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Evaluation of CNS Depressant and Muscle Relaxant Activity of *Lantana Camara* L. Stem and Flowers

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Abstract

CNS stimulant effect of the ethanolic & aqueous stem & flowers extract of *Lantana camara* L. were studied in mice using the benzodiazepine induced sleeping time. Rota rod bar & Actophotometer has been used to observe the extract's effect on muscle coordination & locomotor activity respectively. The extracts effect was compared with caffeine (a well-known stimulant) indicating better contraction & stimulant properties. In comparison with diazepam (well-known sedative), extracts gave shorter duration of sleep; indicating relaxant and less locomotor activity indicating depression action. The results showed that the extracts at all doses tested, reduced the duration of sleeping time, when compared to the control group that received distilled water. This difference in sleeping time was significant ($p<0.05$) and this was also found to be dose dependent. The extracts found least comparable activity with caffeine. On Rota rod, the extract had comparative sedative effect as the animals maintained their balance on the rod through the entire period of the experiment. **Objective:** The purpose of the present study was to investigate ethanolic & aqueous extracts of stem & flowers of *Lantana camara* L. to use it, as a remedy for dizziness and drowsiness (sedation) via benzodiazepine induced sleeping time and the Rota rod to check its effect on muscle co-ordination using mice. **Methods:** The effects on behavioral activity were studied using Rota rod apparatus & Act photometer. The extracts were given orally at a dose of 200 mg/kg & 400 mg/kg. Diazepam (4mg/kg *p.o*) & Caffeine (10mg/kg *p.o*) were used as standard & data were analyzed by ANOVA test followed by Dunnett's t-test. All the results were expressed as Mean (± SEM). $P<0.05$ was considered significant. **Results:** Physicochemical & Phytochemical screening of stem & flowers showed the presence of carbohydrates, triterpenoids, saponins, alkaloids, flavonoids, steroids as major constituents. The results of study showed that ethanolic & aqueous extracts of stem & flowers of *L.camara* decreased locomotor activity, produced muscle relaxation and also showed antianxiety activity. Remarkable stimulant effect has not been observed as comparable to caffeine. **Conclusion:** It has been reported that extracts of *L. camara*exhibit CNS depressant action, muscle relaxant action and significant anxiolytic activity comparable to diazepam.

Keywords
CNS, stimulant, antidepressant, herbal, *Lantana camara,* Extraction, benzodiazepine induced sleeping time, motor coordination, Central / Skeletal Muscle Relaxant.

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INTRODUCTION

According to World Health Report, about 450 million people suffer from a mental or behavioral disorder. This amounts to 12.3 % of the global burden of disease, and predicted to rise up to 15 % by 2020. [(1)]
Over past few decades, the affinity towards the herbal drugs has been grown by utilization of traditional medicinal plant to heal some critical diseases. It is turning out to be better medicine with respect to synthetic drugs that assure numerous side effects for prolong treatment [(2)]. In recent years, focus on plants research has increased all over the world. A large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. [(3-7)]
Depression is a common mental disorder, which affects the personal and social relations of a person. It is a state of low mood and aversion to activity that can affect a person's thoughts, behavior, feelings and physical wellbeing. According to WHO estimation, 121 million people worldwide suffer from clinical depression. The high prevalence of suicide in depressed patients (up to 15 %) coupled with complications arising from stress and its effects on the cardiovascular system have suggested that it will be the second leading cause of death by year 2020.[(8)]
Central nervous system (CNS) stimulation is the primary action of a diverse group of pharmacological agents and an adverse effect associated with the administration of an even larger group of drugs. CNS stimulation consists of a range of behaviors including mild elevation in alertness, increased nervousness and anxiety and convulsions. In general, any hyper excitability associated with drug administration results from an alteration in the fine balance normally maintained in the CNS between excitatory and inhibitory influences. Thus, the bases for CNS stimulation by the class of drugs reside in adjusting the integration of excitatory and inhibitory influences at the level of the individual neuron. [(9)]
A wide variety of agents have the capacity to excite the function of the CNS, such that calming or drowsiness (sedation) is inhibited. Varieties of synthetic antidepressant and stimulant drugs available now a days, however, their effectiveness does not come up with the entire range of population suffering from this disorder. Moreover, the side effects and the drug interactions are major restrictions in their clinical applications. To avoid synthetic medications, herbal medicines are their reasonable substitute and hence widely used across the globe due to their wide applicability and therapeutic efficacy associated with least side effects, which in turn has initiated the scientific research regarding the antidepressant activity from plants.
To our knowledge no any reports are available on the bioactivity of stem & flowers of *L. camara,* even though on ethanolic & aqueous extracts; with the exception of reports that described the other pharmacological activities in leaves.

