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Research Article

STUDY OF PIPERIC ACID FOR ANTIASTHMATIC ACTIVITY IN GUINEA PIGS

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ABSTRACT

Piperic acid an aromatic acid, usually a metabolite of piperine exists naturally in piperaceae and amaranthaceae families. The synthetic derivatives of pipericacid act as promising bioactive molecules. They are anti- oxidant and anti-inflammatory agents. Antihistaminic and anticholinergic studies are used as a part of antiasthmatic study. In the current study antihistaminic and anticholinergic studies were carried out using guinea pig bronchi and ilei in naturally isolated compound piperic acid from the acetone flower extracts of the plant Gomphrena serrata. The results indicate that the compound (2 mg/kg 10.89 ± 2.01*** at $p<0.001$) showed profound anticholinergic activity significantly in acetylcholine induced bronchospasm model compared to standard drug atropinesulphate (2 mg/kg 11.60±1.24). The compound can be further studied for antiasthmatic activity by various other ways to establish its mechanism of action as well as drug development studies to render it a novel antiasthmatic drug.

KEY WORDS: Antiasthmatic, bronchospasm, anticholinergic, isolated.

INTRODUCTION

Bronchospasm, a pulmonary disease caused by constriction of lung muscles usually found associated with respiratory conditions such as asthma or irritants. It makes difficulty in breathing which can be mild to severe (Broide 2001). Presently, no full cure is available, but management methods can help withstand the disease. The drugs currently available show symptomatic and poor response with few side effects. Use of natural drugs is still common and wide spread all over the world. The pathogenesis of asthma is complex, and the use of herbals is also complicated with respect to their role and effective targets. Hence, separation of the effective constituents from herbals and studying their efficiency forms the general basic approach for asthma

study (Ward, et al., 2016). Piperic acid and its derivatives form the innovative source of drugs. It is an organic aromatic acid and a metabolite of piperine, found usually in piperaceae family. It has not gained considerable attention with respect to its therapeutic utility. It was reported for antinociceptive, anti-inflammatory (Oliveira, et al., 2018), antioxidant, antimicrobial (Zarai, et al., 2013) and lipoxygenase inhibitor (Tomy, et al., 2015). In the current study it was assessed for its efficacy in bronchopasmolytic activity by histamine and acetylcholine induced bronchospam and ileum contraction models in guinea pigs. The collected piperic acid was obtained by hydrolysis of piperine isolated from the flower acetone extracts of *Gomphrena serrata* L. belonging to the family Amaranthaceae.

MATERIALS AND METHODS

Chemicals

The compound piperic acid (Fig. 1), obtained from piperine isolated from the plant *G.serrata* was collected from the department of Pharmacognosy, University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Nagarjunanagar, Guntur. The other chemicals used in the study were supplied from MERCK, India. All the chemicals and reagents used were of analytical grade.

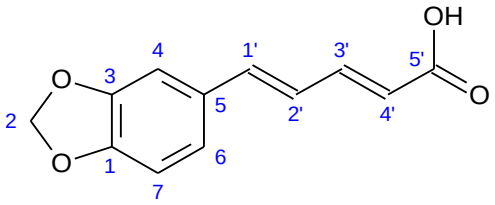

Fig. 1: Structure of Piperic acid

Experimental animals

Guinea pigs (400–600 g) of either sex for carrying out antiasthmatic activity were purchased from Mahaveer enterprises, Hyderabad, Telangana, India, housed in standard conditions of temperature (22 ± 2°C), relative humidity (55 ± 5%), and light (12 h light/dark cycles). They were fed with standard pellet diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Nirmala College of Pharmacy, Atmakur, Mangalagiri, Guntur district, Andhra Pradesh, India, approval no 012/IAEC/NCPA/PhD/2016-17, nominated by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Department of Animal Welfare, Government of India. The animal ethics committee approval certificate had been enclosed.

