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EVALUATION OF INVITRO ANTISPASMODIC ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF *CYANTHILLIUM CINEREUM* (L) H. ROB. ON ISOLATED FROG RECTUS ABDOMINUS MUSCLE

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Abstract

Objective: To evaluate the antispasmodic activity of ethanolic extract of leaves of *Cyanthillium cinereum* on isolated frog rectus abdominus muscle.

Method: *Cyanthillium cinereum* plant has number of medicinal importance as it contains number of active constituents like flavonoids, cardiac glycosides, alkaloids, phenols, and tannins etc. Antispasmodic activity was done by using interpolation bioassay technique on isolated frog rectus abdominus muscle. Activity of acetylcholine, ethanolic extract of leaves of *Cyanthillium cinereum* and acetylcholine along with ethanolic extract of leaves of *Cyanthillium cinereum* were studied on isolated frog rectus abdominus muscle. Finally the results were compared with the standard antispasmodic drug (Atropine).

Results: Concentrations (1µg/ml, 3µg/ml, 10µg/ml, 30µg/ml, 100µg/ml and 300µg/ml) of acetylcholine on frog rectus abdominus muscle showed increased spasmodic action where as ethanolic extract of leaves of *Cyanthillium cinereum* on isolated frog rectus abdominus muscle showed inhibitory effect of acetylcholine. Atropine showed significant antispasmodic action.

Conclusion: Ethanolic extract of leaves of *Cyanthillium cinereum* showed significant antispasmodic activity on isolated frog rectus abdominus muscle against acetylcholine.

Keywords Antispasmodic activity, *Cyanthillium cinereum*, in-vitro bioassay, Acetylcholine, Atropine and frog rectus abdominus muscle.

Introduction

Cyanthillium cinereum(L) H.Rob. Also called as *Vernonia cinerea* belongs to the family of Asteraceae. The species in native to tropical Africa, tropical Asia, India, Indochina, tropical South America, West India and also US state of

Florida. *Cyanthillium cinereum* is an annual herb grows upto 120cm tall and produces flat topped arrays of numerous flower head. It is used in ayurvedic herbal medicine [1]. *Cyanthilium cinereum* containing various chemical constituents like luteolin 7 mono beta D glucopyranoside along with triterpene compounds like beta amyryn acetate, lupeol acetate [2]. Phtochemical screening of this plant showed presence of cardiac glycosides, alkaloids, phenols, flavonoids, steroids, tannins, phlobtannins and saponins [9].These constituents were involved in the treatment of various diseases. Whole plant used in ayurvedic preparation for the treatment of kidney disorders, in the form of decotion used for swellings, stomach pain and diarrhea. It is also used as diuretic and in menstrual pains [2]. Seeds are also used as anthelmintic agents. Leaves are used for the treatment of various diseases such as analgesic, antimicrobial, antipyretic and anti-inflammatory [9].



Leaves and Flowers of *Cyanthilium cinereum* (L) H. Rob.[1]

Antispasmodics are muscular relaxants that are used to relieve cramps or spasms of the stomach, intestines and bladder. They are commonly used for the treatment of different gastrointestinal disorders, including diarrhea and irritable bowel syndrome [4]. The gastrointestinal tract is under the control of the sympathetic and parasympathetic arms of the Autonomic nervous system. Over activity of the parasympathetic arm causes increased peristalsis resulting in gastrointestinal cramps, gastritis, peptic ulcers, diarrhea and ulcers due increased gastric secretions. These disorders results from excessive involuntary muscle movement associated with excess release of Acetylcholine, a neurotransmitter which mediates parasympathetic functions. Antispasmodic agents are substances that suppress muscle spasms. On the gastrointestinal tract their effect is to prevent spasms of the stomach and

intestine mostly by blocking the action of neurotransmitter acetylcholine in the parasympathetic outflow and thereby inhibiting cholinergic nerve impulses by selectively blocking the receptors to which acetylcholine binds. This anti cholinergic property of the antispasmodic agents become useful in the management of disorders associated with over activity of the parasympathetic system on the gastrointestinal system [3,6].

Materials and Methods

Plant collection and authentication

The healthy leaves of *Cyanthillium cinereum* (L) H. Rob. Belongs to the family Asteraceae were collected from the road sides of Thimmapur village, Karimnagar district, Telangana, India and authenticated by the Botanical Survey of India. Reg no: BSI/DRC/2017-18/Tech./699.

