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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *MUSSAENDA INCANA* ON RATS USING BY CARRAGEENAN INDUCED PAW ODEMA METHOD

C. Hari Kumar, Shaik. Afroz Begum*, M. Sravani, P. Chandrakala, B. Pavani

Vasavi Institute of Pharmaceutical sciences, Kadapa, Andhra Pradesh, India.

*Corresponding author E-mail: **fathima.shaik929@gmail.com**

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ABSTRACT

Evaluation of anti-inflammatory activity of methanolic extract of *Mussaendaincana*on rats using by Carrageenen Induced Paw Odema Method. ***Method:*** The plant material was air dried and reduced to coarse powder .The powdered material (2.5kgs) was subjected to solvent extraction in soxhlet apparatus with methanol. The soxhelation was continued until the colourless solution was obtained and the solution was concentrated with rotary evaporator under reduced pressure and the dried extract was weighed (100gms).

Results*: In* the carrageneen induced rat paw edema method, the volume of the paw was measured for the control group,plant extract treated group and Diclofenac treated group by using digital Plethesmometer. From the results it was found thatwith increasing the time period of observation up to four hours, the paw volume was gradually decreased and increasing the concentration of plant extract the paw volume was decreased.The percentage inhibition of plant extract at 100mg/kg showed non-significant anti-inflammatory activity but at the dose of 400mg/kg body weight showed significant ant-inflammatory activity when compared with the standard Diclofenac sodium.So the methanolic extract of the plant showed concentration dependent activity.

Conclusion*:* The methanol extract of the *Mussaendaincana* displayed anti-inflammatory activity and was proved scientifically for the constituents like Quericetrin,epicatechin, saponins, sterols.The plant extracts having the chemical nature like alkaloids,carbohydrates, flavonoids, saponins,steroids and triterpinoids.So the chemical nature of the plant extracts may be responsible for the anti-inflammatory activity.

INTRODUCTION

Traditional medicine has been used for a long time of history which serves peoples all over the world. The ethnobotany provides a rich resource for natural products which provides a step stone for drug research and development. In recent years, the use of traditional medicine of plant source has gained more interest. It has been reported that more than 50% of all modern drugs in clinical usage are of natural products. The medicinal Plants have been comprised about 8000 species and among them 50% accounts for higher flowering plant species of India which is yet to be explored. These plants have been used for the treatment of various diseases for many years. Terrestrial plants have been used as medicines in Egypt, China, India, Greece and it has been proved to be an excellent reservoir of new medicinal compounds. The medicinal activities of many herbal plants

have been well documented in ancient Indian literature as Ayurveda, Siddha, Unani systems of medicine which provides a good base for scientific exploration of new drugs. *Mussaendaincana*is native from India to Malaysia & is much smaller than the above Mussaendas, growing to no more than 3-feet tall,it has flat topped flower clusters (corymbs),with bright yellow corollas and a single enlarged calyx lobe that is yellow to cream. In the landscape it is most effective in mass planting(whistler-2000). The critical analysis of literature review also pinpoints the fact that although the number of diseases for which *mussendaincana*finds use as a medicine in fairly large, yet its therapeutic efficacy has been assessed only in a few cases.The aim of the present investigation is to extract and screen flavonoids (free and bound) from different parts (root, stem, leaf and buds) of *M.Incana*for their anti-inflammatory activity.

Fig no: 1 *Mussendaincana* plant image

Botanical source:*Mussaendaincana* .
Family: Rubiaceae
Common Names: White mussaenda, Whitewing ,Miniature white flag.
Parts used: The whole plant used specially leaves, roots,stem, flowers and fruits.

BOTANICAL CLASSIFICATION:
Kingdom : Plantae
Sub kingdom : Tracheophytes
Division : Angiosperms
Super division : Eudicots
Class : Asterids
Order : Gentianales
Family : Rubiaceae
Sub family : Ixoroideae
Tribe : Mussaendeae
Genus :*Mussaenda*
Species : *Incana*
SYNONYMS: *MussaendaIncana Stem*

OTHER SPECIES:*Mussaendafrondosa,Mussaendaerythrophylla*

TRADITIONAL USES: The genus *Mussaenda (rubiaceae)* is an important source of medicinal natural products, steroids, flavonoids, glycosides and only a few number of species reported positive for alkaloids and tannins. Many *Mussaenda* species were reported to possess anti-oxidant, anti-inflammatory in different models, analgesic, antimicrobial, diuretic, anti-phlogistic and antipyretic, acute gastroenteritis and dysentery**,** anti- fertility activity, antiviral property , antibacterial effect rarely for hepatoprotective activity and Wound healing activity.

