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ANTI-INFLAMMATORY AND ANTI-PYRETIC ACTIVITIES OF THE HYDRO-METHANOL AND PETROLEUM-BENZENE EXTRACTS OF *MICROCOS PANICULATA* BARKS

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Abstract

Inflammation is an automated defensive mechanism in our body which is characterized by swelling, redness and heat formation at the site of tissue damage. And pyrexia is the elevation of body temperature either by inflammatory process or graft rejection of any kind of tissue damage. The aim of this study was to evaluate the anti-inflammatory and anti-pyretic activities of *Microcos paniculata* barks of hydro-methanol extract (HMBE) and petroleum-benzene (PBBE) extract. The anti-inflammatory and antipyretic activities of the extracts were evaluated through xylene-induced ear edema test, cotton pellet-induced granuloma formation in mice and Brewer's yeast induced pyrexia in mice. In the xylene-induced ear edema test, we observed that HMBE 400 mg/kg body weight showed highest (86.07±0.57 %) inhibition of ear edema which was significant also (*P<0.05, vs. control), whereas, in cotton pellet induced granuloma formation test, PBBE 400 mg/kg body weight showed highest (40.84±2.23%) inhibition of granuloma formation which was significant also (*P<0.05, vs. control). Again, post-treatment (up to 1 h) antipyretic activity was found by HMBE 400 and PBBE 200 mg/kg respectively. But PBBE 400 mg/kg showed that antipyretic activity up to 2 h. Taken together, the study results indicate that hydro-methanol and petroleum-benzene extracts of *Microcos paniculata* barks revealed both anti-inflammatory and anti-pyretic activities.

Keywords: *Anti-inflammatory; anti-pyretic; Microcos paniculata; barks.*

Introduction

Since the discovery of medicinal plants or herbs, they are regularly being used in folklore medicine to treat many communicable and non-communicable diseases. Plants synthesize hundreds and thousands of phytochemicals that are used for their defense mechanism acting against different kinds of insects, fungi, disease and herbivorous animals. All these phytochemicals are established with their potential biological activity and the search to find more novel phyto-constituent is still ongoing. Since a single plant contains hundreds of phytochemicals, it is uncertain to predict which activity can be obtained by evaluation of that plant. Still, the phytochemical constituent and biological activity of many plants is to be discovered, though rigorous scientific studies are being conducted to discover their safety and efficacy [1]. About 80% population of developing countries use folklore medicine till now due to their cultural and spiritual importance and this situation provides them an alternative for costly western medicine [2].

Inflammation is an automated defensive and healing mechanism, initiated by chemical mediators released by injured tissue and migrating cells, characterized by pain, heat, swelling, redness and disrupted cellular functions [3]. Though, non-steroidal anti-inflammatory drugs (NSAIDs) are generally used for the management of inflammation, but, they come with a wide variety of side effects [3]. However, drugs can be found from natural sources also which are able to influence the physiological pathways of inflammation [4].

Pyrexia or fever can be initiated by inflammation, graft rejection, malignancy or tissue damage and is characterized by elevation of body temperature. Pyrexia may increase the production of IL (interleukin), TNF-α (tumor necrosis factor-α), interferon and cytokines, which in turn stimulates PGE2 (prostaglandin E2 production) that activates hypothalamus to raise body temperature. Symptoms of fever include depression, inability to concentrate, lethargy, anorexia and sleepiness. These can be accompanied by increased muscle contraction and shivering [5]. The highly COX-2 (cyclooxygenase-2) selective antipyretic drugs irreversibly suppress PGE2 biosynthesis and lowers body temperature by interrupting the feedback loop of PGE2. This irreversible inhibition of COX-2 enzyme demonstrates toxic effect on different organ systems such as glomeruli, heart muscles, cortex of brain and hepatic cells. This problem can be solved by utilizing less selective COX-2 enzyme inhibitors derived from plants [6].

