Section A-Research Paper
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A Comparative Study of *Myrica Esculenta* and Brahmic Acid for Anti-Asthmatic Potential in Anaphylactic Micro Shock Model of Guinea Pig

Neeraj Kumar[1], Nausheen Parveen[2], Bhakti Sudha Pandey[3], Vasim Mansur Aparadh[4], Prateek Kumar Tiwari[5*]

[1]*Galgotias College of Pharmacy, Greater Noida, Knowledge Park II-201310*

[2,3,5]*IIMT College of Pharmacy, Greater Noida, Knowledge Park II-201310*

[4]*Appasheb Birnale college of Pharmacy Sangali Maharashtra*

***Corresponding Author:** Prateek Kumar Tiwari
i.prateektiwari@gmail.com
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Abstract

An estimated 262 million people worldwide were diagnosed with asthma this year, and the disease will be responsible for 455,000 fatalities this year. The current study was based on the evaluation of anti-asthmatic potential of *Myrica esculenta* and Brahmic acid through anaphylactic micro shock model in guinea pig. It also estimated the levels of COX and LOX enzymes. Budesonide was obtained from Farmabios as gift sample. ELISA Kit, (Cusabio Biotech Co. China) and Brahmic acid was purchased from Sigma Aldrich, Mumbai, India. The plant *Myrica esculenta* was obtained from the region of UP West, India and was authenticated by the botanist at National Botanical Research Institute Lucknow with specimen no. NBRI/2022/02/1029. Male Hartley Guinea pigs were obtained from the IVRI Bareilly, India. Animals were divided into 5 groups i.e., group 1 administered only normal saline, group 2 administered 0.8ml crystalline egg albumin solution (5%), group 3 administered Budesonide (50mg/kg, i. p.) + 0.8ml crystalline egg albumin solution (5%), group 4 administered ethanolic extract of *Myrica esculenta* (120mg/kg, orally) + 0.8ml crystalline egg albumin solution (5%), for 14 days and group 5 administered Brahmic acid (100mg/kg, orally) + 0.8ml crystalline egg albumin solution (5%), for 14 days. Anaphylactic micro shock method was used as screening of anti-asthmatic potential. LOX and COX enzyme levels were also estimated. In results, it significantly modulated/ decreased the levels of COX (cyclooxygenase) enzyme and LOX (lipoxygenase) enzyme thus reduced the breathing difficulty in guinea pig as both facilitates the production of autocoids or inflammatory mediators including PGD2, PGE2, PGI1α, TxA2, LT-4

and LT-13. In conclusion, both *Myrica esculenta* and Brahmic acid are significantly effective as anti-asthmatic molecules when observed in guinea pig models. This study suggests, to identify and structure-elucidate of the responsible moiety of *Myrica esculenta* for its anti-asthmatic potential. It might be incorporated into suitable dosage form with better bioavailability and feasible drug delivery.

Keywords: *Myrica esculenta,* anti-asthmatic, anaphylactic micro shock, Brahmic acid.

Introduction

Medicinal plants are an important source of pharmacologically active chemicals and have been utilised as a medicinal treatment for a variety of ailments since antiquity. Medicinal herbs are the foundation of traditional medicine; over 3.3 billion people in developing countries still use them on a regular basis (Davidson-Hunt, 2000). *Myrica esculenta* belonging to Scrophulariaceae family (Nicolson & Stewart, 1974) is an herbaceous plant found in Indo-moist Pakistan's and marshy environments (D., 2017). It is used in folk medicine to treat a variety of diseases such as bronchitis, asthma, inflammation, and nervine issues (Biswas et al., 2012). The plant has been shown to have a wide range of pharmacological properties, including antioxidant (Channa et al., 2006), anti-inflammatory (Bhattacharya & Ghosal, 1998), anxiolytic (Sumathi & Nongbri, 2008), hepatoprotective, and depressive activities (Sairam et al., 2002).

