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Anti-Inflammatory activity of ethanolic extract of leaves of *Mirabilis Jalapa* Linn in Albino Wistar rats

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A B S T R A C T

The ethanolic extract of leaves of *Mirabilis Jalapa* Linn was investigated for anti-inflammatory activity in albino Wistar rats. The ethanolic extract administered by the oral route at a concentration of 200 and 400 mg/kg showed the significant dose dependent anti-inflammatory activity in carrageen in and Formalin-induced paw edema in rats. Anti-inflammatory activity of the tested extract was comparable with that of the standard drug Diclofenac sodium (40 mg/kg). The results lend support to the traditional use of in *Mirabilis Jalapa* Linn. The treatment of inflammatory diseases.

Keywords: Mirabilis Jalapa Linn, ethanolic extract, albino Wistar rats, anti-inflammatory activity

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Contents

1. Introduction

Inflammation is a local response (reaction) of living vascularised tissues to exogenous and endogenous stimuli.[1] The term is derived from latin "Inflammare" meaning to burn. Inflammation is fundamentally destined to localise and eliminate the causative agent and to limit tissue injury. Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells or irritant. Inflammation is a protective immunovascular response that involves immune cells, blood vessels and molecular mediators.[2] The purpose of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and inflammatory process, and to initiate tissue repair[3]**.** Traditional medicine is a very important part of health care. Most of population in the developing countries still relies mainly on indigenous traditional medicine for satisfying their primay health care needs. India has an ancient heritage of traditional medicine[4].

Mirabilis jalapa.L (MJ) commonly known as Four o'clock plant belonging to family Nyctaginaceae is found in India, Tropical South America, throughout the Philippines in settled areas and also in France. MJ characterized as a quick growing much- branched perennial herb with erect, angular, distinctly joined stem, swollen at the nodes. It has been used in traditional medicine which may be due to

presences of some biomolecules of pharmacological importance's. In herbal medicine, parts of the plant may be used as a diuretic, purgative, and for vulnerary wound healing purposes.[5]

2. Materials and Methods

PLANT COLLECTION

Mirabilis Jalapa Linn leaves were collected from region of Pidathapolur,Nellore, Andhra Pradesh. The leaves were authentified by Dr. K. Madhava chetty, Department of Botany, Sri Venkateswara University, Tirupati, Department of Botany.

Preparation of Extract

The collected plant material of *Mirabilis Jalapa* Linn was washed thoroughly under running tap water, shade dried and pulverized, using a grinding machine. Ethanolic extract was prepared by soaking about 100g of the powdered leaves in 800ml of ethanol for 48hours. The extract was filtered and the solvent was removed with rotary evaporation to obtain crude active ingredient.

Drugs and chemicals:

Carrageenan was procured from Sigma Chemical Co.Hyd, diclofenac sodium from Novartis India Ltd, Hyd and formalin from Ranbaxy (Hyd). Vernier caliper purchased from Percision India Ltd, were used in the study.

Experimental animals:

Animals

Female Albino Wistar rats weighing 150−200 g were obtained from National institute of nutrition, Hyderabad. All animals were housed in polypropylene cages (3 in each cage) at an ambient temperature; 25 ± 2°C, relative humidity; 55−65%, and were maintained under a 12 h light/dark cycle each in animal house of Ratnam institute of Pharmacy, Nellore. Ethical clearance for this experimental protocol was obtained from the Institutional Animal Ethics Committee. The animals were fed with standard diet and water *ad libitum* and were deprived of food overnight prior to the experiment. The animal house was well ventilated and maintained in large spacious hygienic cages during the course of the experimental period[6].

(SPSP, No: 1016/a/06/CPCSEA/003/2012).

Acute toxicity study:

The acute toxicity acute toxicity studies were carried out as per fixed dose OECD guidelines No: 420 using albino mice. In brief, albino mice of either sex weighing between 20-30g were used for acute toxicity study. The animals were fasted overnight prior to the experimental procedure. The animals were kept for fasting overnight providing only water, after which the extracts were administered orally at the dose level of 5 mg/kg body weight and observed for 7 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg body weight.[7]

Preliminary phytochemical screening:

The ethanolic extract of leaves of *mirabilis Jalapa* Linn was subjected to preliminary phytochemical screening for detection of various phytochemical constituents such as alkaloids, flavonoids, saponins, tannins, cardiac glycosides, steroids, terpenoids, anthroquinones, phlobatanins, proteins and carbohydrates[8] .

