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Evaluation of Anti-Inflammatory and Analgesic Activity in Three *Morus* Species

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ABSTRACT

Natural products are often a source for bioactive compounds which have great potential for developing novel therapeutic agents. The present study was undertaken to investigate the anti-inflammatory and analgesic effect in different solvent extracts from three species of mulberry, *Morus alba, Morus serrata* and *Morus laevigata.* Plant leaf materials were collected from Mulberry Germplasm Center, Hosur, Tamil Nadu and were shade dried, powdered and subjected for hot soxhlet extraction utilizing petroleum ether, chloroform and methanol sequentially. The anti-inflammatory and analgesic activities were evaluated using Carrageenan-induced paw oedema method and tail immersion test respectively. From the results it is evident that among the three plants *Morus alba* extracts have shown prominent anti-inflammatory and analgesic activities when compared to *Morus serrata* and *Morus laevigata.* The present results also indicated that the methanolic extract of *Morus* species exhibited more significant activity than chloroform and petroleum ether extracts in the treatment of inflammation and pain.

Key words: Anti-inflammatory activity, analgesic activity, carrageenen-induced paw oedema, tail immersion, *Morus* species.

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INTRODUCTION

Inflammation is the way the body deals with infections and tissue damage [1]. It is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can be induced, maintain or aggravate many diseases such as rheumatoid arthritis, chronic asthma, psoriasis, cancer, multiple sclerosis and a host of other diseases [2-4].

Pain has been defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [5]. Drugs that are currently used for the management of pain are opioids or nonopioids and that for inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. All these drugs carry potential toxic effects. Therefore, development of newer and more powerful anti-inflammatory and analgesic drugs with lesser side effects is the order of the day.

On the contrary many medicines of plant origin had been used since ages without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop more effective and cheaper drugs. Plants represent a large natural source of useful compounds that might serve as lead for the development of novel drugs [6].

In the present study, different mulberry species belonging to genera *Morus* were selected because of their medicinal importance. *Morus* plant species possess enormous importance in medicinal, economical, industrial, clinical, and domestic fields [7-8]. Therefore, the main objective of the present study was to compare the anti-inflammatory and analgesic potentials in animal models for various *Morus* extracts in view of its use in the local treatment of some painful inflammatory conditions.

MATERIALS AND METHODS

The plant materials were collected form Mulberry Germplasm Center, Hosur, Tamil Nadu and leaves were shade dried. The dried leaf materials were powdered and subjected for hot soxhlet extraction utilizing petroleum ether, chloroform and methanol sequentially.

Qualitative phytochemical investigation

The preliminary qualitative phytochemical studies were performed for testing the different chemical groups present in three different species of mulberry leaf extracts [9-10].

Experimental animals

Swiss albino mice and rats of both sexes were used in the experiment and they were housed under standard environmental conditions. All animal experiments were carried out in accordance with the guidelines of CPCSEA. The animal ethical committee approval was obtained to conduct the animal experiments (NCP/IAEC/CL/40/12/2011-12 dated 05-01-2012).

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Acute toxicity studies

The acute toxicity studies were carried out as per stair case method [11] and the mortality rates were observed after 48 hours. Accordingly the LD50 of the extracts was found to be 2 g/kg body weight. One tenth and one fifth of this dose was selected for the evaluation of anti-inflammatory and analgesic activity.

Anti-inflammatory activity

Carrageenan-induced paw oedema

The method used was similar to that described by Winter *et al*. 1962 [12]. The albino rats of either sex were divided into eleven groups of six animals each. Group I received only vehicle and Group II received Indomethacin at 50mg/Kg body weight intraperitoneally as a standard drug. Group III-V received Petroleum ether, chloroform and methanol extracts of *Morus alba*. Group VI-VIII received Petroleum ether, chloroform and methanol extracts of *Morus serrata* and group IX-XI received Petroleum ether, chloroform and methanol extracts of *Morus laevigata.* Acute inflammation was induced in all groups by injecting 0.1 ml of 1% carrageenan into the sub-plantar region of the right hind paw of rats. The paw volume was measured 1 h prior to carrageenan injection using plethysmometer and at 60, 120, 180 and 240 minutes after the carrageenan injection. Mean decrease in the paw volume was measured.The percentage inhibition of paw oedema is calculated by,

$$\text{Percentage inhibition of paw oedema} = (1 - Vt/Vc) \times 100$$

Where Vc = average increase in paw volume (average inflammation) of the control group of rats at a given time; and

Vt = average inflammation of the drug treated (i.e. plant extracts) rats at the same time.