The triterpenoid saponins in *Myrica esculenta* are thought to be responsible for the plant extract's medicinal properties. In the family of triterpenoid saponins, bacosides take centre stage. Evidence suggests that they improve the transmission of nerve impulses. Bacosides increase neuronal synthesis and kinase activity, both of which contribute to the healing of injured neurons. Recovery of synapse function, and hence the ability to transmit nerve impulses, is another benefit of the bacosides (K. G. Patel et al., 2010). The proper flow of nerve impulses plays a key role in maintaining normal levels of concentration, memory, learning, and other cognitive abilities. There is evidence that the active ingredients of Myrica esculenta, such as bacosides, affect Serotonin production and availability, and that Myrica esculenta helps maintain neurotransmitter balance (K. Rana & K. Patel, 2016), (T. Patel et al., 2011).

Brahmic (madecassic) acid is a pentacyclic triterpenoid with hydroxyl groups in positions 2, 3, 6,

and 23; the ursane is hydroxylated at position 28. It is a metabolite of plants and plays a part in antioxidant defence. Pentacyclic triterpenoid, tetrol, and monocarboxylic acid; these are its chemical classifications. Originating with an ursane hydrid. Madecassic acid occurs in nature as a component of *Siphoneugena densiflora, Centella erecta, Myrica esculenta* and *Centella asiatica* (Panthari et al., 2013).

An estimated 262 million people worldwide were diagnosed with asthma this year, and the disease will be responsible for 455,000 fatalities this year (Andrade et al., 2022). Poyil et al. (2022) exhibited that the total leukocyte, eosinophil, lymphocyte, neutrophil, and monocyte counts were lower in the animals treated with EHC. In addition to boosting intracellular glutathione (GSH) levels and decreasing malondialdehyde and bicarbonate levels, which indicate a diminished oxidative burden, EHC also enhanced antioxidant activity. In contrast to the sensitised group, the EHC-treated group had less infiltration by inflammatory cells. The EHC treatment group showed a statistically significant decrease in asthma symptoms (Muni Raja Lakshmi et al., 2021).

The current study was based on the evaluation of anti-asthmatic potential of *Myrica esculenta* and Brahmic acid through anaphylactic micro shock model in guinea pig.

Materials and methods

Drugs and instruments

Budesonide was obtained from Farmabios as gift sample, egg albumin, ethanolic extract of *Myrica esculenta*, brahmic acid, ethanol, distilled water, phosphate buffer solution, indomethacin, ultra-centrifuge, ELISA Kit, (Cusabio Biotech Co. China) and Brahmic acid was purchased from Sigma Aldrich of laboratory grade and rotatory evaporator.

Collection and authentication of plant

The plant *Myrica esculenta* was obtained from the region of UP West, India and was authenticated by the botanist at NBRI Lucknow (Botanical Survey of India, Allahabad) with specimen no. NBRI/2022/02/1029. The leaves are washed making dust-free and dried at room temperature or shade. The flowers & leaves were rendered into coarse powders and then finally into fine particles. The powder was weighed and extracted through maceration process using ethanol (Khan et al., 2020).

Preparation of animals

Male Hartley Guinea pigs were obtained from the IVRI Bareilly, India. With room temperatures of 25°C and a 12-hour light/dark cycle, the animals are kept in good health. The animals were fed a typical rodent diet and have unrestricted access to water, and the relative humidity is controlled between 44 & 56 percent(Mani et al., 2022).

Acute Oral Toxicity

As preparation for the experiment, the animals fasted for a full 24 hours. The ethanolic extract and isolated compound were orally administered to groups of guinea pigs at a range of doses (50-3000mg/kg). In accordance with OECD guidelines 425, the animals were monitored continuously for 1 hour, then at 30-minute intervals for 4 hours for any obvious changes in behavior and finally for up to 24 hours for any deaths (Bhajoni et al., 2016).

Group design

All the guinea pigs were divided into 5 groups (n=6) as followings-

Group 1 administered only 1% CMC (2ml) once a day.

Group 2 administered 0.8ml crystalline egg albumin solution (5%) once a day for 14 days.

Group 3 administered Budesonide (50mg/kg, i. p.) once a day for 14 days.

Group 4 administered ethanolic extract of *Myrica esculenta* (120mg/kg, orally) once a day for 14 days

Group 5 administered Brahmic acid (100mg/kg, orally) once a day for 14 days.

