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Pharmacological Screening of Anti-Nociceptive Potential of Methanolic Leaves Extract of *Lawsonia inermis* in Mice

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ABSTRACT

Pain is a warning signal protective in nature but causes discomfort. The recent research work was based on the screening of anti-nociceptive potential of *Lawsonia inermis* (henna) methanolic leaves extract in mice. The leaves of *Lawsonia inermis* were collected in November from Cantt Area, of Bareilly Distt., Uttar Pradesh. The leaves of *Lawsonia inermis* were washed with water to remove dirt. The leaves were further air-dried under shade and made into the fine powder by using a hand homogenizer and sieved through sieve no. 40 to get a fine powder. Extraction of henna was carried out by using the maceration process. Mice of either sex were divided into four groups of six animals in each group. Group I-Normal saline (as per body weight), Group II-Tramadol (25 mg/kg, i.p.), Group III-*Lawsonia inermis* (100 mg/kg, p.o.), Group IV- *Lawsonia inermis* (200 mg/kg, p.o.). All the solutions were freshly prepared daily and animals were treated for 7 days by oral routes, intraperitoneal routes. The result showed that *Lawsonia inermis* extract possesses a significant anti-nociceptive potential at both doses (100mg/kg, 200mg/kg). Finally, it may be concluded that *Lawsonia inermis* extract may be used in the formulation of new analgesic drug better lowest cost of development.

INTRODUCTION

Analgesics relieve pain symptomatically by acting on CNS or peripherally, but they do not affect its cause. The opioid analgesics are usually used to relieve moderate to severe pain of the visceral organs. Pain is a warning signal protective in nature but causes discomfort[1]. Excessive pain may causes-sinking sensation, fear fullness, sweating, nausea, palpitation, and rise or fall in blood pressure and tachypnea, nausea, vomiting, constipation, drowsiness, depression, and hypotension as a common side effect. They are most useful in the control of acute, chronic, and postoperative pain[2]. At the time of disease or injury, a protective signal is given to the body as a warning, this is known as pain. It is a part of the normal healing process. In this process, inflammation occurs, in which protective cells move to the injured area and release chemical mediators that cause fluids and plasma proteins to leak to the surrounding tissue[3]. Estimates suggest that 20% of adults suffer from pain globally and 10% are newly diagnosed with chronic pain each year. Nevertheless, the problem of pain has primarily been regarded as a medical problem and has been little addressed by the field of public health[4]. There is potent confirmation for three major classes of opioid receptors in the central nervous system (CNS), designated as μ, k, and δ. In the past, designating a receptor as an Opioid heavily depends on the antagonist naloxone, which is an antagonist of all subtypes of Opioid receptors[5,6].

Lawsonia inermis (henna extract) contains numerous chemical constituents like lawsone a major constituent found in plants responsible for its dying properties, likewise essential oils are responsible for its fragrance property. The dried leaves contain about 0.5-1.5% Lawsone. Henna contains phenolic glycosides, alkaloids, anthocyanins, phenols, sterols, xanthoproteins[7]. Henna extract represents a wide range of pharmacological activities like spermicidal, antiulcer, antimicrobial, abortifacient, hepatoprotective, anticonvulsant, antiarthritic, analgesic, diuretic, hypoglycemic, antifungal, wound healing, larvicidal, antifertility, anticancer, antibacterial, and protective property[8-10].

A recent study has shown that Bark of *Lawsonia inermis* possess an analgesic effect when tested on mice[11]. Thus, based on the previous literature survey, the present study was designed for the screening of anti-nociceptive potential of *Lawsonia inermis* (henna) methanolic leaves extract in mice.

MATERIALS AND METHODS

Drugs and experimental requirements

Tramadol as a standard drug, distilled water, methanol, and normal saline is taken from the store Department of Pharmacy, M.J.P. Rohilkhand University, Bareilly.

Beaker, funnel, measuring cylinder, glass rod, Soxhlet apparatus, round bottom flask, heating mantel, and weighing balance.

Plant collection and authentication

The leaves of *Lawsonia inermis* were collected in November from Cantt Area, of Bareilly Distt., Uttar Pradesh. The plant was taxonomically identified and authenticated by Dr. Alok Srivastava, (Associate Professor) Department of plant science, M.J.P. Rohilkhand University, Bareilly, 243006, Uttar Pradesh.

Preparation of methanolic extract of *Lawsonia inermis*

The leaves of *Lawsonia inermis* were washed with water to remove dirt. The leaves were further air-dried under shade and made into the fine powder by using a hand homogenizer and sieved through sieve no 40 and the fine powder was for extraction procedure and evaluation. Dried *Lawsonia inermis* leaf powder sample apparatus at 50-60ºC for 6 hours. These methanolic extracts were concentrated under reduced temperature and pressure on a rotary evaporator.

