

Research Article

The phytochemical and central nervous system investigation of aqueous and ethanolic extracts of leaves of *Chromolaena odorata* L.

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ABSTRACT: **Introduction:** The effect of the ethanolic and aqueous extracts of leaves of *Chromolaena odorata* L. on the central nervous system of rats was evaluated using a set of pharmacological screening methods. **Materials and Methods:** A single dose (100 mg/kg) of ethanolic and aqueous extract of leaves of *Chromolaena odorata* was administered to the rats orally to assess the pharmacological activity. Preliminary phytochemical testing preceded pharmacological screening tests for locomotion and spontaneous behavior conducted on the first, fourth and seventh day of administration. The Locomotor activity was evaluated using Rotamex and spontaneous behavior was studied by using Optovarimex. Results were compared using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison's test. **Results:** On continuous administration of the extract, a dose dependent increase in spontaneous activity was observed in Optovarimex apparatus. There was an increase in distance travelled and a decrease in resting time in the Optovarimex instrument but no significant changes in ambulatory time and stereotypic time, while the DT of aqueous extract showed peak activity at 90 min on day 1. **Conclusion:** Preliminary phytochemical screening revealed that the *C. odorata* extract contains flavonoids, saponins, tannins, steroids and proteins. Our findings suggest that the extract has significant CNS stimulant activity. Furthermore, a marked increase in locomotor activity and an improvement in muscle coordination observed with the ethanolic leaf extract indicate that the above CNS activities may be attributed to the various phytoconstituents and antioxidants present in the leaf extract.

KEYWORDS: Autotrack, *Eupatorium odoratum*, Locomotor, Optovarimex, CNS stimulant

INTRODUCTION

Chromolaena odorata Linn. also known as bitter bush or Siam weed, is a scrambling shrub.^[1] It has been proven as an efficacious green manure in cassava cultivation and a nematode control in black pepper.^[2] Medicinal uses of *Chromolaena odorata* are anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory and diuretic agent.^[3] This plant is also used to treat burns, hemorrhages, hemorrhoids, indigestion, skin diseases, and infection.^[4] A decoction prepared from the flowers is used as tonic, antipyretic and heart tonic.^[4]

The modern system of medicine has contributed to the welfare of mankind. However there are limitations of this system. With the onset of scientific research into herbals, it is becoming clearer that the medicinal herbs have a potential in today's synthetic era. Ancient knowledge coupled with scientific principles can come to the forefront and provide us with powerful remedies to eradicate many diseases.^[5]

Anxiety and depression are frequently unrecognized and underdiagnosed by clinicians and thus remains untreated or inappropriately treated in routine clinical practice. Although the symptoms of both are widely acknowledged and recognized by clinicians, numerous epidemiological studies have reported that these disorders are more prevalent than had previously been thought, and that it is challenging to diagnose and treat, particularly because somatic symptoms and comorbid conditions are common in real clinical situations. Optimal treatment should include pharmacotherapy with CNS drugs designed to

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ensure complete remission including psychological and physical symptoms, as well as functional recovery.

CNS agents are invaluable therapeutically, because they can produce specific physiological and psychological effects to correct pathophysiological events of various neurological disorders.^[6] Drugs currently used in treatment of different neurological disorders including anxiety, depression, schizophrenia, epilepsy, Parkinsonism are either refractory, have serious side effects or possess unfavorable drug-drug/drug-food interactions. While the effects caused by CNS stimulants are dramatic, the therapeutic usefulness of these medications is limited due to their side effects.^[7]

Many CNS drugs are metabolized by P450 3A4 or 2D6 pathways, which are common substrates for innumerable CNS and non-CNS concomitant mediations, increasing the drug's risk-to-benefit ratio via potential drug-drug interactions and greater side-effect profiles. This low to no acceptable risk level seems to apply especially to disorders that appear to treat "lifestyle"-type ailments such as depression, anxiety, and attention deficit disorder, which the general public often misperceive as only impacting a person's temperament and quality of life, while having little to no effect on their overall health. However, it is well known that the risks of not treating mood disorders include increased morbidity and mortality from related medical illnesses and suicide, as well as the worsening of other purely "physical" ailments due to stress interactions and treatment noncompliance.

CNS drugs often start off in a relatively poorer position than drugs in other indications, not just because they are viewed as having a relatively higher risk and lower priority, but also because CNS drug developers are not routinely taking advantage of the regulatory tools available to them, such as Priority Review and Fast Track designation. There are innumerable CNS conditions that would be considered serious or life-threatening and therefore eligible for Fast Track designation. Given the lack of effective treatments, the growing number of treatment-refractory CNS patients, and the high degree of intolerable side-effects, many CNS development programmes would be considered to address an unmet medical need and be eligible for Fast Track designation. CNS drugs take longer to reach the market and are subject to greater attrition. The Fast Track designation enables early interaction with the FDA that can help to clarify elements of clinical study design whose deficiency or absence upon the submission of a new drug application (NDA) could delay approval decisions.

Central nervous system (CNS) stimulants are medicines that speed up physical and mental processes. The most

common side effects of CNS stimulants are irritability, nervousness, restlessness, loss of appetite, sleep problems, and a false sense of well-being.^[8] After these effects wear off, other effects may occur, such as trembling, drowsiness, unusual tiredness or weakness, or depression. These side effects and after effects usually go away as the body adjusts to the drug and do not require medical treatment unless they continue, or they interfere with normal activities.

