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ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF LEAF EXTRACT OF *MORINDA CITRIFOLIA* IN EXPERIMENTAL MODELS

T. Sunil Kumar Reddy, G. Balammal

*Sree Vidyanikethan College of Pharmacy, Sree Sainathnagar, Chandragiri (M), Tirupati, Andhra Pradesh, India-517102.

ABSTRACT

Morinda citrifolia is a medium sized evergreen tree belongs to *Rubiaceae* family. The petroleum ether extract of *Morinda citrifolia* (PMC) leaves was investigated for the evaluation of anti- inflammatory and analgesic activity. Acute toxicity studies were performed as per OECD-423 guidelines. Toxicity signs and symptoms were not observed. Anti-inflammatory activity was established by carrageenan induced paw edema and cotton pellet induced granuloma at the dose of 200 and 400 mg/kg. Analgesic activity was carried out by tail immersion method in mice. The activity may be due to the presence of steroids in the extract. The extract exhibited significant analgesic and anti- inflammatory activity, which supports the traditional utilization of the plant. This study established analgesic and anti- inflammatory activity of the leaf of *Morinda citrifolia.*

Key words: *Morinda citrifolia*, anti- inflammatory, analgesic, edema, granuloma.

INTRODUCTION

Morinda citrifolia belongs to the family *Rubiaceae.* It is a Shrub or compacted to twisted small tree up to 8 m high with square stems and large stipules between nodes and petioles. Leaves are opposite, petiolate, glossy, mostly ovate, 15-35 cm long. Flowers white, up to 15 mm long, with a tubular corolla and 5 spreading lobes, the flowers borne on a globose syncarp. Fruit a large fleshy syncarp up to 15 cm long, at first green but becoming white, juicy, and pungent when mature. Flowers and fruits are available throughout the year. Inflammation is local response of living mammalian tissues to injury due to any agent. Inflammation is a protective and defensive mechanism of body. Inflammation types are acute and chronic. There are various components to an inflammatory reaction such as edema formation, leukocyte infiltration and granuloma formation that can contribute to the associated symptoms and tissue injury. Signs of inflammation as: Rubor –Redness; Tumor –Swelling or edema; Color –Heat; Dolor –Pain; 5th sign functio laesa – loss of function was later added by Virchow [1]. The main symptoms of the body are increased body temperature and pain. Analgesia or pain is an ill-defined, unpleasant sensation, usually evoked by an external or internal noxious stimulus [2]. Pain acts as a warning signal against disturbances either in the body or in the external environment of an individual and protective in nature. Pain receptor organs are distributed throughout the body. Clinically, pain can be considered as Superficial or cutaneous pain, deep non- visceral pain, visceral pain, Reffered pain, Deafferentiation or neuropathic pain, Psychogenic or functional pain [3]. Analgesic is a drug that selectively relives pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness. Analgesics are divided into two groups. Opioid analgesics- these are the products obtained from opium poppy; relief from pain and depression of the CNS; some of them induce sleep. Non- Opioid analgesics- they do not interact with Opioid receptors and relieve pain without depression of the CNS. However there are no reports on the anti-inflammatory and analgesic activity of the plant leaves. Hence, the present study was designed to verify the claims of the native practitioners.

MATERIALS AND METHODS

Plant Materials:

The *Morinda citrifolia* leaves were collected in the month of September, 2010 from Thirumala hills in Chittoor district of Andhra Pradesh, India. The leaves were identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi [4].

Correspoding Author: **T. Sunil Kumar Reddy** Email:- drreddysunil@gmail.com

Preparation of Extract:

The leaves of the plant were collected and dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve no.40. Then the powder was extracted with petroleum ether in a Soxhlet extraction apparatus.

Phytochemical Screening:

The *Morinda citrifolia* was tested for the presence of saponins, alkaloids, glycosides, steroids, triterpenoids, flavonoids, tannins and reducing sugars by qualitative and quantitative methods [5].

Animals:

Male Wister rats weighing 160-200gm were used for the study of anti- inflammatory activity. Wister rats of either sex (19-32mg) were used for the study of analgesic activity. They were maintained under standard environmental conditions and were fed with standard pellet diet with water ad libitum.

Preparation of the drug for the experimental study:

Petroleum ether extract was administered in the form of suspension in 2% tween 80 solution and the standard drug in water was used for the study.

Acute Toxicity Studies:

Acute oral toxicity studies were performed as per OECD-423 guidelines. Male Wister mice were used for the study. The animals were divided into six groups containing six animals in each group. The extract was administered orally at the doses from 200- 2000mg/kg. There were no signs of toxicity and mortality was observed up to 2000mg/kg.

Anti- inflammatory activity

Carrageenan induced rat paw edema:

The animals were divided into 4 groups each group contained 6 animals. Animals were weighed and marked for identification. All the groups were treated with extract and standard drugs used for the study. After 30min. of the treatment, 0.1ml of 1% carrageenan in saline was injected into the sub plantar region of the left hind paw of each rat to induce edema [6]. Group I: Control, Group II: PMC -200mg/kg, Group III: PMC -400mg/kg, Group IV: Standard-Indomethacin-10mg/kg

The paw volume was measured initially and at intervals of 30, 60, 120, 180min. after carrageenan injection by volume displacement method using Plethysmometer by immersing the paw in mercury cell. The percentage inhibition of paw volume in drug treated group was compared with control group. Indomethacin (10mg/kg) was used as standard drug. The percentage inhibition of paw edema was calculated by using the following formula;