Acute toxicity testing

The animals were overnight fasted prior to the experiment. Different doses (50–3000 mg/kg, orally) of the isolated compound were administered to groups of guinea pigs. The animals were observed continuously for 1 hr, next half-hourly intervals for 4 hrs for any gross changes in their behavior and then up to 24 hrs for any mortality as per the Organization for Economic Co-Operation and Development (OECD) guidelines 425 (OECD 2008).

Antiasthmatic studies by histamine and acetyl choline induced bronchospasm in guinea pigs

Guinea pigs of either sex of body weight 200–500 g were divided into three groups separately to carry out histamine and acetylcholine induced bronchospasm model studies. Each group comprised of four animals. Animals were exposed to 0.1% w/v of histamine dihydrochloride aerosol in a histamine

chamber (Sigma Scientific). They were also exposed to 0.5% acetylcholine chloride aerosol separately. Progressive dyspnoea was observed in animals when exposed to both the aerosols. Pre convulsion time (PCT) was determined from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsions on day 0 (T_1). As soon as dyspnoea commenced, the animals were removed from the chamber and placed in fresh air. Test group animals were given isolated compound at a dose of 2 mg/Kg orally (*p.o.*) once a day for 7 days. On the seventh day, 2 hrs after the last dose, PCT was recorded (T_2) **(**Chandrakant et al., 2011).

The percentage increase in time of PCT was calculated using the following formula: Percentage increase in $PCT = \left(1 - \frac{T1}{T2}\right) \times 100$

Where T_1 is PCT on day 0 and T_2 is PCT on day 7.

Histamine- induced bronchospasm

Group-1: Control group animals received distilled water

Group-2: Standard group animals received chlorpheniraminemaleate

Group-3: Test group animals received piperic acid isolated (GSC) (Table 1).

Acetylcholine- induced bronchospasm

Group-1: Control group animals received distilled water

Group-2: Standard group animals received atropine sulphate

Group-3: Test group animals received piperic acid isolated (GSC) (Table 2).

Statistical analysis

The experimental data was expresed as a mean ± Standard error of the mean (SEM) and analyzed statistically using One-way analysis of variance, followed by Tukey test, compared with control to find out the level of significance.

Antiasthmatic studies by histamine and acetylcholine induced ileum contaction

Guinea pigs of body weight 200–500 g were selected, divided. They were allowed to starve overnight with free access to water. The animals were killed by a blow on the head and exsanguinated. The isolated ileum was mounted in a 30 ml Organ bath (Lab Tree India) containing a tyrode solution, maintained at 37 ± 1 ^{0}C, and gassed with air. The tissue was equilibrated for 45 min during which the bath solution was replaced every 10 min. A drug tissue contact time of 1 min was maintained and 15 min time cycle was followed by recording the response of histamine. After obtaining a dose response curve of histamine and acetyl choline, the isolated compound (0.5 mg) was added to the reservoir seperately. Histamine (0.5 mg) and acetyl choline (0.1 mg) were given repeatedly in presences of isolated compound. The isolated ileum was cut into individual sections of 1cm, and divided into into three groups; each group consisted of four ileums (Chandrakant et al., 2011).

Histamine- induced guinea pig ileum contraction

Group 1: Control group animals received histamine

Group 2: Standard group animals received chlorpheniramine

Group-3: Test group animals received piperic acid isolated (GSC) (Table 3).

Acetylcholine- induced guinea pig ileum contraction

Group 1: Control group animals received acetylcholine

Group 2: Standard group animals received atropine sulphate

Group-3: Test group animals received piperic acid isolated (GSC) (Table 4)

Statistical analysis

The results of the study were expressed as mean ± SEM and analyzed statistically using One-way Analysis of Variance (ANOVA) followed by Dunnett's test for individual comparison of groups with control. Data were considered statistically significant at *P<0.05 and **P<0.01 respectively.

