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EVALUATION OF *IN VITRO* ANTISPASMDODIC ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF *CLERODENDRUM PHILIPPINUM*

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ABSTRACT

The main aim of this study was to evaluate the in vitro antispasmodic activity by using the Clerodendrum philippinum Schauer (Verbenaceae) leaf extract. In this study frog rectus abdominus muscle was isolated, spasm was produced by using acetylcholine and atropine was used as a standard. The methanolic extract of leaf was produced antispasmodic effect on gastrointestinal and abdominus smooth muscles. The percentage inhibition was also determined. Thus, the Clerodendrum philippinum leaf extract shows prominent anti spasmodic activity.

KEY WORDS

Antispasmodic activity, invitro assay, Clerodendrum philippinum, methanolic extract.

INTRODUCTION

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 [3]. 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 result 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 anticholinergic property of the antispasmodic agents become useful in the management of disorders associated with over activity of the parasympathetic system on the gastrointestinal system. [1]

Clerodendrum philippinum Schauer **(**Verbenaceae**)** known as scent malli in India. It is an erect, ever green shrub with stout branches, it grown upto 2 metres tall. Geographical source of this plant is East Asia, Nepal, Northeast India, Thailand, Myanmar [6]. The genus *Clerodendrum philippinum* is very widely distributed throughout the world. Many species of this genus has been described in various indigenous systems of medicines for the treatment of various life threatening diseases. Root and leaf extract of *Clerodendrum philippinum* have been used for the treatment of rheumatism, and asthma, bronchitis, edema, diuretic, skin diseases, jaundice and antiseptic [7]. Phytochemical analysis of leaves shows presence of flavonoids, steroids, glycosides, phenolic compounds, tannins, saponins, carbohydrates, alkaloids, fixed oils and fats [2].

MATERIALS AND METHOD

Plant collection, Authentication and Extraction:

The fresh leaves of *Clerodendrum philippinum* were collected from karimnagar near Vaageswari College of Pharmacy. The plant was identified and authenticated by BSI/DRC/2017-18/Tech./57. The collected leaves were shade dried at room temperature for 7 days. The dried leaves were grinded to get fine powder using a grinder. Methanolic extract of leaves were prepared by soxhlation method at suitable temperature 60°C. 50gms of powdered leaves were prepared as a thimble separately and placed in the condenser and in the round bottomed flask required amount of methanol was taken. Soxhlation process was carried out for 6-8 hours. The extract obtained was evaporated and dried in desiccators [5].

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. The composition of frog ringers solution is Nacl- 6grms, Kcl- 0.14grms, $Cacl_2$ – 0.12grms, NaHco3 – 0.2grms, glucose- 2grms made with 1000ml distilled water [4].

Invitro Anti spasmodic activity assay procedure:

- Firstly, dose dependent response of acetylcholine were recorded (with the dose of 10,30,100,300,1000 µg/ml) as stock 1 solution by using Sherrington's recording drum with a frontal writing lever. Contact time of 90sec and base line of 30sec time cycle. [1]
- Then same dose dependent response were recorded by using the mixture of acetylcholine and *Clerodendrum philippinum* extract (with a concentration of 10,30,100,300,1000 µg/ml) as a stock 2 solution. [1]
- Lastly the same dose dependent response were recorded by using atropine as standard (with a concentration of 10,30,100,300,1000 µg/ml) when it is compared with plant extract with respective doses as a stock 3 solution. [1]

