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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), sub-family Mimosoideae,

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. It was used to evaluate acute analgesic activity by acetic acid and hot plate 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 reduced the number of acetic acid-induced writhing and significantly increased the latency of paw licking in hot plate method. 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 both chemical induced and thermal induced model .

Key Words: *Prosopis juliflora*; Analgesic; acetic acid model, hot plate model.

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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 basic products 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 system all 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 system of 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 APAP], 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, and rise or fall in BP, tachypnoea. Analgesics relieve pain as a symptom, without affecting its cause [9].

MATERIALS AND METHODS

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 leaves are deciduous, geminate-pinnate, light green, with 12 to 20 leaflets. Flowers 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 *Prosopis cineraria*. 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 leaves have 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.

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.

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

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 Institutional Animal Ethics Committee.

Antinociceptive Activity after Acute Administration

Acetic acid-induced writhing method:

Antinociceptive activity of MEAJ was assessed by counting the number of writhes induced by 0.6% acetic acid (10 mL/kg, I.P.) in the following 20 min. Aspirin (100 mg/kg, P.O.) was used as a reference standard. Percentage protection against writhing was taken as an index of analgesia. % inhibition = [(Number of writhing in control group – Number of writhing in treated) _ 100]/ (Number of writhing in control group).

Hot plate test:

The hot plate was used to measure response latencies according to the method described by Eddy and Leimbach (1953). The rats were placed on a Techno hot plate maintained at 56°C, and the time between placement of the rat on the platform and shaking or licking of the paws or jumping was recorded as the hot plate latency. Rats with baseline latencies higher than 10 s were eliminated from the study. Twenty-four hours later, animals were treated with the aqueous extract of MEAJ (at dose of 300, and 500 mg/kg P.O.) or with diclofenac sodium (10 mg/kg P.O.) 60 min, before the test. Control animals received the same volume of saline solution (10 mL/kg).

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.

Acetic Acid-Induced Writhing Method

Methanolic extract of MEPJ leaves significantly ($P < 0.05$) and dose-dependently reduced the number of acetic acid induced writhing, when compared with vehicle-treated group, indicating significant peripheral antinociceptive activity. The percentage inhibition on single administration of test substance was found to be increased up to 53.52 with MEPJ of 300 mg/kg, P.O., and 61.03 with MEPJ of 500 mg/kg, P.O. Aspirin, used as reference standard, produced maximum inhibition (73%).

Hot plate Test

Diclofenac sodium at a dose of 10 mg/kg and MEPJ produced significantly increased the pain latency, when compared with the control group. MEPJ at doses of 300, and 500 mg/kg, P.O., produced significantly percentage increase in pain (42.40 ± 2.70, 55.34±1.14, and 77.16±0.47) 2 h after drug administration, as shown in Table 3. Two different analgesic testing methods were used in the current investigation with the objective to identifying possible peripheral and central effects of the MEPJ. Using both hot plate test and acetic acid-induced writhing response in rats, it was observed that the plant extracts possessed analgesic effects against both models. The observations also indicated that the extracts exhibit both central and peripheral effects. To evaluate for a possible central antinociceptive effect of the MEPJ the hot plate test was used [14] possibly acting on a descending inhibitory pain pathway [15]. The paw-licking hot plate reaction is a more intricate supraspinally ordered activity [16]. In general, the receptor has been considered as the receptor type related to pain relief and exhibited to be effective in controlling thermal pain [17]. Activation of m^2 opioid subtype results in spinal analgesia and generally via constipation unfavorable effect [18]. Therefore, by considering several reports, the antinociceptive activity of MEPJ is likely to be mediated centrally. The MEPJ also showed antinociceptive activity in the acetic acid test. Acetic acid produces the constriction reaction of abdomen, which forms a sensitive method for peripheral analgesic agents. Acetic acid promotes a raise in the levels of PGEs (PGE2 and PGF2a) in peritoneal fluid, concerning in part with peritoneal receptors [19] and inflammatory pain by inducing capillary permeability [20]. MEPJ exhibited significant reduction in number of writhing, suggesting involvement of PGEs [21]. The presence of alkaloids, triterpenoids, and flavonoids in MEPJ may be responsible for antinociceptive activity as all the three constituents have been reported to possess analgesic and anti-inflammatory activities [22, 23].

