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ANTI-ASTHMATIC ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACT OF *CYCLEA PELTATA*

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ABSTRACT

Cyclea Peltata extract has many important medical uses which makes it a potential extract for researchers prospecting for active compounds in an effort to provide intervention for the global antimicrobial pandemic especially in resource limited setting. The objective is to determine the Anti-asthmatic activity of Aqueous and Etanolic extract of *Cyclea Peltata*. The effect of Antiashmatic activity studied using Guinea pig ileum preparation (*In vitro*), histamine induced bronchospasm in Guinea pig (*In vivo*). *In vitro* study indicated that the alcoholic and aqueous extracts of *Cyclea Peltata* relaxed significantly ileum, pre-contracted by histamine. *In vivo* study of ethanolic and aqueous extracts of *Cyclea Peltata* to guinea pigs had shown the significant increase in preconvulsion time when the guinea pigs were exposed to histamine. The results of present investigation suggest that, ethanolic and aqueous extracts of *Cyclea Peltata* have significant bronchodilatory activity against histamine.

KEYWORDS

Asthma, *Cyclea Peltata*, Histamine and Bronchodilatory.

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INTRODUCTION

Bronchial asthma is a chronic respiratory disorder affecting a large proportion of population throughout the world. The currently used drugs for the treatment of this disease in modern medicine are far from satisfactory as they provide only symptomatic relief, produce several adverse effects and may lose effectiveness on continued use. Asthma is a chronic condition involving the respiratory system in which the airways occasionally constrict, become inflamed, and are lined with excessive amount of mucous, often

in response to one or more triggers. These episodes may be triggered by exposure to environmental stimulants such as an allergen, tobacco smoke, cold or warm air, perfume, pet, dander, moist air, exercise or exertion or emotional stress.

Asthma is characterized by,

Airway inflammation: The airway lining becomes red, swollen, and narrow.

Airway obstruction: The muscles encircling the airway tighten causing the airway to narrow making it difficult to get air in and out of the lungs.

Airway hyper-responsiveness: The muscles encircling the airway respond more quickly and vigorously to small amounts of allergens and irritants. More than 400 medicinal plant species have been used ethno pharmacologically and traditionally to treat the symptoms of asthmatic and allergic disorders worldwide. The World Health Organization (WHO) has recognized herbal medicine as an essential building block for primary health care of vast countries like India and China.

Cyclea peltata, climbing shrubs are about 28 species and which occur in the tropical regions of Asia. About 7 species are found in India. According to Kirtikar KR *et al.*¹, *Cyclea peltata* (LAM) has some Pharmacognostic, antioxidant and antiulcer screening of *Cyclea peltata* roots. Vijayan, *et al.*² has evaluated on the treatment and protective effect of *Cyclea peltata* on cisplatin-induced nephrotoxicity and oxidative damage. Hullatti and Sharada³, reported the diuretic activity on the root extract of *Cyclea peltata*. Lam-Latha *et al.*⁴ evaluated the gastric anti-secretory and antiulcer activities of *Cyclea peltata* Lam. Kirana *et al.*⁵ has reported on the Type II diabetic activity on the roots aqueous extracts of *Cyclea peltata*.

On the other hand, Rukmani *et al.*⁶ analysed the nutritional and toxicological evaluation of *Cyclea barbata* oil Kalyana sundaram *et al.*⁷ reported on the biological active plant extracts of *Cyclea bicristata* as mosquito larvicides. Singh, *et al.*⁸ reported efficacy of plant extracts against *Cyclea ciliata*. Omar⁹, reported flavonoids in *Cyclea ciliata*. Harraz *et al.*¹⁰ isolated dammarantriterpenes from *Cyclea barbata*. Alyand Badran¹¹ reported the mosquito control with extracts

from plants of the Egyptian eastern desert including *Cyclea gracillima*.

Apasara Arkarapanthu., *et al.*¹² investigated the Gel extracted from *Cyclea barbata* Miers leaves chemical composition and gelation properties. Iskandar Muda *et al.*¹³ reported on the protective effect of *Cyclea barbata* Miers leaves against induced gastric ulcer in mice. Jian-Zhongwant., *et al.*¹⁴ isolated cytotoxic bisbenzylisoquinoline alkaloids from the roots of *Cyclea racemosa*.