Male Hartley guinea pigs were exposed to aerosolized 5% ovalbumin solution for 3 minutes on days 0-7 and 14 to create an allergic airway inflammation model. There are two sensitization days (days 0 and 7), followed by a challenge day (day 14). The lungs of the test subjects, the control subjects, the negative control subjects, and the animals that were injected intravenously with budesonide were all collected (Swathi et al., 2013).

Methods

Anaphylactic micro shock model

Intramuscular injections of 0.7-0.8ml of a 5% crystalline egg albumin solution were used to sensitise the animals. The time before the animals began convulsing after being exposed to an aerosolized antigen was measured 14 days after the initial exposure. Since the pre-convulsion time may be very brief (≥40 sec.), and the first signs of dyspnoea may be followed rapidly by convulsions and death, half the usual antigen concentration was used at the first exposure. Shorter intervals of 2-3 hours or longer durations of 24 hours were used for subsequent

exposures. These factors contributed to an elongation of the period before a patient experienced their first convulsive episode. Next exposure occurred 3-4 days after the pre-convulsion time had increased to 80-120 seconds. As time went on, it stayed about the same in some animals while rising in others. The latter group of animals had their interval lengthened to 6-7 days, and this was found to be optimal for achieving and sustaining a constant pre-convulsion period. Once the necessary number of exposures had been repeated at the appropriate intervals, the animals were grouped together at the same intervals, and the drug experiments could begin. For every session under the influence of a drug, there was a corresponding session without the drug to serve as a control. It was compared to the average pre-convulsion period prior to and following drug administration (ARMITAGE et al., 1952).

Estimation of COX level

From all the group of animals, lung tissue was harvested, sliced, and placed in a 6-well plate with RPMI 1640 medium. Lung slices were taken from animals 6 hours after experimental manipulation, spiked with prewarmed medium containing A23187 (final concentration 10M), and incubated for 30 minutes at 37°C to determine stimulated prostanoid release. Lung slices were taken 0.5h after treatment with oval albumin, pre-incubated in RPMI 1640 with arachidonic acid (50M) for 1.5h, and then incubated for 0.25 to 22h in arachidonic acid-free medium to determine the amount of prostanoid released spontaneously. Using an enzyme-linked immunosorbent assay, we measured the supernatant levels of PGD2, PGE2, 6-keto PGF1 (a stable metabolite of prostacyclin), and TxB2 (Oguma et al., 2002).

Estimation of LOX level

It was decided to remove the right upper lobe of the lung and store it in cold storage. All subsequent procedures were carried out between four and zero degrees Celsius. Preparation of frozen guinea pig lung samples for LOX activity was described in detail previously (Hudkova et al., 2021). To determine IL-13 concentration, a frozen lung section was homogenised in liquid nitrogen, then extracted with PBS and centrifuged at 5000g for 5 minutes. Liquid nitrogen was used to freeze the right lower lobe of the lung to analyse its free radical content (FR). Histological examinations were conducted on the left lung after it had been preserved in formalin solution, paraffin-embedded, and frozen. Since alterations in the lung's periphery play a role in the pathogenesis of asthma, it was important to examine both the proximal and distal regions. The concentrations of IL-4 and IL-13 in plasma and lung tissue samples were determined using

commercial enzyme-linked immunosorbent assay kits according to the manufacturer's instructions. Microplate reader Quant absorbance spectra were measured spectrophotometrically between 380 and 600nm with a 5 nm resolution step. With 570nm as the standard, we decided to set the maximum absorbance at 450nm. The levels of IL-4 and IL-13 in plasma and lung tissue were reported in picograms per millilitre. The cut-off points for detecting IL-4/13 were established at 0.78 pg/ml (Farah et al., 2012), (Metzke et al., 2011).

The LT pathway enzyme 5-LOX is a dioxygenase that uses a non-heme iron atom (Smith & Murphy, 2015). It incorporates a molecular oxygen into arachidonic acid (AA) and catalyses the formation of 5(S)-hydroperoxyeicosatetraenoic acid (5-HPETE), which is then dehydrated to leukotriene A4. Asthma and other inflammatory disorders like hay fever and rheumatoid arthritis are believed to have their root cause in 5-LOX (Rådmark et al., 2007), (Rubin & Mollison, 2007).

