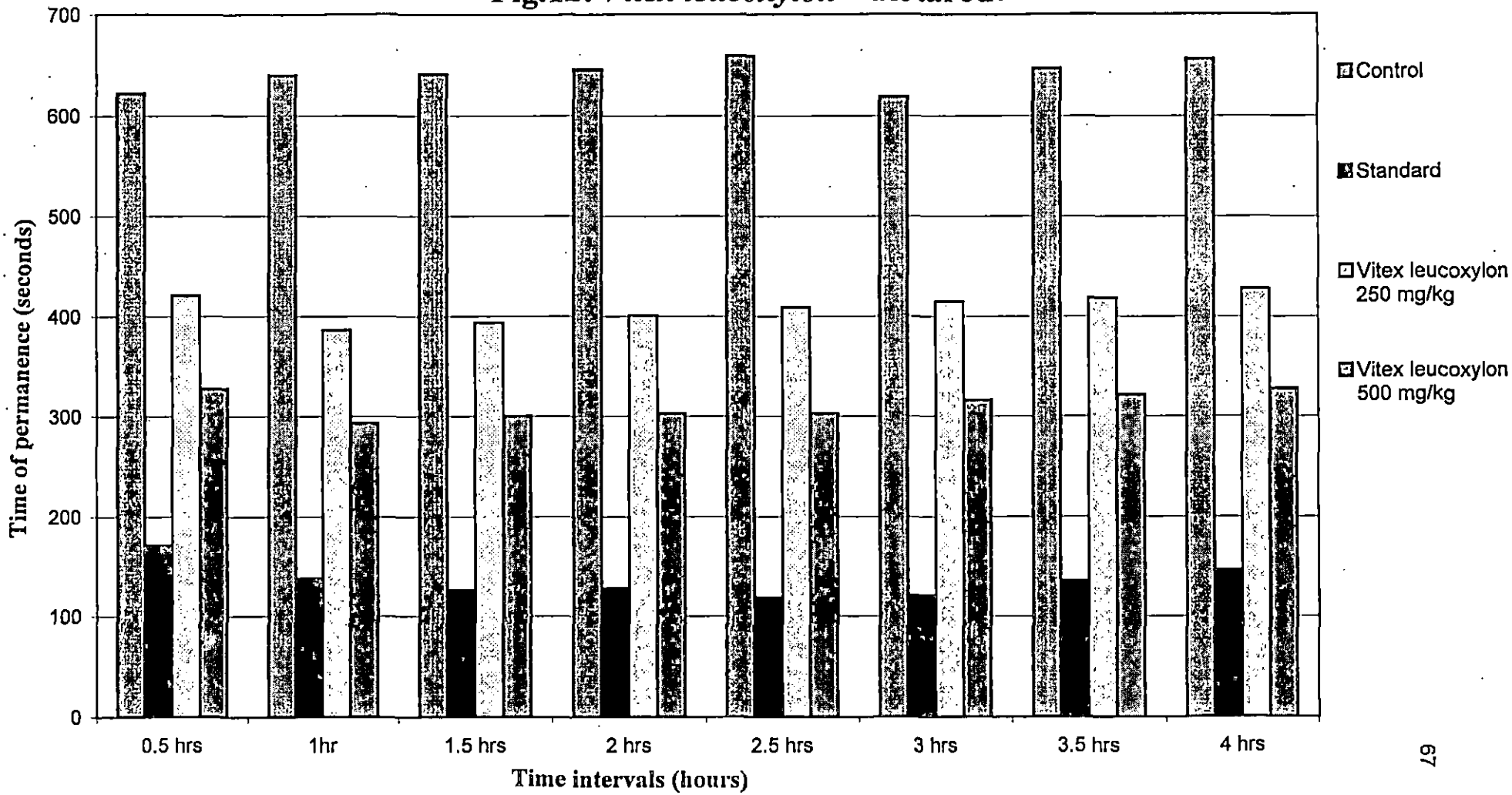


Fig.12: *Vitex leucoxylon* - Rotarod.



