

## Selected neurobehavioural evaluation of *Leucas Aspera* in wistar albino rats

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### ABSTRACT

**Background:** *Leucas Aspera* is used in traditional medicine for many ailments. The present study was designed to evaluate the neuro behavioural effects in Wistar albino rats after oral administration of leaves of *Leucas Aspera* for 28 days.

**Methods:** Wistar albino rats of either sex were treated with the aqueous suspension of dried powdered leaves of *L. aspera*. Three different groups of animals received *L. aspera* suspension in doses of 50 mg/kg/day, 100 mg/kg/day or 200mg/kg/day orally for 28 days. The animals were weighed once in a week and the food and water consumption was monitored every day. Behavioural changes in these animals were monitored every week using Rotarod, Actophotometer and William's maze.

**Results:** A significant increase in body weight, food and water consumption in rats could be recorded after 3<sup>rd</sup> and 4<sup>th</sup> week of treatment with 200mg/kg/day *L.aspera*. However, there were no changes in any of the behavioural responses in *L.aspera* treated groups compared to control.

**Conclusions:** The unaltered neuro behavioural responses after *L. aspera* treatment indicate the safe nature of this medicinal plant. The significant increase in food consumption and body weight changes suggest a potential anabolic/ nutraceutical property of *L. aspera*.

**Keywords:** *Leucas aspera*, Neurobehavioural effects, Nutraceutical property

### INTRODUCTION

*Leucas aspera* (Spreng.) is a wild plant, belongs to Family Labiatae and called as "THUMBAL" in Tamil.<sup>1</sup> The plant has been reported to possess many medicinal properties such as antipyretic<sup>2</sup>, analgesic<sup>3</sup>, and anti-fungal properties.<sup>4</sup> In Ayurvedic medicine it has wide applications in the treatment of common cold, migraine and Sinusitis.<sup>5</sup> The plant is erroneously reputed to be antidote for snake venom.<sup>5</sup> The juice of the leaves of *L. aspera* is being applied externally in Rheumatism, Psoriasis and chronic skin eruption.<sup>6</sup> The leaves of *L. aspera* has been demonstrated to have the anti-inflammatory effect against Carrageenin induced Paw Edema and in cotton pellet

induced Granuloma.<sup>7</sup> *L. aspera* extract has been reported to possess Prostaglandin inhibitory activity and antioxidant property.<sup>8</sup> The extract of *L. aspera* has also been reported to possess anti-bacterial activity against *Micrococcus Pyogenes* and *Escherichia Colli*.<sup>9</sup> Even though the plant has been used to treat various conditions, no attempt has been made to investigate the neuro behavioural effects with oral administration of *L. aspera* and hence the present study.

### METHODS

**Plant materials:** The leaves of *Leucas aspera* were collected from Mangadu, Chennai. The plant was

identified and authenticated by Dr. S.P. Kamala Nalini Ph.D., Department of plant biology & plant biotechnology, SIR The yagaraya College, Chennai 600021, Tamil Nadu, India.

**Preparation of extract:** The leaves *Leucas aspera* were shade dried for 15 days after through washing. The suspension of this coarse powder was evenly made in distilled water by homogenization. Various phytoconstituents like alkaloid, sterols, galactose, oleonolic acid<sup>10</sup>, sitosterols<sup>10</sup>, triterpenoids<sup>11</sup> were detected from various extract of the leaves of *Leucas aspera*.

**Experimental Animals:** Healthy Wistar albino rats weighing 110 to 150 Grams, of either sex, were obtained from the Veterinary College, Madhavaram. Animals were kept in colony cages with husk bedding at  $25 \pm 2^{\circ}$ , relative humidity 50-55%, maintained under 12hr light and dark cycle. The animals were fed with standard rat pellet feed obtained from Gulmohar, India Ltd., Bangalore and given water ad libitum. The study protocol was approved by the Institutional Animal Ethical Committee.