Pharmacological study:

Evaluation of *in-vivo* anti-inflammatory activity and grouping of animals:

Carrageen an-induced paw oedema model

According to the method by Winter et al., 1962, we evaluated our study. Adult wistar rats of either sex (150–180 g) were divided in four groups of 6 animals each. Group I (vehicle) received 0.1 ml of 1% w/v of carrageen an given orally. Group II (standard) received Diclofenac (40mg/kg/i.p) as a reference drug half an hour prior to carrageen an injection. Group III and IV received orally ethanolic extract of Mirabilis Jalapa leaves 200mg/kg and 400mg/kg respectively sixty minutes prior to carrageen an injection. To induce paw edema 0.1ml of 1% suspension of carrageen an in normal saline was injected to the sub planter region of left hind paw[50]. The diameter of paw was measured by using digital vernier caliper before administration of carrageen an at hourly intervals up to six hours after the administration of carrageen an (V The paw volume, up to the tibiotarsal articulation was measured using a plethysmometer at 0, 1,2, 3, 4, 5, & 6 hrs).The difference between the initial and subsequent readings was calculated as mean increase in paw diameter which is a measure of the edema. This was compared with vehicle treatment. The difference of values between treated animals and control group is calculated for each time interval and evaluated statistically. Edema (ΔT) was calculated as follows:[9,10]

$\Delta T = Tt - T0$

Tt is the left hind paw thickness in mm at time t

T0 is the left hind paw thickness before sub plantar injection.

% reduction of edema was calculated as follows,

Mean increase in paw diameter in vehicle group(C) --- mean mincrease in paw diameter in drug treated group (T)/ mean increase in paw diameter vehicle group x 100

([C – T / C] x 100).

The acute anti-inflammatory activity was evaluated by carrageenan – induced rat paw edema. Wistar albino rats were divided into 4 groups (n=6). Acute inflammation was produced by injecting 0.1 mL of 1%carrageenin into sub-plantar surface of rat hind paw.The control group received carrageenan (0.1%) 0.1mL.The test group 1 and 2 received 200 mg/kg ethanolic extract, 400 mg/kg ethanolic extracts suspended in i% CMC respectively by oral route. The standard group received comparator drug diclofenac 40 mg/kg by oral route. All the suspensions were administered 30 minutes before carrageenan injection (0.1mL of 1%). The paw volume, up to the tibiotarsal articulation was measured using a plethysmometer at 0,1,2,3,4,5 & 6 hrs.[11,12]

Grouping of animals:

- Group I Carrageenan control (0.1 ml of 1% w/v/i.p)
- Group II Diclofenac sodium (40 mg/kg/p.o) as standard reference
- Group III Ethanolic extract (200 mg/kg/ p.o)
- Group IV Ethanolic extract (400 mg/kg/p.o)

The paw thickness was measured before injecting the carrageenan and after 60, 120, 180, 240,300,360 min. using vernier caliper. The anti-inflammatory activity was calculated as percentage inhibition of oedema in the animals treated with extract under test in comparison to the carrageenan control group.

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

$$\%\text{inhibition} = To - Tt/To \times 100$$

Where T_t is the thickness of paw of rats given test extract at corresponding time and T_o is the paw thickness of rats of control group at the same time.[13,14]

Formalin-induced paw edema model:

The animals were treated in the same way as in above model except that formalin (0.2 ml of 2% v/v freshly prepared formalin solution prepared in distilled water) was used as edematogenic agent.[15,16]

- Group I Formalin control
- Group II Diclofenac sodium (40 mg/kg/p.o) as standard reference
- Group III Ethanolic extract (200 mg/kg/p.o)
- Group IV Ethanolic extract (400 mg/kg/ p.o)

The thickness was measured before injecting the formalin and after injecting the formalin every day at a fixed time for seven consecutive days using a vernier caliper.

Statistical Analysis:

The data is expressed as mean ± Standard Deviation (SD). Results were analysed using one-way ANOVA followed by Dunnet's test. Differences were considered as statistically significant at $P < 0.05$, when compared with control.

3. Results and Discussion

Table No:1 Phytochemical studies of ethanolic extract of leaves of *Mirabilis jalapa* Linn.