The plant *Cyclea peltata* speculated to possess various medicinal properties. A decoction of the leaves is employed in treatment of jaundice, asthma. Decoction of the roots used for treatment of diabetes. Powdered roots used in toothache. There is also a speculation that the leaves of *Cyclea peltata* have medicinal properties related to Anti-asthmatic property. *Cyclea peltata* also has an important place in indigenous medicine and in view of its usage; an attempt has been made to study the Anti-asthmatic activities of this plant.

Material and Method

Plant Materials

The plant is collected from in Tirunelveli district and was authenticated by the Botanist from Tamilnadu Government siddha college Palayamkottai Tirunelveli.

Preparation of Ethanolic Extract¹⁵⁻²¹

The dried coarse powder of *cyclea peltata* was extracted successively with solvent of increasing polarity. Powder of *Cyclea peltata* was extracted with 2 litres of petroleum ether-chloroform and acetone respectively by continuous hot percolation method using soxhlet apparatus. After 24 hours when the extraction was completed, solvents were redistilled. Dried marc left after the acetone extraction is then again percolated in soxhlet apparatus with 2 litres of ethanol After the completion of the extraction it was filtered and solvent was removed by distillation under reduced pressure, and the yellowish green colored extract was stored in a desiccator. The marc was dried for aqueous extraction.

Preparation of Aqueous Extraction:

The marc left after ethanol extraction was dried and macerated with 2-3 liters of chloroform water (0.25%) in a narrow mouthed bottle for three days. It was

filtered and the solvent was removed by distillation under reduced pressure. The extract was then stored in desiccators.

Experimental Animals

Both sex of Guinea pigs of pir Bright white strain (400-500 g) were used in the studies. Animals were lodged under standard environmental conditions of temperature and humidity for 24 hrs and with water (Figure No.1-5).

Evaluation of Anti-Asthmatic studies

Isolated Guinea pig ileum preparation (*In-vitro*)²²⁻²⁶

Antihistamine effect was studied in isolated guinea pig ileum. The guinea pig are sacrificed by a below on the head and carotid bleeding was performed. The abdomen was cut and the caecum opened to trace the ileo-caecal junction. A few cm length of the ileal position are removed. It was immediately placed in watch glass containing Tyrode solution. The ileum was cut into pieces of 2-3 cm long.

One piece of the ileum was taken and tied with a thread in the top and bottom ends, without closing the lumen. The tissue was mounted in the organ bath containing Tyrode solution maintained at 37°C and bubbled with air. A tension of 0.5 gm was applied on the tissue. The tissue was allowed to stabilize for 30 minutes after placing in the organ bath. The contraction dependent responses of histamine (using Standard stock solution 1µg/ml) using frontal writing lever were recorded. A contact time of 30 seconds and 5 minutes time cycle were followed for recording the responses. At least four concentration responses of histamine (using standard solution 1µg/ml) were recorded.

Tyrode solution containing 250mg/ml of *Cyclea peltata* were charged in the reservoir and the tissue was allowed to stabilize in it for 30 minutes, then the sub- maximal dose of histamine was repeated. The inhibitions of the response were observed by comparing it with the maximal response of histamine.

Histamine Aerosol induced bronchoconstriction in Guinea pigs (*in-vivo*)²⁷⁻²⁹

Animals were divided into 4 groups of 6 animals each.

The groups were as follows:

Group 1 - Standard (Promethazine hydrochloride 300µg/kg).

Group 2 - Ethanolic extract of *Cyclea peltata* (100mg/kg)p.o.

Group 3 - Aqueous extract of *Cyclea peltata* (100mg/kg)p.o.

Animals belonging to each group were subjected to histamine aerosol (0.2% histamine diphosphate in saline) using a nebulizer for 2 seconds in an airtight Perspex Histamine chamber. Aerosolization of the solution was achieved via a compressed air line operating at a pressure of 8psi and flow rate of 5ml/min. After exposure to the histamine aerosol, the animal showed a sign of immediate immobilization, bouts of coughing. This was followed by shallow breathing symptoms, after which the animals collapsed fell on its back and convulsed. The time taken at the animal to fall on its back after exposure to the aerosol was designated as the exposition time. The exposition time for each animal in all the groups were noted. Once the animal fell on its back, it was after exposure to the aerosol was designated as the exposition time. The exposition time for each animal in all three groups were noted. Once the animal fell on its back, it was immediately taken out of the chamber and exposed to fresh air for the recovery of treated animals.