Kathgua or Fattashi is the local name of *Microcos paniculata* (family: Tiliaceae) in Bangladesh that are harvested all over the country. Generally it develops naturally as a shrub or short tree. This plant is known for many traditional uses, for example, to treat diarrhea, wounds, cold, fever, hepatitis, dyspepsia, and heat stroke. Moreover, it has insecticidal activity. However, it is active against the digestive system also. Thorough study of literature revealed that it showed several activities, including analgesic, antimicrobial, neuropharmacological, *α*-glucosidase inhibition, brine shrimp lethality, free radical scavenging, antipyretic, nicotinic receptor antagonistic, larvicidal, cytotoxic, insecticidal, anti-inflammatory, anti-nocieptive and antidiarrheal activities. In addition, it can prevent angina pectoris, coronary heart disease, or coronary artery disease or ischaemic heart disease. Acute toxicity study was also carried out [7-13].

Though anti-inflammatory and anti-pyretic activities [7] of the barks of this plant have already been found using methanol, but, this study has been conducted to evaluate the anti-inflammatory and anti-pyretic activities of this plant with different solvents such as hydro-methanol and petroleum-benzene so that we may ascertain which compounds may become prominent in this plant. And it may tend to initiate partitioning methods for

isolating the desired plant compounds to get expected effects.

Materials and Methods

Plant materials: collection and identification

M. paniculata barks were collected from the Jahangirnagar University campus, Savar, Dhaka, Bangladesh in November, 2013. Species identification was verified by Sarder Nasir Uddin, Principal Scientific Officer at the Bangladesh National Herbarium (accession number 35348). A dried specimen was deposited in the herbarium for future reference.

Extraction

For the extraction procedure, 275 g of powdered bark of M. paniculata was extracted using Hydro-methanol (mixture of 80% methanol and 20% water) and 150 g of powdered bark of M. paniculata was extracted with petroleum benzene. Freshly collected barks were rinsed 3-4 times successively with running water and once with sterile distilled water. Cleansed plant materials were then shade dried for a period of 7 days. The dried plant materials were then ground by using a laboratory grinding mill (MACSALAB 200 Cross Beater, Eriez, Erie, Pennsylvania, USA) and passed through a 40-mesh sieve to get fine powder. Powdered barks (275 g and 150 g) were separately dissolved in hydromethanol (2200 mL methanol and 550 mL water) and petroleum benzene (1500 mL) in closed containers and occasionally stirred for 15 days. Then extractions were completed by using rotary evaporator (RE601, Yamato Scientific America Inc., Santa Clara, California, USA) at a temperature of 40°C. Sterile cottons followed by Whatman No.1 filter papers were used to filter the liquid extracts. The filtrates were then dried in a hot air oven (BST/HAO-1127, Bionics Scientific Technologies Pvt. Ltd., Delhi, India) at 40°C. The extraction yields of HMBE and PBBE were 10.30% (w/w) and 1.39% (w/w), respectively. Both extracts were stored at 4°C for additional studies.

Experimental animals

One hundred and five *Swiss albino* mice of either sex, 6–7 weeks old, weighing 25–30 g (Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh) were used in these experiments. These animals were kept under standard environmental conditions, having relative humidity 55%–65%, 12 h light/12 h dark cycle and (27.0±1.0) °C temperature. Proper supply of foods and water *ad libitum* were ensured. Before the experiment, animals were adapted to the laboratory conditions for 1 week. The Institutional Animal Ethical Committee of Jessore University of Science & Technology, Jessore, Bangladesh approved the protocol used in the experiment conducted with these animals.

In-vivo anti-inflammatory study

Xylene-induced ear edema test

For xylene-induced ear edema test, the procedure performed by Dai *et al.* was followed [25]. Thirty mice were divided into six groups, each group containing five mice. The groups are- control group (normal distilled water), positive control or standard group (diclofenac sodium, DS, 100 mg/kg body weight), and test groups (HMBE & PBBE at 200 mg/kg and 400 mg/kg body weight). Mice in the control group, positive control group and test groups received one dose of normal water, diclofenac sodium, hydro-methanol and petroleum-benzene extracts of *M. paniculata* orally. One hour after the treatment, each animal received 20 µL of xylene on the anterior and posterior surfaces of the right ear lobe. The left ear was untreated, and used as a control. Mice were sacrificed 1 h after xylene application and 3 mm circular sections of the ears were taken and weighed. The weight of xylene-induced edema was considered as the difference between weight of ear treated with xylene (right ear) and the weight of ear without xylene treatment (left ear). The percentage inhibition of ear edema was calculated by the following formula.