Preparation of Plant Extract

The plant material (leaves of the *Cyanthillium cinereum*) was shade dried and powdered by a mechanical grinder. The dried powder was extracted with ethanol as a solvent by using soxhlet apparatus. The powder (100gm) was taken and placed in thimble made up of filter paper and inserted in to the wide central tube of extractor. Ethanol is placed in the round bottom flask and brought to its boiling point up to 78°C for 6-7 hours. Its vapors passed through the larger right hand tube in to the upper part of extractor and then to the condenser. During this period, the active constituents were extracted, when the level of the extract reaches the top of the syphon tube. The process was continued until the drug was completely extracted, then the extract processed for evaporation. After the evaporation the semi-solid jelly is formed. The plant extract was dark green in colour and soluble in distilled water [8].

Drugs and Chemicals

Acetylcholine, CaCl₂ was purchased from Loba chemicals pvt. Ltd. Mumbai, India. NaCl, KCl, NaHCO₃ were purchased from S.D. fine chemicals, Mumbai, India. Atropine (standard drug) was purchased from a Neon Lab. Ltd., India.

Isolation of frog rectus abdominus muscle

Frogs were sacrificed followed by stunning and pithing. Then the frog rectus abdominus muscle was collected by dissecting it. And the collected tissue is immersed in frog Ringers solution and cleaned off. Respective segment of

2-3cm long were mounted in a 25ml tissue organ bath and oxygen is supplied through aerator and maintained at 37°C, isolated tissue was stabilized for 30mins. After stabilization record the responses of normal, acetylcholine, plant extract & Atropine [5].

Composition of frog's ringer solution

NaCl (6g), KCl (0.14g), CaCl₂ (0.12g), NaHCO₃ (0.2g), and glucose (2g) dissolved in 1 liter of distilled water [5].

Assay procedure for in vitro anti-spasmodic activity

- Firstly dose response curve of acetylcholine were recorded (with concentration of 1, 3, 10, 30, 100, 300 µg/ml) as stock 1 solution by using Sherrington's rotating drum with a frontal writing lever. Contact time of 90sec and base line of 30sec time cycle [7].
- The same dose response curve were recorded by using the mixture of acetylcholine and *Cyanthillium cinereum* extract (with concentration of 1, 3, 10, 30, 100, 300 µg/ml) as a stock 2 solution [7].
- The same dose response curve were recorded by using atropine as standard (with concentration of 1, 3, 10, 30,100,300 µg/ml) when it is compared with plant extract with respective doses as a stock 3 solution [7].
- The same dose response curve were recorded by using extract (with concentration of 1, 3, 10, 30,100,300 µg/ml) as a stock 4 solution [7].

Observation and Results

Effect of acetylcholine on frog rectus abdominus muscle showed increases in spasmodic activity an increasing in dose as shown in fig.1.

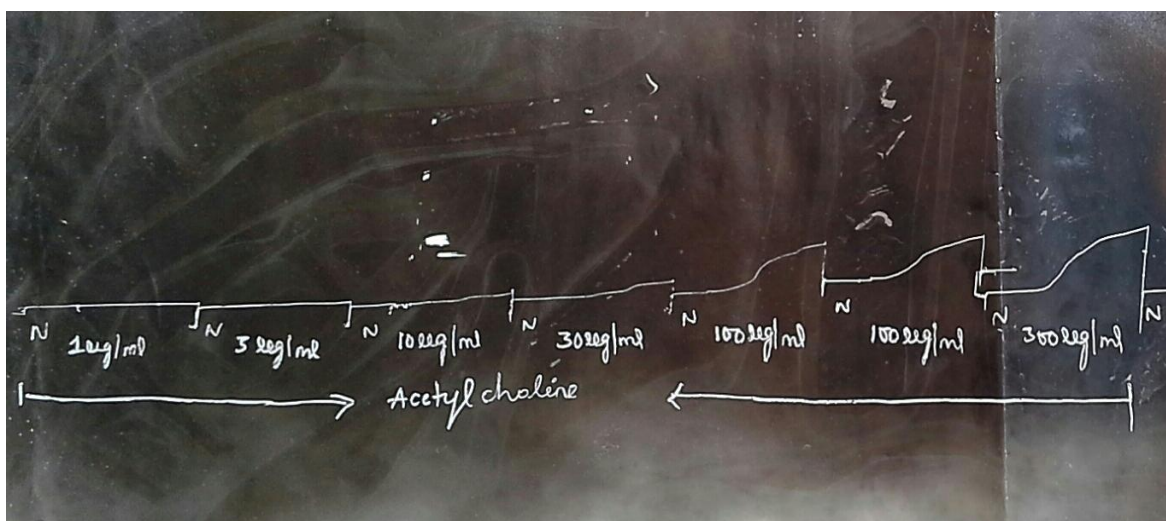


Fig 1: Dose Response Curves of Acetylcholine.