Constituents	Ethanolic Extract of M.J
Alkaloids	+Ve
Saponins	+Ve
Tannins	+Ve
Phlobatannins	_Ve
Flavonoids	+Ve
Steroids	+Ve
Cardiacglycosides	+Ve
Carbohydrates	+Ve
Anthraquinones	_Ve
Proteins	_Ve
Terpenoids	_Ve

Table No:2 Effect of ethanolic extract of Mirabilis jalapa Linn against carragenan induced paw edema in rat

Groups (n=6)	**Change in paw thickness (mm)** ± SD (**% inhibition)**					
	1hr	2hr	3hr	4hr	5hr	6hr
Carrageenan control (0.1 ml of 1% w/v)	1.32 ±0.1	2.37 ±0.11	3.64 ± 0.14	3.23 ± 0.16	2.50 ± 0.16	2.25 ± 0.16
Carrageenan(0.1 ml of 1% w/v) +Test1(200mg/kg)	1.12±0.1 (15.15%)	1.74 ± 0.21* (26.58%)	2.50 ± 0.10* (31.3%)	1.97 ± 0.11* (39%)	1.41± 0.10* (43.6%)	1.20 ± 0.11* (46.66%)
Carrageenan(0.1 ml of 1% w/v) + Test- 2(400mg/kg)	1.00 ± 0.12 (24.24%)	1.54 ± 0.16* (35.02%)	1.81 ± 0.09* (50.09%)	1.41 ± 0.18* (56.34%)	1.11 ± 0.12* (56%)	1.0 ± 0.12* (56%)
Carrageenan(0.1 ml of 1% w/v) + diclofenacsodium(40mg/kg)	0.59±0.18* (55.30%)	0.8 ± 0.12* (66.24%)	1.14 ± 0.12* (68.68%)	0.9 ± 0.11* (72.13%)	0.7 ± 0.12* (72.0%)	0.6 ± 0.12* (73.33%)

Table No: 5 Effect of ethanolic extract of Mirabilis jalapa Linn against Formalin induced paw edema in rats

Groups (n=6)	**Change in paw thickness (mm)** ± SD (**% inhibition)**						
	1st day	**2nd day**	**3rd day**	**4th day**	**5th day**	**6th day**	**7th day**
Formalin control (0.2 ml of 2% v/v)	4.90±0.20	3.90±0.22	3.16±0.24	2.62±0.17	2.00±0.19	1.59±0.25	1.44±0.26
Formalin (0.2 ml of 2% v/v) + Test-1(200mg/kg).	4.16±0.21* (15.10%)	3.05±0.19* (21.89%)	2.31±0.23* (26.89%)	1.76±0.20* (32.88%)	1.45±0.23* (27.6%)	1.21±0.26 (23.87%)	1.15±0.25 (19.90%)
Formalin (0.2 ml of 2% v/v)+Test-2(400mg/kg)	3.82±0.22* (22.12%)	2.76±0.18* (29.23%)	1.99±0.23* (36.83%)	1.44±0.19* (43.0%)	1.23±0.24* (38.3%)	1.05±0.27* (33.62%)	1.02±0.24* (29.17%)
Formalin (0.2 ml of 2% v/v) + diclofenac sodium(40mg/kg)	3.53±0.19* (28.03%)	2.46±0.15* (36.88%)	1.71±0.18* (46.08%)	1.16±0.16* (55.73%)	1.22±0.15* (43.91%)	0.97±0.14* (38.91%)	0.95±0.17* (33.45%)

Discussion

Effect of ethanolic extract of MJL leaves at doses of 200 and 400 mg/kg, and diclofenac sodium as compared to carrageenan control group at different hours in carrageenan-induced paw edema model using vernier caliper. Table-2 shows the effect of ethanolic extract of leaves and standard drug as compared to carrageenan control at different hours in carrageenan-induced paw oedema model using vernier caliper. Ethanolic extract administered at a dose of 200 mg/kg p.o prevented carrageenan-induced paw oedema with a percentage inhibition of 15.15%, 26.58%, 31.3%, and 39.0%, 43.6%, 46.66% at 1, 2, 3, 4,5 and 6 hours, respectively, while 24.24%, 35.02%, 50.09%, and 56.34%, 56%, 56% at a dose of 400 mg/kg p.o. at 1, 2, 3, 4, 5 and 6 hours, respectively. Diclofenac sodium at a dose of 40 mg/kg p.o. prevented carrageenan-induced paw edema with a percentage inhibition of 55.30%, 66.24%, 68.68%, and 72.13%, 72.0%, 73.33% at 1,2,3,4,5,6 hours respectively.[19]

Day wise effect of ethanolic extract of MJL leaves at doses of 200 and 400mg/kg, and diclofenac sodium as compared to formalin control group in formalin-induced paw edema model using vernier caliper. Table-3 shows the day wise effect of ethanolic extract of leaves and standard drug as compared to formalin control group in formalin-induced paw edema model using vernier caliper. Ethanolic extract administered extract prevented formalin-induced paw edema with percentage inhibition of 32.9% and 43.0% on 4 th day at doses of 200 and 400 mg/kg, respectively, while diclofenac sodium (40 mg/kg) showed 57.0% of percentage inhibition of paw edema on 4th day.