After one hour, the animals in the test groups were administered orally 100mg/kg with the ethanolic and aqueous extracts of *Cyclea peltata* respectively, while reference group of animals received 300µg/kg Promethazine hydrochloride by oral route. One hour later, the animals were reexposed on the aerosol and exposition time before and after extract administration was taken as a measure of the protective effort of the drugs and Percent protection afforded by the samples was calculated by the formula.

$$\text{Percentage protection (\%)} = \frac{\text{Eta-Etb}}{\text{Etb}} \times 100$$

Eta - mean exposition time after treatment with the extracts

Etb - mean exposition time before treatment with the extracts.

RESULTS AND DISCUSSION

Isolated Guinea pig ileum preparation (*In-vitro*)

Histamine is one of the important mediators of allergy, inflammation and bronchoconstriction, which were

released after degranulation of mast cell by an antigen exposure. Targeting Histamine, either prevention of its release from mast cell or use of histaminergic receptor antagonist becomes part of antihistaminic therapy in allergic disease. The alcohol and aqueous extracts of *Cyclea peltata* inhibited contractions induced by histamine - the agent which implicate in various ways in the pathogenesis of asthma (Figure No.6-9). The results of this study indicated that the alcoholic and aqueous extracts of *Cyclea peltata* relaxed significantly ileum pre-contracted by histamine, the extracts probably might have acted by blocking the muscarinic receptors. The ability of the alcoholic and aqueous extracts of *Cyclea peltata* to inhibit the contractions induced by the histamine suggests a possible role in the treatment of asthma. The relaxation of histamine pre-contracted ileum by the extracts of *Cyclea peltata* indicates their potency in ameliorating established asthma.

The results of test extracts of *Cyclea peltata* suggested that it is effective in reducing the symptoms of bronchial asthma and also improve the lung function parameters of asthmatic subjects.

Histamine Aerosol induced bronchoconstriction in Guinea pigs (*in-vivo*)

(Aerosol Testing Method)³⁰

In the histamine aerosol study, the control animals showed convulsion during the first 3 minutes of the experiment. Prior treatment of ethanolic and aqueous extracts of *Cyclea peltata* (100mg/kg, p.o) protected the animals (Table No.1) to a significant extent ($P < 0.001$) from the development of asphyxia produced by histamine aerosol confirming that it has antihistaminic activity.

The role of histamine in asthma is well established (Nelson, 2003)³¹. The close resemblance of pulmonary responses to histamine challenge in both guinea pigs and humans, as well as the anaphylactic sensitization made this species the model of choice. In the present study, guinea pigs were used because of the extreme sensitivity of their airways to the primary mediators of bronchoconstriction, including histamine and leukotrienes, and their ability to be sensitized to foreign proteins. Although there are various model of asthma, guinea pig airways react to histamine, acetylcholine, leukotrienes, and other Broncho constrictors in a manner similar to that seen in humans (Agrawal *et al.*, 1991).

Another similarity between the guinea pig model and asthmatic patients is that enhanced bronchoconstriction occurs in both species following sensitization, in response to β -adrenergic antagonists (Matsumoto *et al.*, 1994). Thus, the guinea pig model resembles the human allergic pathology in several aspects, especially in terms of mediator release. Histamine antagonists can be conveniently recognized and assayed by their ability to protect guinea pigs against lethal effects of histamine-induced bronchospasm (Broadbent and Bain, 1964).

In vivo study of ethanolic and aqueous extracts of *Cyclea peltata* to guinea pigs had shown the significant increase in preconvulsion time when the guinea pigs were exposed to histamine. The results of present investigation suggest that, ethanolic and aqueous extracts of *Cyclea peltata* have significant bronchodilatory activity against histamine.