RESULTS AND DISCUSSION

The results of acute toxicity study revealed that isolated compound was safe up to 50-3000 mg/Kg body weight when administered orally in guinea pigs. After 24 hrs, the animals were found to be well tolerated, safe with no signs of mortality and toxicity. Hence a safe and therapeutically effective dose of 2 mg/Kg body weight for carrying out antiasthma tic studies. Piperic acid offered protection against bronchospasm induced by histamine as compared to control. The time of onset of PCT was significantly increased following exposure to histamine (*** p<0.001) aerosol-induced bronchospasm in guinea pigs (Tab 1). The standard drug chlorpheniramine exhibited PCT 8.77 significantly (*p<0.05). The compound piperic acid (GSC) showed increase in PCT 9.81 (*** p<0.001) at 2 mg/Kg body weight than standard drug chlorpheniramine (Table 1). The compound exerted potential antihistaminic bronchospasmolytic activity compared to standard. The compound significantly increased PCT 10.89 (*** p<0.001) following exposure to acetylcholine aerosol-induced bronchospasm in guinea pigs (Table 2) when compared to control. The compound showed anticholinergic bronchospasmolytic activity equivalent to standard atropine sulphate.

Table 1: Effect of histamine-induced bronchospasm

S. No.	Groups	Drug and Dose	PCT Mean ± SEM
1	Control	Distilled water p.o.	2.22±0.24
2	Standard	Chlorpheniramine 2mg/Kg p.o.	8.77±0.43*
3	Test-3	GSC 2mg/Kg p.o.	9.81± 2.08***

Each value was expressed as mean±SEM, where n=4 in each group;*p<0.05, ** p<0.01, *** p<0.001compared to control by one-way ANOVA, Tukeys test.

Table 2: Effect of acetylcholine-induced bronchospasm

S. No.	Groups	Drug and Dose	PCT Mean ± SEM
1	Control	Distilled water p.o.	3.22±0.60
2	Standard	Atropine sulphate 2mg/Kg p.o.	11.60±1.24
3	Test-2	GSC 2mg/Kg p.o.	10.89 ± 2.01***

Each value was expressed as mean±SEM, where n=4 in each group; *p<0.05, ** p<0.01, *** p<0.001 compared to control by one-way ANOVA, Tukey's test

Table 3: Effect of histamine-induced guinea pig ileum contraction

S. No.	Groups	Drug and Dose	Response Mean ± SEM	% Inhibition
1	Control	Histamine 0.5mg	4.9±0.08	0%
2	Standard	Chlorpheniramine 0.5mg	1.8±0.91	63.3%
3	Test-3	GSC 0.5 mg	3.6 ± 0.32*	53.3%

Each value was expressed as mean±SEM, where n=4 in each group at *p<0.05, ** p<0.01, *** p<0.001 compared to control by one-way ANOVA, Dunnett's test.

Table 4: Effect of acetylcholine-induced guinea pig ileum contraction

S. No.	Groups	Drug and Dose	Response Mean ± SEM	% Inhibition
1	Control	Acetylcholine 0.1mg	5.5±0.27	0%
2	Standard	Atropine sulphate 0.5mg	2.2±0.81	60%
3	Test-1	GSC 0.5 mg	2.9 ± 0.25**	53.7 %

Each value was expressed as mean±SEM, where n=4 in each group at *p<0.05, ** p<0.01, *** p<0.001 compared to control by one-way ANOVA, Dunnett's test.

