

ORIGINAL ARTICLE

Preliminary study to evaluate analgesic and behavioural effects of *Lycopodium clavatum* in experimental animals

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ABSTRACT

Background and Aim: In Homoeopathy, the drug *Lycopodium clavatum* is prescribed for digestive upset, inflammations of urinary organs and dermal problems, but not for the treatment of central nervous system disorders unlike its use in traditional and folk medicine for central nervous system conditions. The present study was, therefore, undertaken with an aim to explore the possible analgesic and behavioural activities of the homoeopathic formulations of *Lycopodium clavatum* in animal models.

Material and Methods: Wistar albino rats (120-140 g) of either sex were used to evaluate analgesic effect by using hot plate, ice plate and Randall-Selitto tests and behavioural effect by using rota rod and open field tests. The rats were fed with different potencies (3X, 6X, 12X and 30C) of *Lycopodium clavatum* i.e., 0.5 ml /rat/day for 30 days and response of drug was measured after 30 minutes of drug administration on 10th, 20th and 30th day. Vehicle and saline-treated rats were tested simultaneously along with drug-treated animals for comparison.

Results: The study revealed that all the four potencies of *Lycopodium clavatum* had increased the latency time required to raise and to lick the fore or hind paw for thermal sensation and had also increased the quantum of threshold pressure to mechanical induced pain but depressed the motor coordination and locomotor activity.

Conclusion: This study suggests that the homoeopathic formulations (3X, 6X, 12X and 30C) of *Lycopodium clavatum* possess central nervous system (CNS) depressant activity. So the drug *Lycopodium clavatum* can be taken up for further research for its possible human use.

Keywords: Albino rats, Analgesic activity, Behavioural effect, Homoeopathic medicine, *Lycopodium clavatum*

INTRODUCTION

Traditionally, the entire plant of *Lycopodium clavatum* (Club-moss) is used to relieve muscle cramps, kidney and liver complications.^[1] A decoction of the plant is reported to be analgesic, anti-rheumatic

and carminative and for the treatment of urinary disorders, catarrhal cystitis and gastritis.^[2] Externally, spores are employed in the form of powder as a protective and adsorbent in erysipelas, eczema, herpes between the thighs and armpits of infants.^[3] *Lycopodium clavatum* spores act as diuretic, demulcent

Access this article online

Website:

www.ijrh.org

DOI:

10.4103/0974-7168.123391

Quick Response Code:



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Received: 25-04-2013

Accepted: 21-11-2013

and emmenagogue. Tincture of *Lycopodium* spores is generally given in rheumatism, epilepsy and pulmonary disorders.^[4] It is very beneficial especially in nocturnal micturition in children or adults.^[5]

The aerial part of *Lycopodium clavatum* plant contains dihydrocaffeic acid, which has exhibited blood pressure lowering effect in animals, whereas alkaloids such as lycopodine, chinoline, clavatine, clavatoxine and annotinine have shown to possess hypertensive effect. Lycopodine stimulates the peristaltic movements of the intestine and causes contraction of the uterus in animals.^[6] Chloroform extract and alkaloidal fraction of *Lycopodium clavatum* possess anti-inflammatory activity as it inhibited acid-induced increase in capillary permeability in mice.^[7] *Lycopodium* 30, a homoeopathic formulation prepared from extract of spores of *Lycopodium clavatum* showed considerable protective effect against p-DAB-induced hepatocarcinogenesis in mice.^[8]

The Chloroform: Methanol (1:1) extract^[9] and the active principles viz., lyclavatol,^[10] huperzine^[11] of *Lycopodium clavatum* have been shown to inhibit acetylcholinesterase (AChE) which has therapeutic potential in the treatment of Alzheimer's disease. The alkaloid extracts, lycopodine and acetyldihydrolycopodine of *Lycopodium clavatum* also showed acetylcholinesterase inhibitory and antioxidant activities *in vitro* and *in vivo* models.^[12]