Irrespective of the activity being screened or the kind of screening employed; the purpose of screening is to obtain stable and reproducible inferences with fewer false-positive and false-negative results. Design is a critical step in the formulation of any pharmacological investigation and many modern texts are available that describe in detail important forms, structures and facets of proper experimental design.^[9]

Screening method for motor coordination: Motor coordination is the harmonious functioning of body parts that involve movement, including gross motor movement; fine motor movement, and motor planning. Examples of motor coordination are the ease with which people can stand up, pour water into a glass, walk, and reach for a pen. These are created reliably, proficiently and repeatedly, but these movements rarely are reproduced exactly in their motor details, such as joint angles when pointing or standing up from sitting. The model employed in this study to assess the effect of the extract on motor co-ordination is the Rotarod method.^[10]

Rotarod method

The test is used to evaluate the activity of drugs interfering with motor coordination by assessing the ability of mice or rats to remain on an accelerating revolving rod.^[10] The rotating rod is divided into four lanes separated by screens. The mouse must walk forward to balance on the rotating center bar. This forced motor activity has subsequently been used by many investigators. Only those animals which demonstrate their ability to remain on the revolving rod for at least 1 minute are used for the test. Latency to fall is used as an index to determine activity. Rotarod performance evaluates motor coordination and balance. Improvement in rotarod performance across repeated daily sessions provides a measure of motor learning.^[11] There are other models also described by Vogel to determine muscle co-ordination. Some of them include: inclined plane, chimney test, grip strength and masticatory muscle reflexes.^[10]

Autotrack (Optovarimex): The auto-track is an advanced automated Open Field Activity Monitor system using the

latest technology to quantify locomotor activity and trace the animal's path for behavioural analysis.^[10] The autotrack system senses motion with a grid of infrared photocells placed around a specified arena. Vertical motion is detected by a second array of photocells placed above the animal. The simultaneous interruption of beams along the horizontal axis (X&Y) provides coordinates that identify animal location. Vertical motion is scored and stored with horizontal position data. Autotrack records these coordinates for later playback and analysis. Stereotypic time is automatically recorded. Stereotypy is defined as the repetitive performance of behaviour without apparent purpose and is recorded automatically by the instrument. A break in stereotypy of 1 second or more is required to separate one stereotypic episode from the next. If the animal breaks the same beam (or set of beams) repeatedly then the monitor considers that the animal is exhibiting stereotypy. This typically happens during grooming, head bobbing, etc. The investigation of stereotyped motor activity has elucidated the role of various brain mechanisms in the behaviour of animals and humans.^[10]

The rotarod performance test is a performance test based on a rotating rod with forced motor activity being applied, usually by a rodent. The test measures parameters such as riding time (seconds) or endurance. Some of the functions of the test include evaluating balance and coordination of the subjects; especially in testing the effect of experimental drugs.

The length of time that a given animal stays on this rotating rod is a measure of their balance, coordination, physical condition, and motor-planning. The speed of the rotarod is mechanically driven, and may either be held constant, or accelerated.

Because of concern for impairment in human motor behavior from the use of prescription medications, the rotarod test is frequently used in early stages of drug development to screen-out drugs that might later cause subtle impairments, which might not be detected epidemiologically in a human population for a very long time.^[12] Psychoneural drugs like benzodiazepines are commonly employed in anxiety, depression, epilepsy and insomnia but possess side effects like cognitive function, physical dependence and tolerance.^[13]

Suitable manipulation of the recorded pattern by analysis methods can reveal subtle changes in animal behavior over the course of an experiment, between experiments, and/or between animals.

Drugs acting in the CNS were among the first to be discovered by primitive humans and are still the most widely

used group of pharmacologic agents. In addition to their use in therapy, many drugs acting on the CNS are used without prescription to increase the sense of well-being. Drugs are among the most important tools for studying all aspects of CNS physiology, from the mechanism of convulsions to the laying down of long-term memory. Unraveling the actions of drugs with known clinical efficacy has led to some of the most fruitful hypotheses regarding the mechanisms of disease. For example, information about the action of antipsychotic drugs on dopamine receptors has provided the basis for important hypotheses regarding the pathophysiology of schizophrenia. Studies of the effects of a variety of agonists and antagonists on γ -aminobutyric acid (GABA) receptors have resulted in new concepts pertaining to the pathophysiology of several diseases, including anxiety and epilepsy.

Attention is turning back to natural products as drug sources, since they have been so successful in the past. Modern medicine depends on biological materials as an incomparable source of molecular diversity. Cures as yet undiscovered may exist in plants as yet undescribed—and which may never be described.

Although identifying hits and leads from secondary plant metabolites continues to be a major goal of many drug discovery programs, few concentrate on development of CNS therapies. In addition, only a small number of the studies of neurological activities of herbal extracts and their active constituents have been subsequently evaluated in terms of their potential for identifying structurally or functionally novel CNS drugs. In light of their proven clinical efficacy and their largely unexplained mode of action, these herbal remedies should receive more attention as readily available sources for structurally and functionally novel sources of CNS drugs.

Vernacular names of *Chromolaena odorata* include English (Siam Weed, Bitter bush), Hindi (Tivra gandha), Konkani (Ranmare), Malayalam (Venappacha), Filipino (Agonoi), French (Fleurit-Noel), Kosraean (Mahsrihsrihk) and Spanish (Chimuyo).

Chromolaena odorata has been widely studied for its antimicrobial study viz. antibacterial, antifungal and antiprotozoal studies.^[14] But CNS studies of the plant have not been done. No taxonomic similarities with other plants with known activity was found. Ethno-botanical studies have shown that plant extracts contain flavonoids which may have role in CNS activity.^[14] So the present study was, undertaken to evaluate the potential of the ethanolic and aqueous extract of the leaves of *C. odorata* L. for Central nervous system activity in rats.

MATERIAL AND METHODS

Pharmacological investigation of ethanolic and aqueous extract of leaves of *Chromolaena odorata* Linn. for central nervous system activity

Plant material

The leaves of several plants of various parts of *C. odorata* L. were collected in Panjim. Several equal-sized leaf samples of *C. odorata* L. were collected from field-sites within one day in Feb 2012. Fresh leaves of healthy plants were collected. The crude extracts of the leaves of several plants of various parts of *C. odorata* were prepared separately using ethanol and purified water. They were identified by Dr. Janarthanam Department of Botany, Goa University, Goa, India.

Preparation of extract

The collected leaves were washed thoroughly under running water. They were chopped into small pieces and dried in the shade for 17 days. The pieces were then ground into coarse powder using an electric blender. The coarse powder of the leaves was soaked in the respective solvents (ethanol HPLC Grade (Merck labs) and purified water) and subjected to maceration every 4 days with occasional shaking. After 4 days the solvent layer was decanted off. The process was repeated 4 times with fresh solvent. The solvent from the total extract was evaporated to dryness under rotary evaporator to give the dry extract. The extract was then stored in refrigerator at 12–18 °C until used.

