

***In-vivo* antipyretic activity of methanolic extracts of root and leaves of *Morinda angustifolia* Roxb.**

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ABSTRACT: Bangladesh is a country of vast natural resources and plants of utmost values. Over the years, natural products based medical practitioners have been using numerous plants as the panacea of wide range of ailments. By considering the traditional claims, the present study was conducted to evaluate the antipyretic activity of methanolic extracts of root and leaves of *Morinda angustifolia* Roxb. on Swiss albino mice in which pyrexia was induced injecting brewer's yeast. The experiment was conducted by dividing the test animals into four groups i.e. group I receiving vehicle double distilled water (DDW) at a dose of 10 mL/kg b.w (body weight) p.o (per orally) as control, group II receiving paracetamol at a dose of 150 mg/kg b.w p.o as standard, group III and group IV receiving methanolic extract of root (MERMA) and leaves (MELMA) of *M. angustifolia* respectively; both at a dose of 500 mg/kg b.w p.o. After the administration of the samples, rectal temperature of mice was recorded up to 3 hours. Both the extracts along with paracetamol significantly ($p < 0.05-0.01$) reduced temperature when compared to control.

KEYWORDS – Antipyretic activity, albino mice, *Morinda angustifolia* Roxb., paracetamol, yeast induced pyrexia

I. INTRODUCTION

Fever also termed as pyrexia [1] is a common medical phenomenon which is characterized by an elevation of body temperature above the normal range of 36.5-37.5°C (98- 100°F) due to an increase in the body temperature regulatory set point [2]. This increase in set point triggers increased muscle tone and chilling sensation. Fever is mediated by prostaglandin E2 (PGE2) which is released by prostaglandin synthetase [3]. In general, NSAIDs are believed to produce their antipyretic action by the inhibition of prostaglandin synthetase within the Hypothalamus [4]. The natural products i.e. plants have been being used as the healing agents for thousands of years by the traditional medical practitioners. These plants with therapeutic properties used for treatment of various ailments, can be of considerable interest to modern science in their potential for discovery of lead compounds, which can lead to better drugs [5]. *M. angustifolia* is a plant of Rubiaceae family, locally known as Daru haridra or Rong gach [6]. One of the Bangladeshi tribes named Marma uses root paste and juice of *M. angustifolia* in insect bites and fever [7]. It's an evergreen shrub or small tree, about 6 m tall. Leaves are narrowly lanceolate or oblanceolate. Cymes are terminal, lateral and leaf-opposed; peduncles up to 12 cm long. Flowers are 5-merous, sweet scented, heterostylous, arranged on globose heads which gradually elongates as a small compound fruit. Fruits are turbinate, few fruits set in each head, mostly free, turbinate. Flowering and fruiting period is December to September [6]. The aim of this study is to evaluate the antipyretic activity of methanolic extracts of root and leaves of *M. angustifolia* on Swiss albino mice.

II. MATERIALS AND METHODS

2.1 Plant materials

For the investigation, whole plant of *M. angustifolia* was collected from Ukhiya, Cox's Bazar Hill Tracts, Bangladesh and was identified by Forest Research Institute (FRI), Chittagong, Bangladesh. After collection, the plant parts (root and leaves) were washed with tap water. The root parts were cut into smallest pieces possible. Then the plant parts were shade-dried. Shade-dried root and leaves were crushed into coarse powders and 120 gm of powdered materials of each plant part was subjected for hot extraction with 700 mL methanol in separate soxhlet apparatus (Quickfit, England). The overall extraction process was carried out for a period of 22 hours and then the extracts were filtered through filter paper (Whatman filter paper number 45). The obtained filtrate was made viscous placing the beaker under a non-stop ceiling fan for a period of 72 hours. The yield value of root and leaves extracts were 16.45% and 18.73% respectively.

2.2 Preliminary screening for phytoconstituents

The freshly prepared methanolic extracts of root and leaves of *M. angustifolia* were qualitatively tested for the presence of phytochemicals by using standard procedures [8, 9].

2.3 Animals

Swiss albino mice (20-25 g) of either sex were purchased from Animal Resources Branch (ARB), International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). Besides allowing the animals to have free access to the standard laboratory feed and water, they were kept at room temperature ($26 \pm 2^\circ\text{C}$) with 12 hours light/dark cycle for 1 week to acclimatize to laboratory conditions before starting of the experiment. The food delivered was withdrawn 24 hours before the experiment but allowed free access of water.

