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Analgesic effect of *Lepidium sativum* Linn. *(*Chandrashura*)* in experimental animals

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Abstract

Lepidium sativum Linn, which is known as "*Aselio*" locally, is frequently used by the villagers for the treatment of *Sandhivata* (osteoarthritis), with good therapeutic relief. Here, we have to observe the analgesic activity of the seed of *Lepidium sativum* Linn in albino rats and Swiss albino mice with different parameters.The analgesic study was performed with acetic acid-induced writhing response in mice, formaldehyde-induced paw licking response in rats and tail flick response in mice. Experiments were carried out in two groups – therapeutic dose group and double dose group – with comparison with the control group. In the acetic acid-induced writhing syndrome, latency of onset was highly significantly increased in the therapeutic dose group and significant increase was found in the double dose group. In the formaldehyde-induced paw licking response, the test drug produced significant inhibition of neurogenic pain in the double dose group and significant inhibition of inflammatory pain in the therapeutic dose group. In the tail flick response, the test drug produced a mild to moderate effect in the therapeutic dose group and also in the double dose group.

Key words: Analgesic, paw licking response, tail flick, writhing response, *Lepidium sativum* Linn.

Introduction

The seed powder of *Lepidium sativum* Linn has a strong folklore claim to be effective in the treatment of arthritis. Hence, it was subjected to be explored for analgesic activity by employing different experimental models. An acetic acid-induced writhing test was used for detecting both central and peripheral analgesia. The formaldehyde paw licking test produces both neurogenic pain and pain due to inflammation. Centrally acting analgesic drugs inhibit both phases of the formalin test and peripherally acting analgesic drugs only inhibit the second phase. The formaldehyde injection produces inflammation in the rat paw, which produces pain as well as edema. The first half an hour after the injection of formaldehyde is important for observing the analgesic activity as it is marked by pain. The delay in onset and decrease in the frequency of paw licking after formaldehyde injection in the test drug-treated rats is considered to indicate an analgesic effect. The tail flick test is the most sensitive to centrally acting analgesics. The centrally acting analgesics generally elevate the pain threshold of mice toward heat.

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Materials and Methods

The suspension of Lepidium seed powder was made with sufficient quantity of distilled water according to the required dose. Charles Foster albino rats and Swiss albino mice were selected for the animal study. Drug dose was calculated by referring to the table of Paget and Barnes, (1964)[1] Hence, the calculated dose for the rat was 550 mg/kg body weight and for the mice was 780 mg/kg. Drug was administered through the oral route with the help of a gastric catheter sleeved to a syringe. Animals were randomly divided into the therapeutic dose group and the double dose group with comparison with the control group, which was administered distilled water in the same volume.

Acetic acid-induced writhing syndrome

Acetic acid (1% v/v) was administered intraperitoneally to all the groups at a dose of 1 ml/kg body weight 60 min after the administration of test compounds. The anti-nociception analgesic effect was recorded by counting the number of writhes after the injection of acetic acid for a period of 30 min. A writhe is indicated by abdominal constriction and full extension of the hind limbs.

Formaldehyde-induced paw licking response in rats

The effect of *Lepidium sativum* seed powder on the formaldehyde-induced paw licking response was evaluated by adopting the method used by Bittar *et al.*[2] After the injection of formaldehyde, the animals were kept under observation for half an hour. The time taken for the onset of paw licking and

its frequency was measured in five phases, as 0–5 min, 5–10 min, 10–15 min, 15–20 min and 20–30 min.

Tail flick test

The basal reaction time of animals to radiant heat was recorded by placing the tip (last 1–2 cm) of the tail on the radiant heat source. The tail withdrawal from the heat (flicking response) is taken as the end point. The animals that showed a flicking response within 3–5 s were selected for the study. A cut-off period of 15 s is observed to avoid damage to the tail. After the administration of the drug, the tail flick response was taken at 30 min, 60 min, 120 min, 180 min and 240 min.[3]

Statistical analysis

Students "*t*"-test for unpaired data has been used for analyzing the data generated during the study. However, in case of comparing more than two samples, the analysis of variance (ANOVA) test is applied using the Dunnet's multiple "*t*"-test [Table 1].

Observations and Results

The latency of onset of the writhing syndrome was significantly prolonged in the test drug-administered groups in comparison with the control group. There was also an apparent decrease in the number of writhings in these groups in comparison with the control group. However, it did not reach a statistically significant level [Table 2].

The onset of paw licking response was shortened to a moderate extent in the test drug-administered group in comparison with the control group, but the shortening was found to be statistically non-significant. A statistically non-significant increase in the paw licking response was observed during the first phase in both the test drug-administered groups. During the second phase, a statistically significant decrease was observed at a lower dose level while a statistically significant increase in the paw licking response was observed [Table 3].

The test drug at both the dose levels failed to elevate the threshold for the tail flick response in comparison with the control group. Although a moderate increase and decrease were observed at some time intervals, the observed changes were found to be statistically non-significant.

Discussion

Acetic acid writhing response

Intraperitoneal administration of acetic acid releases prostaglandins and phlogistic mediators like PGE_2 and PGE_2a, and their levels were increased in the peritoneal fluid of the acetic acid-induced mice.[4] The drug in the therapeutic dose and in the double dose group significantly ($P < 0.001$) reduced the number of abdominal constrictions and stretching of the hind limbs induced by the injection of acetic acid in a dose-dependent manner.