Although in homoeopathy, *Lycopodium clavatum* is routinely prescribed for the treatment of constipation, bloating, gas, digestive upsets, heartburn, headaches, inflammations of respiratory and urinary organs, infectious problems, and in various chronic and dermal problems,^[13,14] but not for the treatment of central nervous system disorders unlike its use in traditional and folk medicine for central nervous system conditions. The present preliminary study was, therefore, undertaken with the aim to explore/assess the possible analgesic and behavioural activities of the homoeopathic formulations (3X, 6X, 12X and 30C potencies) of *Lycopodium clavatum*. Positive results if obtained in animal models (rats) could be extrapolated in a meaningful and convincing manner for their possible human use.

MATERIAL AND METHODS

Plant Material

The plant *Lycopodium clavatum* (Family: Lycopodiaceae) was collected and taxonomically identified by the

Survey of Medicinal Plants and Collection Unit, Nilgiri, Tamilnadu.

Drugs

Homoeopathic formulations of *Lycopodium clavatum* in 3×, 6× and 12× potencies in decimal scale and 30C in centesimal scale were prepared and supplied by M/S. Bahola Laboratories, Pondicherry, India according to the standard procedures mentioned in Homoeopathic Pharmacopoeia of India (HPI).^[15] The dilutions (3×, 6×, 12× and 30C) of *Lycopodium clavatum* were selected in this study because these particular potencies are being prescribed/claimed by homoeopathic practitioners for their clinical uses.^[13]

Animals

Studies were carried out on Wistar albino rats (120-140 g) of either sex, obtained from M/S. Jagan animal's breeder and supplier, Hyderabad and housed in polypropylene cages (47 × 34 × 20 cm) under standard laboratory conditions (12/12 hrs, light/dark cycles, room temp. 22 ± 2°C.) and allowed free access to standard pellet diet and water *ad libitum*. The rats were acclimatised to laboratory conditions for 10 days before commencement of experiment.

Experimental Design

The animals were marked on ear pinna for identification. A total of 90 rats were taken and grouped into five batches of 18 each which were further divided into six sub-batches of three each. The animals were accustomed to respective test procedures initially by subjecting them for test trials for three subsequent observations at 10-minute intervals on each day for 3 days before giving them any drug treatment. The test potencies (3×, 6×, 12× and 30C) of *Lycopodium clavatum* and vehicle (91.5%v/v alcohol used as vehicle for preparation of different potencies of test drug) were diluted with distilled water in the ratio of 1:4 and kept as stock solutions. The animals were fed with 2 ml stock solution of different potencies (3×, 6×, 12× and 30C) of *Lycopodium clavatum* i.e., 0.5 ml (drug)/rat/day for 30 days. Vehicle and saline-treated rats were tested simultaneously along with drug-treated animals. The response of drug was measured after 30 minutes of drug administration on 10th, 20th and 30th day. Reading taken just before administration of the drug/vehicle/normal saline on day 1 of study was considered as the initial control value in the same group for comparison. The experimental protocol was duly approved by the Institutional

Animal Ethics Committee (Reg.No. 383/01/a/CPCSEA), Department of Zoology, Osmania University, Hyderabad. Experiments were conducted in an isolated and noiseless air-conditioned room between 10.00 and 15.00 hours. Care of animals was as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Analgesic Activity

For analgesic activity (i) Hot plate, (ii) Ice plate and (iii) Randall-Selitto tests were used.