Effect of Acetylcholine induced spasm followed by treatment of ethanolic extract of leaves of *Cyanthillium*

cinereum showed anti spasmodic activity as depicted in fig.2

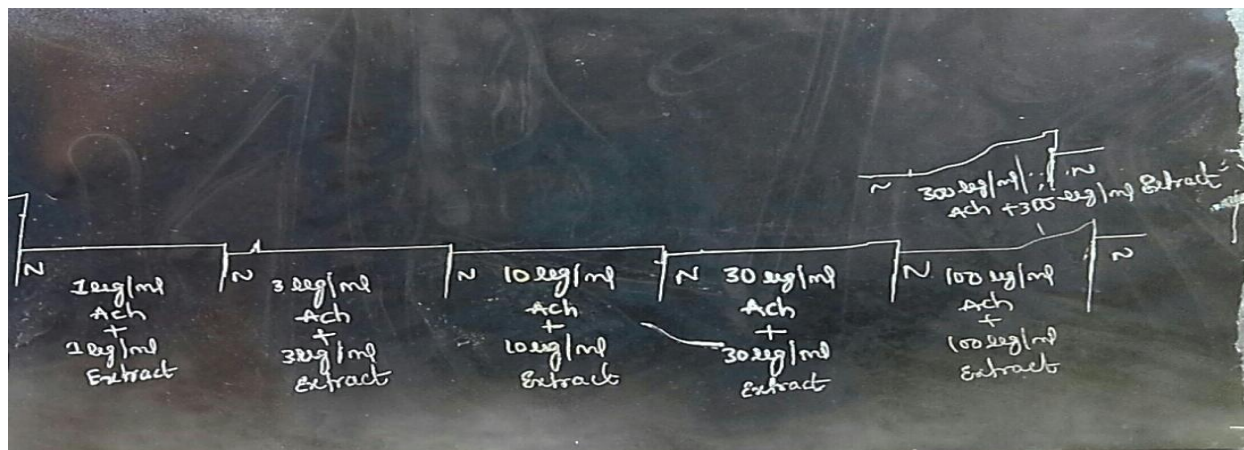


Fig 2: Dose Response Curve of Acetylcholine + Plant Extract.

Effect of Anti-cholinergic drug Atropine (as standard antispasmodic agent) showed receptor blocking action on isolated frog rectus abdominus muscle as shown in Fig: 3

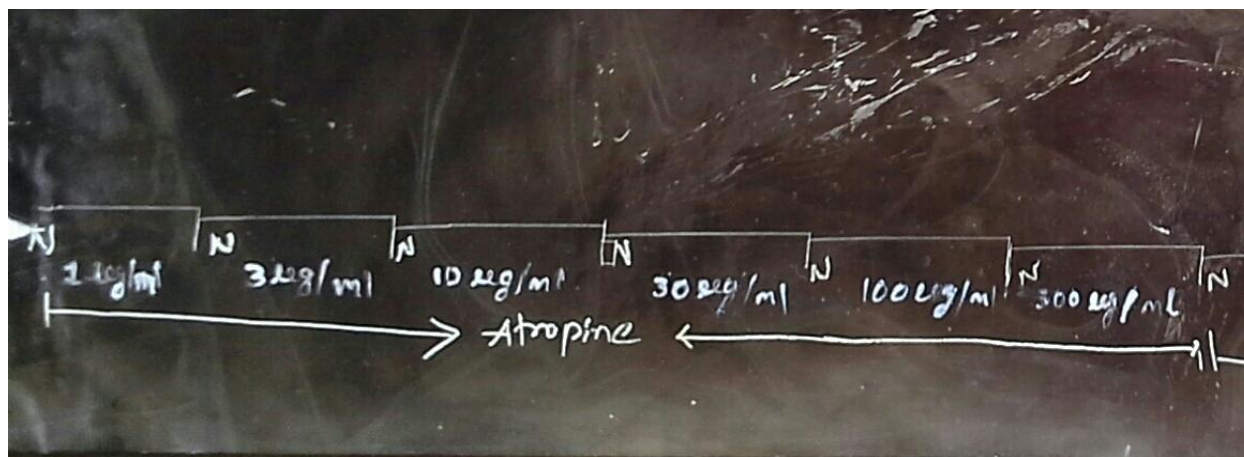


Fig 3: Dose Response Curve of Atropine.