ACETIC ACID-INDUCED WRITHING METHOD

MEPJ significantly ($P <0.05$) and dose-dependently reduced the number of acetic acid-induced writhing, when compared with vehicle-treated group, indicating significant peripheral antinociceptive activity. The percentage inhibition on single administration of test substance was found to be increased up to 53.52 with MEPJ of 300 mg/kg,

P.O., and 61.03 with MEPJ of 500 mg/kg, P.O. Aspirin, used as reference standard, produced maximum inhibition (73%; Table 1).

Table 1: Effect of pj on acetic acid-induced writhing in rats

Sl. No	Treatment	Number of writhes	% Inhibition
1	Control	42.6 ± 0.81	-
2	Aspirin (100)	11.5 ± 1.55*	73
3	MEPJ (300)	19.8 ± 1.15*	53.52
4	MEPJ (500)	16.6 ± 0.93*	61.03

The observations (n = 6) are mean ± SEM.*P o 0.05, compared with control (one-way ANOVA, followed by Turkey's test). MEPJ leaves.

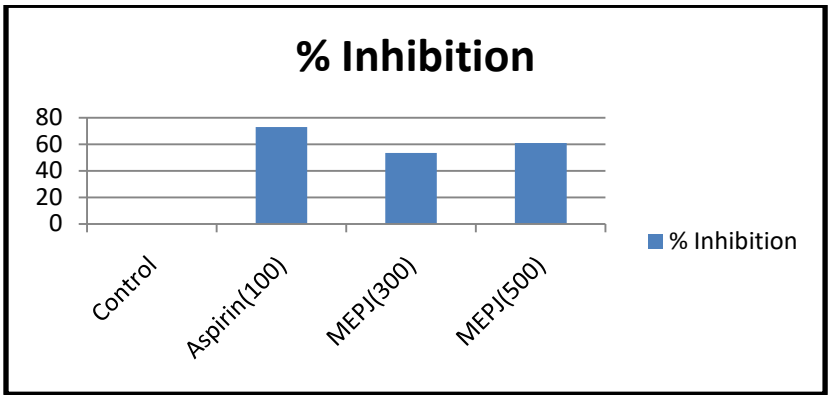

Figure 1 : Representation of % protection of methanolic extract of *Prosopis juliflora* on analgesic

HOT PLATE TEST

Diclofenac sodium at a dose of 10 mg/kg and MEPJ produced significantly increased the pain latency, when compared with the control group. MEPJ at doses of 300, and 500 mg/kg, P.O., produced significantly percentage increase in pain (42.40 ± 2.70 55.34 ± 1.14, and 77.16 ± 0.47) 2 h after drug administration, as shown in Table 2.

Table 2. Analgesic effect of mepj in the hot plate test

Groups	0 min	30 min	60 min	120 min
Control	0.87 ± 0.03	0.97 ± 0.02	1.10 ± 0.02	0.97 ± 0.05
DS	1.0 ± 0.04	1.76 ± 0.02 (9.34 ± 4.81)	2.77 ± 0.02 (46.05 ± 1.22)	4.69 ± 0.16 (61.67 ± 0.81)
MEPJ (300)	1.07 ± 0.02	1.73 ± 0.02 (12.61 ± 4.32)	2.46 ± 0.06 (45.17 ± 1.39)	4.73 ± 0.12 (55.34 ± 1.14)
MEPJ (500)	1.10 ± 0.03	2.83 ± 0.03 (14.15 ± 3.25)	4.89 ± 0.04 (65.75 ± 0.53)	5.39 ± 0.26 (77.16 ± 0.47)

Values are mean ± SD (n = 6); values in bracket indicate percentage inhibition in pain.

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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