Inhibition (%) = [1- Weight of edema (extract or standard drug)/ Weight of edema (normal control)] X 100

Cotton pellet-induced granuloma formation

To perform this test, method of Swingle and Shideman was followed [26]. Cotton pellets, weighing (10±1) mg each, were sterilized and inserted subcutaneously, one on each side of the abdomen of the animal, under light chloroform anesthesia and sterile technique. Mice of each group received treatment doses orally, as described above, once a day for 7 days. The mice were sacrificed on the 8th day. Cotton pellets were removed and dried at 60 ℃ for 24 h. The dry cotton weight was recorded. The weight difference between the removed, dried cotton pellets, and the cotton pellets before insertion was considered to be the weight of granuloma formed. The percentage inhibition of granuloma formation was calculated by the following formula.

Inhibition (%) = [1- Weight of granuloma (extract or standard drug)/ Weight of granuloma (normal control)] X 100

Brewer's yeast-induced pyrexia test

This test was performed with slight modification as described by Turner *et al* [27]. Mice were fasted overnight with water *ad libitum* before inducing pyrexia. Their rectal temperatures were recorded before inducing pyrexia by using an electric thermometer, which was connected with a probe and inserted 2 cm into the rectum. Subcutaneous injection of a 15% (w/v) suspension of brewer's yeast at a dose of 10 mL/kg in the back below the nape of the neck induced pyrexia. The suspension was spread under the skin by massaging the injection site. The increase in rectal temperature was recorded 18 h after injection and the mice that showed an increase in temperature of at least 0.6 ℃ were considered pyretic mice and used for brewer's yeast-induced pyrexia test. The tested samples including paracetamol (100 mg/kg) as standard, normal water as control and plant extracts as described before, which were given orally to the pyretic mice, were investigated for their antipyretic activity. All group's temperatures were recorded at 1, 2, 3 and 4 h.

Statistical analysis

All results are expressed as mean ± standard error (SE). All tests were analyzed statistically by one-way ANOVA followed by Dunnett's *t*-test. In addition, the results of antipyretic test were analyzed by using repeated measure ANOVA (RM-ANOVA). In case of all in vivo studies, pairwise comparison of means among the groups (except control) was done by one-way ANOVA followed by post hoc Tukey's HSD test. $*P < 0.05$ was considered to be statistically significant. All data were analyzed using SPSS software (version 16; IBM Corporation, New York, USA).

Results

Anti-inflammatory Tests

Xylene induced ear edema

Anti-inflammatory activity of HMBE and PBBE on topical xylene-induced ear edema in mice is shown in Table 1. The application of xylene to the mouse ear rapidly induced cutaneous inflammation. All of the groups had significant ear weight differences and inhibition of ear edema when compared to the control. Among the extracts, highest percentage inhibition of ear edema (86.07±0.57 %) was observed by HMBE at 400 mg/kg.

Cotton pellet-induced granuloma formation in mice

The results of the chronic inflammatory test with cotton pellet are displayed in Table 2. All of the groups had significant granuloma weight and percentage inhibition of granuloma formation in mice when compared to the control. Among the plant extracts, PBBE at a dose of 400 mg/kg showed highest percentage inhibition (40.84±2.23 %) of granuloma formation in mice.

