

Aqueous Extract of *Mirabilis Jalapa* Linn. Leaves Have Anti-Inflammatory Properties**Abhilash Sankerneni¹, Sumalatha Gavini², B. Ramesh Chandra³, G. Swathi Kusuma^{***}**¹Associate Professor, Department of Pharmacology, Maheswara Medical College & Hospital, Patancheru, Sangareddy, Telangana State, India²Assistant Professor, Department of Pharmacology, Prathima relief Institute of Medical Sciences, Vangapahad, Hanamkonda, Warangal, Telangana State, India³Associate Professor, Department of Pharmacology, Kakatiya Medical College, Warangal, Telangana State, India⁴Assistant Professor, Department of Pharmacology, Prathima relief Institute of Medical Sciences, Vangapahad, Hanamkonda, Warangal, Telangana State, India

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Conflict of interest: Nil

Abstract:**Background:** The current study's goal was to assess the anti-inflammatory properties of an aqueous extract of *Mirabilis Jalapa* Linn. (MJL) (Nyctaginaceae) leaves in order to provide scientific support for the plant's folkloric claims. In southern Brazil, the leaves are utilized as traditional folk medicine to alleviate painful and inflammatory conditions. MJL-containing cosmetic or dermopharmaceutical formulations are said to be effective in reducing dry skin and irritation.**Methods:** Cold maceration was used to create an aqueous extract of the leaves. Or aqueous extract of the leaves was prepared by cold maceration.**Results:** Carrageenan and formalin-induced paw edema models in Wistar albino rats were used to assess the anti-inflammatory efficacy. In a model of paw edema caused by carrageenan, the anti-inflammatory action was found to be dose dependent. At 100 and 200 mg/kg, the aqueous extract significantly ($P < 0.05$) inhibited paw oedema by 37.5% and 54.0% on the fourth hour, respectively. The formalin-induced paw edema model showed a similar pattern of inhibition. At dosages of 100 and 200 mg/kg, the maximum percentage inhibition in paw edema on the fourth day was 32.9% and 43.0%, respectively.**Conclusion:** The current study's findings show that the leaves' aqueous extract has strong ($P < 0.05$) anti-inflammatory properties.**Keywords:** Paw edema, *Mirabilis Jalapa*, Aqueous extract, Anti-inflammatory action.This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.**Introduction**

Even though it is still an unwritten science, herbal medicine is widely accepted in various cultures and countries and has been ingrained in the lives of nearly 80% of people living in rural areas. Rheumatoid arthritis and other chronic anti-inflammatory illnesses continue to rank among the world's most common health issues. Even though synthetic medications currently dominate the market, it is impossible to completely rule out the possibility that these drugs have some level of toxicity. When used chronically, they can have serious side effects, the most prevalent of which being peptic ulcers and gastrointestinal hemorrhage [1]. [2] As a result, a novel anti-inflammatory drug with few adverse effects must be created. Scientific research in herbal medicine has prioritized the hunt for safe and effective anti-inflammatory medicines. "Maravilla" or "Bonnia" in Brazil, "Marvel of Peru"

in Peru, "Gulambasa" in Ayurveda, "Four o'clock" in English, and "Gul-abbas" in Hindi are all names for *Mirabilis Jalapa* Linn. (Nyctaginaceae; MJL). [3] Originally from tropical America, it is now widely grown as a beautiful plant in many other nations. [4] In southern Brazil, the leaves are used as a laxative and in traditional folk medicine to treat painful and inflammatory conditions. MJL-containing cosmetic or dermopharmaceutical formulations are said to be effective in reducing dry skin and irritation. A number of components have been isolated from the aerial portions and roots, respectively, including β -sitosterol, stigmasterol, ursolic acid, oleanolic acid, brassicasterol, and *Mirabilis* antiviral protein, as well as rotenoids (mirabilalone A-D and boeravinones C and F) [6,7,8,9,10]. Furthermore, a variety of pharmacological actions, including antispasmodic,

antibacterial, antiviral, antifungal, and protein synthesis inhibition, have been documented for various extracts. Total alcoholic and petroleum ether extracts of leaves have already been shown to have anti-inflammatory properties. The goal of the current study was to examine the anti-inflammatory properties of the aqueous extract of leaves because water is a safer and more popular solvent than methanol and petroleum ether for making Ayurvedic formulations [11].

Materials & Methods

Collection of plant material: In Aug 2017, Dr.Suresh. T, Professor & HOD in the Department of Botany at SRR Degree & PG College, collected and authenticated MJL leaves from Kiramnagar, Telangana State, India. The leaves were cleaned with water, shade-dried, coarsely ground, and stored in an airtight container until they were needed.