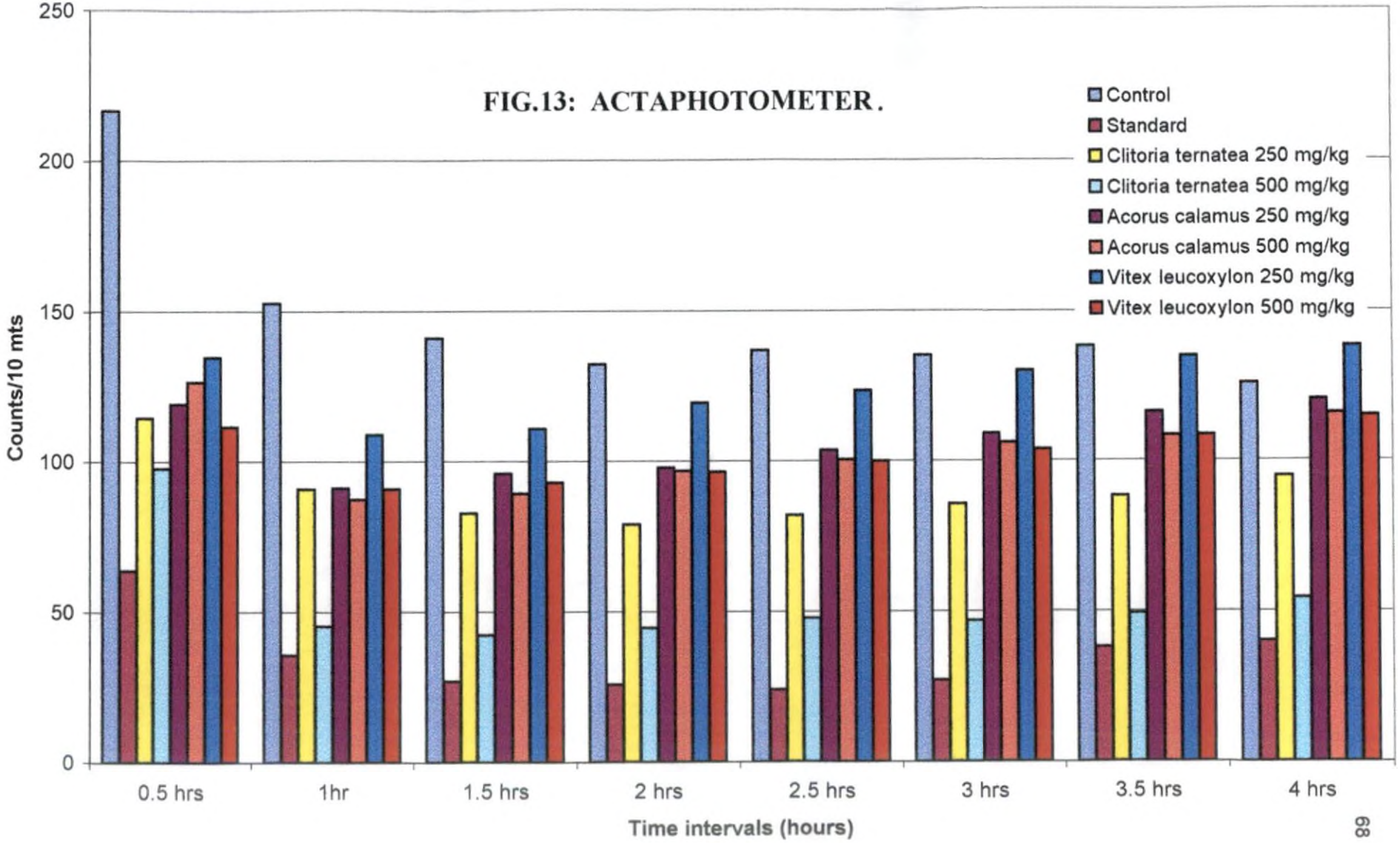
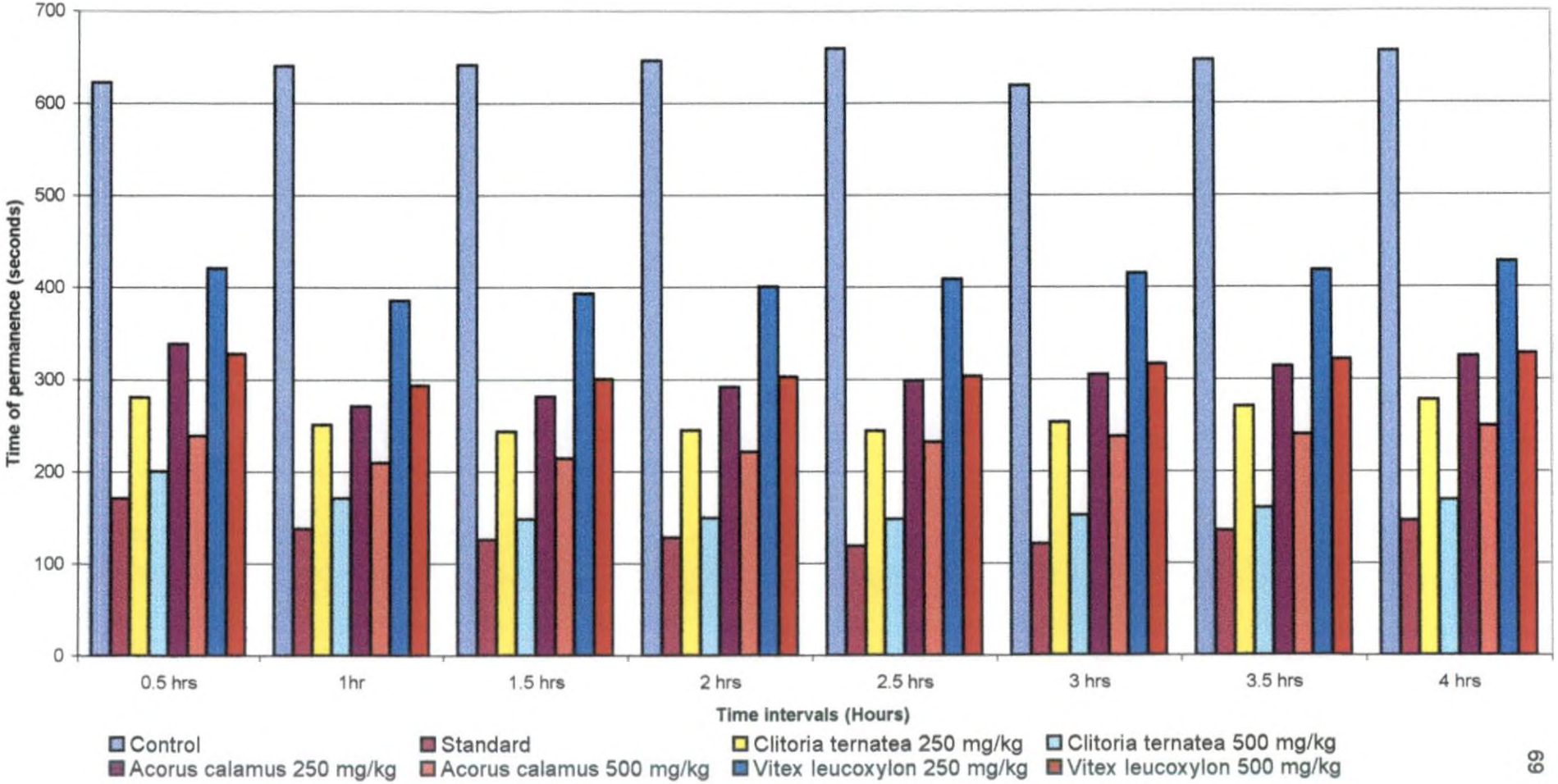