Analgesic activity

Tail immersion test

The basal reaction time to radiant heat source was taken by placing the tip of the tail on the radiant heat source [13]. The mice were screened for the sensitivity test by immersing the tail of the mice gently in hot water maintained at 55^{o}C. The time the animal's tail spent in the water before reacting to the pain is recorded with a stop watch. The animal immersing the tail from hot water with in 5 second was selected for the study. The selected mice were then divided in to eleven groups of six mice each. Group I served as control and received vehicle only intraperitoneally. Group II received aspirin at a dose of 25 mg/Kg which served as standard. Group III-V received Petroleum ether, chloroform and methanol extracts of *Morus alba*. Group VI-VIII received Petroleum ether, chloroform and methanol extracts of *Morus serrata* and group IX-XI received Petroleum ether, chloroform and methanol extracts of *Morus laevigata* at a dose of 200mg/kg & 400mg/kg respectively

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intraperitoneally. After administration of the drugs, the reaction time was measured after 60 minutes.

STATISTICAL ANALYSIS

The observations are reported as mean±SEM. Differences between groups means were assessed by one-way analysis of variance (ANOVA). The results obtained were compared with the control group. P value < 0.01 was considered statistically significant.

RESULTS

Qualitative phytochemical analysis

The phytochemical analysis revealed the presence of steroids, glycosides, flavonoids, tannins and carbohydrates in petroleum ether extract of *Morus alba* leaves, while its chloroform extract showed the presence of steroids, glycosides, terpenoids, saponins, alkaloids, tannins and carbohydrates and methanol extract showed the presence of steroids, glycosides, terpenoids, saponins, alkaloids, flavonoids, tannins, carbohydrates, proteins and aminoacids. Whereas petroleum ether extract of *Morus serrata* leaf showed the presence of glycosides and carbohydrates, chloroform extract showed the presence of steroids, glycosides, terpenoids, alkaloids, tannins, carbohydrates and methanol extract showed the presence of steroids, glycosides, terpenoids, saponins, alkaloids, flavonoids, tannins, carbohydrates, proteins and aminoacids. In regard to petroleum ether extract of *Morus laevigata* leaf showed the presence of glycosides, flavonoids, tannins, carbohydrates, chloroform extract showed the presence of steroids, glycosides, terpenoids, saponins, alkaloids, tannins, carbohydrates and methanol extract showed the presence of steroids, glycosides, terpenoids, saponins, alkaloids, flavonoids, tannins, carbohydrates, proteins and aminoacids (Table 1).

Table 1 Qualitative phytochemical analysis in different species of Mulberry

Tests	MAPE	MACH	MAME	MSPE	MSCH	MSME	MLPE	MLCH	MLME
Steroids	-	+	+	-	+	+	-	+	+
Glycosides	+	+	+	+	+	+	+	+	+
Terpenoids	-	+	+	-	+	+	-	+	+
Saponins	-	+	+	-	-	+	-	+	+
Alkaloids	-	+	+	-	+	+	-	+	+
Flavonoids	+	-	+	-	-	+	+	-	+
tannins	+	+	+	-	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+
Proteins	-	-	+	-	-	+	-	-	+
aminoacids	-	-	+	-	-	+	-	-	+

Where:
MAPE: *Morus alba* petroleum ether extract, MACH: *Morus alba* chloroform extract,
MAME: *Morus alba* methanol extract, MSPE: *Morus serrata* petroleum ether extract,
MSCH: *Morus serrata* chloroform extract, MSME: *Morus serrata* methanol extract,
MLPE: *Morus laevigata* petroleum ether extract, MLCH: *Morus laevigata* chloroform extract,
MLME: *Morus laevigata* methanol extract

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Anti - inflammatory activity