Preparation of extracts and preliminary phytochemical screening:

Cold maceration was used to create the aqueous extract. A rotary evaporator was used to filter and concentrate the extract. To get a consistent weight, the extract was dried in a vacuum desiccator. Norman's description of the phytochemical screening procedure was followed.

Animals: We purchased 150–200 g albino Wistar rats of both sexes from the NIN, Hyderabad. All animals were kept in the animal house of Shadan Institute of Medical Sciences, Hyderabad, and Telangana State, in cages made of polypropylene, three in each, with a temperature of 25°C, a relative humidity of 55–65%, and a 12-hour light/dark cycle. The Institutional Animal Ethics Committee granted ethical clearance for this experimental protocol Reg.No:132/17/IAE. The animals were given a regular meal and unlimited water, and they were fasted the night before the experiment.

Drugs: We purchased formalin from Ranbaxy (Rankem), diclofenac injection (Voveran) from Novartis India Ltd., Bombay, and carrageenan from Sigma Chemical Co. (St. Louis, MO, USA). The study employed a regular chow diet from Ashirwad Industries, Ropar (Punjab), and a vernier caliper that was acquired from Precision India Ltd.

Evaluation of Acute toxicological: The up-and-down approach, as outlined by Bruce, was attempted to determine the LD 50 value of the aqueous extract in order to evaluate the acute toxicity of MJL.[12]

Drug Administration: To administer the test extract, it was suspended in a 1% Carboxymethyl

cellulose (CMC) solution. In the carrageenan model, 30 minutes prior to the carrageenan injection in the sub plantar region of the rat paw, an oral gastric canula was used to give 100 and 200 mg/kg of MJL leaf aqueous extract and 10 mg/kg of diclofenac sodium. In the formalin model, the extract and the normal medication were given in the same manner and at the same dosage as previously described; the only difference was that formalin was only provided on the first day of therapy, whereas the treatment continued for seven days in a row.

Anti-Inflammatory activity [13]:

Carrageenan-Induced Paw Edema Model: Each rat's left hind paw's sub-plantar tissues were injected with 0.1 ml of 1% w/v carrageenan suspended in 1% CMC to cause paw edema [14]. The rats were split up into four groups, each with six rats.

- Group I – Control
- Group II - 100 mg/kg
- Group II – 200mg/kg
- Group IV - Diclofenac sodium (10 mg/kg)

A vernier caliper was used to measure the paw thickness prior to injecting the carrageenan and after 60, 120, 180, and 240 minutes. When comparing the animals administered with the extract under test to the carrageenan control group, the anti-inflammatory activity was computed as the percentage inhibition of oedema.

The percentage (%) inhibition of edema is calculated by using the formula

$$\% \text{ inhibition} = \frac{T_o - T_t}{T_o} \times 100$$

Where T_o to be the paw thickness of the rats in the control group at the same time as T_t , which is the paw thickness of the rats given the test extract at the corresponding time.

Paw edema model caused by formalin With the exception of using formalin as an edematogenic agent (0.2 ml of freshly made formalin solution at 2% v/v in distilled water), the animals were handled as in the model above. [15]

- Group I - Control
- Group II - Aqueous extract of 200 mg/kg
- Group III - Aqueous extract 400 mg/kg
- Group IV - Diclofenac sodium 10 mg/kg

Using a vernier caliper (precision), the thickness was measured both before and after the formalin injection, every day at a set time for seven days in a row.

Analysis of Data:

Table 1:

Group	Dose	Mean $\pm$ SD			
		1 st hour	2 nd hour	3 rd hour	4 th hour
Control (NS)	5ml	1.45 $\pm$ 0.21	2.64 $\pm$ 0.27	3.85 $\pm$ 0.153	3.98 $\pm$ 0.124
Group I	AE100mg/kg	1.29 $\pm$ 0.18	2.36 $\pm$ 0.3	2.99 $\pm$ 0.29	2.75 $\pm$ 0.23
Group II	AE200mg/kg	1.18 $\pm$ 0.24	2.01 $\pm$ 0.17	2.58 $\pm$ 0.24	2.37 $\pm$ 0.16
Group III	10 mg/kg	0.89 $\pm$ 0.03	0.94 $\pm$ 0.04	1.37 $\pm$ 0.02	1.02 $\pm$ 0.20

All values are expressed as mean $\pm$ SD; P value < 0.05 v/s carrageenan control

The mean $\pm$ Standard Deviation (SD) represents the data. Dunnet's test was used after a one-way ANOVA to assess the results. When compared to the control, differences were deemed statistically significant at P < 0.05.