**Acute toxicity studies:** The acute oral toxicity study was done in Wistar Albino rats of either sex according to CPCSEA recommend OECD guidelines 425. There were no changes from non toxic dose to 2000mg/kg, po. The LD<sub>50</sub> calculated is 2000mg/kg for the aqueous suspension of *Leucas aspera*, hence less than of one tenth and one tenth of the maximum tested dose (i.e. 50mg/kg, 100mg/kg, 200mg/kg) were selected for the evaluation of neuro behavioural effect.

**Study design:** Wistar albino rats were selected at random and divided into four groups and each group comprising of six animals. A group of animals were treated with distilled water served as control and three different groups of animals received *L. aspera* suspension in doses of 50mg/kg, 100mg/kg, 200mg/kg per oral daily for 28 days.

The animals were weighed once in a week and noted down. Food consumed by both test and control group animals were monitored daily from the remaining food in the cage and it was weighed and the required food pellet for these animals were given and noted. Water consumed by both test and control group animals were calculated once a day by measuring the remaining water level in the polypropylene container (with calibrations having a capacity of 250 ml). Thus the required quantity of water for these animals were measured and given. The data were noted down.

Once in a week (7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, 28<sup>th</sup> day), the behavioural studies like the, Rotarod, Actophotometer and william's maze observation were carried out in both test and control group animals.

### Behaviour studies

**Hebb-William's maze<sup>12</sup>:** The method is used to test the learning and memory. The treatments were randomized throughout the day, between 08:00 and 13:00 h, to control the diurnal variations in animal activity. Rats were trained for five days before starting the experiments. The time taken for each trial was recorded. Between each trial, the maze was wiped clean with a damp sponge and dried with paper towels. The time taken by the animals (both control and test group) to traverse the maze and to reach the food compartment was the criterion for successful learning and memory activity.

**Rotating Rod<sup>12</sup>:** This method is used to assess the ability of the animals to maintain equilibrium and muscle control on a rotating rod. There is a provision in the Rota rod to mark the time of the falling of the animals. The animals (both control and test group) were placed on the rotating rod and the duration to balance and to maintain itself on the rotating rod was noted.

**Actophotometer<sup>12</sup>:** Locomotor (ambulatory) and behavioural (total) activity was measured using actophotometer. The apparatus consists of a rectangular cage with perforated metal flooring designed for recording the walking and running activity of rats and mice. The number of interruptions on light beams produced by lateral movements of the animals (both control and test groups) during a fixed time interval (2 minutes as cut off period) was recorded by a digital counter.

### Statistical analysis

The data obtained from body weight, food consumption, water consumption and behavioural studies were expressed as mean and standard deviation (SD) and analysed by one way analysis of variance followed by LSD post hoc test. A value of  $P < 0.05$  was considered significant.

## RESULTS

**Body Weight:** Body Weight was found to be within normal limits in the Control group through out the study period. There was no significant change has been observed in the Test groups compared to the Control groups at the end of 7<sup>th</sup> and 14<sup>th</sup> day. In the group treated with *L. aspera* 200 mg / kg, the animals showed significant ( $P < 0.05$ ) increase in body weight at the end of 21<sup>st</sup> day as compared to the control and to the animals treated with *L. aspera* 50 mg/ kg. At the end of the 28<sup>th</sup> day, there was a significant ( $P < 0.05$ ) increase in the body weight in the Group treated with *L. aspera* 200 mg/kg when compared to Control and to the Group treated with *L. aspera* 100 mg/ kg. In the Group treated with *L. aspera* 200 mg/kg, there was a moderate increase in body weight at the end of the 28<sup>th</sup> day when compared to 7<sup>th</sup> day of the treatment (Table 1).

**Table 1: Effect of *Leucas aspera* on changes in body weight.**

Treatment	Changes in body weight (gms)				
	0 Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day	21 <sup>th</sup> Day	28 <sup>th</sup> Day
Vehicle	129.67±12.99	125.67±15.72	133.00±17.15	135.00±13.78	140.8±13.54
Leucas aspera (50mg/kg)	113.33± 5.16	128.33±13.29	136.50±15.41	138.67±17.51	145.00±27.39
Leucas aspera (100 mg/kg)	124.67±12.44	130.00±16.73	135.00±21.68	140.00±21.21	143.60±22.65
Leucas aspera (200 mg/kg)	118.33± 9.83	136.67±12.11	142.00±12.00	159.80*±12.21	176.66*±22.42

Each value is presented as mean ± SD (\*P < 0.05) when compared with vehicle. One way ANOVA followed by LSD post hoc test.