Table No.1: Effect of *Cyclea Peltata* extracts on Histamine induced Bronchospasm in guinea pig

S.No	Treatment	Pre-treatment Exposition time in Seconds \pm SEM	Post-treatment exposition time in Seconds \pm SEM	Percentage Protection
1	Control	108 \pm 1.12	108 \pm 1.56	0%
2	Promethazine Hydrochloride (300 μ g/kg/p.o)	110 \pm 1.29	387 \pm 1.59*	71.57%
3	Ethanolic extract (100mg/kg/p.o)	108 \pm 1.26	310 \pm 1.30	65.2%
4	Aqueous extract (100mg/kg/p.o)	112 \pm 1.53	228 \pm 1.46*	45.5%

n=6; *P<0.001 Vs Control

P value was calculated by comparing with Control by Student "t" Test

PHOTOGRAPHS SHOWING BRONCHODILATOR ACTIVITY AGAINST HISTAMINE AEROSOLISATION



Figure No.1: Housing of Animals



Figure No.2: Oral Administration of Drug

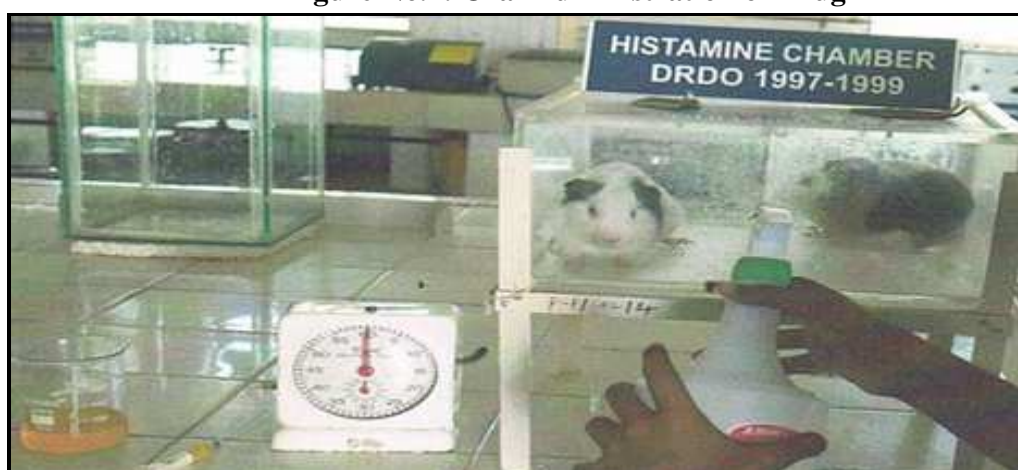


Figure No.3: Chamber Saturated with Histamine Aerosol



Figure No.4: Noted the Recovery time



Figure No.5: Recovery of Animals after Breathing Difficulty

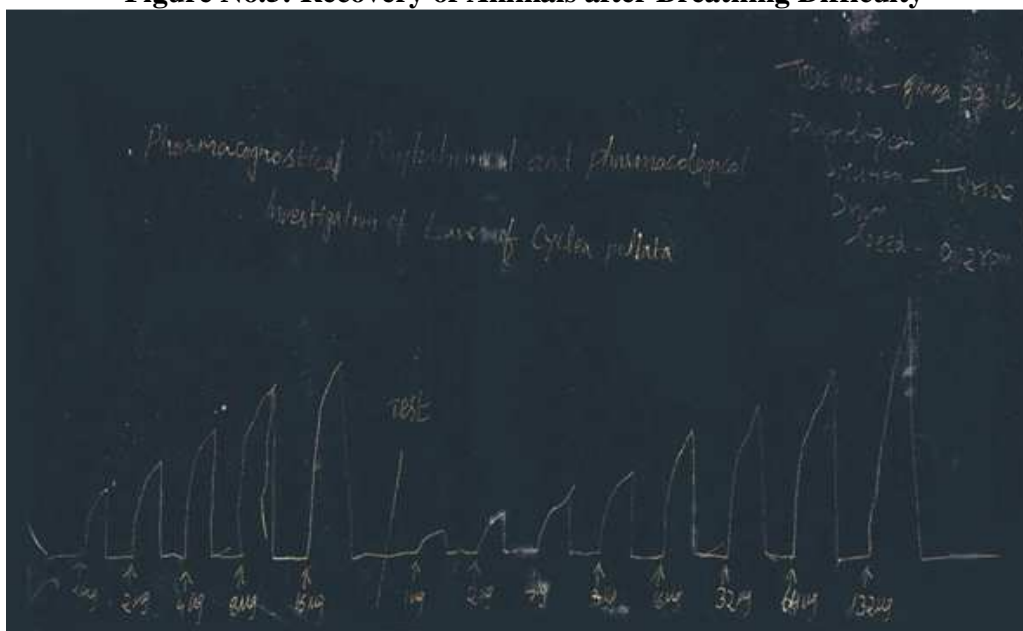


Figure No.6: Effect of ethanolic extract on *Cyclea peltata* on isolated guinea pig ileum