2.4 Evaluation of antipyretic activity

At first, pyrexia was induced injecting aqueous suspension of brewer's yeast into Swiss albino mice with almost uniform body temperature and then antipyretic activity of plant extracts was evaluated on them [10]. The test animals were divided into four groups i.e. group I receiving DDW at a dose of 10 mL/kg b.w p.o as control, group II receiving paracetamol at a dose of 150 mg/kg b.w p.o as standard, group III and group IV receiving methanolic extract of root (MERMA) and leaves (MELMA) of *M. angustifolia* respectively; both at a dose of 500 mg/kg b.w p.o. All the drugs were given as freshly prepared aqueous suspension. The initial rectal temperature of the Swiss albino mice was recorded using digital thermometer. Mice were made hyperthermic by subcutaneous injection of 20% brewer's yeast suspension in double distilled water at a dose of 1 mL/100g b.w. When the temperature was believed to be at its peak (18 hours after yeast injection) the rectal temperature of the mice was recorded again. The experimental animals that showed a rise in rectal temperature of at least 2°F were selected for the test. The plant extracts, paracetamol and control vehicle were given orally and rectal temperature of animals was recorded at 1 hour interval for 3 hours following the administration of the test substances.

2.5 Statistical analysis

The results obtained from antipyretic activity test were expressed as the mean \pm SEM. The results were analyzed using one-way ANOVA followed by using Dunnett's t-test. The statistical analysis was carried out with SPSS software version 15 (Windows). A difference was considered significant at $p < 0.05$.

III. RESULTS AND DISCUSSION

3.1 Preliminary screening for phytoconstituents

In preliminary screening for phytoconstituents, it was found that the methanolic extracts of root and leaves of *M. angustifolia* contained reducing sugar, alkaloids, glycoside, flavonoids, tannins, saponins and amides.

3.2 Evaluation of antipyretic activity

The effect of methanolic extracts of root and leaves of *M. angustifolia* on mice is presented in Table 1. In this test, both the extracts at a dose of 500 mg/kg b.w significantly attenuated hyperthermia up to 3 hours in mice. Throughout the experiment, the root extract reduced temperature from 101.23°F to 99.33°F ($p < 0.01$), 98.10°F ($p < 0.01$) and 98.23°F ($p < 0.01$) in 1st, 2nd and 3rd hour respectively and caused maximum reduction of temperature in 2nd hour. The leaves extract reduced temperature from 101.30°F to 100.13°F ($p < 0.05$), 99.24°F ($p < 0.01$) and 100.37°F ($p < 0.01$) in 1st, 2nd and 3rd hour respectively and caused maximum reduction of temperature in 2nd hour. It was found that the anti-pyretic properties of the extracts were comparable to that of the standard drug paracetamol. It was clearly understood from the study that the observed anti-pyretic effects of the extracts were similar in both magnitude and time course.

In this study, the extracts were observed to inhibit yeast induced pyrexia. The extracts may have reduced temperature of the test animals by decreasing brain concentration of prostaglandin E2 especially in the hypothalamus through its action on COX-3 [11] or by causing the enhancement of the production of the body's own antipyretic substances like vasopressin and arginine [12]. This effect must be due to the presence of the different phytochemical constituents in the extracts.

As mentioned earlier, in preliminary screening, the methanolic extracts of *M. angustifolia* revealed the presence of reducing sugar, alkaloids, glycoside, flavonoids, tannins, saponins and amides. In fact, flavonoid compounds were found to exert powerful antipyretic effects [13]. It has been reported that flavonoids inhibit production of prostaglandin which acts as the mediator of pyrexia [14]. Antipyretic activity of methanolic extracts of root and leaves of *M. angustifolia* may be attributed to the flavonoids identified in this extracts.

Table 1: Effect of methanolic extracts of root and leaves of *M. angustifolia* on brewer's yeast-induced pyrexia in Swiss albino mice

Groups	Oral dose	Rectal temperature in °F at different hours				
		-18 hr	0 hr	1 hr	2 hr	3 hr
Control (DDW)	10 mL/kg	98.28 ± 0.24	101.25 ± 0.15	101.18 ± 0.07	101.38 ± 0.47	101.55 ± 0.27
P'tamol	150 mg/kg	98.51 ± 0.15	101.38 ± 0.21	97.53 ± 0.29**	97.07 ± 0.23**	98.64 ± 0.18**
MERMA	500 mg/kg	98.77 ± 0.27	101.23 ± 0.35	99.33 ± 0.43**	98.10 ± 0.58**	98.23 ± 0.49**
MELMA	500 mg/kg	98.62 ± 0.43	101.30 ± 0.53	100.13 ± 0.25*	99.24 ± 0.16**	100.37 ± 0.32**

Values are expressed as mean ± SEM (n = 3); P'tamol= paracetamol; *p < 0.05, **p < 0.01

MERMA= methanol extract of root of *M. angustifolia*. MELMA= methanol extract of leaves of *M. angustifolia*

IV. CONCLUSION

The methanolic extracts of root and leaves of *M. angustifolia* showed statistically significant activity in antipyretic test. In conclusion, the results of this study verify the traditional use of the plant in the treatment of febrile condition. However, further investigation is necessary not only to isolate and characterize the active principle of the plant responsible for antipyretic activity, but also to elucidate the exact mechanisms of action.

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