The abdominal constrictions produced after the administration of acetic acid are related to sensitization of the analgesic receptors to prostaglandins. It is therefore possible that the drug is effective due to its analgesic effect, probably by inhibiting the synthesis or action of prostaglandins.

Formaldehyde-induced paw licking response

After the injection of formaldehyde, the time taken for the onset of the response and the frequency of paw licking are observed. The latter is observed in two phases, i.e. 0–15 min and 15–30 min. The paw licking observed during the first phase is supposed to be reflective of neurogenic pain while the second phase is supposed to have its origin in inflammation; hence, termed as inflammatory pain. The edema formation that accompanies formaldehyde injection is supposed to represent the proliferative phase of inflammation and hence its suppression is considered to be representative of suppression of the proliferative phase of inflammation. In this test, both neurogenic pain and inflammation continuous pain are produced. Centrally acting drugs inhibit both phases while peripherally acting drugs only inhibit the second phase. A slight delay was observed in the onset of paw licking in both the therapeutic dose and the double dose levels. A significant inhibition of neurogenic pain was observed in the double dose and a mild inhibition was observed in the therapeutic dose

Table 1: Effect of test drug on acetic acid writhing syndrome in mice

Treatment	Dose (mg/kg)	Latency of onset		Frequency of writhing	
		Onset (s)	(%) change	For 30 min after acetic acid injection	(%) change
Control	QS	4.83 ± 0.40	-	36.33 ± 4.72	-
Low-dose A	780	10.17 ± 0.65**++#	110.55↑	29.67 ± 3.38	18.33↓
High-dose B	1,560	7.50 ± 1.12*++	55.27↑	32.08 ± 3.89	11.99↓

**P < 0.001; *P < 0.05; ++ ANOVA test (F, 11.62; P < 0.01; df [2,15]), #Dunnets test; P < 0.05, Data: Mean ± SEM; ↑, increase; ↓, decrease; QS, quantity suffice

Table 2: Effect of test drug on the formaldehyde-induced paw licking syndrome in rats

Treatment	Dose (mg/kg)	Frequency of paw licking after formaldehyde injection (mean ± SEM)					
		Onset (s)	(%) change	Neuro. 0–15	(%) change	Impo. 16–30	(%) change
Control	QS	46.67	-	3.83 ± 0.83		9.67 ± 1.54	
Low-dose A	550	36.17	↓19.02	5.50 ± 0.96++	↑43.60	5 ± 0.89*++	↓48.29
High-dose B	1100	36.67	↓17.90	6.50 ± 0.76++	↑69.71	13.17 ± 13.0++*	↑36.19

*P < 0.05; ++ ANOVA test (F, 6.81; P < 0.01; df [2, 15 for 0–15 min]) (F, 10.33; P < 0.01; df [2, 15 for 16–30 min]); #Dunnets test; P < 0.05, Data: Mean ± SEM; ↑, increase; ↓, decrease

Table 3: Effect of *Lepidium sativum* on the tail flick response at different intervals in mice

Group	Dose (mg/kg)	30 min	60 min	120 min	180 min	240 min
C	QS	11.17 ± 2.18	11.50 ± 1.65	11.33 ± 1.05	10.50 ± 1.84	10.67 ± 1.69
A	780	↓10.33 ± 1.33	↓10.33 ± 1.33	↓9.33 ± 1.02	↓9.50 ± 1.38	↓7.83 ± 0.79
B	1,560	↑13.00 ± 2.33	↑14.00 ± 2.46	↓9.00 ± 1.63	↑11.17 ± 0.70	↑10.67 ± 9.50

Data: Mean ± SEM; ↑, increase; ↓, decrease

group. However, a significant inhibition of inflammatory pain is observed in the therapeutic dose group while the double dose group showed a marginal increase.

Tail flick

This test is most sensitive to centrally acting analgesics. These drugs generally elevate the pain threshold of mice toward heat. In the tail flick response, the test drug at the therapeutic dose level shows a decrease at different intervals, while in the double dose group it increases at 30 min, 60 min and 180 min and decreases at 120-min and 240-min intervals.

Conclusion

In acetic acid writhing, a moderate 18.33% suppression at a lower dose level and a marginal 12% suppression at a higher dose level were seen. This indicates that the test drug has only a weak peripheral analgesic activity. This may be due to the presence of a moderate anti-inflammatory activity. The formaldehyde paw licking response in phase I was not affected, indicating a lack of effect on neurogenic pain. However, a significant suppression of paw licking was observed during phase II at a lower dose level, while at a higher dose level, a significant increase was observed.

The test drug at the dose level studied did not produce consistent significant elevation of threshold for the tail flick response, indicating a lack of central analgesic activity in it.

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हिन्दी सारांश

लेपिडिअम सटाइवम(चंद्रशुर) के वेदनाहर प्रभाव का प्रायोगिक अध्ययन

नीता रावल बी. रविशंकर

लेपिडिअम सटाइवम (चंद्रशुर), स्थानिक गुजराती भाषा में 'असेलियो' के नाम से जाना जाता है। यह संधिवात में प्रयुक्त होता है। प्रस्तुत अध्ययन में इस वनस्पति के बीज का स्विस अल्बिनो चूहों में वेदनाहर प्रभाव विभिन्न मापदण्डों पर परीक्षित किया गया। परिणामस्वरूप इसका औषधिय मात्रा वर्ग में तथा दो गुना ज्यादा मात्रा वर्ग में मंद से मध्यम वेदनाहर प्रभाव पाया गया।