JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

PHYTOCHEMICAL ANALYSIS AND ANALGESIC ACTIVITY OF THE EXTRACT OF PROSOPIS JULIFLORA

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Abstract :

Background: *Prosopis juliflora* is one of the most economically and ecologically important tree species in arid and semi-arid zones of the world. *Prosopis juliflora* belongs to the family Leguminosae (Fabaceae).

Aims and Objectives: To evaluate the analgesic activity of methanolic extract of dried leaves of *Prosopis juliflora* Linn.

Materials and Methods: Adult male Wistar rats (100–150 g body weight) were used in this study. methanolic extract of *Prosopis juliflora* Linn. was used to evaluate acute analgesic activity by tail flick method by oral administration at doses of 300, and 500 mg/kg body weight in healthy albino rats.

Result: In acute studies, the methanolic extract showed significantly and dose-dependently did not show any significant difference in the reaction time on tail-flick throughout the 60 min. Statistical analysis was carried out by one-way ANOVA, followed by Turkey's test.

Conclusion: Methanolic extract of *Prosopis juliflora* Linn. possesses analgesic activity in a dose-dependent manner in thermal induced model .

KEY WORDS: *Prosopis juliflora*; Analgesic; tail flick model.

I. INTRODUCTION:

Nature always stands as golden mark to exemplify the outstanding phenomena of symbiosis. Nature serves humans with medicines which were used to maintain health, to treat and heal many ailments. For the treatment of human diseases a basic product from Natural products like plant, animal and minerals were used[1]. Medicinal plants are of great importance to the health of individuals and communities. Medicinal plants has a potential source of therapeutic aid has attended a significant role in health syleavesall over the world for both human and animals not only in the diseased condition but also has potential material for maintaining proper health[2]. Man ever since his first appearance on earth, has used plant throughout his historical development as a source of medicines. Herbal medicine is a triumph of popular therapeutic diversity[3]. The world is now moving towards the herbal medicine or system, which can then properly fight foreign invaders, and help to destroy offending pathogens without toxic side effects[4]. The world health organization in the early 1970's had encouraged government to effectively utilize local knowledge of herbal medicines for disease prevention and health promotion[5]. WHO has showed great interest in documenting the use of medicinal plants used by tribal's from different parts of the world[6]. The plant kingdom still holds many species of plants containing substances of medicinal values, which have yet to be discovered. We are all aware that India is one of the richest sources of medicinal plants. Interest in medicinal plants has increased enormously over the last two decades. From the academic view point it is apparent that students of botany, phytochemistry and pharmacology have now also come to expect some in –depth studies relating to medicinal plants. The use of modern isolation techniques and

pharmacological testing procedures means that new plant drugs usually find their way into medicine as purified substances rather than in the form of galenical preparations. For these new drugs it is important that the pharmacist, rather than be fully conversant with the macroscopically and histological characters of the dried plant, is able to carry out the chromatographic and other procedures necessary for the identification and determination of purity of the preparation supplied. The plants used in the traditional syleavesof medicine of India and China as now receiving much scientific attention[7].

An analgesic, or painkiller, is any member of the group of drugs used to achieve analgesia-relief from pain[8]. Analgesic drugs act in various ways on the peripheral and central nervous systems. They are distinct from anesthetics, which reversibly eliminate sensation, and include Paracetamol [known in the US as Acetaminophen or simply , the non-steroidal anti-inflammatory drugs [NSAIDs] such as the salicylates, and opioid drugs such as morphine and opium. An analgesic is a drug that selectively relieves pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness.Pain is a warning signal, primarily protective in nature, but causes discomfort and suffering; may even be unbearable and incapacitating. Excessive pain may produce other effects- sinking sensation, apprehension, sweating, nausea, palpitation, rise or fall in BP, tachypnoea. Analgesics relieve pain as a symptom, without affecting its cause[9].

II. MATERIALS AND METHODS :

2.1. PLANT MATERIALS: *Prosopis juliflora* is one of the most economically and ecologically important tree species in arid and semi-arid zones of the world. *Prosopis juliflora* belongs to the family Leguminosae (Fabaceae), sub-family Mimosoideae, and it having 44species of which 40 are native to the Americas, three to Asia and one to Africa. The tropical Andean region is home to six species and eight species are found in the texas area, seven of them being endemic. These species are having the several properties such as soil binders, sand stabilizers, as well as its ability to grow in the poorest soils. It is a shrub or tree having 8-12 metres long. Growing to a height of up to 12 metres (39 ft), *P. juliflora* has atrunk diameter of up to 1.2 metres (3.9 ft). Its leavesare deciduous, geminate-pinnate, light green, with 12 to 20leaflets. Leavess appear shortly after leaf development. The tree reproduces solely by way of seeds, not vegetative. Seeds are spread by cattle and other animals, which consume the seed pods and spread the seeds in their droppings. The tree is said to have been introduced to Srilanka in the 19th century, where it is now known as vanni-andara, or katu andara in Sinhala. It is claimed that *P.juliflora* existed and was recognized even as a holy tree in ancient India, but this is most likely confusion with Prosopiscineraria. The tree is believed to have existed in the Vanni and Mannar regions for a long time[10]. In the western extent of its range in Ecuador and Peru, *Prosopis juliflora* readily hybridizes with Prosopispallida and can be difficult to distinguish from this similar species or their interspecific hybrid strains[11].