The compound inhibited (53.3% at *p<0.05) the contraction induced by histamine in isolated guinea pig ileum studies (*** p<0.001). It had exhibited meaningful antihistaminic activity on ileum contraction compared to control. The standard compound chlorpheniramine showed 63.3% inhibition. Standard drug chlorpheniramine showed better results than compound GSC (Table 3). The compound piperic acid inhibited the contraction induced by acetylcholine as compared to control significantly (*** p<0.001). The standard drug atropine sulphate exhibited 60% inhibition. The compound piperic acid (GSC) showed 53.7% inhibition (*** p<0.001) equivalent to standard (Table 4). During allergy immunoglobulin-E mediated mast cells get activated and release inflammatory mediators like interleukins, eosinophils, neutrophils, histamines, bradykines that cause inflammation in the throat and bronchial hyper secretions (Sagar et al., 2014). Histamine is one of the important mediators of broncho constriction and inflammation present in mast cells, and varius body fluids. It participates in various cell physiological processes like allergic reaction, inflammation, gastric acid secretion, central and peripheral neuro transmission (Goyal 2003). Histamine release from mast cells and basophils by antigenic stimulation causes smooth muscle contraction, increased vascular permeability, and mucus formation by stimulation of H1 receptors, a common feature of asthma. Hence use of antihistamines is a part of antiallergic therapy. Apart from it parasympathetic nerves which release acetyl choline as neuro transmitter also control the symptoms and inflammation of allergic diseases through peripheral muscarinic receptors which are present on air way smooth muscle and secretory glands. So, muscarinic antagonists are also used in the treatment of asthma (Kubo et al., 1989). Therefore, the drugs which block the effects of inflammatory mediators like histamine, acetyl choline are used in the treatment of asthma. Antiasthmatic drugs act on the contraction of the ileum muscle through several mechanisms,including stimulation of β-adrenergic receptors, inhibition of histamine (H1) receptors, or through an anticholinergic property (Stephen et al., 2012; Sagar et al., 2012). Hence, use of natural drugs with antioxidant, immune modulatory and anti-inflammatory properties would be beneficial. The excessive generation of reactive oxygen species (ROS) induced by various stimuli and which exceeds the antioxidant capacity of the organism leads to variety of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer (Mc Clements et al., 2000). The compound piperic acid acts as an antioxidant and antimicrobialsubstance (Zarai et al., 2013). In the current study the compound exhibited better antihistaminic and anticholinergic activities which may be due to inhibition of COX-2, NF-κB and TNF-α receptors where the triazolyl derivatives of piperic acid exhibited anti-inflammatory activity (Ali et al., 2015). It was observed that GSC showed better anticholinergic activity in both bronchospasm and ileum contraction study models. It showed more bronchodilator activity when compared to ileum contraction studies. GSC exhibited equipotent spasmolytic or bronchodilator activity compared to standard drugs chlorpheniramine and atropine. The support to the observation can be attributed to

anti-inflammatory activity studies involving cholinergic systems (Oliveira et al., 2018). The study conducted by Oliveira et al., indicates that piperic acid exhibited anti-inflammatory activity by inhibiting leukocyte migration and cytokine migration similar to standard drug atropine. According to the study conducted by Tomy et al., 2015, it can also be added that the compound may act by inhibition of lipoxygenase which is responsible for the production of inflammatory leukotrienes (Tomy et al., 2015). The results of the bronchospasmolytic study are in line with the findings of bronchoprotective effect of a novel MABA compound in the experimental bronchospasm model in anaesthetised guinea pigs (Daniela et al., 2017). The effects of ileum contraction study agree with the results of anticholinergic properties of progesterone in the isolated ileum of the guinea-pig (Rodolfo et al., 1996).

CONCLUSION

The compound piperic acid showed anticholinergic activity in both bronchospasm and ileum contraction models. The compound can be further studied by more models in order to establish its mechanism of action and assess its efficacy in treating bronchial problems to make it a suitable drug candidate.