**Table 2: Effect of *Leucas aspera* on food consumption.**

Treatment	Food consumption in gms			
	7 <sup>th</sup> Day	14 <sup>th</sup> Day	21 <sup>th</sup> Day	28 <sup>th</sup> Day
Vehicle	50 ±6.32	49.17 ±6.65	59.17 ±11.14	50.00±8.37
Leucas aspera (50mg/kg)	60.83*±5.85	52.50±10.84	62.50±7.58	55.83±3.76
Leucas aspera (100 mg/kg)	65.83**±9.70	57.50±12.14	68.33±10.33	52.50±6.12
Leucas aspera (200 mg/kg)	73.33**±7.53	70.83*±15.94	89.33*±8.16	83.00**±13.78

Each value is presented as mean ± SD (\*P < 0.05\*\* P < 0.01) when compared with vehicle. One way ANOVA followed by LSD post hoc test.

**Food Consumption:** Food consumption was found to be within the normal limits through out the experimental period in the Control Group. In the Group treated with *L. aspera* 50 mg/ kg, there was a significant (P < 0.05) increase in the consumption of food at the end of 7<sup>th</sup> day and there was a moderate increase (P < 0.01) increase in the food consumption in Group treated with *L. aspera* 100 mg/kg and in Group treated with *L. aspera* 200 mg/kg as compared to the Control group. At the end of the 14<sup>th</sup> day, the Group treated with *L. aspera* 200mg/kg showed significant (P < 0.05) increase in food consumption as compared to the Control group and Group treated with *L. aspera* 50 mg/kg. Though there was an increase in food consumption at the end of 21<sup>st</sup> day in the test groups, it was not statistically compared to control animals. In the Group treated with *L. aspera* 200mg/ kg the animals showed significant (P < 0.01) increase in food consumption when compared to the control and to the Group treated with *L. aspera* 50mg/kg and to the Group treated with *L. aspera* 100 mg/kg. In the Group treated with *L. aspera* 200 mg kg, there was a significant (P < 0.01) increase in food consumption at the end of the 28<sup>th</sup> day, compared to the Control and to the Group treated with *L. aspera* 50mg/kg and to the Group treated with *L. aspera* 100mg/kg (Table 2).

**Water Consumption:** Water consumption was normal through out the experimental period in the control group. But the Group treated with *L. aspera* 200 mg/kg showed significant (P < 0.01) increase in water consumption as compared to the Control and a mild increase (P < 0.05) as compared to the Group treated with *L. aspera* 50 mg/kg and Group treated with *L. aspera* 100 mg/kg. At the end of the 14<sup>th</sup> day, the Group treated with *L. aspera* 200mg/kg showed a significant (P < 0.05) increase in water consumption as compared to the Control, Group treated with *L. aspera* 50 mg/kg and Group treated with *L. aspera* 100mg/kg. There was a significant (P < 0.01) increase in water consumption in the Group treated with *L. aspera* 200 mg/kg at the end of 21<sup>st</sup> and 28<sup>th</sup> days as compared to Control, Group treated with *L. aspera* 50 mg/kg and Group treated with *L. aspera* 100mg/kg (Table 3).

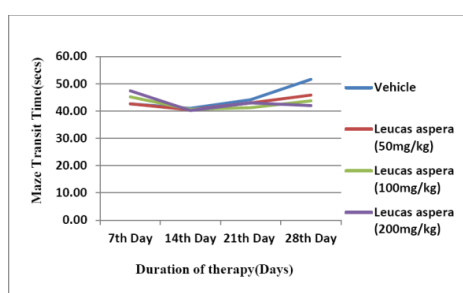
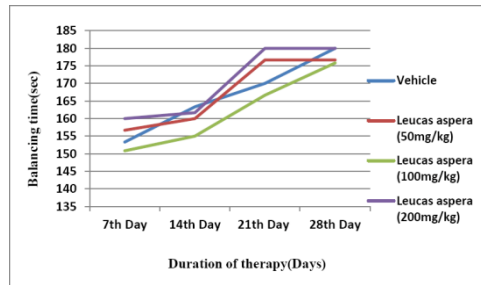
**William's Maze (Learning and Memory):** Comparison of the time interval recorded in the test groups with Control groups did not reveal any statistically significant change (Figure 1).