Hot plate test

The hot plate latency assay was based on the method of Eddy *et al.*^[16] The rats were gently placed individually on a hot plate maintained at a temperature of $55 \pm 2^\circ\text{C}$. The time taken by the animals to lick the fore or hind paw or jump out of the hot plate was considered as the reaction (in seconds) time. The latency (licking or jumping) time was recorded 30 minutes after the administration of drug, vehicle or saline. The reaction time noted just before administration of the drug, alcohol or saline on day 1 of the study was considered as the initial control values for comparison. A cut-off reaction time of 15 seconds was chosen in order to avoid physical injury to the animal.^[17]

Ice plate test

Ice plate test was carried out as described by Sundaram *et al.*^[18] After 30 minutes of drug, vehicle or saline administration, all rat, one at a time was gently placed on the ice cubes ($0-4^\circ\text{C}$) filled in a transparent container ($20 \times 20 \times 20$ cm) and covered with a plastic cover. The rat was visualised to record the latency time (in seconds) taken to lick the fore or hind paws to cold sensation. The latency time taken just before administration of drug, vehicle or saline on day 1 of the study was considered as initial control values for comparison. A cut-off reaction time was set at 15 seconds in order to avoid tissue damage to the animals due to frozen temperature.

Randall-selitto assay

The analgesic activity of drug against mechanically induced pain was measured by Randall-Selitto assay^[19] (Randall-Selitto apparatus, Ugo Basile, Italy). After 30 minutes of drug, vehicle or saline administration, the rats were gently held in the hand and paw of the right foot of rat was placed on the rubber base of the apparatus and pressure (in ponds;

expressed in g) was applied either on 2nd – 3rd or 3rd – 4th metatarsal region through a pointed tip and increased gradually until vocalisation elicited which was considered as threshold pressure to mechanical induced pain. Control threshold pressure to mechanical induced pain was taken on day 1 before the administration of drug, alcohol or normal saline for comparison.

Behavioural Activity

Behavioural activity of the animal was studied by (i) Rota - rod (ii) Open field tests.

Rota-rod test

To observe behavioural strategy adopted by the rats to maintain motor coordination, grip strengths of the rats were measured by using the automated rota-rod apparatus (Dolphin™ instrument).^[20] The rotor was divided into three compartments which allowed three rats to test simultaneously at a time. After 30 minutes of drug, vehicle or saline administration, the rats were gently placed on the rotor with the body axis perpendicular to the rotor's long axis with the head directed opposite to the direction of rotating rod (5 rpm) and the fall-off time from the rod was noted for each rat. In the beginning, each rat was trained on the rota rod until rat achieved the criteria of remaining on the rotating spindle for about 60 seconds. The control grip strengths of the rats were measured on day 1 just before administration of the drug, alcohol or normal saline for comparison.

Open field test

Open field test was studied for recording the locomotor activity. The floor of the apparatus (a wooden box, $96 \times 96 \times 6$ cm) was divided in to 36 equal squares which were coloured with black and white alternatively. The apparatus was illuminated with low-intensity diffuse light (40 W) placed at a height of 100 cm and it was cleaned using 5% alcohol after every test trial. After completion of the training, the rats were placed individually in the apparatus and the number of squares visited by the animals in 5 minutes was counted^[21] before and 30 minutes after drug, vehicle or saline administration.

Statistical Analysis

The data were expressed as Mean \pm S.E.M. The difference between mean values of groups were statistically analysed by Student's *t* test. $p < 0.05$ were considered as statistically significant.^[22]

RESULTS

Analgesic Activity

Hot plate test

The results of the analgesic effect of *Lycopodium clavatum* using hot plate assay are summarised in Table 1. The initial latency time recorded on day 1 before administration of drug, alcohol or normal saline and 30 minutes after the administration of normal saline on different days of experimentation to noxious thermal stimulus was more or less constant (3.38-3.72 seconds). On the other hand, there was an increase in the latency time (5.22-5.45 seconds) to thermal noxious stimulus when measured 30 minutes after the administration of different potencies (3X, 6X, 12X and 30C) of *Lycopodium clavatum* or alcohol at a dose of 0.5 ml/rat/day on 10th day. The difference was significant ($p < 0.05$) only with those rats treated with 3× potency when compared to initial latency time taken just before administration of drug on day 1 of the study. Thereafter, the increase in the duration of latency time to thermal noxious stimulus was tapered off gradually on 20th day and 30th day on continuation of the treatment [Figure 1].