RESULTS

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

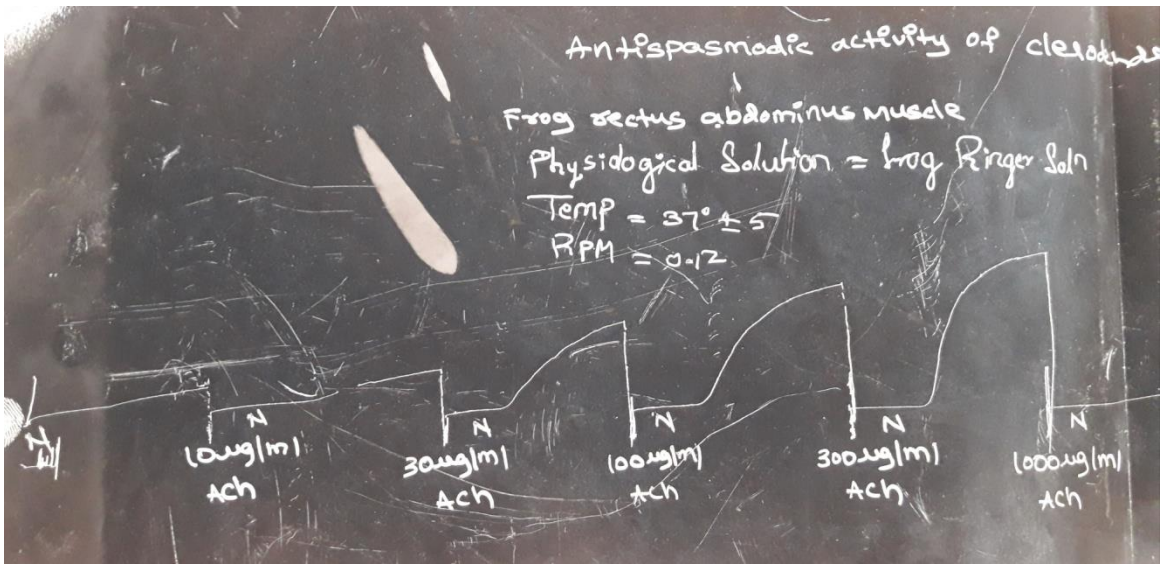


Fig 1. Response Curve of Acetylcholine

Table: 1 Dose Response Relationship Observations of Acetylcholine

S.No.	Drug	Dose (µg/ml)	Response (cm)
1	Ach	10	0.4
2	Ach	30	0.9
3	Ach	100	1.8
4	Ach	300	2.5
5	Ach	1000	3.15

Effect of Acetylcholine induced spasm followed by treatment of methanolic extract of *Clerodendrum philippinum* showed anti spasmodic activity as depicted in Fig.2

Fig 2: Response Curve of Acetylcholine + Leaf extract

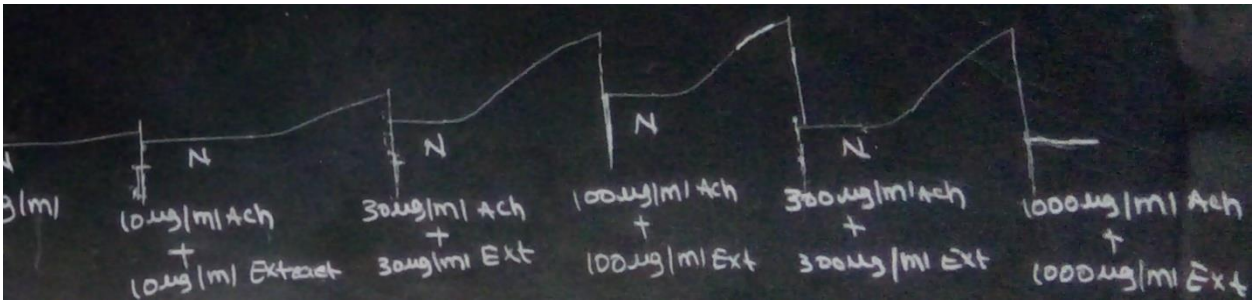


Table 2: Dose Response Relationship Observation of Acetylcholine and Extract

S.No.	Drug	Dose (µg/ml)	Response (cm)
1	Ach+ extract	10	0.15
2	Ach + extract	30	0.5
3	Ach + extract	100	1.0
4	Ach + extract	300	2.0
5	Ach + extract	1000	2.1

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

Fig 3: Response Curve of Atropine

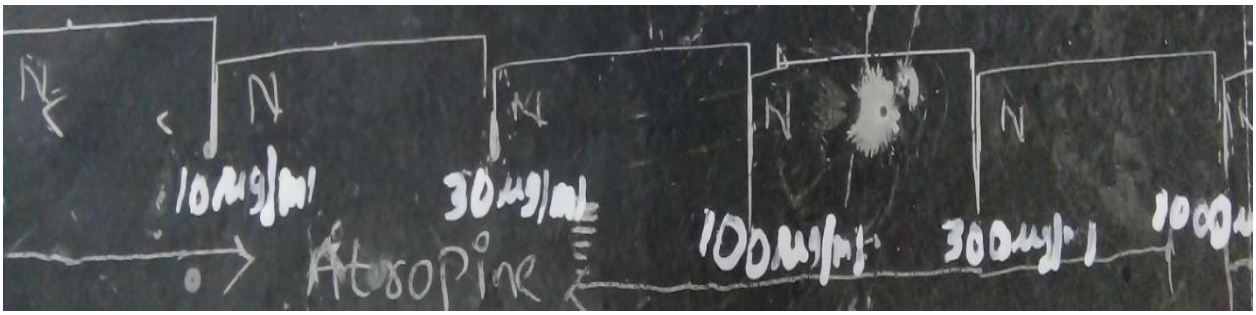


Table 3: Dose response relationship observation of Atropine

S.No.	Drug	Dose (µg/ml)	Response (cm)
1	Atropine	10	0
2	Atropine	30	0
3	Atropine	100	0
4	Atropine	300	0
5	Atropine	1000	0.1

Effect of Methanolic Extract of *Clerodendrum philippinum* showed receptor blocking action (anti spasmodic) as that of standard agent on isolated frog rectus abdominus muscle as shown in Fig:4