Table 2:

Group	Dose	Mean $\pm$ SD			
		1 st day	2 nd day	3 rd day	4 th day
Control (NS)	5ml	5.12 $\pm$ 0.28	4.87 $\pm$ 0.20	4.34 $\pm$ 0.15	3.98 $\pm$ 0.11
Group I	Formalin+AE 100mg/kg	4.98 $\pm$ 0.81	4.20 $\pm$ 0.62	3.82 $\pm$ 0.90	3.4 $\pm$ 0.38
Group II	Formalin+AE 200mg/kg	3.92 $\pm$ 0.42	3.28 $\pm$ 0.43	2.98 $\pm$ 0.87	2.66 $\pm$ 0.82
Group III	10 mg/kg	3.87 $\pm$ 0.32	3.28 $\pm$ 0.41	2.73 $\pm$ 0.25	1.92 $\pm$ 0.16

All values are expressed as mean $\pm$ SD; P Value < 0.05 v/s formalin control

Day-by-day effects of diclofenac sodium and MJL leaf aqueous extract at 100 and 200 mg/kg in comparison to the formalin control group in a vernier caliper-based model of formalin (0.2 ml)-induced paw edema

Results

An aqueous extract yield of 16.2% w/w was determined. The results of the phytochemical screening were the same as those previously published by Lakshminath et al. and showed that the aqueous extract contained carbohydrates, proteins, amino acids, flavonoids, alkaloids, tannins, and phenolic chemicals.[16]

Anti-inflammatory properties: In a carrageenan-induced paw edema model employing a vernier caliper, Table 1 compares the effects of aqueous leaf extract and a conventional medication to the carrageenan control at various hours.

With percentage inhibitions of 18.5%, 29.1%, 35.8%, and 38.65% at 1, 2, 3, and 4 hours, respectively, aqueous extract given at a dose of 100 mg/kg p.o. prevented carrageenan-induced paw edema, whereas a dose of 200 mg/kg p.o. at 1, 2, 3, and 4 hours produced 23.5%, 31.03%, 49.08%, and 58.7%, respectively. At doses of 10 mg/kg p.o., diclofenac sodium prevented paw edema caused by carrageenan with percentage inhibitions of 52.10%, 61.42%, 65.5% and 70.01% at 1, 2, 3, and 4 hours, respectively.

Discussion

One of the best test methods for identifying anti-inflammatory drugs is carrageenan-induced acute inflammation. A biphasic curve typically depicts the progression of edema in a rat model of carrageenan-induced paw edema.[17] Within an hour of receiving a carrageenan injection, the first stage of

inflammation sets in, partially brought on by the trauma of the injection as well as the histamine and serotonin components. According to Table 1, paw edema was not significantly inhibited by aqueous extract at 100 and 200 mg/kg, respectively, in the early hours of the investigation (18.5% and 23.5%). Therefore, it can be said that serotonin and histamine are not inhibited. The efficacy of non-steroidal anti-inflammatory drugs, which mainly block the cyclo-oxygenase involved in prostaglandin synthesis, has been assessed using a carrageenan-induced paw edema model in rats, which is known to be susceptible to cyclo-oxygenase inhibitors.

The development of the second phase of the inflammatory reaction, which is measured in the third hour, is significantly influenced by it.[18] Table 1 indicates that the aqueous extract significantly (P < 0.05) inhibits paw edema at the third hour at doses of 100 and 200 mg/kg, respectively, by 35.8% and 58.7%. Consequently, it can be concluded that the aqueous extract's inhibitory effect on inflammation caused by carrageenan may result from its inhibition of the cyclo-oxygenase enzyme, which in turn inhibits the creation of prostaglandins.

Since formalin-induced paw edema closely mimics human arthritis, it is one of the best test procedures to assess chronic anti-inflammation. Aqueous extract treatment inhibited formalin-induced paw edema in a dose-dependent manner, as seen in Table 2, with a notable anti-inflammatory impact on the fourth day.

The percentage inhibition was 30.45% and 45.70% at doses of 100 and 200 mg/kg, respectively. Therefore, it is hypothesized that MJL leaf aqueous extract could help with arthritis treatment. [19]

Conclusion

MJL's aqueous extract has strong anti-inflammatory properties. These results provide credence to the extract's application in traditional medicine for the treatment of inflammatory diseases.

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