MATERIALS & METHODS

Collection &Authentication of Plant Parts

Plant stem & flowers were obtained locally from Indore M.P. Proper identification of plant sample was done by an expert Botanist Dr. S. N. Dewedi, Professor and Head, Department of Botany, Janta PG College, APS University, Rewa, M.P. (Voucher No.J/BOT/L-251) for *L. camara.*

Washing and Drying of Plant Parts

At first stem was thoroughly washed with tap water to remove dust, soil, bird's droppings etc. within them. Stem was sun dried for 3 months days in day light. Then stem parts were dried in oven for 24 hours at considerably low temperature for better grinding. Flowers were sun dried only. The dried stem & flowers were then ground separately in coarse powder using high capacity grinding machine.

Grinding and Storage of Dried Samples

The dried parts were ground to coarse powder with the help of home blender machine. This process breaks the plant parts into smaller pieces thus exposing internal tissues and cells to solvents and facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the grinder.

Extraction of the Dried Powdered Sample

The fine powder of *L. camara* stem & flowers were dissolved in ethanol and it was thoroughly shaken to dissolve the powder into the solvent. Then it was

kept in a closely covered glass jar for 7 days and shaken several times during the process for more interaction between the powdered particles and the solvent. This process is termed as maceration. The cover of the jar was closed properly to resist the entrance of air in the jar.

Filtration of the extracts

After the extraction process the extracts were filtered with sterilized cotton filter and filter paper. The filtrate was collected in a beaker. The filtration process was repeated three times by using cotton and filter paper. Then the filtrate was taken into a volumetric flask and covered with aluminum foil paper was prepared for rotary evaporation.

Evaporation and Condensation of extracts

The extracts were transferred to the round bottle flask of rotary evaporator. Then excess amount of solvents in the extracts were removed by rotary evaporator, with reduced pressure which was done by using a vacuum pump. The temperature of the rotary evaporator was set 50ºC. It run for 1 hour 10 minutes and the RPM was set 80 for evaporation process. After evaporation extract was transferred in a beaker. Rest of the extract was removed from the round bottle flask. Then extract was kept in hot air oven to get more dried extract. All beakers were covered with aluminum foil. Then extracts have been collected and stored in a cool (4˚C) dry place for further assay.

Ethical approval &Experimental Protocols

Protocol has been approved by Institutional Animal Ethical Committee (IAEC) before initiating the study. Protocol approval reference number (PBRI/IAEC/PN-17047a).

Swiss albino mice of 3-4 weeks of age, weighing between 20–25 gram have been used for activity and females of the same strain for LD_{50} calculation. The mice were kept in animal house at a standard environmental condition (temperature-22 ± 1°C relative humidity-55 ± 5% and 12h light/12 h dark cycle). Animals were fed *ad libitum* with standard food and water. Experiments were performed after an overnight fast and it is acceptable with protocols for use of animals in experiment.

Acute toxicity study

Acute oral toxicity studies have been conducted separately followed by using OECD guideline 423. The method used defined doses of 5, 50, 300, 2000 mg/kg *p.o.* body weight. Results were allowed substance rank and classify according to the Globally Harmonized System (GHS) for classification of chemicals which causes acute toxicity. From LD_{50} determination, $1/10^{th}$ of the dose was focused as the medial for pharmacological screening. Since all animals were alive & no toxicity and no significant changes in the body weight between the control and treated group were demonstrated at doses up to 2000 mg. [7]

Drugs and chemicals

The ethanolic & aqueous extracts of *L. camara* stem & flowers were analyzed at different doses (200mg/kg and 400 mg/kg, *p.o.*).

Diazepam (Roche) and Absolute ethanol (Sigma-Aldrich), Caffeine.

Stock solutions of drug and the ethanolic & aqueous extract of *L. camara* were prepared fresh for each model.