Effect of ethanolic extract of leaves of *Cyanthillium cinereum* showed receptor blocking action (anti spasmodic) on isolated frog rectus abdominus muscle as shown in Fig: 4

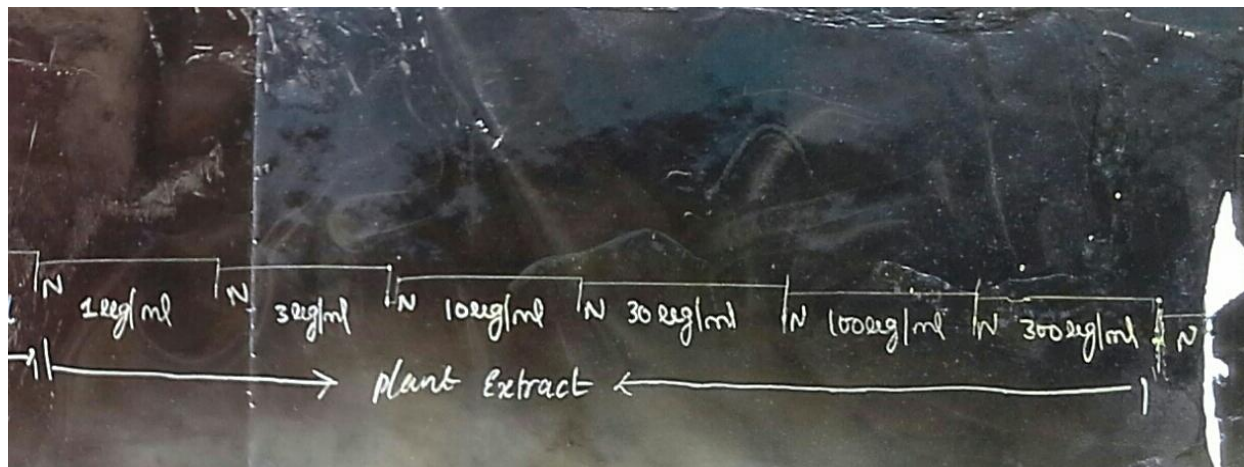


Fig 4: Dose Response Curves of ethanolic extract of leaves of *Cyanthillium cinereum*.

Table-1: Dose response relationship observation of acetylcholine.

S. No	Drug	Concentration ($\mu\text{g/ml}$)	Response (cm)
1	Acetylcholine	1 ($\mu\text{g/ml}$)	0.1
2		3 ($\mu\text{g/ml}$)	0.3
3		10 ($\mu\text{g/ml}$)	0.5
4		30 ($\mu\text{g/ml}$)	0.6
5		100 ($\mu\text{g/ml}$)	1.2
6		300 ($\mu\text{g/ml}$)	1.5

Table-2: Dose Response relationship observation of Ach and *Cyanthillium cinereum* ethanolic extract.

S. No	Drug	Concentration ($\mu\text{g/ml}$)	Response (cm)
1	Ach + plant extract	1 $\mu\text{g/ml}$ + 1 $\mu\text{g/ml}$	0.1
2		3 $\mu\text{g/ml}$ + 3 $\mu\text{g/ml}$	0.2
3		10 $\mu\text{g/ml}$ + 10 $\mu\text{g/ml}$	0.3
4		30 $\mu\text{g/ml}$ + 30 $\mu\text{g/ml}$	0.4
5		100 $\mu\text{g/ml}$ + 100 $\mu\text{g/ml}$	0.6
6		300 $\mu\text{g/ml}$ + 300 $\mu\text{g/ml}$	0.8

Table-3: Dose Response relationship observation of Atropine.

S. No	Drug	Concentration ($\mu\text{g/ml}$)	Response (cm)
1	Atropine	1 ($\mu\text{g/ml}$)	-
2		3 ($\mu\text{g/ml}$)	-
3		10 ($\mu\text{g/ml}$)	-
4		30 ($\mu\text{g/ml}$)	-
5		100 ($\mu\text{g/ml}$)	-
6		300 ($\mu\text{g/ml}$)	-

Table-4: Dose response relationship observation of *Cyanthillium cinereum* ethanolic extract.

