

ANALGESIC AND ANTI-INFLAMMATORY STUDIES OF *MYRICA NAGI* BARK

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ABSTRACT

The crude extract of *Myrica nagi* bark was tested for its analgesic activity by writhing and hot plate methods and its anti-inflammatory activity by formalin test method in Swiss albino mice. In analgesic activity assessment by writhing test, the extract showed effectiveness at 300mg/kg (77.24 and 58.88% inhibition respectively) in both early and late phases in comparison with 500mg/kg dose which showed non-significant activity (33.56 and 22.22% inhibition) in both phases. Standard drug aspirin showed 41.83% inhibition of first phase and 74.07% inhibition of second phase. *M. nagi* bark also exhibited negligible analgesic activity in comparison with standard drug aspirin. It showed significant reduction in tail flicking and biting response. *M. nagi* bark @ 300 and 500 mg/kg dose showed 40.0 and 11.81 and 41.64 and 6.36% of inhibition of licking and biting respectively at early and late phases.

Key words: *Myrica nagi*, analgesic, anti-inflammatory, mice

INTRODUCTION

Myrica nagi Thunb belongs to family *Myricaceae* and locally known as Kaiphali in Urdu and Hindi and Box Myrtle in English. It is a small evergreen tree had brown grey bark. The flowers are fleshy, red composed of spindle shaped fleshy fibers radiating from the rugose stone (Chopra *et al.* 1986; Nadkarni, 1976; NIIR, 2004). It is distributed in subtropical Himalayas, Simla, Sylhet (Bangladesh) and Southwards to Singapore, Khasi mountains and hills of Burma. It is very commonly cultivated in China and Japan (Chopra *et al.* 1986; Nadkarni, 1976). The plant can be grown from late winter to mid summer. Its bark and fruit have great medicinal value. The bark reveals the presence of steroids, reducing sugars, tannins, glycosides, saponins and volatile oils. It is used both, internally as well as externally. Its uses for wound healing, ulcer healing, facial palsy and paralysis, as gargle for various dental problems, for piles for respiratory tract infections, diarrhea, dysentery, typhoid and diuresis, menorrhagia, epilepsy, cardiac debility, edema, haemoptysis anti-rheumatic, antiseptic, aromatic, astringent, carminative, ophthalmic and stimulant. It has proved useful in the treatment of fever, asthma and coughs (Krishnamurthy and Seshadri, 1966; Nadkarni, 1976; Watt, 1889; Chopra *et al.*, 1986). The bark is said to contain 60 - 80% tannin. Wood - hard, close-grained a good fuel is used mainly for fuel, though it is sometimes used for making poles for construction (Krishnamurthy and Seshadri, 1966; Nadkarni, 1976; Watt, 1889).

MATERIALS AND METHODS

Swiss albino mice (25-30 gm) of either sex obtained from H.E.J Research Institute of Chemistry, University of Karachi, Pakistan, were used to determine the analgesic and anti inflammatory activities of *M. nagi* bark. Animals were kept in colony cages (five animals in each group) with access to food and water. They were maintained in a climate and light controlled room (30°C ± 1°C and 12/12hours light/dark cycle). Standard drug, Aspirin was used for the comparison. Inflammation was induced in right paw injecting formalin in standard dose to adjudge anti-inflammatory activity of the bark.

EXTRACTION PROCEDURE

The dry plant material (*M. nagi* bark) was chopped into small pieces then macerated with methanol and kept for 15 days at room temperature for percolation. The methanol extract was then filtered. After filtration once again

methanol was added in the remaining material and kept for 15 days at room temperature for further percolation. Later same procedure was repeated for filtration. The methanol extract was evaporated under reduced pressure in a rotary evaporator to obtain a residue. Two residues were combined together and used for experiments.

ASSESSMENT OF ANALGESIC ACTIVITY

By writhing and hot plate method

The tests were performed according to the modified method of Koster *et al.* (1959) and Elaine *et al.* (1995). Mice were used as the test animals in this method. According to this method writhes were induced by intraperitoneal administration of the acetic acid solution 10ml/kg thirty minutes prior to the administration of the acetic acid, the animals were treated orally with the test substance. Numbers of writhes was counted for a period of 5 minutes after 30 and 60 minutes of acetic acid administration which are termed as early phase and late phases. A reduction in the number of writhing as compared to the control animals was considered as evidence for the presence of analgesia and expressed as percent inhibition of writhing. For hot plate analgesic activity mice were placed in an empty beaker (2 liter) on a hot plate ($55\pm 1^{\circ}\text{C}$) and their reaction to heat was observed. When the animal raised and licked the front paws they were quickly removed from the hot plate.

Mice were divided into 4 groups (i.e., Group-A for control, Group-B and Group-C for 300mg/kg and 500mg/kg oral doses of crude extract respectively, and Group-D for standard). Each group comprised of 5 animals, weighing 25-30 g. Aspirin as 300mg/kg orally was used as the reference compound. The crude drug and the Aspirin was diluted in distilled water and administered orally. The control animals were treated orally with the same volume of saline as the crude extract.

ASSESSMENT OF ANTI-INFLAMMATORY ACTIVITY

Anti inflammatory activity by formalin test method

Swiss albino mice (25-30 g) were divided into different groups of 5 animals each. They were injected with 20 μ l of 1% Formalin in the right hind paw and the left hind paw was injected with an equal volume of normal saline. Two distinct phase of intensive licking and biting of right hind paw were observed during 0-10 minutes (early phase) and 10-30 minutes (late phase) after formalin injection. These phases were scored separately for studying drug effect. Vehicle or drugs were administered orally 30 minutes before formalin injection (Modified method of Rathee *et al.*, 2002).