In the present study, acute inflammatory condition is produced in the animals by carrageenan induced pedal inflammation and the results are presented in Table 2. Administration of carrageenan to the rats showed a rise in paw volume at different time intervals in control group. However, from the results it is evident that among the three solvents used, methanol extract has shown greater anti-inflammatory activity with respect to each time intervals (22.95%, 37.87%, 49.25%, and 55.22%) when compared to chloroform extract (13.11%, 30.30%, 38.80% and 47.76%) and petroleum ether extract (47.540%, 62.12%, 70.14%, and 76.11%) in *Morus alba*. Among the extracts of *Morus serrata*, methanol extract showed greater effect with respect to each time intervals (14.75%, 33.33%, 47.76%, and 52.23%) than chloroform (11.47%, 27.27%, 35.82%, and 46.26%) and petroleum ether (11.47%, 24.24%, 32.83%, and 38.80%). Similar trend is continued with respect to different solvent extracts of *Morus laevigata*, where Methanol extract showed 13.11%, 24.24%, 34.32% and 41.79% of anti- inflammatory effect, chloroform extract showed 9.83%, 21.21% 31.34% and 37.31% and petroleum ether extract showed 9.83%, 19.69%, 28.35% and 31.34% of anti- inflammatory effect. Among the three species under study the order of anti-inflammatory effect were *Morus alba* > *Morus serrata* > *Morus laevigata.*

Table 2: Anti-inflammatory activity in three different species of Mulberry

	Group	Initial	I	II	III	IV
Control	I	0.57±0.02	0.61±0.06	0.66±0.03	0.67±0.03	0.67±0.02
Standard	II	0.52±0.01	0.32±.02	0.25±0.05	0.20±0.02	0.16±0.03
% Decrease		-	47.540%	62.12%	70.14%	76.11%
Mape	III	0.54±0.04	0.55±0.01	0.53±0.01	0.44±0.01	0.41±0.01
% Decrease		-	9.83%	19.69%	34.32%	38.80%
Mach	IV	0.56±0.02	0.53±0.01	0.46v0.01	0.41±0.01	0.35±0.01
% Decrease		-	13.11%	30.30%	38.80%	47.76%
Mame	V	0.55±0.01	0.47±0.01	0.41±0.01	0.34±0.01	0.3±0.01
% Decrease		-	22.95%	37.87%	49.25%	55.22%
Mspe	VI	0.53±0.01	0.54±0.01	0.5±0.02	0.45±0.02	0.41±0.01
% Decrease		-	11.47%	24.24%	32.83%	38.80%
Msch	VII	0.54±0.03	0.54±0.01	0.48±0.02	0.43±0.01	0.36±0.01
% Decrease		-	11.47%	27.27%	35.82%	46.26%
Msme	VIII	0.53±0.03	0.52±0.02	0.44±0.01	0.35±0.01	0.32±0.01
% Decrease		-	14.75%	33.33%	47.76%	52.23%
Mlpe	IX	0.56±0.02	0.55±0.01	0.53±0.01	0.48±0.01	0.46±0.01
% Decrease		-	9.83%	19.69%	28.35%	31.34%
Mlch	X	0.57±0.01	0.55±0.01	0.52±0.02	0.46±0.01	0.42±0.01
% Decrease		-	9.83%	21.21%	31.34%	37.31%
Mlme	XI	0.55±0.04	0.53±0.01	0.5±0.01	0.44±0.01	0.39±0.01
% Decrease		-	13.11%	24.24%	34.32%	41.79%

Values are Mean ±SE, N=6, P<0.01 Vs. Control

Analgesic activity

Analgesic effects of different solvent extracts of three species of mulberry are presented in Table 3 at different concentrations (*i.e.,* 200 and 400 mg/Kg). Among the three plants, *Morus alba* extracts have shown prominent analgesic activity when compared to *Morus serrata* and *Morus laevigata.* At 200 mg/ kg concentration, methanol extracts of respective plants showed considerable increase in reaction time (8.97±0.21, 8.7±0.19 and 7.88±0.16) when compared to chloroform extracts (7.03±0.19, 6.33±0.14 and 6.22±0.2) and petroleum ether extracts (4.33±0.13, 3.78±0.18 and 3.27±0.18). At 400mg/kg concentration also similar effect was observed where, methanol extract showed the highest analgesic effect of 10.58±0.16, 9.92±0.27 and 9.37±0.15 than chloroform extract (8.23±0.15, 7.52±0.16 and 6.93±0.13) in *Morus alba, Morus serrata* and *Morus laevigata* respectively. Among the petroleum ether extracts of three plants, *Morus alba* extract have the effect of 5.15±0.13 seconds *Morus serrata* extract with 4.8±0.13 seconds and *Morus laevigata* with 4.38±0.15 seconds of increased reaction time.