Antipyretic study

Brewer's yeast-induced pyrexia test

Subcutaneous injection of yeast caused the elevation of rectal temperature after 18 h of its administration. 30 mice of all of the groups

showed a mean increase of 1.23 °C of rectal temperature 18 h after brewer's yeast injection. The antipyretic effect of the different doses of the extracts (200 and 400 mg/kg), standard (paracetamol, 100 mg/kg), and control are shown in Table 3. Moreover, post-treatment (up to 1 h) antipyretic activity was found by HMBE 400 and PBBE 200 mg/kg respectively. But, PBBE 400 mg/kg showed that antipyretic activity up to 2 h.

Discussion

Phospholipase A2, which is the precursor of all inflammatory activity, is inhibited by anti-inflammatory steroidal drugs and non-steroidal anti-inflammatory drugs. This action be evaluated by xylene induced ear edema test, as acute inflammation is initiated by xylene in this test, which initiates the release of inflammatory mediators such as bradykinin, histamine and serotonin. These agents stimulate ear edema by increasing vascular permeability and vasodilation [14]. The inhibition of fluid accumulation created by xylene at the treatment site is regarded as anti-inflammatory activity [15]. The significant inhibition of ear edema by HMBE (200 mg/kg and 400 mg/kg doses) and PBBE (200 mg/kg and 400 mg/kg doses) in table 1 may be due to the hindrance of phospholipase A2 and by decreasing vascular permeability and vasodilation. In this study, 400 mg/kg dose demonstrated better result than 200 mg/kg dose which is relevant to a previous study conducted by Aziz *et al* which also demonstrated better activity at 400 mg/kg dose [7]. But, more extended study is required at molecular phase to ensure the mechanism by which the plant extracts inhibited edema.

In the cotton pellet induced granuloma model (an in-vivo chronic inflammatory test), synthesis and release of inflammatory cells like neutrophils, macrophages and fibroblasts is responsible for granuloma formation [16]. In this test, implantation of cotton subcutaneously leads to the induction of a minimum of three phases which are denoted as transudative phase, exudative phase and proliferative phase. The implanted materials lead to host inflammatory response which finally results in tissue proliferation and granuloma formation [17, 18]. NSAIDs, such as diclofenac sodium, provide inhibition in the late phase, by inhibiting PG (prostaglandin) synthesis, whereas, steroidal anti-inflammtory drugs show strong inhibition in transudative and proliferative phases [16, 19]. Diclofenac sodium and the plant extracts reduced the weight of wet cotton pellet (Table 2), an indication of reduction in accumulation of exudates at the inflammatory site [20]. Therefore, the decrease in the weight of granuloma by the plant extracts may be due to the suppression of proliferative phase. Analysis of additional biochemical pathways may confirm the significant reduction of granuloma weight by the plant extracts.

Brewer's yeast is an exogenous pyrogen which initiates the synthesis and release of various endogenous cytokine factors like IL-1, IL-6, TNF-α [21, 22]. These endogenous cytokines activate the arachidonic acid pathway by crossing the blood brain barrier easily and acting on the preoptic/anterior hypothalamus which results in the synthesis and release of prostaglandins (PGE2) and set the thermoregulatory center at a higher temperature [23]. The effect of HMBE and PBBE in lowering the body temperature is similar to that of standard paracetamol in the observation time intervals (table 3). The extracts are likely to reduce pyrexia which may be by reducing the brain concentration of prostaglandin E2 especially in the hypothalamus through its action on cyclooxygenase enzyme or by enhancement of the production of the body's own antipyretic substances like vasopressin and arginine [24].

Conclusion

From the existing study, it could be suggested that the hydro-methanolic and petroleum-benzene extracts of *Microcos paniculata* barks might possess anti-inflammatory and antipyretic activities. Nevertheless, further

quantitative chemical studies are now under way to isolate and determine the structure of the active constituents. Similarly, we are pursuing biological testing of the specific compounds thought to be responsible for anti-inflammatory and antipyretic activities presented in the barks extracts of *M. paniculata*.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1. Xylene induced ear edema results of hydro-methanolic and petroleum-benzene extract of *Microcos paniculata* barks.