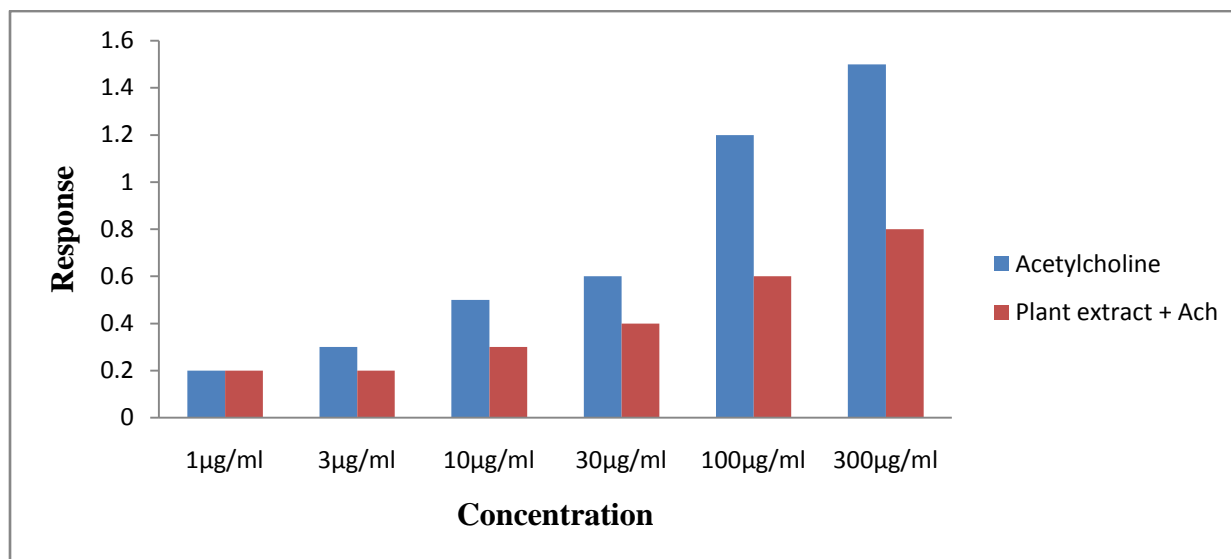
S. No	Drug	Concentration ($\mu\text{g/ml}$)	Response (cm)
1	<i>Cyanthillium cinereum</i> ethanolic leaves extract	1 ($\mu\text{g/ml}$)	-
2		3 ($\mu\text{g/ml}$)	-
3		10 ($\mu\text{g/ml}$)	-
4		30 ($\mu\text{g/ml}$)	-
5		100 ($\mu\text{g/ml}$)	-
6		300 ($\mu\text{g/ml}$)	-

Table-5: Comparative dose responses of Ach and Ach followed by *Cyanthillium cinereum* leaves extract.

S.No	Drug	Concentration ($\mu\text{g/ml}$)	Response (cm)	%Decrease in response
1	Ach	1 ($\mu\text{g/ml}$)	0.1	-
2		3 ($\mu\text{g/ml}$)	0.3	
3		10 ($\mu\text{g/ml}$)	0.5	
4		30 ($\mu\text{g/ml}$)	0.6	
5		100 ($\mu\text{g/ml}$)	1.2	
6		300 ($\mu\text{g/ml}$)	1.5	
7	Ach + <i>Cyanthillium cinereum</i> ethanolic leaves extract	1 ($\mu\text{g/ml}$) + 1 ($\mu\text{g/ml}$)	0.1	0
8		3($\mu\text{g/ml}$) + 3($\mu\text{g/ml}$)	0.2	33.3
9		10($\mu\text{g/ml}$) + 10 ($\mu\text{g/ml}$)	0.3	40.0
10		30($\mu\text{g/ml}$) + 30 ($\mu\text{g/ml}$)	0.4	33.33
11		100($\mu\text{g/ml}$) + 100 ($\mu\text{g/ml}$)	0.6	50.0
12		300($\mu\text{g/ml}$) + 300 ($\mu\text{g/ml}$)	0.8	46.6

$$\% \text{ decrease in response} = \frac{\text{Response of Ach} - \text{Response of extract + Ach}}{\text{Response of Ach}} \times 100$$

Fig 5: Comparative dose response relationship of Acetylcholine and ethanolic extract of leaves of *Cyanthillium cinereum* on isolated frog rectus abdominus muscle.



Discussion

By the present results it was observed that acetylcholine alone causes dose dependent contractions on isolated frog rectus abdominus muscle. When acetylcholine and extract combination was given there was marked decreased in contractions were observed. Individual leaf extract also showed marked decreased in contractions on frog rectus abdominus muscle as compared with standard atropine drug. This showed that ethanolic extract of the leaves of *Cyanthillium cinereum* possess a high degree of spasmolytic (antispasmodic) activity by blocking cholinergic receptors.

Conclusion

From the obtained results it was concluded that ethanolic extract of leaves of the *Cyanthillium cinereum* showed anti-spasmodic activity when compared with standard antispasmodic agent like Atropine. 100µg/ml concentration of plant extract showed 50% inhibition of acetylcholine action. *Cyanthillium cinereum* being a herbal origin drug with high degree of safety and efficacy could be suitable alternative to existing drugs. It is also included as a new member of antispasmodics family.

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