Rats were individually weighed and the required dose was calculated for every rat based on their body weight. Since the dose selected was 100 mg/kg, for a rat weighing 200 g, 20 mg of the extract was administered. For this 100 mg of the extract was dissolved in 5 ml of Tween 80 thereafter 1 ml of the extract was given to rats. This was used to test for muscle coordination and locomotor activity.

Chemicals

All commercial solvents were of analytical grade except ethanol (HPLC Grade) were used without any further purification. Biochemical reagents used were freshly prepared. All reagents obtained from Merck labs Goa, India.

Phytochemical screening

The freshly prepared extract of the leaves of *C. odorata* L. was subjected to phytochemical screening.^[15]

Experimental animals

Wistar albino rats of either sex, 12–14 weeks old, weighing 180 ± 20 g were employed for this study. The animals were housed in polypropylene cages maintained under standard condition (temperature 25 ± 2 °C, relative

humidity $55 \pm 10\%$ and 12 h. light : 12 h. dark cycle). All rats had free access to standard pelleted rat feed and water ad libitum. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All experimental protocols were reviewed and accepted by the Institutional Animal Ethical Committee (IAEC) prior to the commencement of the experiment.

Dose selection

A single dose of 100 mg/kg of the dose was selected for the screening of various pharmacological activities. As per OECD guidelines maximum tolerable dose that can be given to rats is 2 g/kg. Hence one tenth of that is considered to be 200 mg. Since no relevant study data was available half of the selected dose was selected as safe and single dose of 100 mg/kg was taken.

Treatment groups

The animals were randomly divided into 4 groups containing 6 animals in each group:

- group I – received 2% Tween 80 and served as control.
- group II – received diazepam (2 mg/kg), which served as standard for locomotor and spontaneous behavior activity.
- group III – received aqueous extract of leaves of *C. odorata* (100 mg/kg).
- group IV – received ethanolic extract of leaves of *C. odorata* (100 mg/kg).

Dose administration

The ethanolic and aqueous extract of leaves and 2% Tween 80 (control) were administered to the rats by the per oral route using an Oral feeding needle. The standard diazepam was administered intraperitoneally.

The CNS activity of ethanolic & aqueous extract of leaves of *C. odorata* L. was compared using the following screening methods.

Locomotor activity (muscle coordination and behavioral activity).

a) Optovarimex method b) Rotamex method.

Experimental protocol for central nervous system activity in rats

The animals were tested (by the screening methods mentioned above) during the light period and observed in a closed room at constant temperature by the screening methods mentioned above. The ethanolic and aqueous leaf extract was suspended in 2% Tween 80 and administered for a continuous period, of 7 days at a single dose of 100 mg/kg per orally.

Effect on spontaneous activity using Optovarimex method

The effect of the ethanolic and aqueous leaf extract on spontaneous activity was evaluated in rats using Optovarimex apparatus. The observations were made after 30 min, 60 min, 90 min and 120 min of oral administration. The vehicle control and the test extract were administered continuously for a period of seven days and the method was repeated on the fourth day and seventh day.

The rats were placed individually in the apparatus, subsequent to administering and the ambulation was recorded for 1 minute. The parameters monitored were the distance travelled (DT) in cm, resting time (RT) in sec, stereotypic time (ST) in sec, ambulatory time (AT) in sec.

Statistical analysis

The Statistical analysis was carried out to determine the significance of the result using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison's test using the software graph pad instat 3.1. *p* value of <0.05, <0.01 and <0.001 was considered as statistically significant.

RESULTS

Phytochemical screening

Phytochemical screening of the ethanolic and aqueous extracts showed that the crude extract contained alkaloids, flavonoids, saponins, cardiac glycosides, triterpenoids, phenolic compounds and tannins which are tabulated in Table 1.

Herbal medicines play an important role in the healthcare system worldwide.^[16] The search for novel pharmacotherapy from medicinal plants for CNS related disorders has progressed significantly in the past decade and their therapeutic potential has been assessed in a variety of animal models. In the present study, the effect of single dose (100 mg/kg) of the ethanolic leaf extract of *C. odorata* was studied using several behavioral animal models to investigate possible CNS activity.

The Autotrack Method was used to test the effect of the ethanolic and aqueous extract of leaves of *Chromolaena odorata* Linn. on spontaneous behavioural activity in rats. Four indices were used to quantify this activity viz. distance travelled (DT), resting time (RT), ambulatory time (AT) and stereotypic time (ST) as measured on the Optovarimex (Columbus) instrument. These were compared with the values obtained for the group-I and group-II. The results obtained are presented in Table 2.

Table 1: Phytochemical screening of extracts

Biochemical Tests	Ethanolic	Aqueous
Tests for Carbohydrates		
Molisch test	+	+
Test for Reducing Sugars		
Fehlings Test	+	+
Benedicts Test	+	+
Test for Monosaccharide's		
Barfoed's test	-	-
Test for Hexose sugar		
Cobalt Chloride Test	+	+
Test for Non Reducing Polysaccharides (starch)		
Iodine test	-	-
Tannic Acid test	-	-
Tests for Proteins		
Biuret Test	+	+
Millon's test	+	+
Xanthoprotein test	+	+
Tests for Amino Acids		
Ninhydrin test	+	+
Test for Tyrosine	+	+
Test for Tryptophan	+	+
Test for Cystein	+	+
Tests for Steroids and Triterpenoids		
Salkowski's reaction	+	+
Liebermann burchard reaction	+	+
Liebermann reaction	-	-
Tests for Glycosides		
Baljet test	+	+
Legal test	+	+
Keller killiani test	+	+
Liebermann test for Bufadienolides	-	-
Tests for Tannins	+	+
Tests for Alkaloids		
Dragendorff's test	+	+
Mayer's test	+	+
Wagner's test	+	-
Hager's test	+	+
Tests for Flavonoids		
Shinoda test	+	+
Alkaline reagent test	+	+
Test for Saponin glycosides		
Foam test	+	+
Test for Anthraquinone glycosides		
Borntragers test	-	-
Modified Borntragers test	-	-

+ Positive, - Negative

Table 2: Effect of extract of leaves of *Chromolaena odorata* Linn. on spontaneous behavior using Optovarimex Instrument on Days 1, 4, and 7 showing distance travelled, resting time, stereotypic time and ambulatory time