4. Conclusion

The Ethanolic extract of MJL possesses significant anti-inflammatory potential. These findings support the use of the extract in traditional system of medicine for the management of inflammatory conditions. In the present study of *Mirabilis Jalapa Linn* ethanolic extracts of leaves of phytoconstituents responsible for the activity were isolated. The scientific research on Mirabilis jalapa. L suggests a huge biological potential of this plant. The acute toxicity studies of MJ have been found that the no mortality or any signs of behavioral changes was observed. It is strongly believed that detailed information as presented in this review might provide detailed evidence for the use of this plant in different medicines.[21]

5. References

[1] Kirtikar KR, Basu BD, Indian medicinal plants, Delhi: Sri Satguru Publications, 2000

[2] K. Mukerjee, Quality control of herbal drugs, An approach of evaluation of botanicals I edition, 2001, Page: 17-20

[3] http//www.ayurveda india.com

[4] http//www.unani herbal.org

[5] B. R. Meher1, B. G. Rath and S. Biswal- Evaluation of anti-inflammatory activity of ethanolic extract of Sphaeranthus indicus. J. Chem. Pharm. Res., 2011, 3(3):831-834

[6] K. Kanagasanthos, S. Shanmugapriyan- Evaluation of acute toxicity, anti-inflammatory activity and phytochemical screening of ethanolic extract of azadirachta indica leaves. 2015, Vol. 4, No.5, PP: 1737-1742.

[7] Novartis, Voltaren (diclofenac sodium enteric-coated tablets) prescribing information. East Hanover, NJ; 2006 Jan.

[8] Reynolds JEF, ed. Martindale: The Extra Pharmacopeia. 28th ed. London: The Pharmaceutical Press; 1989:250.

[9] Goodman Gilman's, The Pharmacological Basis of therapeutics. 11th edn, pp. 671-712.

[10] Subin mary Zachariah - "Evaluation Of Antioxidant and total flavanoid content of Mirabilis Jalapa linn using in vitro model"s. International research journal of pharmacy, page no:187.

[11] Sujata mahapatra and Bhaskar padhy- "Ethano pharmacological review of four o clock flower plant".Journal of biological and scientif ic opinion, page no:344-348

[12] Mahajan neerajan shishir - "Use of Mirabilis Jalapa linn flower extract as a natural indicator in acid base titrations".Journal of pharmacy research, Page no:159-162

[13] Subin mary Zachariah- " Free radical scavenging and anti-bacterial activity of Mirabilis jalapa". Asian journal of pharmaceutical and clinical research, page no:115-119.

[14] Bibin baby Augustine- "Effect of Mirabilis Jalapa linn flowers in experimentally induced arthritis and consequtive oxidative stress". International journal of pharmacy and pharmaceutical research, page no:190-193.

[15] Grace Annapoorani, Divya sundar raj – "Dyeing of cotton and wool fabric using Mirabilis Jalapa linn flower". International journal of science and research, page no:1126-1129.

[16] Shrutika kumthekar and jessy pius – " HPTLC finger print profile and characterisation of dopamine from different parts of four cultivors of Mirabilis Jalapa linn". Journal of Chemical and Pharmaceutical Research, 2015, 7(11):117-120.

[17] Irena sobolev1, Phyllis G. weintraub, Abdullah gera – "Phytoplasma infection in the four o clock flower(mirabilis jalapa)".Bulletin of insectology, Bulletin of Insectology 60 (2): 281-282, 2007.

[18] Rozina rozina- "pharmacological and biological activities of mirabilis jalapa linn".International journal of pharmacological research, page no: 2277-3312.

[19] Rout Soumya Prakash, KarDurgaMadhab, Swain Rosalin -"Antihyperglycemic Activity of Aerial parts of Mirabilis jalapa l. In Normoglycemic and Streptozotocin-Induced Hyperglycemic Rats" Int. J. Drug Dev. & Res., October-December 2012, 4(4): 334-341.

[20] Wicaksono PA, Name S, Martien R, Ismail H - Formulation and cytotoxicity of ribosome inactivating protein mirabilis jalapa linn- nano particles using alginate low viscosity chitosan conjugated".Asian,pacific Journal of Cancer prevention,page no:2277.