FIG.13: ACTAPHOTOMETER.
Control
Standard
Clitoria ternatea 250 mg/kg
Clitoria ternatea 500 mg/kg
Acorus calamus 250 mg/kg
Acorus calamus 500 mg/kg
Vitex leucoxylon 250 mg/kg
Vitex leucoxylon 500 mg/kg
Counts/10 mts
250
200
150
100
50
0
0.5 hrs
1hr
1.5 hrs
2 hrs
2.5 hrs
3 hrs
3.5 hrs
4 hrs
Time intervals (hours)

FIG : 14 ROTA-ROD.



DISCUSSION

5.DISCUSSION

The objectives of present study were to (1) assess the tranquillizing properties of the plants namely *Clitoria ternatea, Acorus calamus* and *Vitex leucoxylon* and (2) to compare the tranquillizing properties of above plants among themselves and to that of standard drug chlorpromazine.

The methods used to assess tranquillizing properties were measurement of spontaneous motor activity, measurement of forced motor activity and aggressive behavioral test score.

Spontaneous activity is measured by counting the spontaneous movement of the treated rat in an isolated cage. As the degree of tranquillization increases, the number of spontaneous movements decreases. Turner (1965) described one method to assess tranquillization as counting of spontaneous motor activity, which is counted by an actaphotometer as number of light beam interruptions, which gave the number of spontaneous movement. It was then analysed statistically for significance.

Forced locomotor activity can also be used as a method of assessing the degree of tranquillization (Turner, 1965) as it directly indicate the motor in co-ordination as an effect caused by the action of tranquillization on the motor centres of brain. This is done with a rota rod. Interpretation of result is done in two ways. In the first method if the time of permanence in less than a particular cut off time, the animal is said to be tranquillized. (Dunham and Miya, 1957 quoted by Turner, 1965). In the second method, the time of permanence of the test group is compared with a control group and analysed statistically for significance. (Morais *et al.* 1998).

Another method that can be used to assess tranquillization property is aggressive behaviour test score. Baldessarini (1996) reported that

tranquillizers could reduce aggression and behavioural activation either environmentally or pharmacologically induced. The aggressive behavioural test scores thus directly indicate a decrease in aggressive behaviour of rats and this gives a measure of tranquillization (Garin-Anguilar, 2000).

Clitoria ternatea possess prominent central nervous system depressant effects. At the dose rate of 500 mg/kg, it considerably reduced spontaneous locomotor activity as much as the standard drug did by one to four hours. With the same dose a significant reduction in forced locomotor activity was also observed when compared to control group. At the dose rate of 250mg/kg, significant reduction in spontaneous locomotor activity was observed when compared to control group, but this effect was lesser than the effects made by the standard drug. Also there was a significant reduction in the forced locomotor activity at this dose rate. Aggressive behaviour was reduced at 250 mg/kg and 500 mg/kg dose rates.

At the dose rate of 250mg/kg maximum depression of spontaneous motor activity was observed at two hours and maximum depression of forced locomotor activity was observed at 1.5 – 2 hours. At the dose rate of 500 mg/kg, maximum depression of spontaneous activity and forced motor activity was observed at 1.5 hours.

The above results confirms the finding of Kulkarni *et al.* (1988) that alcoholic extract of *Clitoria ternatea* possess tranquillization property as proved by increased sedation, inhibition of conditioned avoidance response, prolongation of time for spatial discrimination, dose dependant reduction in spontaneous motor activity and reduction in body temperature. Its antipyretic (Nadkarni, 1976) and antipsychotic properties (Vasudevan, 1982 quoted by Kulkarni, 1988) are well known in Ayurveda. The exact mechanism of tranquillization is yet to be explored. Chemical analysis of

plant parts yielded certain glycosides as anthocyanines and ternatins, whose activity is not investigated (Terahara *et al.*, 1998). The central nervous system depressant activities of *Clitoria ternatea* is mostly similar to the effects produced by chlorpromazine except that it does not produce cataleptic effect even at higher dose rates (Kulkarni *et al.*, 1988). So further investigation are required to find out exact mechanism of action.

Acorus calamus possesses significant central nervous system depressant activity at 250 mg/kg and 500 mg/kg does rate when compared to control. But even at higher dose level, the effect on depression of spontaneous and forced motor activity was significantly less than the standard drug. Maximum depression of spontaneous and forced motor activities were observed at one hour after administration of drug at both dosage levels, which indicate the peak tranquillization effect at both dosage rates at one hour. Panchal *et al.* (1989) reported that maximum effect was obtained at 1.5 hours when 25 mg/kg and 50 mg/kg dose rates were administered.

At both dose rates, aggressive behaviour was much reduced. This coincides with the findings of Vohora *et al.* (1990).

