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In-vitro Antispasmodic Activity Analysis of Methanolic Leaves Extract of *Lantana camara* Linn. on Excised Rat Ileum.

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ABSTRACT

This study was aimed to provide the pharmacological basis for medicinal use of *Lantana camara* Linn. as an antispasmodic agent using in-vitro pharmacological assay. *Lantana camara* Linn. (*Verbenaceae*), is a widely growing shrub which found to be toxic to some animal species, has been used in the traditional medicine for treating many ailments. The purpose of the present study was to evaluate the antispasmodic effects of *Lantana camara* leaf constituents on rat ileum. Antispasmodic activity was assessed by the interpolation method on isolated rat ileum. Effects of acetylcholine, methanolic extract of *Lantana camara* leaves and acetylcholine along with methanolic leaves extract were studied on isolated rat ileum; which later compared with atropine as standard anti-spasmodic agent. The present study results revealed that methanolic leaves extract of *Lantana camara* Linn. showed promising antispasmodic action on excised rat ileum.

Keywords: Antispasmodic activity, *In-vitro* assay, *Lantana camara* Linn, Methanolic extract.

1. Introduction

Lantana camara Linn. (*Verbenaceae*) is an aromatic shrub, native to tropical America and was introduced in India as an ornamental and hedge plant [1]. Dutch explorers introduced it into the Netherland from Brazil in the late 1600s and later explorers from other countries brought seeds to Europe, Great Britain and North America. Following its introduction into Hawaii as a garden flower, it soon spread to the Islands of the Pacific, Australia and Southern Asia. In a similar way, from Natal it was rapidly spread by birds to the warmer areas of South Africa [2]. Amongst the traditional use of this plant, various parts of it are used in the treatment of cold, headache, uterine haemorrhage, chicken pox, eye injuries, whooping cough, asthma [3], bronchitis and arterial hypertension [4,5]. The root of this plant is also used for the treatment of malaria, rheumatism and skin rashes [6]. The methanolic leaves extract of *Lantana camara* Linn. is claimed to possess biological activities like healing of gastric ulcers and also prevents development of duodenal ulcers in rats [7]. Previously, *L. camara* has been extensively investigated for the phytochemical compositions. Several triterpenoids, naphthoquinones, flavonoids, alkaloids and glycosides isolated from this plant are known to exert diverse biological activities including cytotoxic and anticancer properties. It has been claimed that a steroid, lancamarone from the leaves exhibited cardiotoxic properties and lantamine, an alkaloid from the stem bark and roots showed antipyretic and antispasmodic properties, but the validity of these claims has not been confirmed [2]. The different parts of plant extract were useful in various diseases like diaphoretic, tonic, antispasmodic, wounds, ulcers, swelling, carminative, tumors and rheumatism, anti-tumor [8], anti-inflammatory [9], anti-malarial [10], anti-ulcerogenic, treatment of emotional stress and trauma [11], anti-microbial, nematicidal, insecticidal, fungicidal [12], influenza, asthma [13], antidote to snake venom, eczema [14], gastrointestinal disorders anti-nociceptive, anti-pyretic, inhibitor of acetyl cholinesterase [15], abortifacient [16], anthelmintic, febrifuge, carminative, anti-rheumatic [17], to treat various skin diseases [18], adulticidal activity, larvicidal, biological control activities [19].

2. Materials and Methods:-

2.1 Plant Collection, Authentication and Extraction:

The leaves collection was done in the month of August from side land of green Sahyadri Hills at Degaon village, Dist. Satara, (MS) India. Plant authentication was done by a team of botanists under supervision of Dr. A. B. Pawar (Head of Botany Department, Y. C. Institute of Science), Satara, (MS) India. Collected leaves were shade dried at room temperature for 7 days. The dried leaves of *Lantana camara* Linn. were then grinded to get fine powder using a grinder (Voltas-300). The grinded leaf powder was then assembled in a Soxhlet apparatus for removing impurities with the help of petroleum ether, later powder was removed from Soxhlet apparatus and dried for 5-10min for evaporation of petroleum ether. After evaporation of petroleum ether, powder was again placed in Soxhlet apparatus for extraction with the help of methanol this time.