Statistical Analysis

Values for observations were expressed as mean after drug administration \pm SEM. The significance of difference between means was determined by Dunnett's t-test and values of $P < 0.05$ were considered significant and $P < 0.01$ as highly significant. All statistical procedure was performed according to the method of Alcaraz and Jimenez (1989).

RESULTS AND DISCUSSION

The literature search shows the presence of gum, resins, albumen, starch (Newall *et al.*, 1996), flavonoids, tannins, terpenoids, wax of palmitic, myristic and lauric acid (Felter & Lloyd, 1893), saponins, glycosides, steroids etc. (Chopra *et al.*, 1986). Medicinal properties like gastritis, diarrhea, and dysentery (Felter & Lloyd, 1893), fever, cold, sore throat, flu, infection (Cook, 1869), antioxidant, cardiovascular, hepatoprotective, anti-inflammatory activities (Alam *et al.*, 2000; Ohta, 1992; Njung'e *et al.*, 2002) confirmed by the literature search.

According to our screening the bark of *M. nagi* possesses significant analgesic and anti-inflammatory activities. *M. nagi* crude extract has less potential for analgesic response, at 300 mg/kg less significant results obtained at $P \leq 0.05$. This data might be used in the evaluation of medicinal properties of *M. nagi* extract. It also gave valuable information regarding the use and development of drug from crude extract.

Anti-inflammatory results of crude extract of *M. nagi* bark exhibited mild but significant reduction in licking and biting response (Table 1). At 300 and 500 mg/kg percentage of inhibition of first phase and second phase after *M. nagi* bark administration showed 40 and 11.81; and 41.64 and 6.36% of inhibition respectively at 300 and 500mg/kg. The test extract showed mild but significant anti-inflammatory effect at first phase in comparison with standard drug aspirin. Table 2 shows the results of analgesic activity by writhing test. Crude extract of *M. nagi* bark showed effectiveness at 300mg/kg (77.24 and 58.88% inhibition of both phases respectively) in comparison of

500mg/kg dose (33.56 and 22.22% inhibition). Standard drug aspirin showed 41.83% inhibition of first phase and 22.22% inhibition of second phase. Table 3 shows the results of analgesic activity by hot plate analgesiometer. *M. nagi* bark also exhibited negligible analgesic activity in comparison with standard drug aspirin. The test drug exhibited significant analgesic activity by writhing test at 300mg/kg body weight. The response was short term i.e decreased after one hour. At higher dose the test drug did not exhibit noticeable response. By the other method (Hot plate method) it did not exhibit any analgesic activity at any dose. This shows that *M. nagi* bark can be used as an analgesic for short term local response for gastrointestinal cramps in low dose. Further search can be carried out at other doses.

Table 1. Anti-inflammatory activity of *M. nagi* bark by formalin test.

Treatments	Dose mg/kg orally	Mean No. of licking and biting± S.E.M		Inhibition %	
		1 st Phase	2 nd phase	1 st Phase	2 nd phase
<i>M. nagi</i> bark	300 mg/kg	43.8±1.74	24.6±3.7	40.0	11.81
	500 mg/kg	42.6±1.66	20.6±5.1	41.64	6.36
Aspirin	300 mg/kg	57±.19	19±0.29	21.91	13.63
Control	0.5ml saline	73±1.16	22±2.01	-	-

Values represents mean ± S.E.M statically significant from control and standard drug

*Significant at $p \leq 0.05$, **highly significant at $p \leq 0.005$

Table 2. Analgesic activity of *M. nagi* bark by writhing syndrome method.

Treatments	Dose mg/kg orally	Mean No. of writhes± S.E.M		Inhibition %	
		1 st Phase	2 nd phase	1 st Phase	2 nd phase
<i>M. nagi</i> bark	300 mg/kg	19.8±4.87	22.2±6.59	77.24**	58.88*
	500 mg/kg	57.8±1.93	42±2.23	33.56	22.22
Aspirin	300 mg/kg	50.6±1.35	14±0.45	41.83	74.07**
Control	0.5ml saline	87±5.56	54±4.67	-	-

mean ± S.E.M n=5; significant with respect to control (*Significant results, **highly significant results)

Table 3. Analgesic activity of *M. nagi* bark on hot plate Analgesiometer in mice.

Variation reaction time with ± S.E.M (time in sec at 55± 1°C)										
Group	0hr	0.5 hr	1 hr	1.5 hr	2 hr	2.5 hr	3 hr	3.5 hr	4 hr	4.5 hr
<i>M. nagi</i> bark 300 mg/kg	11.2± 1.31	10.4± 0.51	10.6± 1.17	10.2± 1.11	10.6± 1.17	11± 1.0	9.2± 1.3	10.4± 1.2	11± 1.0	10.6± 1.5
<i>M. nagi</i> bark 500 mg/kg	13.2± 1.91	13.2± 1.36	14.6±2.91	14.2± 3.47	12.8± 1.53	14.4± 2.48	13.2± 0.75	12± 1.76	12.8± 1.73	12.1± 1.8
Aspirin 300 mg/kg	15± 1.43	39± 0.36	38± 0.82	39± 0.12	44± 0.51	45± 0.86	33± 0.81	22± 0.91	22± 0.91	22± 0.91
Control 0.5ml saline	11.6± 1.029	12± 1.04	11.8± 1.583	10.8± 1.969	*12.6± 1.777	*11.8± 2.26	11.6± 1.02	11.6± 1.01	11.6± 1.013	11.6± 1.01

Values represents mean ± S.E.M statically significant from control and standard drug *Significant at $p \leq 0.05$.

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