Ice plate test

Table 2 depicts the results of the analgesic effect of different potencies of *Lycopodium clavatum* using ice plate assay. The initial latency time to cold sensation recorded on day 1 before administration of drug, vehicle or normal saline and 30 minutes after the administration of normal saline on different days of experimentation was more or less same

(5.70-6.09 seconds). Similar to the effect on hot plate, there was an increase in the latency time (7.56-8.31 seconds) to cold sensation when measured 30 minutes after the administration of different potencies (3X, 6X, 12X and 30C) of *Lycopodium clavatum* or vehicle at a dose of 0.5 ml/rat/day on 10th day. The difference in the increase in latency time to cold sensation was significant ($p < 0.05$) with those groups which were treated with 3× and 6× potencies of *Lycopodium clavatum*. Thereafter, the increase in the duration of latency time to cold sensation was tapered off gradually on 20th day and 30th day on continuation of the treatment [Figure 2].

Randall-Selitto Test

The results of the analgesic effect of different potencies of *Lycopodium clavatum* on Randall-Selitto assay are presented in Table 3. The quantum of threshold pressure required to elicit vocalisation to applied mechanical pain was more or less same (131.33-150.00 g) on day 1

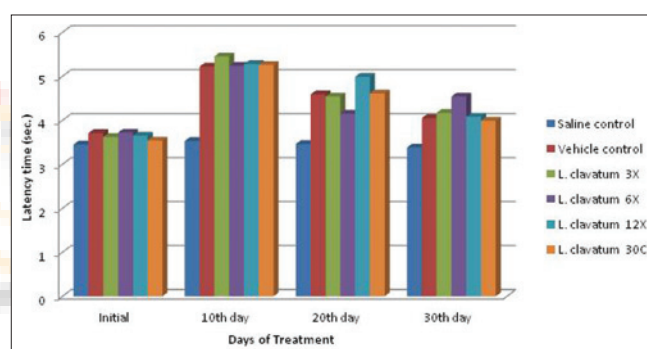


Figure 1: Analgesic effect of *Lycopodium clavatum* (0.5 ml/rat/day) on hot plate test

Table 1: Analgesic effect of *Lycopodium clavatum* (0.5 ml/rat/day) on hot plate test

Group	Latency time to pain response in seconds			
	Initial	On days of treatment		
		10 th day	20 th day	30 th day
Control (normal saline)	3.45±0.52	3.53±0.34	3.46±0.36	3.38±0.39
Vehicle (91.5% alcohol)	3.71±0.42	5.22±0.48	4.59±0.32	4.05±0.40
<i>Lycopodium clavatum</i> 3x	3.62±0.45	5.45±0.48*	4.54±0.42	4.17±0.42
<i>Lycopodium clavatum</i> 6x	3.72±0.40	5.24±0.42	4.15±0.40	4.54±0.35
<i>Lycopodium clavatum</i> 12x	3.65±0.43	5.28±0.48	4.99±0.53	4.08±0.45
<i>Lycopodium clavatum</i> 30C	3.54±0.46	5.26±0.48	4.61±0.45	3.99±0.39

Values are mean±SEM; *Significantly different at $p < 0.05$

Table 2: Analgesic effect of *Lycopodium clavatum* (0.5 ml/rat/day) on ice plate test

Group	Latency time to pain response in seconds			
	Initial	On days of treatment		
		10 th day	20 th day	30 th day
Control (normal saline)	5.91±0.45	6.09±0.37	5.80±0.55	6.04±0.43
Vehicle (91.5% alcohol)	5.85±0.51	7.76±0.56	7.36±0.48	6.44±0.37
<i>Lycopodium clavatum</i> 3x	5.70±0.51	8.31±0.40*	7.27±0.37	6.56±0.44
<i>Lycopodium clavatum</i> 6x	5.88±0.43	7.82±0.49*	7.35±0.45	6.39±0.45
<i>Lycopodium clavatum</i> 12x	5.94±0.52	7.56±0.52	6.89±0.51	6.18±0.43
<i>Lycopodium clavatum</i> 30C	6.03±0.45	7.78±0.52	7.56±0.48	7.01±0.45