Percentage of edema inhibition= $[(V_c - V_t) / V_c] \times 100$

Vc- Volume of edema in control group

Vt- volume of edema in treated group

Cotton pellet granuloma

The rats are divided into 4 groups, 6 animals in each group. The cotton pellet granuloma model investigated the proliferation phase of inflammation [7]. The animals of each group were treated with standard and test drugs orally. After 30 minutes the animals were anaesthetized with diethyl ether. The 20mg of sterile cotton pellets were inserted in each axilla of rats by making small subcutaneous incision. The incisions are sutured with sterile catgut [8]. The extract and standard drugs were administered for seven days. After 8^{th} day the animals were sacrificed with excess anaesthesia and cotton pellets were removed surgically and separated from extraneous tissues. The pellets were weighted and dried at 70°C for 6 hour and weighted again. The dry weight of the pellets was taken and granuloma formation was measured. Group I: Control, Group II: PMC - 200mg/kg, Group III: PMC - 400mg/kg, Group IV: Standard- Indomethacin- 10mg/kg. The percentage inhibition of granuloma was calculated by using the following formula;

Percentage inhibition= $[(Wc – Wd) / Wc] \times 100$

Wc = Pellet weight in control group

Wd = Pellet weight in drug treated group

Analgesic activity

Tail immersion method:

The mice were divided into 4 groups, 6 animals in each group. Mice were weighed and 3-4cm. area of the tail was marked and immersed in the thermo- statically maintained water bath at 51^0c. The withdrawal time of the tail from the hot water in seconds was noted as the reaction time. The maximum cut off time for immersion was 150 seconds to avoid the injury of the tail tissue. Then control group was treated with vehicle, standard group was treated with Indomethacin 10mg/kg and test groups were treated with PMC 200 and 400 mg/kg orally. The initial readings were taken immediately before the administration of the test and standard drugs and at then 60, 90, 120, 150 min after the administration. Tail withdrawal time difference after drug administration was used to indicate the analgesia produced by standard and test drugs [9].

RESULT AND DISCUSSION

General qualitative and quantitative tests showed the presence of steroids. The plant extract did not exhibit any mortality up to the dose level 2000mg/kg. The petroleum ether extract of leaf part of *Morinda citrifolia* was evaluated for anti- inflammatory activity in acute and chronic experimental animal models and analgesic activity was evaluated for centrally acting analgesic effect in animal model. The *Morinda citrifolia* exhibited significant anti-inflammatory and analgesic activity at the doses of 200, 400mg/kg. The *Morinda citrifolia* showed 34% inhibition at the dose of 200mg/kg and 40% inhibition at the dose of 400mg/kg 3hr after the drug treatment in carrageenan induced paw edema, where as the standard drug showed 46% of inhibition (table 1).

In chronic model i.e., cotton pellet granuloma the *Morinda citrifolia* showed decreased formation of granuloma tissue of 30.24% at the dose of 200mg/kg, 42.42% at 400mg/kg, whereas standard showed 45.74% at the dose of 10mg/kg (table 2). The *Morinda citrifolia* exhibited analgesic activity at the dose 200 and 400mg/kg. The duration as well as the intensity of analgesia produced by *Morinda citrifolia* was dose dependent. It showed significant analgesic activity at 200mg/kg even in the first hour of the test after the 120min the activity began to decrease. The analgesic activity was almost comparable to that produced by Indomethacin (table 3).

Table 1. Effect of the *Morinda citrifolia* on carrageenan induced paw edema

Treatment	Dose (mg/kg)	Paw edema volume (ml)					Percentage of inhibition
		0min	30min	60min	120min	180min	
Control	0	0.29±0.01	0.32±0.01	0.39±0.01	0.42±0.01	0.50±0.01	_
PMC	200	0.29±0.02	0.34±0.01	0.39±0.01	0.36±0.02***	0.33±0.01***	34
PMC	400	0.31±0.01	0.34±0.01	0.39±0.01	0.34±0.01***	0.30±0.01***	40
Indomethacin	10	0.30±0.02	0.35±0.02	0.37±0.01	0.34±0.01***	0.27±0.01***	46

Table 2. Effect of *Morinda citrifolia* on cotton pellet induced granuloma

Treatment	Dose Mg/kg	Weight of cotton pellet		Percentage of inhibition
		Before	After	
Control	0	21±0.02	45.16±1.01	_
PMC	200	21±0.02	31.5±0.25***	30.24
PMC	400	21±0.01	26±0.58***	42.42
Indomethacin	10	21±0.02	24.5±0.56***	45.74

Table 3. Effect of *Morinda citrifolia* in mouse tail immersion method

Treatment	Dose Mg/kg	Tail withdrawing in sec				
		Before	After			
			60	90	120	150
Control	0	1.6±0.42	1.89±0.30	1.17±0.31	1±0.36	0.72±0.21
PMC	200	1.6±0.42	5.52±0.25***	5.5±0.22***	4.85±0.16***	4.52±0.21***
PMC	400	1.6±0.43	5.62±0.22***	6.5±0.25***	5.68±0.21***	4.63±0.21***
Indomethacin	10	1.73±0.48	6.32±0.42***	7.5±0.34***	5.97±0.17***	4.12±0.30***

CONCLUSION

In conclusion, the results of the present study support to the traditional use of *Morinda citrifolia* in inflammation and analgesia. This study established anti-inflammatory activity of the leaf extract of *Morinda citrifolia*. Acute oral toxicity studies were performed and mortality was not observed up to 2000mg/kg. *Morinda citrifolia* exhibited significant anti-inflammatory and analgesic activity at the doses of 200 and 400mg/kg. The results produced by *Morinda citrifolia* were comparable with that of standard Indomethacin. The activities may be due to the presence of steroids in the extract.

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