Identification test of *Lawsonia inermis*

Color **:** Dark Greenish

Melting Point: 195 to 196º

Molar Mass **:** 174.15g/mol

Solubility **:** Insoluble in water

Animal preparation

In the present prospective experimental study, healthy Swiss albino mice of either sex weighing 25-30gms were used for the analgesic activity. Albino mice were selected from the animal's house of the Department of Pharmacy, M.J.P. Rohilkhand University, Bareilly-

243006, U.P. and animals were kept at an ambient temperature of 25±2^{0}C and 55-65% relative humidity with 9-12 hour light and dark cycle. They were fed with a standard diet and water *ad libitum.* Animals were housed in polypropylene cages in groups for at least 1 week before using them for the experiment.

Experimental protocol

Four groups and each group comprising of six mice of either sex were employed in this present study.

(Group I, n=6): Vehicle group

Normal control was treated with normal saline for seven days.

(Group II, n=6): Standard drug (Tramadol)

Animals treated with standard drug tramadol 25mg/kg, intraperitoneal, before 30 min starting the experiment.

(Group III, n=6): Extract I

Animals were treated with methanolic extract of *Lawsonia inermis* (100 mg/kg) orally for seven days.

(Group IV, n=6): Extract II

Animals were treated with methanolic extract of *Lawsonia inermis* (200 mg/kg) orally for seven days.

Firstly, animals were weighed, numbered, and divided into three groups: (a) Reference (b) Control.

Noted the reaction time of mice by flicking its tail from thermal stimuli source. A cut-off time was kept for about 10-12 sec to avoid unneeded pain and damage. Injected the drug (Plant extract) in experimental animals and allowed the drug to be engrossed, and again placed them to thermal stimulus source and noted down the basal reaction time. Reaction time was noted after 15, 30, 45, and 60 min of drug treatment[12].

RESULTS AND DISCUSSION

Test group consists of six animals, in each group treated with *Lawsonia inermis* leaves extract of dose 100 mg/kg, 200 mg/kg and the standard group treated with Tramadol of dose 25 mg/kg respectively for seven days. After treatment, they were tested in analgesiometer apparatus to evaluate its analgesic effect. *Lawsonia inermis* (100mg/kg, p.o.) and *Lawsonia inermis* (200 mg/kg, p.o.) administered group was found to be a significant decrease in reaction time as compared to tramadol (25mg/kg, i.p.) administered group. However higher dose of 200 mg/kg demonstrated an increased reaction time than a lower dose 100 mg/kg treated group.

Table 1: Day-1 Effect of Tramadol (25 mg/kg), Normal saline (5 ml/kg), *Lawsonia inermis* (100 mg/kg) and *Lawsonia inermis* (200 mg/kg) on analgesic activity

OBSERVATION TIME (Min)		15	30	45	60
REACTION TIME (Sec)	Control (5 ml/kg)	3.91±0.74	3.63±0.51	3.71±1.02	3.86±0.73
	Standard (25 mg/kg)	7.49±0.07	8.45±0.11	8.12±0.10	7.25±0.12
	Test-1 (100 mg/kg)	5.55±0.27	5.45±0.17	4.94±0.31	4.86±0.18
	Test-2 (200 mg/kg)	5.35±0.86	4.82±0.11	5.32±0.25	5.55±0.31

Each value represent mean ± SEM (n=6).

When control group (normal saline) was compared to test-1 (100 mg/kg), test-2 (200 mg/kg) and tramadol (25 mg/kg) groups. The result was found to be a significant increase in reaction time. Each value represents mean ± SEM (n=6). The test group consists of six animals, in each group treated with *Lawsonia inermis* leaves extract of dose test-1(100 mg/kg), test-2 (200 mg/kg), and the standard group treated with tramadol of dose 25 mg/kg respectively for seven days. When tramadol (25mg/kg) compare to test-1 (100 mg/kg) and test-2 (200 mg/kg) group, reaction time increase & because tramadol is a standard drug so it is more potent than test-1 and test-2 group.

Table 2: Day-4 Effect of Tramadol (25 mg/kg), Normal saline (5 ml/kg), *Lawsonia inermis* (100 mg/kg) and *Lawsonia inermis* (200 mg/kg) on analgesic activity

OBSERVATION TIME (Min)		15	30	45	60
REACTION TIME (Sec)	Control (5 ml/kg)	3.07±0.31	2.48±0.22	3.06±0.23	3.04±0.16
	Standard (25 mg/kg)	6.49±0.06	6.48±0.05	6.70±.08	6.42±0.09
	Test-1 (100 mg/kg)	4.99±0.11	5.02±0.79	5.117±0.08	5.16±0.07
	Test-2 (200 mg/kg)	4.64±0.21	4.74±0.07	4.35±0.09	4.38±0.12

Each value represent mean ± SEM (n=6)

Tramadol (25 mg/kg) administered group was found to be a significant increase in reaction time as compared to the normal saline (5mg/kg p.o.) administered group. Because tramadol is the standard drug so it is more potent. Each value represents mean ± SEM (n=6). Tramadol treated group consist of six animals, each animal treated with tramadol for seven days. After treatment, they were tested in analgesiometer apparatus to evaluate its analgesic effects.