Values are mean±SEM; *Significantly different at $p < 0.05$

before administration of drug, vehicle or normal saline and 30 minutes after administration of normal saline on different days of experimentation. There was an increase in the quantum of applied threshold pressure (145.33-152.66 g) required to elicit vocalisation to mechanical pain when measured 30 minutes after the administration of different potencies (3×, 6×, 12× and 30C) of *Lycopodium clavatum* or vehicle at a dose of 0.5 ml/rat/day on 10th day. The difference was significant ($p < 0.05$) only with those rats treated with 3× potency of *Lycopodium clavatum*. Afterwards, the increase in the quantum of threshold pressure required to elicit vocalisation to applied mechanical pain did not persist but gradually tapered off on 20th day and 30th day of experiments on further continuation of the treatment [Figure 3].

Behavioural Activity

Rota-rod test

The effect of different potencies of *Lycopodium clavatum* on motor coordination activity of rats using

grip strength test are given in Table 4. The average grip strength of the rats determined on day 1 before administration of drug, vehicle or normal saline and 30 minutes after administration of normal saline on different days of experimentation on the rotating rod was 'more or less' same (48.31-51.55 seconds). On the other hand, there was a decrease in the grip strength of the rats when measured 30 minutes after the administration of the different potencies (3X, 6X, 12X and 30C) of *Lycopodium clavatum* or vehicle at a dose of 0.5 ml/rat/day for 30 days. On 10th day of experiments, drug or vehicle treated rats fell between 33.71 and 35.59 seconds from the rota rod when they were subjected to test after 30 minutes of drug administration. The decrease in grip strength was significant ($p < 0.05$) only with those rats treated with 3× potency of *Lycopodium clavatum*. Afterwards, there was a progressive reversal in the grip strengths of drug or vehicle treated rats on further continuation of the treatment as the rats stayed for longer duration but still for less duration on the rota rod that was observed on day 1 before

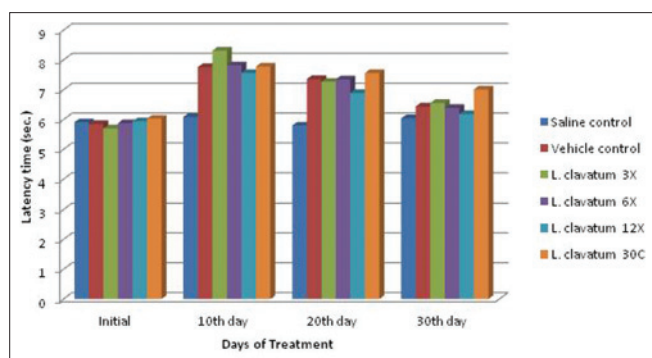


Figure 2: Analgesic effect of *Lycopodium clavatum* (0.5 ml/rat/day) on ice plate test

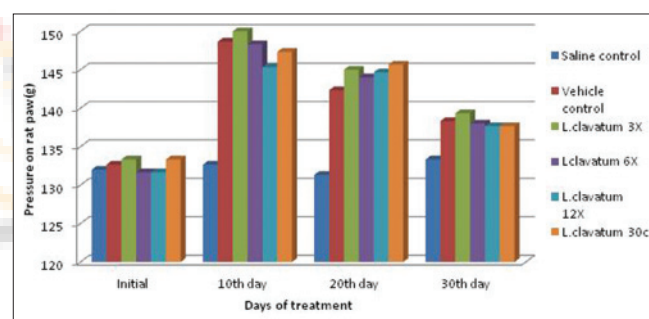


Figure 3: Analgesic effect of *Lycopodium clavatum* (0.5 ml/rat/day) on Randall-Selitto test

Table 3: Analgesic effect of *Lycopodium clavatum* (0.5 ml/rat/day) on Randall-Selitto test