Treatment groups	Time in mins	Day 1				Day 4				Day 7			
		DT	RT	ST	AT	DT	RT	ST	AT	DT	RT	ST	AT
		30	225.33 ± 25.17	12.17 ± 3.67	14.17 ± 2.15	21.33 ± 2.33	117.33 ± 13.58	20.67 ± 5.74	13.83 ± 1.45	23.17 ± 4.9	112.33 ± 42.04	21.17 ± 7.24	14.33 ± 1.56
group I (Control)	60	143 ± 22.39	17.33 ± 5.34	16.17 ± 1.81	20.17 ± 4.61	99.33 ± 26.3	20.17 ± 4.03	19.17 ± 2.29	18.83 ± 5.11	136.17 ± 23.29	17.83 ± 3.45	16.33 ± 8.17	15.83 ± 1.62
	90	88 ± 17.23	32.33 ± 4.7	13.17 ± 2.09	13.83 ± 2.97	67 ± 21.15	21.83 ± 3.03	15.83 ± 2.67	14.83 ± 5.27	74.17 ± 43.93	25.17 ± 5.47	15.66 ± 0.81	14.33 ± 5.62
	120	81.17 ± 25.98	30.83 ± 4.56	14.67 ± 1.28	14.5 ± 4.09	144 ± 47.37	25.83 ± 6.22	14.83 ± 2.83	18.33 ± 3.95	113 ± 40.08	20.17 ± 3.90	13.5 ± 2.08	12.33 ± 4.58
	30	27.83 ± 5.95	35.67 ± 3.35	12.33 ± 1.38	5.33 ± 0.95	17.17 ± 1.92	47.5 ± 1.96	6.17 ± 0.79	5.5 ± 0.99	16 ± 2.67	44.83 ± 1.92	6.67 ± 1.14	4.5 ± 0.96
group II (Diazepam)	60	20 ± 3.92	43.33 ± 3.59	6.33 ± 0.81	7.33 ± 1.05	14.83 ± 2.98	45.83 ± 2.24	7.17 ± 0.65	5.83 ± 0.79	13.33 ± 3.46	47.33 ± 2.11	8.17 ± 0.71	6.17 ± 0.48
	90	15.5 ± 1.94	40.17 ± 2.89	7.33 ± 0.67	6.17 ± 1.01	12 ± 1.7	45.67 ± 2.56	7.5 ± 0.76	5.67 ± 0.88	15.67 ± 2.03	44.5 ± 2.4	7.33 ± 1.05	5.17 ± 0.94
	120	19.83 ± 2.89	42.17 ± 4.47	6.83 ± 0.61	5.67 ± 0.95	20 ± 2.97	44.33 ± 3.25	8.33 ± 0.843	5.5 ± 0.74	13.33 ± 2.91	37.67 ± 2.95	5.83 ± 0.91	4.83 ± 0.94
	30	279.33 ± 47.37	7.33 ± 2.73	19.33 ± 2.03	34.5 ± 4.16*	255 ± 33.23**	11.5 ± 3.27	19.17 ± 2.01	26.67 ± 4.36	259 ± 42.94*	12.83 ± 3.91	15.33 ± 1.54	28.5 ± 4.59
group III (Aqueous 100 mg/Kg)	60	226.5 ± 26.12	12.33 ± 2.46	18.83 ± 1.25	25.83 ± 4.59	169.83 ± 28.98	16.16 ± 2.96	18.5 ± 2.23	21.83 ± 4.21	204.67 ± 46.51	19.67 ± 6.25	15.83 ± 0.71	25.5 ± 5.71
	90	210.67 ± 19.61***	17.17 ± 4.41**	16.17 ± 2.12	26.83 ± 4.02*	130.67 ± 40.86	20.5 ± 4.88	17.83 ± 1.79	18.33 ± 4.53	180.67 ± 29.32	14.5 ± 2.64	17.33 ± 1.63	24.17 ± 3.12
	120	194.83 ± 40.41*	20.17 ± 5.54	15.5 ± 1.33	24.33 ± 5.79	200.17 ± 26.03	14.5 ± 2.21	17.83 ± 1.79	24.17 ± 2.07	148.5 ± 28.35	15.67 ± 3.84	20.17 ± 1.33*	24.17 ± 4.46
	30	227.83 ± 35.8	15.83 ± 4.28	17.17 ± 1.79	25.17 ± 3.75	169 ± 30.66	16.17 ± 3.53	20.67 ± 2.12	23.33 ± 3.59	151 ± 25.43	20.17 ± 2.14	19.67 ± 1.93*	20.17 ± 2.86
group IV (Ethanolic 100 mg/Kg)	60	222.5 ± 48.03	17.83 ± 4.96	15.33 ± 1.02	24.67 ± 5.51	204 ± 68.64	16.5 ± 4.05	17.67 ± 1.84	23.5 ± 3.76	120.83 ± 19.25	24.17 ± 5.15	19.67 ± 1.96	16.17 ± 3.99
	90	152.67 ± 16.40*	21.17 ± 2.64*	15.67 ± 2.09	20.17 ± 2.83	186.67 ± 40.82	20.33 ± 4.94	17.5 ± 2.26	15.83 ± 4.57	166.66 ± 25.85	18.5 ± 2.38	22.17 ± 1.99**	23.5 ± 3.14
	120	120.17 ± 36.37	22.83 ± 5.11	17.67 ± 2.51	17.5 ± 4.09	163.67 ± 37.29	14.17 ± 2.6	17.5 ± 2.26	19.17 ± 3.39	118.83 ± 28.11	21.17 ± 1.97	22.33 ± 1.78**	12.67 ± 2.17

Values are expressed as mean ± SEM (n = 6) *P < 0.05, **P < 0.01, ***P < 0.001 vs. Control.

On the *first* day at 30 min post administration, distance travelled (DT), resting time (RT), ambulatory time (AT) and stereotypic time (ST) for group-III were observed to be $279.33 \text{ cm} \pm 47.37$, $7.33 \text{ sec} \pm 2.73$, $34.5 \text{ sec} \pm 4.16$ at $p < 0.05$ and $19.33 \text{ sec} \pm 2.03$ resp. and for group-IV were $227.83 \text{ cm} \pm 35.8$, $15.83 \text{ sec} \pm 4.28$, $25.17 \text{ sec} \pm 3.75$ and $17.17 \text{ sec} \pm 1.79$ resp. These results were then compared with the results obtained for group-I ($225.33 \text{ cm} \pm 25.17$, $12.17 \text{ sec} \pm 3.67$, $21.33 \text{ sec} \pm 2.33$ and $14.17 \text{ sec} \pm 2.15$ resp.) and group-II ($27.83 \text{ cm} \pm 5.95$, $35.67 \text{ sec} \pm 3.35$, $5.33 \text{ sec} \pm 0.95$ and $12.33 \text{ sec} \pm 1.38$ resp.). In contrast, all indices of group-IV results were insignificant to group-I while only ST results of group-III were significant at $P < 0.05$ to group-I.