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REFERENCES

1. Ali, Y., Alam, M.S., Hamid, H., Husain, A., Bano, S., Dhulap, A., Kharbanda, C., Nazreen, S., Haider, S. (2015). Design, synthesis and biological evaluation of piperic acid triazolyl derivatives as potent anti-inflammatory agents. Eur. J. Med. Chem., 6, 92, 490-500.
2. Broide, D.H. (2001). Molecular and cellular mechanisms of allergic disease. J. Allergy. Clin. Immunol., 108, 2, S65–S71.
3. Chandrakant, N., C, DattatraY, P. S., Dinesh, K.B. (2011). Anti-asthmatic and anti-anaphylactic activities o*f Blatta orientalis* mother tincture. Homeopathy, 100, 138–143.
4. *Daniela, M.,, ChiarA, C., Franco, B., Marina, T., Annalisa, M.,Valentina, M.,* Benedetta, R., Riccardo, P., Maurizio, C., Gino, V. (*2017).* CHF6366: Characterisation of the bronchoprotective effect of a novel MABA compound in the experimental bronchospasm model in anaesthetised guinea pigs. Eur. Respir. J., *50, OA4405*.
5. Goyal, R.K. (2003). Practical in Pharmacology, 3rd edition. B.S. Shah Prakashan, India. 103 p.
6. Kubo, N., Minami, T., Hori, Y., Yamashita, T., Kumazawa, T. (1989). Enhanced parasympathetic nerve activities in experimentally induced nasal hyper sensitivity. Acta. Otolaryngol. Suppl., 463, 14-20.
7. MC Clements, J., and Decker, E.A. (2000). Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. J. Food .Sci. 65, 1270.

8. OECD guidelines for the testing of chemicals-425. 2008. Acute oral toxicity-up and down procedure (UDP).
9. Oliveira, P.A., De, Almeida, T.B., De, Oliveira, R.G., Gonçalves, G.M., De, Oliveira, J.M., Neves, DOS, Santos, B.B., Laureano-Melo, R., Côrtes W.D.S., França, T.D.N., Vasconcellos, M.L.A.A., Marinho, B.G. 2018. Evaluation of the antinociceptive and anti-inflammatory activities of piperic acid: Involvement of the cholinergic and vanilloid systems. Eur.J.Pharmacol*.,* 5, 834, 54-64.
10. Rodolfo**,** R., Gabriela, F., Rafael, R., Martha, M. (1996). Anticholinergic properties of progesterone in the isolated ileum of the guinea-pig. Drug Dev Res.. 38, 1, 50-55. SAGAR, R., SAHOO, H. B. 2012. Evaluation of the antiasthmatic activity of ethanolic extract of *Elephantopus scaber L*. leaves. *Indian. J. Pharmacol*., 44, 398–401.
11. Sagar, R., Sahoo, H.B., Kar, B., Mishra, N.K., Mohapatra, R., Sarangi, S.P. (2014). Pharmacological evaluation of *Calendula officinalis* L. on bronchial asthma in various experimental animals. *Int. J. Nutr. Pharmacol. Neurol. Dis.,* 4, 95, 103.
12. Stephen, O. O., Gerald, I.E., Ifeanyi, H.A., Ogochukwu, L.I., Dickson, O.U., Viona, O. *et al*. (2012). Evaluation of the anti-asthma activity of aqueous root bark extract of *Ficus exasperata* Vahl (Moraceae). International Journal of Health Research, 5, 5–12.
13. Tomy, M. J., Sharanya, C. S., Dileep, K. V., Prasanth, S. , Sabu, A. , Sadasivan, C. and Haridas, M. (2015). Derivatives Form Better Lipoxygenase Inhibitors than Piperine: *In Vitro* and *In Silico*Study. Chem. Biol. Drug. Des*.,* 85, 715-721.
14. Ward, C. E., Baptist, A .P. (2016). Characteristics of Complementary and Alternative Medicine (CAM) use among older adults with asthma. *J. Asthma*, 53, 5, 546–552.
15. Zarai, Z., Boujelbene, E., Ben Salem, N., Gargouri, Y., & Sayari, A. (2013). Antioxidant and antimicrobial activities of various solvent extracts, piperine and piperic acid from *Piper nigrum*. LWT - Food Science and Technology, 50, 2, 634-641.