The various chemical agents that are present in it show the medicinal value that may alters certain physiological actions in the human body. The several biochemicals present in the plant are terpenes, alkaloids, flavonoids and phenolic compounds. Terpenes are used as insecticides and their pharmacological properties include antibacterial, antifungal, anthelmintic, antimalarial and molluscicidal[12]. Extracts of *P. juliflora* seeds and leaveshave several in vitro pharmacological effects such as anti-bacterial, anti-fungal and anti-inflammatory properties[13].

Since it is a main source of fuel for both urban and rural poor in the country, this plant provides more than 90% of the fuel wood in some Indian villages because *P. juliflora* wood has excellent burning qualities. Thus, it is called wooden anthracite. It also has high calorific value. The wood obtained from this plant doesn't need storage and drying process[14]. *Prosopis juliflora* (Sw.) DC contains many alkaloids such as juliflorine, julifloricine and julifloridine, juliprosine, juliprosinine and juliflorinine are found to be responsible for the biological activity.

2.2 Preparation of Plant Extract: We have collected methanolic extract of *Prosopis juliflora* through Soxhlet apparatus by hot continuous extraction method. The use of commercially available Soxhlet apparatus is a convenient way to prepare crude plant extract. The dried and powered drug was packed. Soxhlet apparatus is an automatic, continuous method that does not require further manipulation. This method is not time-consuming, as, for a standard-sized sample (50 g), extraction time is 48 h. The yield of the aqueous extract was 9.52%. The extract was stored in refrigerator until further studies.

2.3 Drugs: Ibuprofen, Diclofenac sodium, Asprin (Cipla), acetic acid (ASES Chemical Works, Jodhpur), and Sodium chloride (ASES Chemical Works).

2.4 Procurement of Animals: Male Wistar rats weighing (100–150 g) were obtained. They were housed in ventilated cages and fed with a normal pellet diet and water ad libitum. All experiments were in agreement with ethical guidelines for investigations of experimental plant in conscious animal. Research protocol was approved by the Animal Ethics Committee.

2.5 Anti-nociceptive Activity after Acute Administration

2.5.1. Tail-Flick Test

Antinociceptive (analgesic) activity of the extract was evaluated by the tail-flick method described . About 5 cm from the distal end of the tail of each rat was immersed in warm water maintained at 50°C. The reaction time (in seconds) was the time taken by the rat to flick its tail due to pain. The first reading was omitted and reaction time was taken as the average of the next two readings. The reaction time was recorded before (0 min) and at 15, 30, 45, and 60 min after the administration of the treatments. The maximum reaction time was fixed at 15 sec to prevent any tail tissue injury. If the reading exceeds 15 sec, it would be considered as maximum analgesia. The maximum possible analgesia (MPA) was calculated as follows:

$$\text{MPA}=\frac{\text{Reaction time for treatment}-\text{reaction time for saline}}{15\,\text{sec}-\text{reaction time for saline}}\times 100.$$

2.6. Statistical Analysis:

The results are expressed as mean ± SD (n = 6). Statistical significance was determined by ANOVA and subsequent Turkey's test. P values less than 0.05 were considered as indicative of significance.

III. Results:

3.1. Tail-Flick Test

The results of the analgesic activity of the methanol extract of the galls of *Prosopis juliflora* are shown in table.1. Rats treated with normal saline (negative control) did not show any significant difference in the reaction time on tail-flick throughout the 60 min observation. In comparison with the baseline values within the same treatment groups, the increase in reaction time at different time points significantly differed ($P < 0.05$) for morphine sulfate only. Duration of the reaction time in morphine sulfate and extract treated animals was significantly higher compared to saline treated animals, except for the extract group at 60 min. The highest reaction time for the extract treated group was 8.0 sec at 30 min, while it was 4.4 sec and 11.9 sec for saline and morphine sulfate groups, respectively. At all time points, the tail-flick latency time differed significantly between the extract and morphine sulfate groups, being greater for the latter group. No significant difference in reaction time was observed between the extract and sodium salicylate. Observation in rats treated with sodium salicylate did not give any significant analgesic effect in comparison with baseline values, saline, or extract (except for 30 min after treatment).