At 60 min post administration, DT, RT, AT and ST values for group-III were $226.5 \text{ cm} \pm 26.12$, $12.33 \text{ sec} \pm 2.46$, $25.83 \text{ sec} \pm 4.59$ and $18.83 \text{ sec} \pm 1.25$ resp. for group-IV were $222.5 \text{ cm} \pm 48.03$, $17.83 \text{ sec} \pm 4.96$, $24.67 \text{ sec} \pm 5.51$ and $15.33 \text{ sec} \pm 1.02$ resp. The group-I values were $143 \text{ cm} \pm 22.39$, $17.33 \text{ sec} \pm 5.34$, $20.17 \text{ sec} \pm 4.61$ and $16.17 \text{ sec} \pm 1.81$ and group-II values were $20 \text{ cm} \pm 3.92$, $43.33 \text{ sec} \pm 3.59$, $7.33 \text{ sec} \pm 1.05$ and $6.33 \text{ sec} \pm 0.81$. It was seen that as compared to group-I, no indices of group-III or group-IV showed any significance.

At 90 min, DT, RT, AT and ST observations recorded for group-III were $210.67 \text{ cm} \pm 19.61$ at $p < 0.001$, $17.17 \text{ sec} \pm 4.41$ at $p < 0.01$, $26.83 \text{ sec} \pm 4.02$ at $p < 0.05$ and $16.17 \text{ sec} \pm 2.12$ resp., for group-IV were $152.67 \text{ cm} \pm 16.40$ at $p < 0.05$, $21.17 \text{ sec} \pm 2.64$ at $p < 0.05$, $20.17 \text{ sec} \pm 2.83$ and $15.67 \text{ sec} \pm 2.09$ resp. The corresponding readings for group-I were $88 \text{ cm} \pm 17.23$, $32.33 \text{ sec} \pm 4.7$, $13.83 \text{ sec} \pm 2.97$ and $13.17 \text{ sec} \pm 2.09$ resp. and for group-II were $15.5 \text{ cm} \pm 1.94$, $40.17 \text{ sec} \pm 2.89$, $6.17 \text{ sec} \pm 1.01$ and $7.33 \text{ sec} \pm 0.67$. The readings of group-III was found to be extremely significant for DT with respect to group-I and very significant for RT with respect to group-I and significant for AT w.r.t. group-I.

At 120 min, group-III readings were $194.83 \text{ cm} \pm 40.41$ at $p < 0.05$, $20.17 \text{ sec} \pm 5.54$, $24.33 \text{ sec} \pm 5.79$ and $15.5 \text{ sec} \pm 1.33$ resp., group-IV readings were $120.17 \text{ cm} \pm 36.37$, $22.83 \text{ sec} \pm 5.11$, $17.5 \text{ sec} \pm 4.09$ and $17.67 \text{ sec} \pm 2.51$ resp. While group-I readings were $81.17 \text{ cm} \pm 25.98$, $30.83 \text{ sec} \pm 4.56$, $14.5 \text{ sec} \pm 4.09$ and $14.67 \text{ sec} \pm 1.28$ resp. And group-II readings were $19.83 \text{ cm} \pm 2.89$, $42.17 \text{ sec} \pm 4.47$, $5.67 \text{ sec} \pm 0.95$ and $6.83 \text{ sec} \pm 0.61$ resp. In comparison, only DT of group-III showed significance at $P < 0.01$ while ST, RT and AT of group-IV were not significant w.r.t. group-I.

On the *fourth* day, at 30 min of administration, group-III values were $255 \text{ cm} \pm 33.23$ at $p < 0.01$, $11.5 \text{ sec} \pm 3.27$, $19.17 \text{ sec} \pm 2.01$ and $26.67 \text{ sec} \pm 4.36$ for DT, RT, ST and

AT resp. while group-IV values were found to be $169 \text{ cm} \pm 30.66$, $16.17 \text{ sec} \pm 3.53$, $20.67 \text{ sec} \pm 2.12$ and $23.33 \text{ sec} \pm 3.59$ resp. group-I readings were $117.33 \text{ cm} \pm 13.58$, $20.67 \text{ sec} \pm 5.74$, $13.83 \text{ sec} \pm 1.45$ and $23.17 \text{ sec} \pm 4.9$ resp. whereas group-II were $17.17 \text{ cm} \pm 1.92$, $47.5 \text{ sec} \pm 1.96$, $6.17 \text{ sec} \pm 0.79$ and $5.5 \text{ sec} \pm 0.99$ resp. When compared, ST, RT, AT of group-III showed no significance while DT of group-III showed significance at $P < 0.01$ w.r.t. group-I. No indices of group-IV showed any significance.

Subsequently at 60 min post administration, group-III values obtained were $169.83 \text{ cm} \pm 28.98$, $16.16 \text{ sec} \pm 2.96$, $18.5 \text{ sec} \pm 2.23$ and $21.83 \text{ sec} \pm 4.21$ resp. while group-IV values were $204 \text{ cm} \pm 68.64$, $16.5 \text{ sec} \pm 4.05$, $17.67 \text{ sec} \pm 1.84$ and $23.5 \text{ sec} \pm 3.76$ resp. as compared to group-I values which were $67 \text{ cm} \pm 21.15$, $21.83 \text{ sec} \pm 3.03$, $19.17 \text{ sec} \pm 2.29$ and $18.83 \text{ sec} \pm 5.11$ resp. and group-II values were $14.83 \text{ cm} \pm 2.98$, $45.83 \text{ sec} \pm 2.24$, $7.17 \text{ sec} \pm 0.65$ and $5.83 \text{ sec} \pm 0.79$ resp. At 60 min post administration none of the indices of group-III or group-IV were found to be significant.