METHODOLOGY

Plant collection and extraction

Collection and extraction of Stem

- The stem of the plant "*Mussendaincana*" were collected from Tirupathi, Chittoor District, Andhra Pradesh and authenticated by Dr.K.MadhavaChetty, Department of Botany, Sri Venkateswara University, Tirupathi.
- Stem of *MussaendaIncana* plant were collected (2.5 kg) and was air dried and reduced to coarse powder.
- The materials were weighed and transfer in to a clean round bottom flask.
- Sufficient quantity of methanol was added till it totally immersed and soxhelation it for seven days.
- The obtain Solution was concentrated with rotary evaporator under reduced pressure Until it become like dried extract and was weighed (100gms).

PHYTOCHEMICAL ANALYSIS

Methanolic extract of *Mussaendaincana stem* were subjected to qualitative Phytochemical tests for different constituents such as alkaloids, carbohydrates, glycosides, flavonoids, Phenolic compounds, tannins, proteins and free amino acids, saponins, steroids and terpenoidsand found that for the presence of alkaloids, carbohydrates, saponin glycosides, flavonoids, steroids and tri terpenoids and tannins was given in the tableno.1 .

ACUTE ORAL TOXICITY STUDIES:

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method). Wistar albino rats of either sex were selected randomly and treated with methanolic extract of plant up to the dose of 2000 mg/kg body weight. The animals were observed for any toxic signs for initial period of 2h and further 24 h for mortality study. The animal does not show any signs of toxicity and mortality up to the dose of 2000 mg/kg.

EXPERIMENTAL WORK:

Animals:A total of 25 Wistar albino rats of either sex weighing 150-250 g were used for carregeenen induced paw edema method. All the animals were obtained from the Animal house, Department of Pharmacy, Annamacharyacollege of pharmacy, Rajampeta. All the animals received standard laboratory diet, reverse osmosis water.

PREPARATION OF DRUG SAMPLE: The extract was suspended in distilled water and it is used for the anti-inflammatory study.

REFERENCE DRUG: Diclofenac Sodium (10mg/kg) was prepared by dissolving them in normal saline at concentration of 15mg/ml.

CARREGEENEN INDUCED PAW EDEMA METHOD:

The anti-inflammatory activity was evaluated by the Carregeenen induced paw edema method. All Formulations for anti-inflammatory activity were administered orally. The standard drug Diclofenac sodium was administered in the form of solution in water for injection as vehicle. For the assessment of anti-inflammatory activity the animals five were divided into five groups each composed of five animals. All groups received subplanar injection (maximum 0.1 ml as per ethical norms).The paw volumes were measured using a digital plethesmometer before as well as 15min, 30min, 1hr, 2hr, 3hr after the injection of carrageenan.

Group I served as control.

Group II served as standard and was injected Diclofenac sodium (15 mg/kg) subplanar.

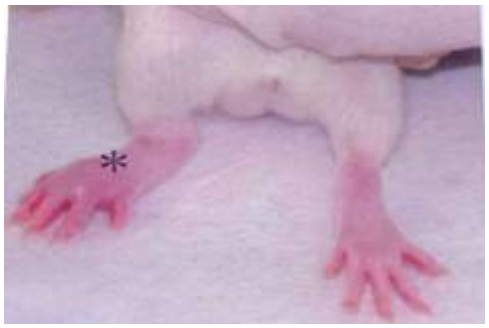
Group III served as extract low dose 100 mg/kg

Group IV served as extract at medium dose 200 mg /kg

Group V served as extract at high dose 200 mg /kg

Figno: 2- Plethesmometer

Figno: 3 - Rat handling



Figno: 4- Inflamed paw

EVALUTION OF ANTI-INFLAMMATORY ACTIVITY

Evaluation of anti-inflammatory activity of the extract was also carried out using Carrageenan induced paw edema method.Paw oedema was induced by subplanar injection of 0.1 ml of freshly prepared 1% carrageenin suspension into the right hind paw of each rat. After hour of carrageen in injection , the first group was control that which only carrageenan was injected and normal saline was given. Then the second group was treated with diclofenac sodium which acts as astandard.To the 3rd 4th

5th groups of the rats treated with doses 100mg/kg, 200mg/kg, 400mg/kg of the methanol extract.