TableNo:1 Analgesic effect of methanolic extract from the *Prosopis juliflora* by tail flick method in rats

S.No	Treatments	Reaction time in seconds (mean ± SEM)				
		0 min	15 min	30 min	45 min	60 min
1	**Control (normal saline)**	**4.25 ± 0.57**	**4.50 ± 0.34**	**4.42 ± 0.45**	**4.58 ± 0.44**	**5.17 ± 0.80**
2	**Morphine sulfate**	**6.50 ± 1.22**	**11.04 ± 0.73*[ab]**	**10.92 ± 0.84*[ab]**	**11.83 ± 0.35*[ab]**	**13.33 ± 0.83*[ab]**
3	**Sodium salicylate**	**4.13 ± 0.54**	**5.19 ± 0.57**	**6.75 ± 0.62*[a]**	**5.65 ± 0.56**	**6.79 ± 1.24**
4	**MEPJ**	**6.57 ± 0.85[a]**	**7.04 ± 0.67[a]**	**8.04 ± 0.73[a]**	**7.00 ± 0.92[a]**	**6.57 ± 0.86**

All values by Student's *t*-test, significant at $P < 0.05$, and SEM = standard error mean. *$P < 0.05$ versus baseline of the respective treatment, [a] $P < 0.05$ treatment versus control, [b] $P < 0.05$ extract versus morphine sulfate, extract versus sodium salicylate was not significant at all time points.

IV. Discussion:

4.1. Analgesics are drugs that act on peripheral or central nervous selectively relieve pain without significantly altering consciousness. Centrally acting analgesics act by raising the threshold for pain and also altering the physiological response to pain. On the other hand, peripherally acting analgesics act by inhibiting the generation of impulses at chemoreceptor site of pain. The animal models employed for screening of analgesic activity in this study are pain-state models using thermal stimuli which include tail-flick methods. Both methods are useful in illustrating centrally mediated antinociceptive responses which focus generally on changes above the spinal cord level. While the tail-flick method mediates a spinal reflex to a nociceptive stimulus, hot plate method involves higher brain functions and is regarded a supraspinally organized response.

4.2 **In tail-flick model**, the methanol extract from the *Prosopis juliflora* exhibited significant analgesic activity by increasing the reaction time of the rats compared to control (saline treated rats) at all time points, except at 60 min. Sodium salicylate and morphine sulfate were used as reference drugs, which are considered mild and moderate to severe analgesics, respectively. In comparison with control, morphine produced the most significant antinociception effect during all observation times, followed by the extract, while no significant analgesic effect was observed for sodium salicylate. The tail-flick method is based on the observation that morphine-like compounds are selectively able to prolong the reaction time of typical tail-withdrawal effect in rats. This method is also useful in differentiating central opioid-like analgesics from peripheral analgesics. Analgesic drugs which are centrally acting elevate pain threshold of animals towards heat and pressure. Therefore, the analgesic effect of the extract on this pain-state model indicates that it might be centrally acting. With reference to the MPA value, the analgesic effects of both the extract and morphine sulfate were evident within 15 min following intraperitoneal administration. However, the extract showed short-lived analgesia as the MPA gradually decreased after 30 min compared to morphine sulfate. The tail-flick latency of the extract at all time points was less than that of reference drug, morphine sulfate, which is a slow onset opioid with long duration of action [17]. Although there was no significant analgesic effect between the reaction time of the extract and sodium salicylate, the extract exhibited a non-significant trend of higher reaction time compared to sodium salicylate. Both treatments produced comparable reaction times, suggesting that the galls of *Quercus infectoria* could be a better natural alternative for mild pain relief.

A number of alkaloids, flavanoids, steroids, and tannin isolated from medicinal plants have been reported to possess significant analgesic activity. The major constituent of the galls from *Prosopis juliflora* is tannin, which comprises up to 60% of its total content. Thus, analgesic activity observed with this extract might be attributed to the presence of this compound. Furthermore, there are reports on the role of tannin in analgesic activity. According to, preliminary phytochemicals which were screened from *Prosopis juliflora* L. including tannin might be responsible for the observed analgesic activity. Another research suggested that the presence of tannin and flavonoid in the methanol extract of *Prosopis juliflora* leavesseems to inhibit prostaglandin synthesis and exerts the anti-inflammatory and analgesic effects.

V. Conclusion:

In this study, methanolic extract of MEPJ (500mg/kg, p. o.) significantly reduced the number of acetic acid-induced writhing and significantly increased the latency of paw licking in hot plate method.

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