At 90 min, DT, RT, ST and AT for group-III were $130.67 \text{ cm} \pm 40.86$, $20.5 \text{ sec} \pm 4.88$, $17.83 \text{ sec} \pm 1.79$ and $18.33 \text{ sec} \pm 4.53$ resp. whereas corresponding results for group-IV were $186.67 \text{ cm} \pm 40.82$, $20.33 \text{ sec} \pm 4.94$, $17.5 \text{ sec} \pm 2.26$ and $15.83 \text{ sec} \pm 4.57$ resp. group-I results obtained were $67 \text{ cm} \pm 21.15$, $21.83 \text{ sec} \pm 3.03$, $15.83 \text{ sec} \pm 2.67$ and $14.83 \text{ sec} \pm 5.27$ resp. and group-II were $12 \text{ cm} \pm 1.7$, $45.67 \text{ sec} \pm 2.56$, $7.5 \text{ sec} \pm 0.76$ and $5.67 \text{ sec} \pm 0.88$ respectively. At 90 min post administration none of the indices of group-III or group-IV were found to be significant.

Later at 120 min, the corresponding readings were $200.17 \text{ cm} \pm 26.03$, $14.5 \text{ sec} \pm 2.21$, $17.83 \text{ sec} \pm 1.79$ and $24.17 \text{ sec} \pm 2.07$ for group-III and $163.67 \text{ cm} \pm 37.29$, $14.17 \text{ sec} \pm 2.6$, $17.5 \text{ sec} \pm 2.26$ and $19.17 \text{ sec} \pm 3.39$ for group-IV whereas $144 \text{ cm} \pm 47.37$, $25.83 \text{ sec} \pm 6.22$, $14.83 \text{ sec} \pm 2.83$ and $18.33 \text{ sec} \pm 3.95$ for group-I and $20 \text{ cm} \pm 2.97$, $44.33 \text{ sec} \pm 3.25$, $8.33 \text{ sec} \pm 0.843$ and $5.5 \text{ sec} \pm 0.74$ for group-II. At 120 min post administration none of the indices of group-III or group-IV were found to be significant.

On the *seventh* day, DT, RT, ST and AT values at 30 min post administration for group-III were $259 \text{ cm} \pm 42.94$ at $p < 0.05$, $12.83 \text{ sec} \pm 3.91$, $15.33 \text{ sec} \pm 1.54$ and $28.5 \text{ sec} \pm 4.59$ resp., for group-IV were $151 \text{ cm} \pm 25.43$, $20.17 \text{ sec} \pm 2.14$, $19.67 \text{ sec} \pm 1.93$ at $p < 0.05$ and $20.17 \text{ sec} \pm 2.86$ resp. group-I results at this time were $112.33 \text{ cm} \pm 42.04$, $21.17 \text{ sec} \pm 7.24$, $14.33 \text{ sec} \pm 1.56$ and $19.83 \text{ sec} \pm 6.62$ and group-II results were $16 \text{ cm} \pm 2.67$, $44.83 \text{ sec} \pm 1.92$, $6.67 \text{ sec} \pm 1.14$ and $4.5 \text{ sec} \pm 0.96$ resp. At this time point, DT values of group-III and ST values of

group IV showed significance at $P < 0.05$ while RT and AT values showed no significance.

At 60 min, the corresponding values were $204.67 \text{ cm} \pm 46.51$, $19.67 \text{ sec} \pm 6.25$, $17.33 \text{ sec} \pm 1.63$ and $24.17 \text{ sec} \pm 3.12$ for group-III and 120.83 ± 19.25 , $24.17 \text{ sec} \pm 5.15$, $19.67 \text{ sec} \pm 1.96$ and $16.17 \text{ sec} \pm 3.99$ for group-IV. group-I values were $136.17 \text{ cm} \pm 23.29$, $17.83 \text{ sec} \pm 3.45$, $16.33 \text{ sec} \pm 8.17$ and $15.83 \text{ sec} \pm 1.62$ and group-II values were $13.33 \text{ cm} \pm 3.46$, $47.33 \text{ sec} \pm 2.11$, $8.17 \text{ sec} \pm 0.71$ and $6.17 \text{ sec} \pm 0.48$. None of the indices of group-III or group-IV were found to be significant.

At 90 mins post administration, DT, RT, ST and AT readings for group-III were $180.67 \text{ cm} \pm 29.32$, $14.5 \text{ sec} \pm 2.64$, $17.33 \text{ sec} \pm 1.63$ and $24.17 \text{ sec} \pm 3.12$ resp. and for group-IV were $166.66 \text{ cm} \pm 25.85$, $18.5 \text{ sec} \pm 2.38$, $22.17 \text{ sec} \pm 1.99$ at $p < 0.01$ and $23.5 \text{ sec} \pm 3.14$. In contrast, group-I readings obtained were $74.17 \text{ cm} \pm 43.93$, $25.17 \text{ sec} \pm 5.47$, $15.66 \text{ sec} \pm 0.81$ and $14.33 \text{ sec} \pm 5.62$ and group-II readings were $15.67 \text{ cm} \pm 2.03$, $44.5 \text{ sec} \pm 2.4$, $7.33 \text{ sec} \pm 1.05$ and $5.17 \text{ sec} \pm 0.94$ resp. It was seen that ST of group-IV showed significance at $P < 0.01$ while rest of the values of group-IV and group-III were not significant.

At 120 min post administration group-III results for the four indices parameters were $148.5 \text{ cm} \pm 28.35$, $15.67 \text{ sec} \pm 3.84$, $20.17 \text{ sec} \pm 1.33$ at $p < 0.05$, $24.17 \text{ sec} \pm 4.46$ resp., for group-IV results were $118.83 \text{ cm} \pm 28.11$, $21.17 \text{ sec} \pm 1.97$, $22.33 \text{ sec} \pm 1.78$ at $p < 0.01$ and $12.67 \text{ sec} \pm 2.17$ resp. in contrast to group-I results which were $113 \text{ cm} \pm 40.08$, $20.17 \text{ sec} \pm 3.90$, $13.5 \text{ sec} \pm 2.08$ and $12.33 \text{ sec} \pm 4.58$ and group-II results were $13.33 \text{ cm} \pm 2.91$, $37.67 \text{ sec} \pm 2.95$, $5.83 \text{ sec} \pm 0.91$ and $4.83 \text{ sec} \pm 0.94$ resp. ST of group-IV showed significance at $P < 0.01$. It was further observed that while the DT, RT and AT results of group-IV were not significant w.r.t. group-I. However ST of group-III showed significance at $P < 0.05$ while rest of the indices did not show any significance.