2.2 Isolation of Rat Ileum:-

Rats were anesthetized and sacrificed by cervical displacement followed by exsanguinations. The ileum was dissected out, immersed in Tyrode's solution and cleaned off the mesentery. Respective segments of 2-3cm long were mounted in a 25ml tissue organ bath, filled with a mixture of 95% O₂ and 5% CO₂ and

maintained at 37 °C. The composition of Tyrode's solution (in mM for 1 lit) was 9 mg KCl, 0.1 mg NaCl, 0.1mg NaHCO₃, 0.42mg NaH₂PO₄, 0.6 mg Glucose and pH value was 7.4.^[20]

2.3 Anti-Spasmodic Activity Assay Procedure:-

1. Firstly concentration dependent responses of acetylcholine were recorded (with dose of 0.1ml, 0.2ml, 0.4ml, 0.8ml, 1.6ml, 3.2ml) using Sherrington's recording drum with a frontal writing lever. Contact time of 60 sec, and base line of 30sec time cycle were opted for proper recording of the responses in presence of plane Tyrode's solution as stock-I solution.
2. Then same concentration dependent responses of acetylcholine (Ach) using same procedure for a mixture of Tyrode's solution+ *Lantana camara* extract (with a concentration of 1mg/ml) as a stock-II solution were recorded.
3. Lastly the same concentration dependent responses of Ach for a mixture of Tyrode's solution+ Atropine (as a standard anti-spasmodic agent) as a stock-III solution were recorded.

3. Observations and Results:-

Effect of Acetylcholine on excised rat ileum reflected an increase in spasmodic activity (response) with an increase in dose as shown in Fig.-1.



Fig 1: Response Curves of Acetylcholine

Table 1: Dose Response Relationship Observations of Acetylcholine.

Sr. No	Drug	Dose	Response (cm)
1	Acetylcholine	0.1 ml	1.5 cm
2		0.2 ml	2.3 cm
3		0.4 ml	3.3 cm
4		0.8 ml	3.8 cm
5		1.6 ml	4.1 cm

Acetylcholine induced spasm followed by treatment of methanolic extract of *Lantana camara* showed prominent antispasmodic activity as depicted in Fig.-2.

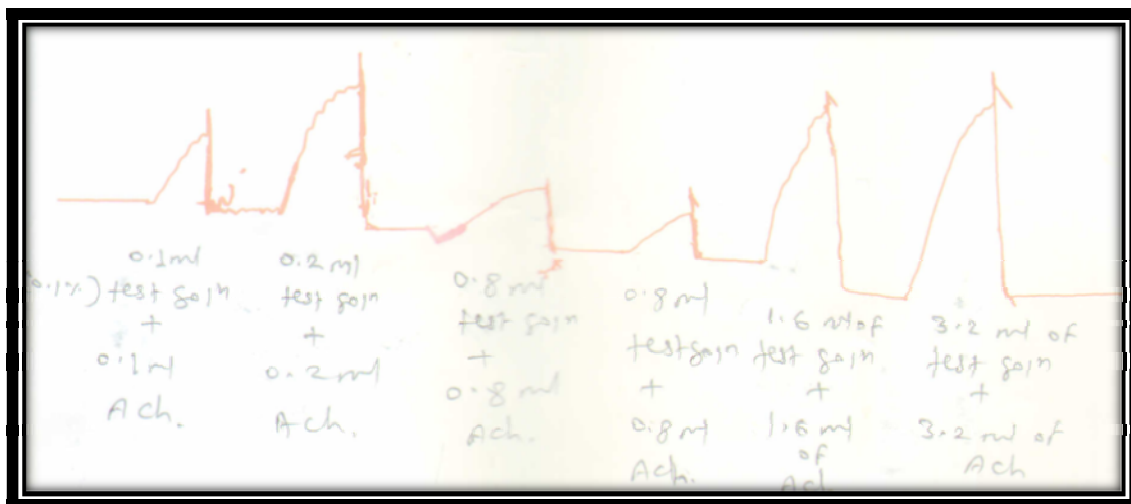


Fig 2: Response Curves of Acetylcholine + Leaves Extract

Table 2: Dose Response Relationship Observations of Acetylcholine and Extract.