Experimental Design

Animals were weighed and randomly divided into the following 10 groups with 6 animals in each group. The experimental findings are noted below. ***(Table 1)***

Table 1:

Group	Category	Drug administered
1.	Normo Control	Distilled water
2.	Negative Control	Diazepam (4mg/kg *p.o.*)asstandard depressant drug
3.	Positive Control	Caffeine(10mg/kg *p.o*) as standard stimulant drug
4.	Test 1 200 mg/kg	*L. camara stem extract (ethanol)*
5.	Test 2 400 mg/kg	*L. camara stem extract (ethanol)*
6.	Test 3 200 mg/kg	*L. camara stem extract (Aqueous)*
7.	Test 4 400 mg/kg	*L. camara stem extract (Aqueous)*
8.	Test 5 200 mg/kg	*L. camara flower extract (ethanol)*
9.	Test 6 400 mg/kg	*L. camara flower extract (ethanol)*
10.	Test 7 200 mg/kg	*L. camara flower extract (Aqueous)*
11.	Test 8 400 mg/kg	*L. camara flower extract (Aqueous)*

Central / Skeletal Muscle Relaxant by Rota Rod apparatus [10, 11]

Rota rod is a horizontal metal rod coated with rubber. Its diameter is 3cm, rotates with 25 rpm. The metal rod is about 50 cm above the surface to prevent the animal from jumping off from the roller. The mice have been placed on the revolving rod. Mice remains on Rota-Rod for 2 minutes or more in

low successive trials after the administration of test drugs & control vehicle has been selected for the test. The test and standard drug (diazepam, 4mg/kg, *i.p.*) has been administered 1 hour before placing the mice on the Rota rod. The fall off time from the rotating rod was noted. The difference in the fall off time from the rotating rod between the control and treated rats was taken as an index of muscle relaxation. The animals were divided into 10 groups of 6 mice per group. ***(Table 1)***

Groups received the extracts at 100 & 200 mg/kg doses *p.o.* One group received distilled water (2 ml/kg orally) served as normo control group. Group received Diazepam (4mg/kg *p.o.*)Served as positive control group. The onset of sleep and duration of sleeping time for each animal was determined and mean for each group calculated. The sleeping time is the time interval between onset of loss of righting reflex and regain of righting reflex. The fall off time was recorded in all the groups before and 30 minutes after drug administration. Decrease in fall off time is suggestive of CNS depression. ***(Table 2)***

CNS Stimulant / Depressant activity by Actophotometer [12,13]

In digital actophotometer, continuous beam of light falls on photoelectric cells. When the reading is considered as zero, any cut off in the continuity of light by the animal has been recorded on a digital counter in the form of counts. Depending on CNS action of the drug, the animals show locomotor activity either increased or decreased. Each animal has been placed in the actophotometer for 10 minutes and the initial locomotor reading has been taken. The animals have been placed in the actophotometer and allowed to move for a given period of time (10 minutes). The locomotor activity has been observed after 30 minutes & 01 hour of drug administration. The mice have been divided into 10 groups of 6 mice per group as per experimental design. Hence locomotor activity has been measured. Diazepam (4mg/kg *p.o.*) has been used as standard drug. Rest groups received test extracts in 100 & 200 mg/kg doses *p.o.* The locomotor activity of each animal was measured and results of test drug were compared with standard. ***(Table 3)***

DISCUSSION

Two test models were employed for evaluation of CNS stimulant or depressant effect of ethanolic & aqueous extracts of *L. camara* stem & flowers by using two standard drugs diazepam & caffeine of opposite category, to find out suitable comparable effect. This was so essential to justify its nonexistent use in state of dizziness and drowsiness (sedation). [14] Sedation indicates a decrease in activity, moderate excitement and drowsiness. [15]

Caffeine, a mild stimulant is widely used psychoactive drug in the world. [16] It increases norepinephrine secretion and enhances neural activity in numerous brain areas. [17] Diazepam, a benzodiazepine with anxiolytic, sedative, muscle relaxant and amnesic properties and has long duration of action. Its actions are mediated by enhancement of gamma-amino butyric acid activity. [18-20]

Anxiety and hypno sedation are chiefly mediated in CNS by $GABA_A$ receptor complex, which is also involved in other physiological functions related to behavior, psychological & neurological disorders. [21] GABAergic activity in the brain can be increased by three ways such as, by GABA agonists; barbiturates and benzodiazepines directly increase inhibitory chloride conductance and / or up regulate the effect of GABA release at synapse on the $GABA_A$ receptor respectively. [22] Secondly many psychological and neurological disorders might be modifying the GABA system with respect to GABA synthesis. This potentiation of GABA mediated postsynaptic inhibition through an allosteric modification of GABA receptors induces anxiolysis or hypnosis in animals. [23] Thirdly by direct increase in chloride conductance or indirectly by potentiating GABA-induced chloride conductance with simultaneous depression of voltage activated Ca^{++} currents like barbiturates [24], as consequence of Ca^{++} channel blockage, Ca^{++} entry into presynaptic nerve terminals is blocked. It leads to inhibition of excitatory neurotransmitters release such as glutamate. This causes complete reduction of excitatory synaptic transmission. [25]