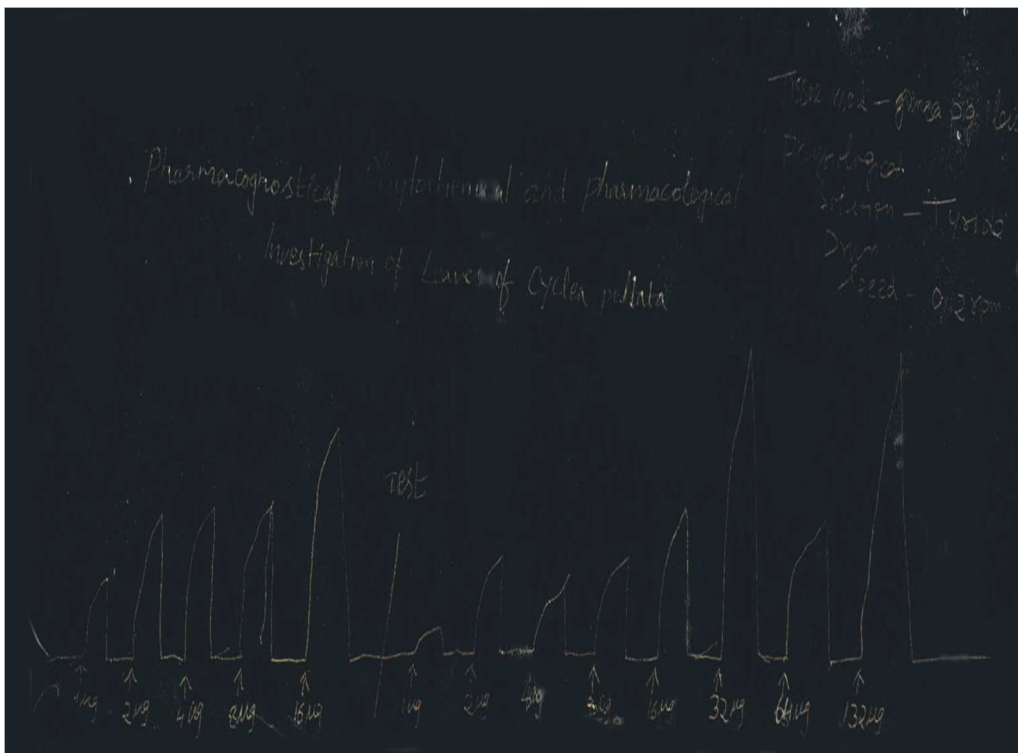


Figure No.7: Effect of Aqueous extract on *Cyclea peltata* on isolated guinea pig ileum