DISCUSSION

To the methanolic extract of stems of *Mussaendaincana* acute toxicity studies was performed and did not found any toxic signs up to the dose 2000mg/kg body weight. Based on this, 1/20th, 1/10th, 1/5th doses were selected for the anti-inflammatory activity.In the carrageneen induced rat paw edema method, the volume of the paw was measured for the control group,plant extract treated group and Diclofenac treated group by using digital Plethesmometer in the table no2 and the percentage inhibition calculation results were presented in the table no3. and the graphically represented in the figure no.5. From the results it was found thatwith increasing the time period of observation up to four hours, the paw volume was gradually decreased and increasing the concentration of plant extract the paw volume was decreased.So the methanolic extract of the plant showed concentration dependent activity.

Table: 1PHYTOCHEMICAL STUDY OF METHANOLIC EXTRACT OF MUSSAENDA INCANA

S. No	Class of compound	Plant part (Stem)	Test performed
1.	Alkaloids	+	Dragendorff's test, Mayers test
2.	Carbohydrates	+	Molish test, Fehling test
3.	Anthra-quinone glycosides	_	Borntrager`s test
4.	Cardiac glycosides	_	Kedde`s test
5.	Saponin glycosides	+	Froth formation test
6.	Flavanoids	+	Shinoda Test, Lead acetate test
7.	Steroids and triterpenoids	+	Salkowski test, LibermannBurchard test
8.	Tannins	+	Ferric chloride test
9.	Proteins and Aminoacids	_	Ninhydrin test, Biuret test
10.	Gums and mucilage	_	Mucilage test

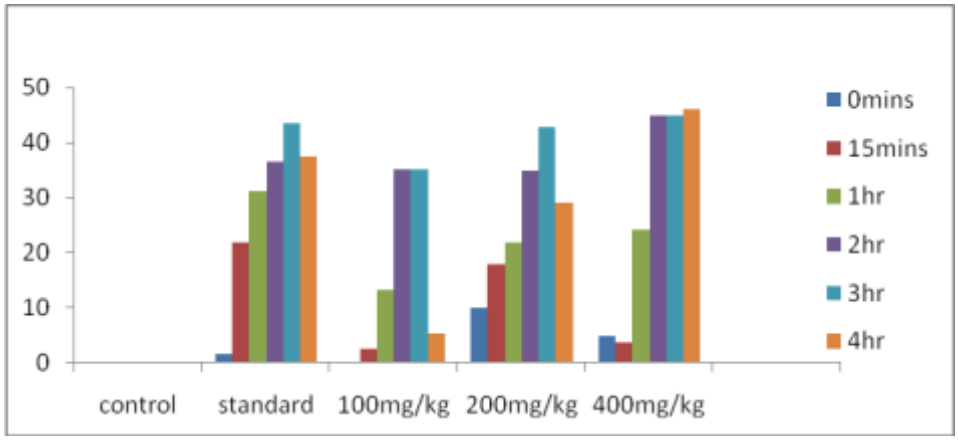
Table:2Evaluation of anti-inflammatory activity ofmethanolic extract of *MussaendaIncana* on rats by using Carrageenan induced paw edema method

Treatment	0mins	15mins	1hour	2 hours	3 hours	4hours
Control	1.18±0.028	1.25±0.042	1.28±0.054	1.34±0.015	1.38±0.016	1.12±0.035
Standard	1.16±0.044	0.94±0.007	0.887±0.028	0.85±0.011	0.78±0.011	0.70±0.020
MEMI 100mg/kg	1.16±0.07	1.19±0.066	1.11±0.66	1.07±0.069	1.02±0.084	1.06±0.015
MEMI 200mg/kg	1.06±0.065	1.00±0.055	1.00±0.043	0.87±0.046	0.78±0.034	0.80±0.073
MEMI 400mg/kg	1.12±0.058	1.20±0.057	0.97±0.009	0.85±0.007	0.74±0.03	0.72±0.022

MEMI: Methanolic extract of *Mussaendaincana*

Table:3 Percentage Inhibitionof anti-inflammatory activity

Treatment	0 min	15 min	1 hour	2 hours	3 hours	4 hours
Control	0	0	0	0	0	0
Standard	1.69	21.8	31.2	36.5	43.5	37.5
MEMI 100mg/kg	0	2.5	13.2	35.2	35.2	5.35
MEMI 200mg/kg	10.1	18	21.8	35	42.7	29
MEMI 400mg/kg	5	3.8	24.2	45	45	46



Figno:5 Graphical representation of Anti-inflammatory activity of methanolic extract of *MussaendaIncana*

CONCLUSION

In conclusion, the methanol extract of the *Mussendaincana* displayed anti-inflammatory activity and supported the traditional use of this plant in inflammation. Further study is warranted to identify the active compounds present in this extract and to elucidate the mechanisms involved in its anti-inflammatory properties.

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