The effects of *Acorus calamus* @ 250 mg/kg on spontaneous and forced motor activities were less than *Clitoria ternatea* @ 250 mg/kg. Also the effect of *Acorus calamus* @ 500 mg/kg was less effective than *Clitoria ternatea* 500 mg/kg. In fact *Acorus calamus* @ 500 mg/kg was less effective than *Clitoria ternatea* @ 250 mg/kg in reducing spontaneous motor activity.

These results coincide with the findings of Panchal *et al.* (1989) that alcoholic extract of *Acorus calamus* can antagonize spontaneous motor activity. Also it coincides with the reports of Vohora *et al.* (1990) that ethanolic extract of *Acorus calamus* rhizomes produce sedative and tranquillizing action.

There are studies done on active ingredients of *Acorus calamus* extract. Santhiavathy *et al.* (1976) reported that some plants of Araceae such as *Acorus calamus*, *Asarum eurpoeum* etc., are found to contain an alkaloid called α-asarone (1 – propanyl-2,4,5 methoxybenzol) etherophenol and another compound β-asarone. α-asarone and β-asarone prolonged hypnosis caused by pentobarbital and α-asarone in small quantity can potentiate the effect of chlorpromazine while β-asarone did not. Belova *et al.* (1985) reported that the LD_{50} of asarone for mice was 417.6 mg/kg for enteral administration and it had activities as tranquillizer, sedative, spasmolytic and anti ulcer. Vohora *et al.* (1990) found out that ethanolic extract of *Acorus calamus* rhizomes exhibited a large number of actions similar to α - asarone but differed in several other respects as response to electroshock, aggressive behaviour, behavioural despair syndrome etc. They proposed that *Acorus calamus* might contain other chemical substances yet to be isolated. Liao *et al.* (1998) reported that some components but not α-asarone binds with D_1 and D_2 dopamine receptor and $GABA_A$ receptors.

Vitex leucoxylon also found to possess central nervous system depressant effect. At both dose rates of 250mg/kg and 500 mg/kg, it reduced both spontaneous and forced locomotor activity significantly when compared to control group with maximum effect at one hour. But overall effect and peak effect were very less when compared to the standard drug. This drug possessed least effect among the three drugs. But it reduced the aggressive behaviour, the effect almost comparable to standard drug group.

These findings coincides with the findings of Makwana *et al.* (1994) that cold aqueous infusion and ethanol extract of *Vitex leucoxylon* has central nervous system depressant properties.

Hematological examination of animals revealed all animals in normal hematological range and comparable to control group. The standard group showed a decrease in total erythrocytic count.

The exact mechanism by which these plants extracts produce central nervous system depressant effect is not yet documented. Locomotor system is under the control of substantia nigra and basal ganglia, where the predominant excitatory neurotransmitter is dopamine. This is evidenced by the fact that chronic intracerebral infusion of dopamine into mesolimbic system in rats resulted in an enhanced locomotor activity (Costal *et al.*, 1982). It was supported by findings of Miyamoto *et al.* (1984) that same dopamine agonists exert motor stimulatory actions by enhancing the release of dopamine from nerve terminals in the mesolimbic dopamine system and findings of Campbell *et al* (1985) that dopamine administered intracerebrally into nucleus accumbans septi induced strong locomotor arousal effect in rats. Bradbury *et al.* (1984) reported that small doses of dopamine and its agonists can influence dopamine receptors which are sensitive to neuroleptic drugs to effect inhibition of motor activity. Schaeffer and Michael (1984) reported that chlorpromazine and haloperidol, which act as antidopaminergic drugs in brain could produce graded decrease in spontaneous locomotor activity. Kiraly and Ree (1984) quoted Seeman's classification of dopamine receptor system as D_1, D_2, D_3 and D_4 receptor sites. The D_4 is responsible for hypolocomotion, which is sensitive to small doses of dopamine agonists and antagonists. The D_2 receptor site is sensitive to larger doses of dopamine and mediates hyperlocomotion, which is sensitive to small doses of dopamine agonists and antagonists. D_3 receptors are also involved in

inhibition of dopamine release and locomotor activity (Levant, 1997). In another classification they quoted that the dopamine receptors as pre-synaptic and post synaptic Activation of pre-synaptic result in diminished dopamine output and so diminished locomotor activity. Iversen (1984) quoted that 5-hydroxy tryptamine system is also involved as forebrain 5-HT raphae/nigro striatal mechanisms inhibit general output of basal ganglia. Moreover there are opiate receptors and inhibitory neurotransmitters as GABA in the central nervous system, which can suppress the motor activity when stimulated. Benton *et al.* (1984) reported that opiate receptor antagonists could produce a "non-emotional" animal and decrease social investigation and latency to attack. Besides, nor epinephrine system may also involved, where reduced nor-epinephrine in the system may produce hypolocomotion.

Dopamine is the major neurotransmitter related to behaviour of the animals as it forms the major neurotransmitter in the limbic system, the area that controls the behaviour and emotions. Silverstone and Cookson (1988) reported the key role of dopamine pathways in the pathogenesis of maniac disorders in humans. Mascarenhas (1978) reported that electrical stimulation of caudate nucleus, where the main receptors are dopaminergic, could induce an aggressive behaviour in cats manifested by piloerection, hissing, growling and attacking.