**Actophotometer (Motor Activity):** There were no significant changes to be reported in the motor activity in all the test groups in comparison to the control Group (Figure 2).

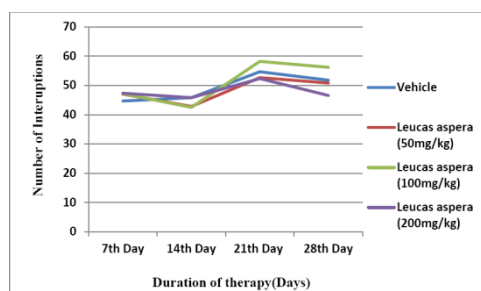
**Table 3: Effect of *Leucas aspera* on water consumption.**

Treatment	Water consumption in ml			
	7 <sup>th</sup> Day	14 <sup>th</sup> Day	21 <sup>th</sup> Day	28 <sup>th</sup> Day
Vehicle	77±16.55	72.14±17.76	65.00±14.43	62.86±13.50
Leucas aspera (50mg/kg)	89.29±10.97	80.71±10.58	62.14±12.86	66.43±9.00
Leucas aspera (100 mg/kg)	94.29±18.35	75.71±10.18	68.57±9.88	69.29±13.67
Leucas aspera (200 mg/kg)	120.00**±25.3	97.86*±18.22	102.14**±14.10	118.57**±21.93

Each value is presented as mean ± SD (\*P < 0.05\*\* P < 0.01) when compared with vehicle. One way ANOVA followed by LSD post hoc test.

**Figure 1: Effect of *Leucas aspera* on Learning and Memory by Hebb-William's maze.****Figure 2: Effect of *Leucas aspera* on Motor Co-ordination by rotarod.**

**Rotarod (Motor Co-ordination):** There was no statistical difference noted in the Rotarod performance between the control and the Test groups (Figure 3).

**Figure 3: Effect of *Leucas aspera* on motor activity by actophotometer.**

## DISCUSSION

*L. aspera* is being used in India to treat Chronic Rheumatism, Psoriasis and Chronic skin eruptions<sup>6</sup>. Ayurvedic Medicine claims include antipyretic effect, usefulness in common cold, Sinusitis, migraine. *L. aspera* has been claimed to possess anti-inflammatory property and anti-oxidant property.<sup>8</sup> The present Study with *Leucas aspera* in Wistar albino rats was carried out for the evaluation of learning and activities which have shown that there was no gross deviation between the test groups in comparison with the Control group.

The results of studies with Actophotometer have shown that the normal activity during the course of exposure remained unchanged. The ability of the animals to learn was not affected with the administration of plant material as observed by evolution with William's maze. Administration of *L. aspera* has also not interfered with the neuro-muscular co-ordination as observed by Rotarod evaluation. Similar to the Control animals, all the groups exposed to *L. aspera* have shown improvement in the form of staying for longer time after 28 days over the Rotarod compared to the time of stay during the 7<sup>th</sup> day.

Apart from this, observations with reference to food and water consumption indicate that this plant possesses appetizing property and its potential in these lines needs to be investigated further. Food consumption parallels that of increase in body weight and at the same time, increase in water consumption has not resulted in any observable water retention (or) water logging condition like Pedal edema, Ascites etc.

The observation thus recorded indicates that the plant has got the potential to improve the body weight and food consumption. It has been reported that the sterolic compounds like, Alpha Sitosterol and B – Sitosterol have been isolated from the leaves of this plant and any possibility of an anabolic effect by these compounds to be explored. Further investigations in this aspect is recommended. This property without any untoward effect as seen by the neurobehavioral parameters makes the

plant *L. aspera* to be a potential candidate for being an ingredient in the nutraceutical formulations.

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*Ethical approval: This study was approved by the Institutional Animal Ethics Committee.*

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