Group	Pressure on rat paw (g)			
	Initial	On days of treatment		
		10 th day	20 th day	30 th day
Control (normal saline)	132.00±4.55	132.66±3.89	131.33±3.75	133.33±4.40
Vehicle (91.5% alcohol)	133.66±4.71	145.66±5.33	142.33±4.33	138.33±4.63
<i>Lycopodium clavatum</i> 3x	133.33±4.37	150.00±5.36*	145.00±5.77	139.33±4.91
<i>Lycopodium clavatum</i> 6x	131.66±4.40	148.33±4.48	144.00±4.00	138.00±5.00
<i>Lycopodium clavatum</i> 12x	131.66±3.66	145.33±3.71	144.66±4.66	137.66±4.33
<i>Lycopodium clavatum</i> 30C	133.33±3.84	147.33±3.92	145.66±4.97	137.66±4.33

Values are mean±SEM; *Significantly different at $p < 0.05$

Table 4: Behavioural effect of *Lycopodium clavatum* (0.5 ml/rat/day) on rota-rod test

Group	Grip strength in seconds			
	Initial	On days of treatment		
		10 th day	20 th day	30 th day
Control (normal saline)	51.53±2.84	51.55±2.39	49.97±2.61	50.70±3.45
Vehicle (91.5% alcohol)	48.31±3.87	34.81±3.21	39.92±2.90	47.74±2.46
<i>Lycopodium clavatum</i> 3x	50.52±3.71	33.71±2.48*	38.47±3.58	45.59±3.38
<i>Lycopodium clavatum</i> 6x	48.93±3.72	34.36±4.09	37.82±3.58	46.88±2.48
<i>Lycopodium clavatum</i> 12x	48.83±3.67	35.38±3.68	40.74±2.82	45.26±2.96
<i>Lycopodium clavatum</i> 30C	49.52±3.79	35.59±3.70	43.32±3.20	41.00±2.33

Values are mean±SEM; *Significantly different at $p < 0.05$

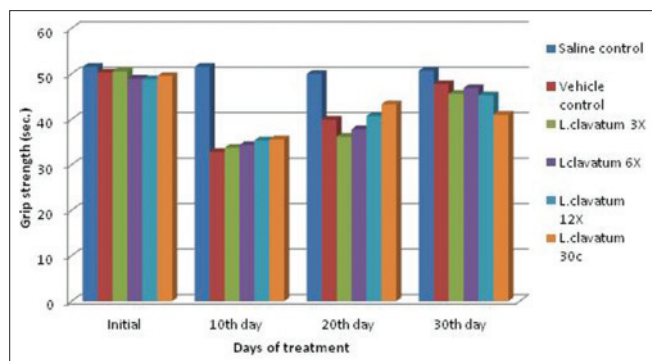


Figure 4: Behavioural effect of *Lycopodium clavatum* (0.5 ml/rat/day) on rota-rod test

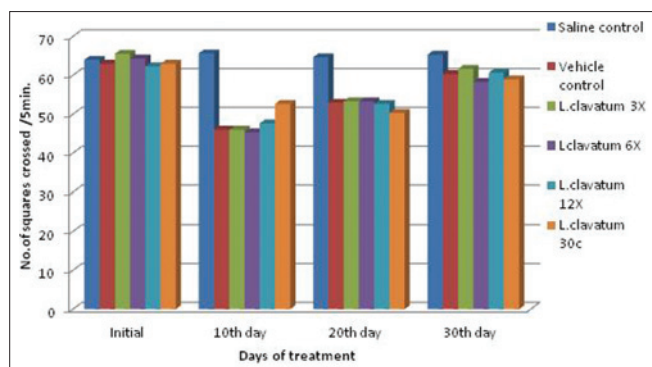


Figure 5: Behavioural effect of *Lycopodium clavatum* (0.5 ml/rat/day) in the open field test

administration of drug when tested on 20th and 30th day of experiment [Figure 4].