Table 3: Day-7 Effect of Normal saline (5 ml/kg), Tramadol (25 mg/kg), *Lawsonia inermis* (100 mg/kg) and *Lawsonia inermis* (200 mg/kg) on analgesic activity

OBSERVATION TIME (Min)		15	30	45	60
REACTION TIME (Sec)	Control (5 ml/kg)	3.91±0.74	3.63±0.51	3.71±1.02	3.86±0.73
	Standard (25 mg/kg)	7.49±0.07	8.45±0.11	8.12±0.10	7.25±0.12
	Test-1 (100 mg/kg)	5.55±0.27	5.45±0.17	4.94±0.31	4.86±0.18
	Test-2 (200 mg/kg)	5.35±0.86	4.82±0.11	5.32±0.25	5.55±0.31

Each value represents mean ± SEM (n=6).

In the tail-flick model, the methanol extract from the *Lawsonia inermis* exhibited significant analgesic activity by increasing the reaction time of the mice compared to the control (Normal saline) at all time points. Tramadol was used as a standard drug, which is very potent, respectively. In comparison with control, tramadol produces the most significant effect during all observation times.

Analgesic drugs that are centrally acting elevated the pain threshold of the animal towards heat and pressure[13]. The tail-flick latency of the extract at all reaction times was less than that of the standard drug tramadol which is a more potent opioid with a long duration of action[14].

In the present study, the analgesic activity of *Lawsonia inermis* extract in mice was studied by the Tail flick method using analgesiometer apparatus. The present research demonstrates the leaves of *Lawsonia inermis* contained a higher level of the active component. Various chemical constituents like lawsone (2- Hydroxy-1:4 Naphthoquinone) are principal constituents responsible for coloring properties. *Lawsonia inermis* leaves extract 100mg/kg given orally to the group of mice for continue 7 days at same environmental condition and experiment was start 30 min after drug treatment on analgesiometer. Normal saline (5ml/kg) was given orally to the groups of mice for continue 7 days. *Lawsonia inermis* leaves extract 200 mg/kg given orally to the group of mice for continue 7 days at the same environmental condition. Tramadol (25 mg/kg) given i.p. to the group of mice for continue 7 days. From the obtained result, the following salient finding may be possible. The following parameters are noted down reaction time and each value expressed as mean ± SEM. According to these parameters, graphs were plotted and their activities were evaluated. Normal saline (5 ml/kg) administered group exhibit no significant difference in analgesic activity. This group is administered from day first today seventh of the experiment and results show that normal saline does not affect the analgesic activity of mice.

Tramadol (25 mg/kg) administered group exhibits significant increases in reaction time as compared to normal saline administered group (5ml/kg) and *Lawsonia inermis* extract (100 mg/kg, 200 mg/kg) administered group by using analgesiometer apparatus.

Lawsonia inermis leaves extract (100 mg/kg, 200 mg/kg) administered group was found to significantly increase in reaction time as compared to normal saline (5 ml/kg) administered group. When *Lawsonia inermis* leaves extract (100 mg/kg) and *Lawsonia inermis* extract (200 mg/kg) are administered group compared to tramadol (25 mg/kg) administered group it will significantly decrease the reaction time, by the tail-flick method using analgesiometer. The methanol extract of the *Lawsonia inermis* displayed analgesic activity and supported the traditional use of this plant in pain relief. Further study is warranted to identify the active compounds present in this extract and to elucidate the mechanisms involved in its analgesic properties.

CONCLUSION

Tramadol (25 mg/kg) administered group shows a significant increase in reaction time as compared to normal saline administered group and *Lawsonia inermis* extract administered group. This result shows that tramadol is more potent as compared to *Lawsonia inermis*

leaves extract. However, a higher dose of 200 mg/kg demonstrated an increase in reaction time than a lower dose of 100 mg/kg treated group.

Lawsonia inermis extract (100 mg/kg) and (200 mg/kg) administered group was found to be significant increases in reaction time as compared to normal saline (5ml/kg) administered group. The result stated that *Lawsonia inermis* extract improves the analgesic activity in mice.

Finally, it may be concluded that *Lawsonia inermis* extract exhibits a protective effect and may attribute to its analgesic effect.

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