Results and discussion

Anaphylactic micro shock model

Brahmic acid ethanolic extract of *myrica esculenta* exhibited the anti-asthmatic activity when compared with control group of guinea pig. Pre-convulsion was observed at day 0 and day 14 and thus mean % protection was calculated using following formula-

$$\text{Percentage protection} = (1 - C/T) \times 100$$

The pre-convulsion time was estimated as 367.18±0.24 sec, 354.48±0.62 sec in group 4, and group 5 respectively, at day 14 while, it was estimated as 127.24±0.52 sec in group 2. Thus, it might be seen for its significant differences in pre-convulsion time in test groups of both Brahmic acid and ethanolic extract of *myrica esculenta* that indicate for their anti-asthmatic potential. In the same order, mean % protection was found optimum in std. group and marked in test group when compared with vehicle and negative control group.

The effects are enumerated in below table-

Table 1. Pre-convulsion time and mean % protection of control, std. and ethanolic extract of *Myrica esculenta* and Brahmic acid in anaphylactic micro shock model in guinea pig

Group/ Treatment	**Pre-convulsion time (Sec)**		**Mean % Protection**
	Day 0	**Day 14**	
Group 1 (Vehicle Control)	83.74±0.62*	96.52±0.31*	13.24±0.41*
Group 2 (0.8ml crystalline egg albumin solution (5%)	109.21±0.51**	127.24±0.52**	14.17±0.18*
Group 3 (Budesonide (50mg/kg, i. p.) + 0.8ml crystalline egg albumin solution (5%)	209.64±0.57*	457.32±0.37*	54.40±0.37**
Group 4 (Ethanolic extract of *Myrica esculenta* (120mg/kg, orally) + 0.8ml crystalline egg albumin solution (5%)	214.53±0.58**	367.18±0.24***	41.57±0.56***
Group 5 (Brahmic acid (100mg/kg, orally) + 0.8ml crystalline egg albumin solution (5%)	227.67±0.28***	354.48±0.62***	35.77±0.17***

P< 0.01 compared to the standard drug are significant, and data were given in mean ± SEM of % protection and analyzed by ANOVA.

Estimation of COX level

In this parameter, all the inflammatory mediators i.e., PGD2, PGE2, PGF1α and TxB2 were found reduced in guinea pig when estimated. Group 4 and 5 demonstrated the level of PGD2 as 18.03±0.05 and 19.09±0.08, respectively while it was estimated as 41.01±0.05 in negative control. Thus, it might be understood that PGD2 as potent inflammatory mediator and precursor of asthma got reduced significantly.

Similarly, the levels of PGE2, PGF1α and TxB2 were found declined that confirmed for the anti-asthmatic potential of Brahmic and Brahmic acid when separately administered.

Table 2. Estimation of COX (PGD2, PGE2, PGF1α and TxB2) levels of control, std. and ethanolic extract of *Myrica esculenta* and Brahmic acid in guinea pig

Group/ Treatment	PGD2	PGE2	PGF1α	TxB2
Group 1 (Vehicle Control)	37.04±0.02*	2.72±0.04*	19.31±0.05*	0.34±0.03*
Group 2 (0.8ml crystalline egg albumin solution (5%)	41.01±0.05*	3.61±0.02*	17.20±0.06	0.39±0.02*
Group 3 (Budesonide (50mg/kg, i. p.) + 0.8ml crystalline egg albumin solution (5%)	13.07±0.02**	0.53±0.07**	7.31±0.03	0.09±0.07**
Group 4 (Ethanolic extract of *Myrica esculenta* (120mg/kg, orally) + 0.8ml crystalline egg albumin solution (5%)	18.03±0.05*	1.36±0.04*	11.43±0.06**	0.14±0.06**
Group 5 (Brahmic acid (100mg/kg, orally) + 0.8ml crystalline egg albumin solution (5%)	19.09±0.08**	1.26±0.02**	12.05±0.03***	0.16±0.07**

P< 0.01 compared to the standard drug are significant, and data were given in mean ± SEM of % protection and analyzed by ANOVA.

Estimation of LOX

Interleukins are inflammatory mediators synthesized under the influence of LOX enzyme. IL-4 and IL-13 were estimated to confirm the anti-asthmatic activity of *myrica esculenta* and Brahmic acid in guinea pig.