Open Field Test

Table 5 shows the effect of different potencies of *Lycopodium clavatum* on locomotor activity of rats using open field test. The average locomotor activity as measured in terms of crossing of the squares of a open-field apparatus during 5 minutes of observations on day 1 before administration of drug, vehicle or normal saline and 30 minutes after the administration of normal saline on different days of experimentation was ‘more or less’ same (62.33-65.66 squares in 5 minutes). There was a decrease in the locomotor activity of the rats (45.33-52.66 squares in 5 minutes) when measured on 10th day of the experiment 30 minutes after administration of different potencies (3X, 6X, 12X and 30C) of *Lycopodium clavatum* or vehicle at a dose of 0.5 ml/rat/day. The difference in locomotor activity was significant ($p < 0.05$) with those rats treated with 3× and 6× potencies of the drug when compared than that of normal saline treated rats or initial locomotor activity taken just before administration of drug on day 1 of the study.

Table 5: Behavioural effect of *Lycopodium clavatum* (0.5 ml/rat/day) in the open field test

Group	Numbers of squares crossed in 5 min.			
	Initial	On days of treatment		
		10 th day	20 th day	30 th day
Control (normal saline)	64.00±3.51	65.66±4.70	64.66±3.52	65.33±4.80
Vehicle (91.5% alcohol)	63.00±4.00	46.00±4.93	53.00±3.21	60.33±4.17
<i>Lycopodium clavatum</i> 3x	65.53±4.37	46.00±4.58*	53.33±4.40	61.66±4.17
<i>Lycopodium clavatum</i> 6x	64.33±3.84	45.33±3.75*	53.33±4.25	58.33±3.48
<i>Lycopodium clavatum</i> 12x	62.33±4.48	47.66±4.91	52.66±3.71	60.66±5.36
<i>Lycopodium clavatum</i> 30C	63.00±4.50	52.66±3.17	50.33±4.09	59.00±3.05

Values are mean±SEM; *Significantly different at $p < 0.05$

However, such depressant effect on locomotor activity slowly vanished off when tested subsequently on 20th and 30th day of the study [Figure 5].

DISCUSSION AND CONCLUSIONS

In homoeopathy, the entire plant of *Lycopodium clavatum* is widely used to treat variety of ailments^[13] but not for the treatment of central nervous system diseases unlike its use in traditional medicine for muscle cramps, rheumatism and epilepsy besides other disorders.^[4]

The objective of the present preliminary study was to examine the analgesic and behavioural activities of homoeopathic formulation of *Lycopodium clavatum* (3X, 6X, 12X and 30C) in albino rats. Positive results if obtained in animal models, rats it could be extrapolated in meaningful and convincing manner for their possible human use in homoeopathy.

The analgesic activities of different potencies (3X, 6X, 12X and 30C) of *Lycopodium clavatum* in rats were evaluated by using hot plate assay, ice plate assay and by Randall - Selitto assay. The data obtained for its analgesic effects revealed that all the four potencies of *Lycopodium clavatum* had increased the latency times for both thermal noxious stimulus and cold sensation and had also increased the quantum of threshold pressure to mechanical induced pain when measured on 10th day of study 30 minutes after the administration of the drug. The level of significance varied not only between the potencies of the drug but also between different sets of experiments when compared than that of normal

saline group or initial values taken just before administration of drug on first day of the study.

The behavioural activities of different potencies of *Lycopodium clavatum* (3X, 6X, 12X and 30C) in rats were evaluated by using rota-rod (for motor coordination) and open field test (for locomotor activity). The present results showed that different potencies of *Lycopodium clavatum* (3X, 6X, 12X and 30C) had decreased the grip strength and locomotor activity when measured on 10th day of study, 30 minutes after the administration of the drug. Further continuation of drug treatment resulted into wearing off their depressive responses on the central nervous system of the rats when tested on 20th and 30th day of the study.