In present study, CNS depressant activity of *L. camara* extracts were evaluated by Rota rod test, which has been clearly indicated CNS depressant activity with an evidenced of decreased of fall off time. Another vital step in evaluation of drug action on CNS is to observe its effect on locomotor activity. This activity is a measure of excitability level of CNS with decreased locomotor activity, indicated depression of CNS. [26]

Several plants reported CNS depressant and anxiolytic activity because of the presence of triterpenoids, saponins and flavonoids. [27, 28] Triterpenoid & saponins are reported to have agonistic/facilitatory activities at $GABA_A$ receptor complex. [29, 30] CNS depressant and anxiolytic activity reflected in results of the present study is attributed to these phytochemicals found in all extracts. Phytochemical analysis of *L. camara* also showed the presence of triterpenoid & saponins, which leads to

the hypothesis that they act as benzodiazepine-like molecules. This is supported by the behavioral effects in animal models of CNS depression, relaxation and anxiety.

This could provide a rationale for the use of this particular plant in situations of dizziness, drowsiness and sedation in folk medicine.

Statistical analysis

All data were expressed as mean ±SEM. Where applicable, the data were analyzed statistically by Student's t-test using graph pad instant version 2.05a. The level of significance was $P < 0.05$ and n represents six animals per group.

RESULTS

Physicochemical & Phytochemical analysis: The stem & flowers yielded carbohydrates, triterpenoids, saponins, alkaloids, flavonoids, steroids as major constituents. ***(Table 2, 3, 4)***

Table 2: Physiochemical Parameters of *L. camara*L.

S.N	Parameters	*L. camera*Stem (*w/w*)	*L. camera* Flowers (*w/w*)
1.	Loss on drying	11.20 %	6.6 %
2.	Total ash	4.12 %	1.58 %
3.	Acid insoluble ash	2.26 %	2.08 %
4.	Water soluble ash	2.58 %	1.52 %
5.	Ethanol soluble Extractive	12.2 w/w	10.4 w/w
6.	Water soluble Extractive	17.4 w/w	14.7 w/w
7.	Foreign matter	0.3 %	0.5 %
8.	Moisture content	8.0 %	5.5 %

Table 3: Micromeretic Parameters of *L. camara*L.

S.N	Parameters	*L. camera*Stem	*L. camera* Flowers
1.	Angle of Repose	0.46	0.40
2.	Bulk Density	1.56 gm/ml	1.27 gm/ml
3.	Tapped Density	2.21 gm/ml	1.52 gm/ml

Table 4: Phytochemical Screenings of *L. camara*L.

S. N	Chemical Constituents	Ethanolic (Stem)	Aqueous (Stem)	Ethanolic (Flowers)	Aqueous (Flowers)
1.	Alkaloids	+	-	+	-
2.	Carbohydrates	+	+	+	+
3.	Glycosides	-	+	-	+
4.	Steroids	+	-	+	-
5.	Flavonoids	+	+	+	+
6.	Saponins	+	-	+	-
7.	Fixed oils and fats	+	-	+	-
8.	Tannins	+	-	+	-
9.	Proteins and amino acids	-	-	-	-
10.	Terpenoids / triterpenoids	+	-	+	-

Acute toxicity study: The results of acute toxicity study showed no clinical signs of toxicity and mortality in the *L. camara* treated animals. Lethal dose was calculated and was found to be more than 2000 mg/kg. $1/10^{th}$ of this lethal dose (200 mg/kg) was taken as effective dose for the study.

Rota-rod model: Diazepam (4mg/kg*p.o.*) and *L. camara* extracts were (200 mg/kg *p.o.*& 400 mg/kg *p.o.*) treated groups showed significant CNS depressant activity when compared to control groups. ***(Table 5)***