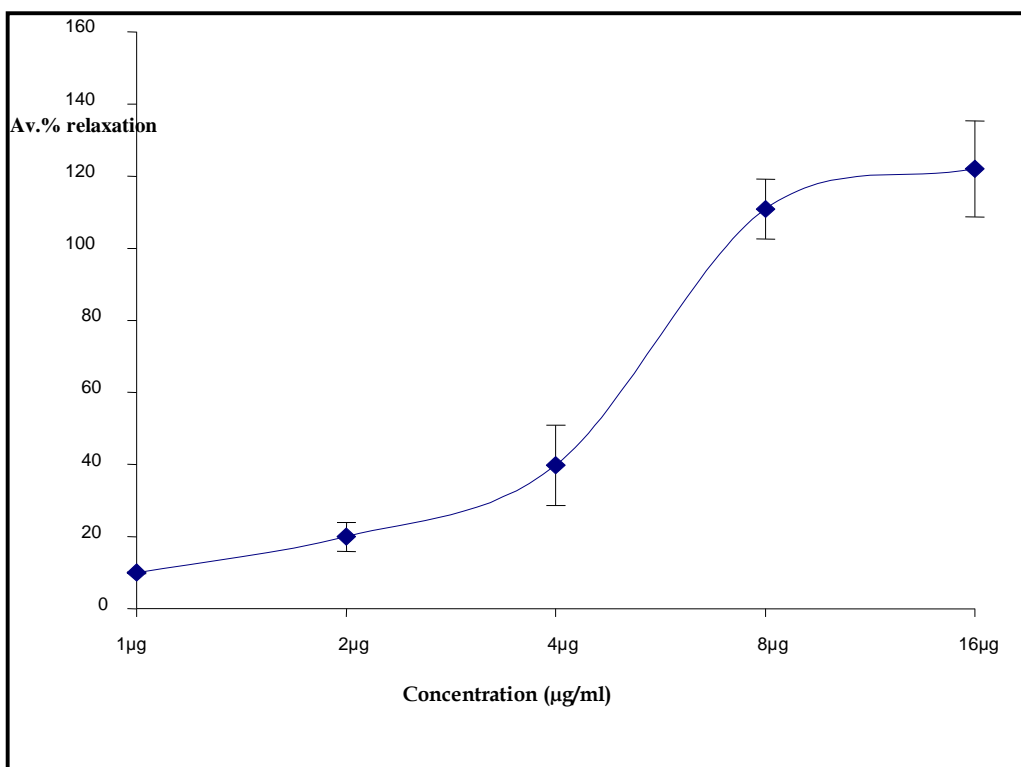


Figure No.8: Effect of the alcoholic extract on histamine induced contraction of the isolated guinea pig ileum

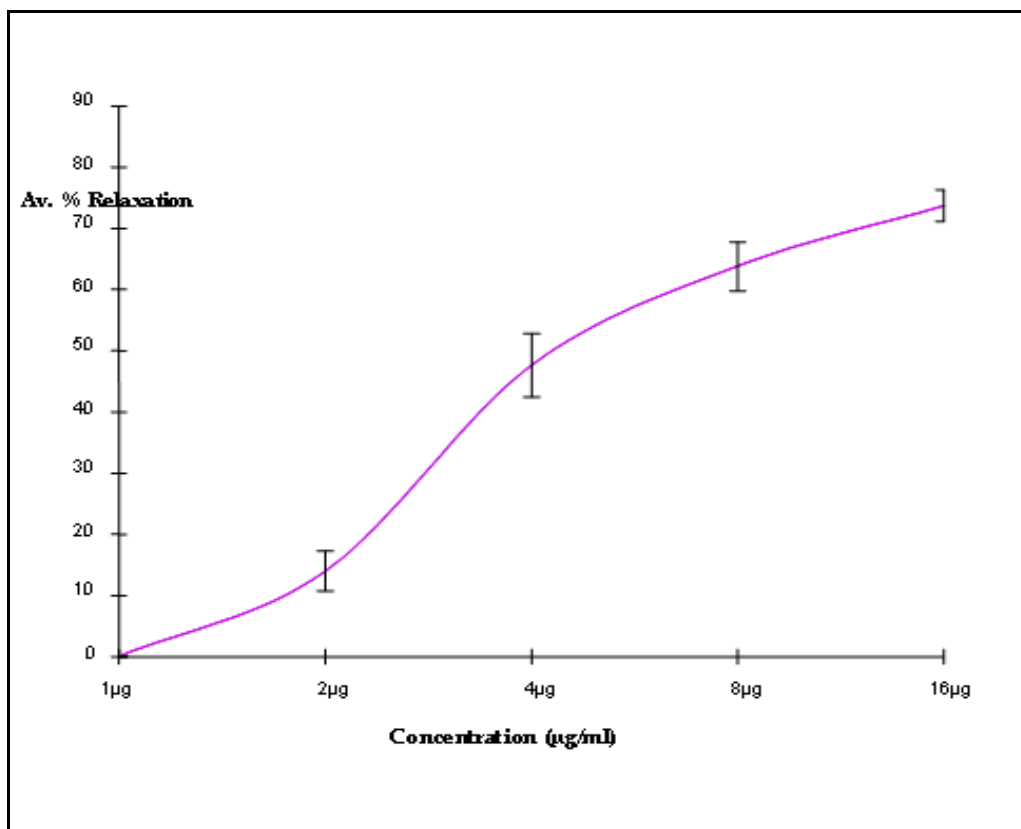


Figure No.9: Effect of the aqueous extract on histamine induced contraction of the isolated guinea pig ileum

CONCLUSION

Our study suggests that both aqueous and ethanolic extract of the leaves of *Cyclea Peltata* possess significant anti-asthmatic activity. The results obtained from this study serve as the basic data for further progress and application on these plants. However, further studies are needed to establish the molecular mechanism, to isolate and characterize the active principle which are responsible for anti-asthmatic action.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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