Convincing evidences are obtained to believe that the plant extracts administered can alter the neurotransmitter levels in brain. Sathiavathy *et al.* (1976) quoted that sedative effect of asarone, the active principle of *Acorus calamus* was dependant on depression of ergotropic division of hypothalamus. Viola *et al.* (1994) demonstrated pharmacologically active benzodiazepine receptor ligand from *Tilia tomentosa*. Liao *et al.* (1998) reported the presence of components that bind with D_1 and D_2 dopaminergic

receptors and $GABA_A$ receptor from *Acorus gramineus*. Nalini *et al.* (1995) reported that celastrus oil, isolated from *Celastrus paniculatus* when given orally reduced the nor-epinephrine, dopamine and serotonine levels in the brain. Hsieh *et al.* (1996) reported that water fraction of *Hemerocallis flava* when administered orally reduce the motor activity, the mechanism may be related to decrease in catecholaminergic activity in brain stem. The reason for tranquillization produced by active ingredient/ ingredients of plant extracts may be any of the above mechanism.

Based on the results of experimentation it can be concluded that alcoholic extract of three plants tested possess tranquillizing properties. The order of effect is *Clitoria ternatea* with the maximum, *Acorus calamus* slightly lesser and *Vitex leucoxylon* with least tranquillizing effect. All the plants are less effective than standard drug, chlorpromazine (Table 13 and 14).

SUMMARY

6.SUMMARY

The present study was undertaken in albino rats to evaluate tranquillizing effect of alcoholic extract of whole plant of *Clitoria ternatea*, roots and rhizomes of *Acorus calamus* and leaves of *Vitex leucoxylon* each at dose rate of 250 mg/kg and 500 mg/kg and to compare with a standard tranquillizing drug chlorpromazine at the dose rate of 7 mg/kg.

Sixty-four adult healthy albino rats in eight groups half of them males and half females were used for the study with eight rats in each group. The parameters observed to assess tranquillization were spontaneous locomotor activity, forced locomotor activity and reduction in aggressive behaviour. Spontaneous locomotor activity was measured using actaphotometer, forced locomotor activity measured using rota rod and reduction in aggressive behaviour using behavioural test score. Haematological studies like total erythrocytic count, total leucocytic count, differential leucocytic count and haemoglobin estimation were also done in control, standard and test group.

Clitoria ternatea at the dose rate of 250 mg/kg produced statistically significant level of tranquillization when compared to control group. Peak effect was observed at 1.5-2 hours with an actaphotometer count of 79.00 ±6.523 at two hours compared to 132.25 ±10.686 of control and rota rod permanence time of 243.625 ± 11.052 seconds when compared to 641.375 ± 15.793 of control group. But the effect was significantly less than standard drug. Aggressive behaviour was reduced as indicated by a score of three, compared to 8-11 of control group. Haematological parameters were normal.

Clitoria ternatea at the dose rate of 500 mg/kg produced maximum level of tranquillization when compared to control group. Peak effect was observed at 1.5 hours with an actaphotometer count of 42.25 ± 2.043 compared to 140.875 ± 11.148 of

control and rota-rod permanence time of 148.25 ± 11.621 seconds when compared to 641.375 ± 15.793 of control group. At certain time intervals the effects were comparable to the standard drug. The effect was significantly greater than *Clitoria ternatea* 250 mg/kg. Aggressive behaviour was reduced as indicated by a score of three, compared to 8-11 of control group. Haematological parameters were normal.

Acorus calamus at the dose rate of 250 mg/kg produced peak effect at 1 hour with actaphotometer count of 91.125±5.662 and rota-rod time of 271.00±13.612 seconds when compared to 172.625±5.105 and 640.25±12.219 seconds of control group. The effect was significantly less than standard drug. Aggressive behaviour was reduced as indicated by a score of three, compared to 8-11 of control group. Haematological values were normal.

Acorus calamus at the dose rate of 500 mg/kg produced peak effect at 1 hour with actaphotometer count of 87.371±4.674 and rota-rod time of 209.375±6.753 seconds when compared to 172.625±5.105 and 640.25±12.219 seconds of control group. The effect was significantly less than standard drug. The effect was significantly greater than *Acorus calamus* 250 mg/kg. Aggressive behaviour was reduced as indicated by a score of three, compared to 8-11 of control group. Haematological parameters were normal.

Vitex leucoxylon at the dose rate of 250 mg/kg produced peak effect at 1 hour with actaphotometer count of 109.00±3.468 and rota-rod time of 386.125±7.474 seconds when compared to 172.625±5.105 and 640.25±12.219 seconds of control group. The effect was significantly less than standard drug. Aggressive behaviour score was reduced to four. This was the drug with least effect when compared to the standard. Aggressive behaviour was reduced as

indicated by a score of three, compared to 8-11 of control group. Haematological values were normal.

Vitex leucoxylon at the dose rate of 500 mg/kg produced peak effect at 1 hour with actaphotometer count of 90.875±3.907 and rota-rod time of 293.25±8.927 seconds when compared to 172.625±5.105 and 640.25±12.219 seconds of control group. The effect was significantly less than standard drug. The effect was significantly greater than *Vitex leucoxylon* 250 mg/kg. Aggressive behaviour was reduced as indicated by a score of three, compared to 8-11 of control group. Haematological values were normal.

From the effect, it can be concluded that all the plant parts possess tranquillizing property. Maximum effect was produced by *Clitoria ternatea* followed by *Acorus calamus* and *Vitex leucoxylon* possess least effect, their higher doses were more effective in producing tranquillization than their respective lower dose levels. All of them were less effective than chlorpromazine.

The exact mechanism of tranquillization by these plant parts and their possible toxicities needs further detailed study.

REFERENCES

REFERENCES

Abraham, M.E. and Gogate, M.G. (1989). Effect of stress on behaviour in rats. *Indian J. Physiol, Pharmacol.* 33(2):84-88.