Increased in the latency time to noxious thermal stimulus and/or cold sensation, and increased in the quantum of threshold pressure to mechanically induced pain and decreased locomotor activity and motor coordination by the drug is the sign of CNS depression.^[23,24] Wearing off the depression on prolonged and continuous use of the drug may be either due to decreased sensitivity of the central nervous system or due to increased metabolising enzymatic activity in the liver.

The present preliminary study suggests that the homoeopathic formulations of *Lycopodium clavatum* possess central nervous system depressant activity; however, detailed studies are mandatory in order to arrive at a definitive conclusion.

ACKNOWLEDGMENT

The authors are thankful to Head, Department of Zoology, Osmania University, Hyderabad for extending laboratory facilities and Mrs. Maya Padmanaban, Statistical Assistant, CCRH, New Delhi for preparation of histogram.

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How to cite this article: Sundaram EN, Singh KP, Reddy PK, Kumar S, Nair KR, Khurana A, Singh H, Nayak C. Preliminary study to evaluate analgesic and behavioural effects of *Lycopodium clavatum* in experimental animals. Indian J Res Homoeopathy 2013;7(4):168-74.

Source of Support: Nil, **Conflict of Interest:** None declared.

पृष्ठभूमि एवं उद्देश्य: होम्योपैथी में लायकोपोडियम क्लेवेटम नामक औषधि का पाचक विकार, मूत्रीय अंगों का सूजन व त्वचीय विकारों में उपयोग किया जाता है लेकिन केन्द्रीय तन्त्रिका तन्त्र में विकार के उपचार के लिए उपयोग नहीं किया जाता है। इसके विपरीत केन्द्रीय तन्त्रिका तन्त्र की स्थिति के लिए पारम्परिक व लेख औषधियों में इसका उपयोग किया जाता है। इसलिए, पशु मॉडलो में लायकोपोडियम क्लेवेटम के होम्योपैथिक योगों के संभावित वेदनासार और व्यावहारिक गतिविधियों का पता लगाने के उद्देश्य से वर्तमान अध्ययन किया गया।

सामग्री एवं विधियाँ: दोनों लिंगों के विस्टर एलबिनो चूहों (120–140) में लायकोपोडियम क्लेवेटम औषधि के प्रभाव का आकलन, गर्म प्लेट, बर्फ की प्लेट और रेन्डाल-सेलिटो परीक्षण और व्यावहारिक प्रभाव, रोटा छड़ एवं खुली क्षेत्र परीक्षण के उपयोग द्वारा किया गया। 30 दिनों के लिए 0.5 एमएल लायकोपोडियम क्लेवेटम औषधि की विभिन्न पोटेंसिया (3X, 6X, 12X और 30C) चूहों को खिलायी गई और औषधि की प्रतिक्रिया को 10वें, 20वें, और 30वें दिन पर दवा प्रशासन के तहत 30 मिनट के बाद मापा गया। अनुपान और लवणयुक्त उपचारित चूहों की तुलना हेतु इस दवा से उपचारित अन्य जानवरों के साथ परीक्षण किया गया।

परिणाम: इस अध्ययन के अनुसार लायकोपोडियम क्लेवेटम औषधि की सभी चार पोटेंसियों को बढ़ाने पर आवश्यक विलंबता समय बढ़ गया और उष्ण अनुभूति के लिए आगे और पीछे के पैरों का चाटना और यांत्रिक प्रेरित दर्द की सीमा दबाव की मात्रा में भी वृद्धि हुई लेकिन गामक समन्वय और गतिशीलता गतिविधि में कमी थी।

निष्कर्ष: इस अध्ययन से पता चलता है कि लायकोपोडियम क्लेवेटम औषधि के होम्योपैथिक योगों की विभिन्न पोटेंसिया (3X, 6X, 12X और 30C) केन्द्रीय तन्त्रिका तन्त्र क्रिया की क्षमता को कम करती है। इसलिए मानव के सम्भव उपयोग हेतु लायकोपोडियम क्लेवेटम औषधि का और अधिक अनुसंधान किया जा सकता है।