There was an increase in DT as compared to the control group, group-I. On the first day at 90 mins DT of GROUP III showed extremely significant result and RT also showed very significant difference to the control. On the first day at 90 mins DT and RT of GROUP IV showed significant results. On the fourth day at 30 mins DT of GROUP III showed very significant result at $p < 0.01$. On the seventh day, the ST of group-IV showed very significant results at 90 and 120 mins and group-III also showed significant results at 120 min. Resting time on the other hand was found to decrease as compared to the control. This shows that leaf extract of *Chromolaena odorata* L. has CNS stimulant effect in rats.

The four groups of rats were tested on Rotamex (Columbus) system for this particular activity. Time of fall from the rotating rods was used as an index to determine activity. The results obtained were as tabulated in Table 3.

On the first day, at 30 min post administration, the time of fall recorded for group-III was $34.86 \text{ sec} \pm 4.23$ at $p < 0.05$, while that of group-IV was $33.45 \text{ sec} \pm 3.15$ at $p < 0.05$, as compared to the control group, group-I which was $17.67 \text{ sec} \pm 2.13$ and the positive control group, group-II which was $11.82 \text{ sec} \pm 0.63$. group-III and group-IV results showed significance at $P < 0.05$, at this time point. At 60min post administration, the time of fall observed for group-III was $30.08 \text{ sec} \pm 4.22$ at $p < 0.05$, for group-IV was $28.77 \text{ sec} \pm 3.01$ at $p < 0.05$ as compared to the control group, group-I for which the time of fall was $17.27 \text{ sec} \pm 0.82$, group-II which was $8.1 \text{ sec} \pm 0.61$. At this time, group-III and IV showed significance at $P < 0.05$.

At 90 min, the time of fall for group-III 28.5 ± 3.36 at $p < 0.01$, for group-IV was $21.75 \text{ sec} \pm 3.75$ at $p < 0.05$. In comparison, the time of fall for group-I was $12.9 \text{ sec} \pm 3.11$ and for group-II was $8.52 \text{ sec} \pm 0.73$. group-III and group-IV were significant at $P < 0.01$ and $P < 0.05$ as compared to group-I. At 120 min, group-III readings

Table 3: Effect of extract of leaves of *Chromolaena odorata* Linn. on muscle co-ordination using Rotamex Instrument on Days 1, 4 and 7

Treatment groups mg/Kg	Time in Mins	Day 1	Day 4	Day 7
		Time of fall (sec)	Time of fall (sec)	Time of fall (sec)
group I (Control)	30	17.67 ± 2.13	13.97 ± 2.10	20.32 ± 4.99
	60	17.27 ± 0.82	13.98 ± 0.65	17.3 ± 1.85
	90	12.9 ± 3.11	15.18 ± 2.72	15.17 ± 3.12
	120	12.63 ± 0.67	16.07 ± 3.14	16.97 ± 2.54
group II (Diazepam)	30	11.82 ± 0.63	8.8 ± 0.95	8.8 ± 0.77
	60	8.1 ± 0.61	8.83 ± 0.76	9.05 ± 0.69
	90	8.52 ± 0.73	8.42 ± 1.21	8.73 ± 0.74
	120	9.43 ± 0.58	8.08 ± 0.62	9.12 ± 0.69
group III (Aqueous 100 mg/Kg)	30	$34.86 \pm 4.23^*$	33.08 ± 10.47	37.18 ± 11.93
	60	$30.08 \pm 4.22^*$	34.82 ± 8.53	33.07 ± 4.77
	90	$28.5 \pm 3.36^{**}$	35.9 ± 2.98	34.48 ± 7.95
	120	$32.88 \pm 2.08^*$	$36.48 \pm 5.20^*$	34.13 ± 4.27
group IV (Ethanolic 100 mg/Kg)	30	$33.45 \pm 3.15^*$	38.78 ± 11.85	29.28 ± 5.97
	60	$28.77 \pm 3.01^*$	$42.27 \pm 10.55^*$	$41.13 \pm 9.71^*$
	90	$21.75 \pm 3.75^*$	$54.27 \pm 10.52^{***}$	$39.77 \pm 7.85^*$
	120	$42.65 \pm 8.98^{**}$	$39.37 \pm 6.71^*$	$38.52 \pm 8.74^*$

Values are expressed as mean \pm SEM (n = 6) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. Control.

were $32.88 \text{ sec} \pm 2.08$ at $p < 0.05$ group-IV readings were $42.65 \text{ sec} \pm 8.98$ at $p < 0.01$ as compared to group-I which was $12.63 \text{ sec} \pm 0.67$ and group-II which was $9.43 \text{ sec} \pm 0.58$. group-III & group-IV were significant at $P < 0.01$ & $P < 0.05$ resp. as compared to the control group, group-I.

On the *fourth* day of administration, the time of fall at 30 min for group-III was observed to be $33.08 \text{ sec} \pm 10.47$, for group-IV was $38.78 \text{ sec} \pm 11.85$. In comparison the time of fall for group-I was $13.97 \text{ sec} \pm 2.10$ and for group-II was $8.8 \text{ sec} \pm 0.95$. None of the results were significant. Further, at 60min post administration, the time of fall for group-III was $34.82 \text{ sec} \pm 8.53$, for group-IV was $42.27 \text{ sec} \pm 10.55$ at $p < 0.05$ and group-I was $13.98 \text{ sec} \pm 0.65$ and group-II which was $8.83 \text{ sec} \pm 0.76$. On this day, only group-IV results were significant at $P < 0.05$ as compared to group-I. Rest of the results were not significant to that obtained for the control, group-I.