Asuzu, I.U., Ezejiofor, S. and Noicku, C.J. (1998). The pharmacological activities of *Olax viridis* root bark extract on central nervous system. *Fitotherapia*, Vol. LXIX, No.3, pp. 260 – 264.

Baldessarini, R.J. (1996). Drugs and treatment of psychiatric disorders-psychosis and anxiety in Goodman and Gilman's The Pharmacological basis of therapeutics. Ed. Hardman. J.G. Limbird, L.E. 9th edn. McGraw Hill. pp. 399-412.

Barns, C.D and Eltherington, L.G. (1973). Drug dosage table-Chlorpromazine in Drug Dosage in laboratory animals. A hand book 2nd revised edn. University of California, Press, Berkeley and London.p.

*Belova, L.F., Alibekov, S.D., Baginskaia, A.I., Sla, S., Pokrovskaia, G.V. (1985). Asorone and its biological properties. *Farmkol Toksikol.* 48(6): 17-20.

Benton, D., Brain, S and Brain, P.F. (1984). Comparison of the influence of the opiate delta receptor antagonist, IC154, 129 and naloxone

on social interaction and behaviour in an open field. *Neuropharmacology*. **23**(1): 13-17.

Bradbury, A.J., Costall, B and Naylor R.J. (1984). Inhibition and facilitation of motor responding of the mouse by actors of dopamine agonist in the forebrain. *Neuropharmacology*. **23**(9): 1025-1031.

Campbell, A., Baldessarini, R.J., Teicher, M.H. and Neumeyer, J.L. (1985). S(+) Apomorphines – selective inhibition of excitatory effect of dopamine injected into the limbic system of rats. *Neuropharmacology*. **24**(5): 391-399.

Costal, B., Domeney, A.M. and Naylor, R.J. (1982). Behavioural and biochemical consequences of persistant overstimulation of mesolimbic dopamine systems in the rat. *Neuropharmacology*. **21**: 327-335.

Council of Scientific and Industrial Research (1950). The Wealth of India, Vol. I. pp. 28, 233, 522.

Dodman, N.H. and Shuster, L. (1994). Pharmacologic approaches to managing behavioural problems in small animals. *Veterinary Medicine*. **89**(10): 960-969.

Garin – Anguilar, M.E., Luna, J.E.R, Hernandez, M.S., Toro, G.V.D. and Vazquez, M.M. (2000). Effect of crude extract of *Erythrina*

americana Mill. on aggressive behaviour in rats. *J. Ethnopharmacol.* **69**: 189-196.

Gross, M.E. and Booth, N.H. (1995). Tranquillizers,alpha-2adrenergic agonists and related agents in Veterinary pharmacology and therapeutics. Ed. Adams, R. 7th Edn. IWOA state University press/AMES. pp. 311-318.

*Gupta M., Mazumder U.K.and Bhawal S.R. CNS activity of *Vitex negundo* Linn. in mice. *Indian J. Exp. Biol.* 1999 Feb;37(2): 143-146.

Hellion–Ibarrola, M.C., Iburrola, D.A., Montalbetti, Y., Villalba,D., Heinichien, O. and Ferro, E.A (1999). Acute toxicity and general pharmacological effect on central nervous system of crude rhizome extract of *Kyllinga brevifolia* Rottb. *J. Ethnopharmacol.* **66**: 271-276.

Henriques, A.T., Melo, A.A., Moreno, P.R.H., Ene, L.L., Henriques, J.A.P and Schapoval, E.E.S. (1996). *Ervatamia coronaria*: Chemical constituents and some pharmacological activities. *J. Ethnopharmacol.* **50**: 19-25.

Hrapkiewics,K., Medina,L and Holms,D.D (1998).Appendix one in Clinical laboratory animal medicine. 2nd edn. .Iowa state university press. p. 259.

Hsieh, M.T., Ho, Y.F., Peng, W.H., Wu, C.R., Chen, C.F. (1996). Effect of *Hemerocallis flava* on motor activity and concentration of central monoamines and its metabolites in rats. *J. Ethnopharmacol.* 52: 71-76.

Iversen, S.D. (1984). 5-HT and anxiety. *Neuropharmacology.* 23(12b): 1553-1560.

Jaiswal, A.K., Bhattacharya, S.K. and Acharya, S.B. (1994). Anxiolytic activity of *Azadirachta indica* leaf extract in rats. *Indian. J. Exp. Biol.* **32**: 489 – 491.

Kamboj,V.P.(2000). Herbal medicines. *Curr. Sci.* **78**: 35-39.

Khory, R.N.S. and Katrak, N.N. (1984). Materia medica of India and their therapeutics. 2nd edn. Neeraj Publishing House, Delhi. pp. 205,627.

Kiraly, I. and Ree, J.M.V. (1984). Non-opiate B-endorphine fragments and dopamine – IV. *Neuropharmacology.* 23(5): 511-516.

Kulkarni, C., Pattanshetty, J.R.and Amruthraj, G. (1988). Effect of alcoholic extract of *Clitoria ternatea* Linn. on central nervous system in rodents. *Indian. J. Exp. Biol.* 26(12): 957-960.

Lees, P (1993). Sedative, anticonvulsant, central muscle relaxant and analgesics. Veterinary applied pharmacology and therapeutics.

Ed. Brander G.C., Pugh, D.M., Bywater, R.J., Jenkins W.L. 5th edn. ELBS. pp. 331.

Levant, B. (1997). The D_3 dopamine receptor: Neurobiology and potential clinical relevance. *Pharmacological Reviews*. **49**(3): 231-252.