Table 3: Analgesic activity at 200mg/Kg and 400mg/Kg concentration of different solvent extracts of *Morus alba, Morus serrata* and *Morus laevigata*

Extract	Reaction time in Sec	
	200mg/Kg	400mg/Kg
control	2.28±0.06	2.43±0.08
Standard	3.17 ± 0.19	3.17 ± 0.19
Mape	4.33±0.13	5.15±0.13
Mach	7.03±0.19	8.23±0.15
Mame	8.97±0.21	10.58±0.16
mspe	3.78±0.18	4.8±0.13
msch	6.33±0.14	7.52±0.16
msme	8.7±0.19	9.92±0.27
Mlpe	3.27±0.18	4.38±0.15
Mlch	6.22±0.2	6.93±0.13
Mlme	7.88±0.16	9.37±0.15

Values are Mean ±SE, N=6, P<0.01 Vs. Control

DISCUSSION

The result of present investigation revealed that, the preliminary phytochemical analysis of three different species of mulberry leaf extracts are bestowed with the presence of several bioactive compounds *viz.* steroids, glycosides, terpenoids, saponins, alkaloids, flavonoids, tannins which therefore encourages further pharmacological studies.

The carrageenan-induced paw oedema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis [14]. Carrageenan-induced hind paw oedema is the standard experimental model of acute inflammation. Carrageenan is a sulphated polysaccharide obtained from seaweed (Rhodophyceae) which is commonly used to induce acute inflammation [15]. Carrageenan-

induced oedema involves the synthesis or release of mediators at the injured site. These mediators include prostaglandins, especially the E series, histamine, bradykinins, leucotrienes and serotonin, all of which also cause pain and fever [16]. Inhibitions of these mediators from reaching the injured site or from bringing out their pharmacological effects normally ameliorate the inflammation and other symptoms. This study has shown that the methanolic leaf extracts of *Morus* species possessed a significant anti-oedematogenic effect on paw oedema induced by carrageenan. Development of oedema induced by carrageenan is commonly correlated with early exudative stage of inflammation [17-18]. Carrageenan oedema is a multimediated phenomenon that liberates diversity of mediators. It is believed to be biphasic; the first phase involves the release of serotonin and histamine while the second phase is mediated by prostaglandins, the cyclooxygenase products, and the continuity between the two phases is provided by kinins [18-19]. Since carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation [20-21], the results of this study are an indication that *Morus alba* can be effective in acute inflammatory disorders compared to *Morus serrata* and *Morus laevigata.*

Preliminary qualitative phytochemical screening reveals the presence of alkaloids, flavonoids, carbohydrates, glycosides, terpenoids, tannins *etc*. in *Morus species.* Therefore, it is assumed that these compounds may be responsible for the observed anti-inflammatory and analgesic activity. Analgesic and anti-inflammatory effects of flavonoids, steroids and tannins have been reported [22-24]. It has been reported that flavonoids possess anti-inflammatory [25] and analgesic activity [26]. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins [27]. They inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase and reported to produce anti-inflammatory effects [28]. Since, prostaglandins are also involved in the pain perception; inhibition of their synthesis might be the possible reason for the analgesic activity of the extracts. There are also reports on the role of tannins in anti-nociceptive activity [29]. Besides this, alkaloids are well known for their ability to inhibit pain perception [30]. Hence the analgesic and anti-inflammatory effects produced by the mulberry extracts may be attributed individually or collectively to the flavonoids, steroids, alkaloids and tannins.

CONCLUSION

Plants are exemplary source of medicines and several drugs have been derived directly or indirectly from them. Mulberry was traditionally claimed for a large number of pharmacological action and medicinal uses. The present study offered a scientific proof to the traditional use of mulberry. It can be concluded that the three different species of mulberry *viz. M. alba, M. serrata, M. laevigata* possess anti-inflammatory and analgesic activity against carrageenan induced paw oedema in rats and tail immersion test in mice. These activities may be attributed due to their phytoconstituents present in them. Hence the study demonstrates the efficacy of three different species of mulberry as analgesic and anti-inflammatory agents. Efforts of further phytochemical to isolate the active compounds responsible for the anti-inflammatory and analgesic activities will be therefore highly rewarding.