The IL-4 was estimated as 1.40±0.01 and 1.54±0.05 in group 4 (treated with ethanolic extract of *Myrica esculenta* (120mg/kg, orally) + 0.8ml crystalline egg albumin solution (5%) and group 5

(treated with Group 5 (Brahmic acid (100mg/kg, orally) + 0.8ml crystalline egg albumin solution (5%) and IL-13 was estimated as 1.57±0.06 and 1.69±0.03 in group 4 and group 5, respectively. Whereas, negative group was demonstrated IL-4 and IL-13 levels as 2.13±0.04 and 2.61±0.03, respectively.

Table 3. Estimation of LOX (IL-4 and IL-13) levels of control, std. and ethanolic extract of *Myrica esculenta* and Brahmic acid in guinea pig

Group/ Treatment	**IL-4**	**IL-13**
Group 1 (Vehicle Control)	0.91±0.06**	0.97±0.02***
Group 2 (0.8ml crystalline egg albumin solution (5%)	2.13±0.04***	2.61±0.03**
Group 3 (Budesonide (50mg/kg, i. p.) + 0.8ml crystalline egg albumin solution (5%)	1.16±0.03**	1.31±0.01***
Group 4 (Ethanolic extract of *Myrica esculenta* (120mg/kg, orally) + 0.8ml crystalline egg albumin solution (5%)	1.40±0.01***	1.57±0.06***
Group 5 (Brahmic acid (100mg/kg, orally) + 0.8ml crystalline egg albumin solution (5%)	1.54±0.05***	1.69±0.03***

P< 0.01 compared to the standard drug are significant, and data were given in mean ± SEM of % protection and analyzed by ANOVA.

In the early (provocation) and late (effector) stages of asthma, airway inflammation is caused by Th2-generated cytokines, IL-4 and IL-13 (Vacca & Le Gros, 2022). In addition, the symptoms of atopic asthma include a rise in FR, iNOS content in the lungs, and NO-producing cells in BAL of asthmatic animals, all of which are indicators of oxidative/nitrosative stress (Prado et al., 2011),

(Cho & Moon, 2010). Together, these findings suggest the emergence of acute respiratory hypersensitivity, and the data from histology and morphometry confirm the occurrence of inflammation and fibrosis in the lung tissue of animals with BA (Sánchez-Jiménez et al., 2013). Because BA triggers the release of pro-inflammatory mediators, lung epithelial and endothelial cells become dysfunctional and eventually apoptosis, taking on the phenotype of mesenchymal cells in the process (myofibroblasts and fibroblasts). Collagen and elastin are two examples of ECM components that are secreted in large amounts by actively proliferating fibroblasts. Fibrosis foci develop as a result of LOX-mediated intermolecular cross-linking between these molecules. That is why it is clear that BA's pulmonary fibrosis is linked to elevated LOX activity (Zibadi et al., 2009), (Li et al., 2012).

In anaphylactic micro shock model, at 14^{th} day of observation pre-convulsion time was found statistically significantly increased in ethanolic extract of *Myrica esculenta* and Brahmic acid treated guinea pig. The anti-asthmatic effect of Brahmic acid extract was almost near to standard group when compared with *Myrica esculenta*. Thus, Brahmic acid was considered as much potent than *Myrica esculenta*. It might be due to reduction in sudden precipitation of inflammatory mediators that leads to constriction of bronchi. It also significantly modulated/ decreased the levels of COX (cyclooxygenase) enzyme and LOX (lipoxygenase) enzyme thus reduced the breathing difficulty in guinea pig as both facilitates the production of autocoids or inflammatory mediators including PGD2, PGE2, PGI1α, TxA2, LT-4 and LT-13.

Conclusion

In conclusion, both *Myrica esculenta* and Brahmic acid are significantly effective as anti-asthmatic molecules when observed in guinea pig models. After successful clinical experiments for safety and efficacy, they might be given in the management of asthma among human beings. This study suggests, to identify and structure-elucidate of the responsible moiety of *Myrica esculenta* for its anti-asthmatic potential. It might be incorporated into suitable dosage form with better bioavailability and feasible drug delivery.

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Conflict of Interest

Authors have declared for none conflict of interest.

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