Group	Dose	Ear weight differences (mg)	Inhibition (%)
Control	10 mL/kg	6.80±0.34	0.00
Standard (DS)	100 mg/kg	3.56±0.16*	46.88±4.39*
HMBE	200 mg/kg	1.46±0.03*△	78.35±0.92*△
HMBE	400 mg/kg	0.94±0.02*△	86.07±0.57*△
PBBE	200 mg/kg	3.13±0.20*▲□■	53.63±2.93*▲□■
PBBE	400 mg/kg	1.30±0.15*△	80.49±2.97*△

Values of ear weight differences are present as mean ± standard error of mean. *n*=5 mice in each group. *P<0.05, *vs* control (Dunnett's *t* test); △P<0.05, vs Standard (DS); ▲P<0.05, vs HMBE 200 mg/kg; □P<0.05, vs HMBE 400 mg/kg; ■P<0.05, vs PBBE 400 mg/kg (pair-wise comparison by post-hoc Tukey's HSD test). DS: diclofenac sodium; HMBE: Hydro-methanol extract of *Microcos paniculata* barks; PBBE: Petroleum-benzene extract of *Microcos paniculata* barks.

Table 2. Effect of standard and different extract of *M. paniculata* barks on cotton pellet induced granuloma formation test.

Group	Dose	Granuloma weight (mg/mg cotton)	Inhibition (%)
Control	10 mL/kg	33.66± 0.43	0.00
Standard (DS)	100 mg/kg	10.46± 0.23*	68.80±0.75*
HMBE	200 mg/kg	24.43±0.43*△	27.36±0.66*△
HMBE	400 mg/kg	23.17± 0.35*△	31.09±0.38*△
PBBE	200 mg/kg	26.45±0.31*△▲□	21.35±2.58*△□
PBBE	400 mg/kg	19.88±0.56*△▲□■	40.84±2.23*△▲□■

Weights of cotton pellets are present as mean ± standard error of mean. *n*=5 mice in each group. *P<0.05, *vs* control (Dunnett's *t* test); △P<0.05, vs Standard (DS); ▲P<0.05, vs HMBE 200 mg/kg; □P<0.05, vs HMBE 400 mg/kg; ■P<0.05, vs PBBE 200 mg/kg (pair-wise comparison by post-hoc Tukey's HSD test). DS: diclofenac sodium; HMBE: Hydro-methanol extract of *Microcos paniculata* barks; PBBE: Petroleum-benzene extract of *Microcos paniculata* barks.

Table 3: Antipyretic effect of various extracts of *Microcos paniculata* barks

Group	Dose	Initial rectal temperature	Rectal temperature after 18 h of yeast injection				
			0 h	1 h	2 h	3 h	4 h
Control	10 mL/kg	37.21±.49	38.34±.17	38.26±.16	38.57±.15	38.09±.53	38.20±22
Paracetamol	100 mg/kg	37.10±.24	38.81±.11	38.33±.31	38.24±.29	38.25±.33	38.43±.21
HMBE	200 mg/kg	37.22±.45	38.43±.14	38.53±.16	38.84±.16	38.65±.22	38.70±.14
HMBE	400 mg/kg	36.68±.17	38.12±.28	37.41±.90	38.12±.51	38.28±.37	37.66±.47
PBBE	200 mg/kg	37.48±.38	38.38±.33	36.66±.34	37.62±.39	37.42±.65	38.19±.25
PBBE	400 mg/kg	37.48±.65	38.44±.53	37.32±.44	37.25±.66	37.48±.49	37.66±.63

Rectal temperature values (°C) are present as mean ± standard error of mean. *n*=5 mice in each group. Tests of within subjects effects reveal that for the factor 'Temparature' calculated F= 18.642 for all methods and P value = 0.000 in every case. So time is highly significant at any level of significance. *P<0.05, vs control. Repeated measure analysis of variance with Dunnett's multiple comparison was performed to analyze this data set. DS: diclofenac sodium; HMBE: Hydro-methanol extract of *Microcos paniculata* barks; PBBE: Petroleum-benzene extract of *Microcos paniculata* barks.