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REFERENCES

[1] Simmons DL. Drug Discovery Today, 2006; 11(5-6): 210-219.
[2] Balkwill F, Mantovani A. Lancet 2001; 357: 539-545.
[3] Coussens LM, Werb Z. Nature 2002; 420: 860.
[4] Sosa S, Balick MJ, Arvigo R, Esposito RG, Pizza C, Altinier G. J Ethanopharmacol 2002; 8: 211-215.
[5] Michel YB, Dubois, Christopher G, Allen HL. Wylie and Churchil-Davidson's A practice of Anaesthesia, 7t h edition. London, Hodder Arnold, 2003; pp. 1139-1235.
[6] Amberkar MV, Tara S, Meena KK, Bairy KL, Smita S. Indian J Physiol Pharmacol 2011; 55(1): 13-24.
[7] Sanchez MD, FAO Electronic Conference on Mulberry for Animal Production. 2000.
[8] Andreoni N, First International Conference on Crop Wild Relative Conservation and Use, Agrigento, Sicily, Italy, 2005; pp. 121.
[9] Trease GE, Evans WC, A Text book of Pharmacognosy, 11th edition, Bailliere Tidall London, 1978, 530.
[10] Khandelwal KR, Practical Pharmacognosy Techniques and Experiments, 16th Edition, Nirali Prakashan, 2006, 149-156.
[11] Ghosh MN, Fundamentals of Experimental Pharmacology, 2nd edn. Scientific book agency, Calcutta, 1984.
[12] Winter CA, Risley EA, Nuss GW. Exp Biol 1962; 111: 544-7.
[13] Kulkarni SK. Handbook of Experimental Pharmacology, first edition vallabh prakashan, Delhi 1987; 63-64.
[14] Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, Perkins W, Lee L, Isakson P. Acadamic Sciences 1994; 91: 12013-12017.
[15] Dirosa M. J Pharm Pharmacol 1972: 24: 89-102.
[16] Asongalem EA, Foyet HS, Ngogang J, Folefoc GN, Dimo T, Kamtchouing P. J Ethnopharmacol 2004; 91(2-3): 301-308.
[17] Ozaki Y. Chem Pharmaceut Bull 1990; 38:1045-1048.
[18] Silva GN, Martins FR, Matheus ME. J Ethnopharmacol 2005; 100: 254-259.
[19] Perianayagam JB, Sharma SK, Pillai KK. J Ethnopharmacol 2006; 104: 410-414.
[20] Mossai JS, Rafatullah S, Galal AM, Al-Yahya MA. Int J Pharmacol 1995; 33: 242-246.
[21] Sawadogo WR, Boly R, Lompo M, Some N. Int J Pharmacol 2006; 2: 435-438.
[22] Collier HO, Dinneen LC, Johnson CA, Schneider C. Br J Pharmacol 1988; 32: 295-310.
[23] Maruthi T Ekbote, Ramesh CK, Riaz Mahmood, Thippeswamy BS and Veeresh Verapur. J Pharm Res 2010,3(3):478-481.
[24] MN Prabha, CK Ramesh, IJ Kuppast, KL Mankani. Int J Chem Sci 2008;6(4); 1781-1787.
[25] Hossinzadeh, H, Ramezani M, Fedishei M, Mahmoudi M. Phytomedicine 2002; 9: 135-41.

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[26] Ramaswamy S, Pillai NP, Gopalkrishnan V, Parmar NS, Ghosh MN. Indian J Exp Biol 1985; 23: 219.
[27] Rao MR, Rao YM, Rao AV, Prabhkar MC, Rao CS, Muralidhar N. J Ethnopharmacol 1998; 62: 63-66.
[28] Alcaraz MJ, Jimenez M., Fitoterapia 1988; 59: 25.
[29] Vanu MR, Palanivelu S, Panchanatham S. Biol Pharm Bull 2006; 29: 693-700.
[30] Uche FI, Aprioku JS. J App Sci Environm Manag 2008; 12(4): 99–102.