At 90 min, the time of fall for group-III was 35.9 ± 2.98 , for group-IV was $54.27 \text{ sec} \pm 10.52$ at $p < 0.001$ and for group-I was $15.18 \text{ sec} \pm 2.72$ and group-II was $8.42 \text{ sec} \pm 1.21$. It was seen that group-IV results were extremely significant at $P < 0.001$ while group-III results were not significant compared to the control group. Later at 120 min, the time of fall for group-III was $36.48 \text{ sec} \pm 5.20$ at $p < 0.05$, while group-IV was $39.37 \text{ sec} \pm 6.71$ at $p < 0.05$. The time of fall for group-I was $16.07 \text{ sec} \pm 3.14$ and for group-II was found to be $8.08 \text{ sec} \pm 0.62$. group-III and group-IV results were significant at $P < 0.05$ in comparison to group I.

On the *seventh* day, the time of fall observed at 30 min for group-III was $37.18 \text{ sec} \pm 11.93$, for group-IV was $29.28 \text{ sec} \pm 5.97$ and for group-I was found to be $20.32 \text{ sec} \pm 4.99$ and for group-II was $8.8 \text{ sec} \pm 0.77$. None of the results were significant as compared to the control group. At 60 min, the time of fall for group-III was observed to be $33.07 \text{ sec} \pm 4.77$, for group-IV was $41.13 \text{ sec} \pm 9.71$ at $p < 0.05$ and group-I results at this time was found to be $17.3 \text{ sec} \pm 1.85$ and for group-II was found to be $9.05 \text{ sec} \pm 0.69$. group IV showed significance at $P < 0.05$, while the results of group-III were not significant in comparison to group I.

At 90 mins post administration, the time of fall observed for group-III was found to be $34.48 \text{ sec} \pm 7.95$, for group-IV was $39.77 \text{ sec} \pm 7.85$ at $p < 0.05$. In contrast, the time of fall for group-I was $15.17 \text{ sec} \pm 3.12$ and for group-II was $8.73 \text{ sec} \pm 0.74$. group-IV was significant at $P < 0.05$; group-III results were not significant as compared to the control, group-I. At 120 mins, the time of fall was observed to be $34.13 \text{ sec} \pm 4.27$ for group-III, $38.52 \text{ sec} \pm 8.74$ at $p < 0.05$ for group-IV. The time of fall for group-I was $16.97 \text{ sec} \pm 2.54$ and that for group-II was $9.12 \text{ sec} \pm 0.69$.

group-IV was significant at $P < 0.05$. At this time group-III did not show any significant result as compared to group-I.

Hence it was observed that, there was an increase in the time of fall. In case of group III it showed significant results at 30, 60 and 120 mins on 1st day and very significant results seen at 90 mins; group III also showed significant results on day 4 at 120 mins. The rats were revolving on the rotating rod for a longer time as compared to the control group, group-I. On 1st day group-IV showed significant results at 30, 60, 90 mins and very significant result at 120 mins, group-IV showed significant results at 60, 120 mins and extremely significant at 90 mins on day 4.

On day 7 group-IV continued to show significant results at 60, 90, and 120 mins. Thus it may be established that the leaf extract of *Chromolaena odorata* L. has an effect on motor co-ordination. On the whole ethanolic extract showed profound results as compared to aqueous extract.

DISCUSSION

A set of screening tests was conducted over a period of seven days to assess the effects of the ethanolic and aqueous extracts of *Chromolaena odorata* Linn. on the CNS of rats at a dose of 100 mg/kg p.o. The models chosen were Opto-Varimex (Autotrack) instrument and rotarod instrument. The tests were carried on the first, fourth and seventh day at 30, 60, 90 and 120 min of administration and statistically compared with the values obtained for the control group (Group-I) and diazepam treated rats (Group-II).

Spontaneous behaviour was quantified by the distance travelled, resting time, ambulatory time and stereotypic time of the rat in the autotrack instrument. On continuous administration, it was seen that there was a considerable increase in DT as compared to the control group and decrease in Resting Time. AT also showed significant results on first day. This shows that leaf extract of *Chromolaena odorata* L. has CNS stimulant effect in rats.

Time of fall from the rotating rods was used as an index to determine motor co-ordination in rats. It was seen that there was an increase in time of fall on each subsequent day of testing, particularly ethanolic extracts showed extremely significant results as compared to control. Aqueous extract treated rats also showed increase in time of fall as compared to control. It can be concluded that the extract possess some muscle contraction properties by virtue of which they substantially increase motor co-ordination.

From the pharmacological investigation, we can suggest the use of *Chromolaena odorata* Linn. leaf extract has

CNS stimulant effects as can be seen from the results obtained on the autotrack instrument. The CNS activity of *Chromolaena odorata* Linn. has never been reported earlier. The plant material can be used as a template to develop new drugs. Further studies are required to identify the components responsible for this activity. This was based on evaluation of CNS activity. It is further recommended to perform pharmacokinetics and bioavailability studies on the extract to further ascertain improved activity.

Statistical significance of the study

All results obtained by statistical methods suffer from the disadvantage that they might have been caused by pure statistical accident. The level of statistical significance is determined by the probability that this has not, in fact, happened. P is an estimate of the probability that the result has occurred by statistical accident. Therefore a large value of P represents a small level of statistical significance and vice versa. Small the value of P, there is always a finite chance that the result is a pure accident. A typical level at which the threshold of P is set would be 0.01, which means there is a one percent chance that the result was accidental. The significance of such a result would then be indicated by the statement $P < 0.01$. A level frequently quoted is $P < 0.05$. This means that there is a one in twenty chance that the whole thing was accidental. On the whole $P < 0.01$ would normally be considered significant and $P < 0.001$ highly significant. Popular levels of significance are 10% (0.1), 5% (0.05), 1% (0.01), 0.5% (0.005), and 0.1% (0.001). If a test of significance gives a *p*-value lower than or equal to the significance level α then null hypothesis is rejected. Such results are informally referred to as 'statistically significant'. For example, if someone argues that "there's only one chance in a thousand this could have happened by coincidence", a 0.001 level of statistical significance is being implied. The lower the significance level chosen, the stronger the evidence required.

CONCLUSION

The aqueous and ethanolic extract showed an improvement in muscle coordination and spontaneous behaviour. The CNS activity of the ethanolic and aqueous extracts

of *C. odorata* L. may be due to the presence of various bioactive constituents present in the extract detected during its preliminary phytochemical screening. The plant merits further investigation to determine the exact phytoconstituents responsible and mechanism of action involved for the above mentioned CNS activities.

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