Liao, J.F., Huang, S.Y., Jan Y.M., Yu, L.L. and Chen C.F. (1998). Central inhibitory effect of water extract of *Acori graminei* rhizoma in mice. *J. Ethnopharmacol.* **61**: 185-193.

Lu, M.C. (1998). Studies on the sedative effect of *Cistanche deserticola*. *J. Ethnopharmacol.* **59**: 161-165.

Lutterodt, G.D. and Maleque, A. (1988). Effect on mice locomotor activity of a narcotic like principle from *Psidium guajava* leaves. *J. Ethnopharmacol.* **24**:219-231.

Makwana, H.G., Ravishankar, B., Shukla, V.J., Nair, R.B., Vijayan, N.P., Sasikala, C.K., Saraswathy, V.N. and Bhatt, S.V. (1994). General Pharmacology of *Vitex leucoxylon* Linn leaves. *Indian J. Physiol. Pharmacol.* **38**(2): 95-100.

Mascarenhas, J.F., Dhume, M.G., Gogate, M.G. and Gopalakrishnan, R. (1978). Modulation of hypothalamically induced aggressive behaviour following electrical stimulation of caudate nucleus. *Indian J. Med. Res.* **67**: 835-843.

Miyamoto, M., Narumi, S., Nagai, Y., Saji, Y and Nagawa, Y(1984). A TRH – analogue (DN – 1417): Motor stimulation with rearing related to catecholaminergic mechanism in rats. *Neuropharmacology*. **23**(1): 61-72.

Monteiro, F., Abraham, M.E., Sahakari, S.D. and Mascarenhas, J.F., (1989). Effect of immobilization stress on food intake, body weight and weights of various organs in rats. *Indian J. Physiol. Pharmacol.* **33**(3): 186-190.

Morais, L.C.S.L., Barbosa-Filho, J.M. and Almeida, R.N. (1998). Central depressant effect of reticuline extracted from *Ocotea duckei* in rats and mice. *J. Ethnopharmacol.* **62**: 57-61.

Mukerjee, P.K., Saha, K., Baksubramanian, R., Pal., M. and Saha, B.P. (1996). Studies on psychopharmacological effects of *Nelumbo Nucifera* Gaertn. rhizome extract. *J. Ethnopharmacol.* **54**: 63-67.

Nadkarni, A.K. (1976). Dr. K.M. Nandkarni's Indian Materia Medica. pp. 35,355,1278.

Nalini, K., Karanth, K.S., Rao, A., Aroor, A.R. (1995). Effect of *Celastrus paniculatus* on passive avoidance performance and biogenic amine turnover in albino rats. *J. Ethnopharmacol.* **47**: 101-108.

N'gouemo, P., Nguemby–Bina, C and Baldy–Moulinier,M.(1994). Some neuropharmacological effects of an ethanolic extract of *Maprounea africana* in rodents. *J. Ethnopharmacol.* **43**: 161-166.

Panchal, G.M., Bhatt, V.H., Doctor, R.B. and Vajpayee, S. (1989). Pharmacology of Acorus calamus L. *Indian J. Exp. Biol.* **27**(6): 561-567.

Perez G, R.M., Perez L, J.A., Garcia D,L.M and Sossam, H (1998). Neuropharmacological activity of *Solanum nigrum* fruit. *J. Ethnopharmacol.* **62**: 43-48.

Reynolds, J.E.F. (1993). Anxiolytic sedatives hypnotics and neuroleptics in Martindale The extra pharmacopoeia. 13th edn. The pharmaceutical Press, London. pp. 564-566.

*Ripperger, H (1978). Isolation of stigmast-4-ene-3,6-dione from *Hamelia patens* and *Clitoria ternatea*. *Pharmazie.* 33(2): 82-83.

Sahakari, S.D., Abraham, M.E., and Mascarenhas, J.F. (1989). Effect of stress on maternal behaviour. *Indian J. Physiol. Pharmacol.* **33**(2): 93-96.

Sathiavathi, G.V., Raina, M.K., Sharma, M. (1976). Medicinal plants of India, ICMR, New Delhi. pp. 19-20, 260.

Saxena, M.J. and Madan, P. (1997) Herbals for stress management in pets. *The Veterinarian.* **21**(1). pp. 11-14.

Schaefer, G.J. and Michael, R.P. (1984). Drug interactions on spontaneous locomotor activity in rats. *Neuropharmacology.* **23**(8): 909-914.

Schalm, O.W., Jain N.C and Caroll E.J.,(1975). Veterinary Haematology 3rd edn. Lea and Febriger, Philadelphia. p.54.

Silverstone, T and Cookson, J. (1988). Examining the dopamine hypothesis of schizophrenia and of mania using the prolactin response to antipsychotic drugs. *Neuropharmacology.* **22**(4): 539-544.

Singh, R.C.P. Yadava, K.P. and Roy. B.K. (1998). Preliminary studies on the central sedative and hypothermic effect of *Bumbusa bambos* leaves. *J. Medicinal. Arom. Plant. Sci.* **20**: 22-24.

Soulimani, R., Younos, C., Jarmouni, S., Bousta, D., Misslin, R. and Mortier,F (1997). Behavioral effect of *Passiflora incarnata* L and its indole alkaloid and flavonoid derivatives and maltol in the mouse. *J. Ethnopharmacol.* **57**: 11-20.

Taesotikul, T., Panthong, A., Kanjanapothi, D., Verpoorte, R., Scheffer. J.J.C (1998). Neuropharmacological activities of crude alkaloidal fraction from stems of *Tabernaemontana pandacaquie* Poir. *J. Ethnopharmacol.* **62**: 229-234.