Sr. No	Drug	Dose	Response (cm)
1	Acetylcholine + Extract	0.1 ml + 0.1 ml	1 cm
2		0.2 ml + 0.2 ml	1.8 cm
3		0.8 ml + 0.8 ml	0.9 cm
4		1.6 ml + 1.6 ml	2.2 cm
5		3.2 ml + 3.2 ml	2.4 cm

While treatment of anti-cholinergic drug Atropine (which is referred here as standard antispasmodic agent) showed expected receptor blocking action (antispasmodic) on isolated rat ileum as shown in Fig. : 3.

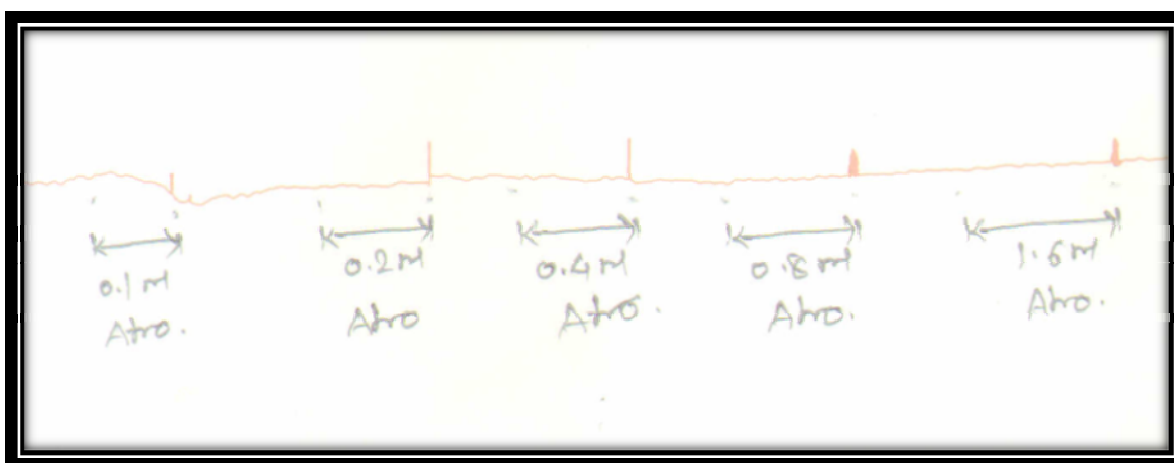
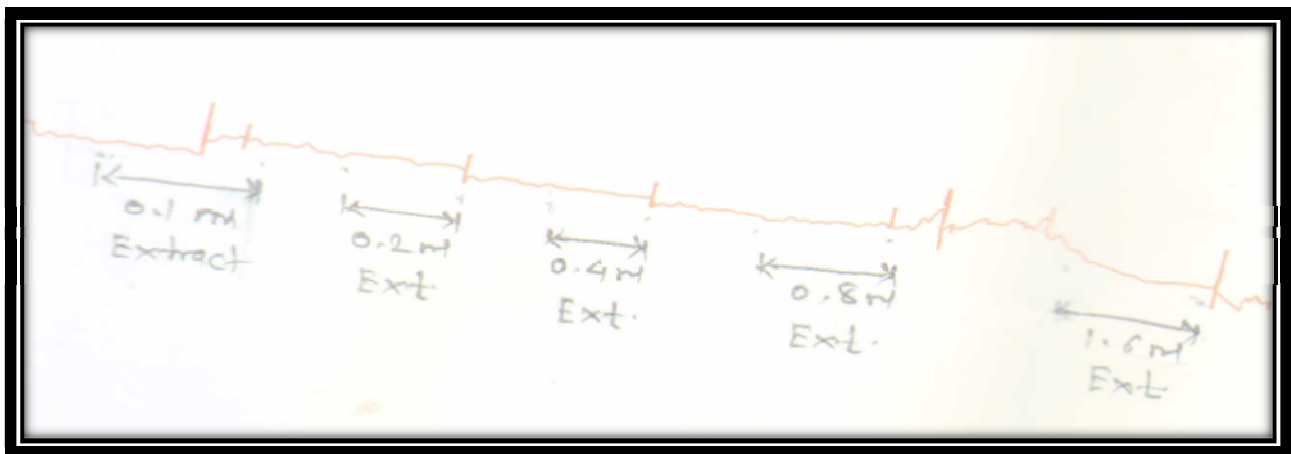


Fig 3: Response Curves of Atropine.