Fig 4: Response curve of leaf extract

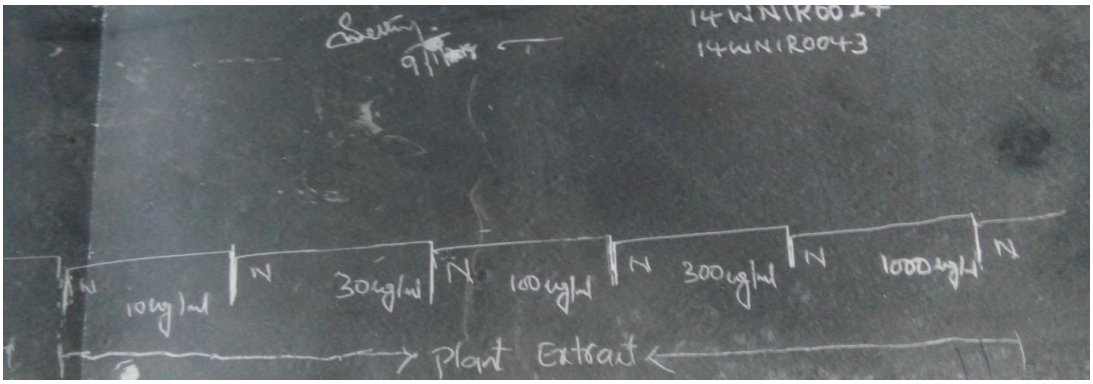


Table 4: Dose Response relationship observation of *Clerodendrum philippinum*.

S.No.	Drug	Dose (µg/ml)	Response (cm)
1	Extract	10	0
2	Extract	30	0
3	Extract	100	0
4	Extract	300	0.1
5	Extract	1000	0.1

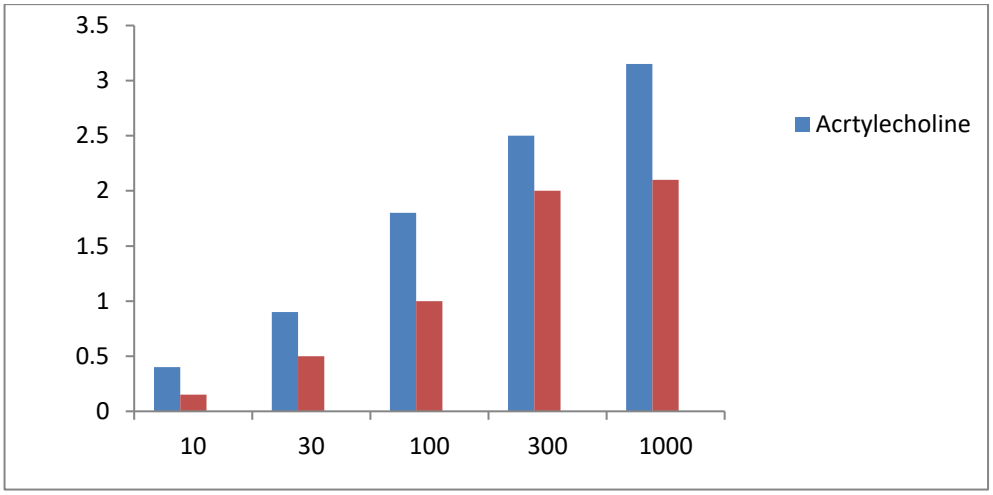
Table: 5 comparative dose responses of Ach followed by Methanolic extract of leaves of *Clerodendrum philippinum*.

S.No.	Treatment Given	Dose (µg/ml)	Response (cm)	% decrease in response
1	Ach	10	0.4	-
2	Ach	30	0.9	-
3	Ach	100	1.8	-
4	Ach	300	2.5	-
5	Ach	1000	3.15	-
6	extract+Ach	10	0.15	62.5
7	extract+Ach	30	0.5	44.4
8	extract+Ach	100	1.0	44.4
9	extract+Ach	300	2.0	20.0
10	extract+Ach	1000	2.1	33.3

$$\% \text{ response} = \frac{\text{Test response} - \text{standard response}}{\text{Standard response}} \times 100$$

Plot a graph between concentration (µg/ml) on x-axis and response (cm) on y-axis

Fig: 5 comparative dose response relationship between acetylcholine and Methnolic extract of the leaves of *Clerodendrum philippinum*



DISCUSSION

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

CONCLUSION

From the above results the present study it was concluded that methanolic extract of the leaves of *Clerodendrum philippinum* exhibits anti-spasmodic activity when compared with a standard antispasmodic agent (atropine). As many anti spasmodic drugs available in market show side effects such as urinary retention, mydriasis, tachycardia, blurred vision and hypersensitivity reactions. Thus, *Clerodendrum philippinum* is a herbal origin drug with high degree of safety and efficacy.

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