Table 5: Muscle Relaxant Property of L. camara in mice on rotarod apparatus

Group with dose *(p.o.)*	Treatment mg/kg, *(p.o.)*	Time spent on revolving rod (in seconds)			% Reduction in Activity	
		Before Treatment	After 30 min Treatment	After 1hour Treatment	After 30 min	After 1 hour
Normo Control	Distilled water	735.10±	732.20±	835.14±	--	--
Positive control	Diazepam(4mg/kg)	717.20±	145.10±	19.33±	80.18	97.68
Negative control	Caffeine (10mg/kg)	747.10±	840.12±	985.00±	14.73	18.00
Test 1 200 mg/kg	Stem extract *(ethanol)*	800.20±	423.00±	110.32±	42.22	86.79
Test 2 400 mg/kg	Stem extract *(ethanol)*	780.10±	200.15±	50.61±	72.66	93.93
Test 3 200 mg/kg	Stem extract *(Aqueous)*	755.15±	402.23±	103.05±	45.06	87.66
Test 4 400 mg/kg	Stem extract *(Aqueous)*	822.22±	210.10±	55.00 ±	71.30	93.41
Test 5 200 mg/kg	Flower extract *(ethanol)*	830.20±	305.25±	111.35±	58.31	86.66
Test 6 400 mg/kg	Flower extract *(ethanol)*	740.15±	145.12±	47.41±	80.18	94.32
Test 7 200 mg/kg	Flower extract *(Aqueous)*	805.25±	325.10±	99.05±	55.59	88.13
Test 8 400 mg/kg	Flower extract *(Aqueous)*	738.10±	122.21±	51.00±	83.30	93.89

Locomotor activity model*:* Diazepam (4mg/kg *p.o.*) and *L. camara* extracts (200 mg/kg *p.o.* & 400 mg/kg*p.o.*) significantly exhibited reduction in movements as compared to standard drug groups. ***(Table 6)***

Table 6: CNS Stimulant / Depressant activity of L. camara using actophotometer in mice

Group with dose *(p.o.)*	Treatment mg/kg, *(p.o.)*	Number of movements (for 10 minutes)			% Reduction in Activity	
		Before Treatment	After 30 min Treatment	After 1hour Treatment	After 30 min	After 1 hour
Normo Control	Distilled water	240.50 ± 1.50	235.40 ± 1.40	238.50 ± 1.45	--	--
Positive control	Diazepam(4mg/kg)	237.00 ± 1.52	35.50 ± 1.35	05.10 ± 1.50	85.00	97.86
Negative control	Caffeine (10mg/kg)	240.00 ± 1.30	305.20 ± 1.10	355.10 ± 1.25	29.65	48.88
Test 1 200 mg/kg	Stem extract *(ethanol)*	214.15 ± 1.00	113.00 ± 1.75	72.00 ± 1.40	52.00	70.00
Test 2 400 mg/kg	Stem extract *(ethanol)*	237.10 ± 1.45	122.10 ± 1.25	69.20 ± 1.10	48.13	71.00
Test 3 200 mg/kg	Stem extract *(Aqueous)*	212.20 ± 1.90	110.00 ± 1.20	73.20 ± 1.25	53.27	69.30
Test 4 400 mg/kg	Stem extract *(Aqueous)*	222.10 ± 1.25	104.15 ± 1.35	48.50 ± 1.50	56.00	79.66
Test 5 200 mg/kg	Flower extract *(ethanol)*	233.25 ± 1.40	119.15 ± 1.65	80.20 ± 1.30	49.38	66.37

Group with dose *(p.o.)*	Treatment mg/kg, *(p.o.)*	Number of movements (for 10 minutes)			% Reduction in Activity	
		Before Treatment	After 30 min Treatment	After 1hour Treatment	After 30 min	After 1 hour
Test 6 400 mg/kg	Flower extract *(ethanol)*	231.20 ± 1.35	116.15 ± 1.35	65.30 ± 1.30	51.00	73.00
Test 7 200 mg/kg	Flower extract *(Aqueous)*	240.10 ± 1.45	122.10 ± 1.30	77.40 ± 1.40	48.13	67.54
Test 8 400 mg/kg	Flower extract *(Aqueous)*	227.20 ± 1.50	83.12 ± 1.45	38.10 ± 1.35	65.00	84.00

n=6, The percent inhibition for each group was calculated by comparison with the control group. Values indicate Mean ± S.E.M. (ANOVA test followed by Dunnett's t-test). Significance variation against control at,* p<0.001, ** p<0.05. Percent reduction in parenthesis calculated with reference to basal score.

CONCLUSION

From the results obtained, we can conclude that ethanolic & aqueous extracts of stem & flowers of *L. camara* decreased locomotor activity, produced muscle relaxation and also showed antianxiety effects. Remarkable stimulant effect has not been observed as comparable to caffeine. Hence it can be concluded that *L. camara* stem and flowers has potential CNS depression & antianxiety effect that can be explored for therapeutic advantage as an alternative treatment in medical conditions associated with dizziness, drowsiness and sedation.

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CONFLICTS OF INTEREST

Not have any conflict of interest.

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