*Terahara, N., Toki, K., Saito, N., Honda, T., Matsui, T. and Osajima, Y. (1998). Eight new anthocyanins, ternatins C_1-C_5 and D_3 and preternatins A_3 and C_4 from young *Clitoria ternatea* leaves. *J. Nat. Prod.* **61**(11): 1361-1367.

Tortoriello, J. and Anguilar-Santamaria, L. (1996). Evaluation of the calcium antagonist, antidiaorheic and central nervous system activities of *Baccharis serraefolia*. *J. Ethnopharmacol.* **53**: 157-163.

Tripathi, A.K. and Singh, R.H. (1995). Clinical study on an indigenous drug Vaca (*Acorus calamus*) in the treatment of depressive *illness*. *J. Res. Ayurveda. Siddha.* Vol. XVI, No. 1-2: 24-34.

Turner,R.A. (1965).Ataractic(tranquillizing, neuroleptic)agents in Screening methods in pharmacology, Academic press, New York and London. pp. 88-89.

Vale, T.G., Matos, F.J.A., de Lima, T.C.M. and Viana, G.S.B. (1999). Behavioural effects of essential oils from *Lippia alba* (Mill) NE Brown chemotypes. *J. Ethnophrmacol.* **167**: 127-133.

Viola, H., Wolfman, C., de Stein, M.L., Wasowski, C., Pena, C., Medina, J.H. and Paladini, A.C. (1994). Isolation of pharmacologically active benzodiazepine receptor ligands from *Tilia tomentosa* (Tiliaceae). *J. Ethnopharmacol.* **44**: 47-53.

Vohora, S.B., Kumar, I., Shah, S.A and Khan, M.S.Y. (1980). Effect of biflavonoids of *Taxus baccata* on the central nervous system. *Indian J. Med. Res.* **71**: 815-820.

*Vohora, S.B., Shah, S.A. and Dandia, P.C. (1990).. Central Nervous System studies on an ethnoal extract of *Acorus calamus* rhizomes. *J. Ethnopharmacol.* **28**: 53-62.

Wu, L.J., Sun.L.L., Li, M., Yang, H., Jiang, Z.R., Lu, Y., Tian, Z., Zheng, Q., Miyase, T. and Ueno, A(1994). Studies on the constituents of the roots of *Acorus calamus* L. *Yakugaku Zasshi.* **144**(3): 182-185.

Zia, A., Siddiqui, B.S., Begum, S., Siddiqui, S. and Suria, A. (1995). Studies on constituents of leaves of *Nerium oleander* on behavioural pattern in mice. *J. Ethnopharmacol.* **49**:33-39.

* - Originals not consulted.

TRANQUILLIZING PROPERTY OF *Clitoria ternatea* Linn. (Shankupushpam), *Acorus calamus* Linn. (Vayampu) AND *Vitex leucoxylon* Linn. (Atta nocchi) IN RATS

By
SURESH N. NAIR

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Pharmacology and Toxicology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680651
KERALA

2001

ABSTRACT

The study was conducted in sixty four adult albino rats of either sex to assess tranquillizing property of alcoholic extract of whole plant of *Clitoria ternatea*, roots and rhizomes of *Acorus calamus* and leaves of *Vitex leucoxylon* at two dose levels of 250 mg/kg and 500 mg/kg of body weight and compare the effect to the standard tranquillizer chlorpromazine at dose rate of 7 mg/kg. The control group was given gum acacia. The dose levels of these drugs were as follows

Plants	Amount of Extract (mg/kg)	
Clitoria ternatea	250(G1)	500(G2)
Acorus calamus	250(G3)	500(G4)
Vitex leucoxylon	250(G5)	500(G6)
Chlorpromazine	7(G7)	
Control (gum acacia)	7ml/kg(G8)	

Level of tranquillization was measured using three parameters (1) depression of spontaneous motor activity, measured using actaphotometer (2) depression of forced locomotor activity measured by decrease in time of permanence in a rota-rod and (3) decrease in aggressive behaviour, measured by aggressive behaviour test score.

Haematological parameters like total erythrocytic count, total leucocytic count, differential leucocytic count and haemoglobin percentage were determined to assess any change in haemogram by these drugs.

The results of present study were as follows. *Clitoria ternatea* possess maximum tranquillizing property among the three. At 250 mg/kg it produced significant depression of spontaneous motor activity and forced locomotor activity than control but less than the standard drug. At 500 mg/kg it produced almost similar effect, as standard at certain time intervals but altogether the effect was less than the standard. Aggressive behaviour was also reduced at both dose rates. *Clitoria ternatea* at 500 mg/kg was more effective than *Clitoria ternatea* 250 mg/kg.

Acorus calamus follows *Clitoria ternatea* in producing tranquillization. At 250 mg/kg as well as 500 mg/kg dose rates it produced significant depression of spontaneous motor activity, forced locomotor activity and aggressive behaviour. *Acorus calamus* at 500 mg/kg was more effective than *Acorus calamus* 250 mg/kg.

Vitex leucoxylon possess least effect among the three in producing tranquillization. But at 250 mg/kg as well as 500 mg/kg dose rates it produced significant depression of spontaneous motor activity, forced locomotor activity and aggressive behaviour. *Vitex leucoxylon* at 500 mg/kg was more effective than *Vitex leucoxylon* 250 mg/kg.

Haematological studies revealed no significant change in haemogram by any of the plant drugs.

The exact mechanism of tranquillization by these plants and their possible toxicities needs further detailed study