Table 3: Dose Response Relationship Observations of Atropine.

Sr. No	Drug	Dose	Response (cm)
1	Atropine	0.1 ml	–
2		0.2 ml	–
3		0.4 ml	–
4		0.8 ml	–
5		1.6 ml	–

Also treatment of methanolic extract of *Lantana camara* showed receptor blocking action (antispasmodic) as that of standard agent on isolated rat ileum as shown in Fig. No.:-4.

**Fig 4:** Response Curves of Methanolic Extract of *Lantana camara*.**Table 4:** Dose Response Relationship Observations of *Lantana camara*.

Sr. No	Drug	Dose	Response (cm)
1	<i>Lantana camara</i> Methanolic Extract	0.1 ml	–
2		0.2 ml	–
3		0.4 ml	–
4		0.8 ml	–
5		1.6 ml	–

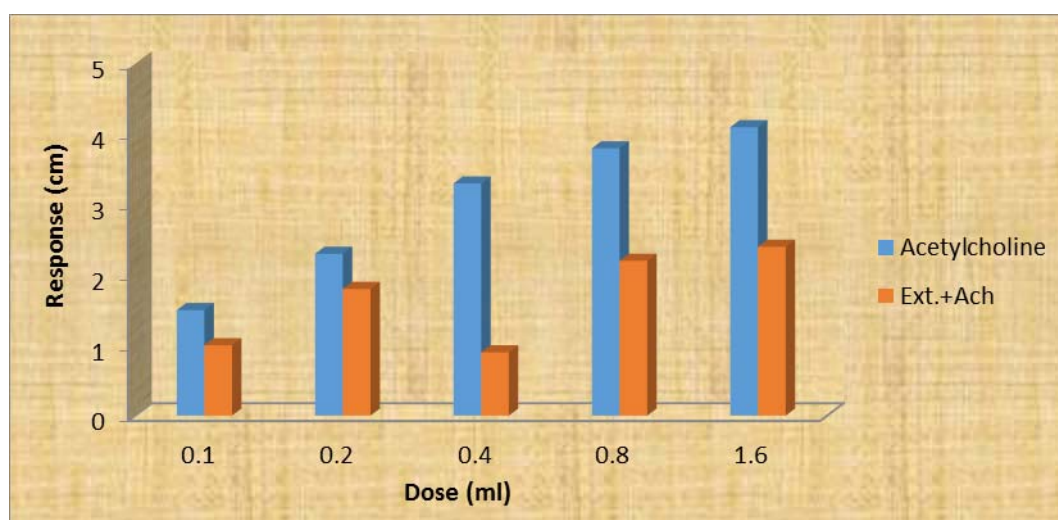
4. Discussion

From the present study results it was observed that acetylcholine (Ach) alone causes contraction of excised rat ileum but when acetylcholine was given in presence of methanolic leaves extract of plant *Lantana camara* Linn., there was a marked decrease in

contraction of ileum was observed. This revealed that methanolic leaves extract of *Lantana camara* possess a high degree of spasmolytic (anti-spasmodic) activity by blocking cholinergic receptors.

Table 5: Comparative Dose Responses of Ach and Ach Followed by Methanolic Leaves Extract of *Lantana camara*.

Sr. No.	Treatment Given	Dose (ml)	Response	% Decrease in Response
1	Acetylcholine	0.1	1.5 cm	-
2		0.2	2.3 cm	
3		0.4	3.3 cm	
4		0.8	3.8 cm	
5		1.6	4.1 cm	
6	Methanolic extract + Acetylcholine	0.1 + 0.1	1 cm	33.34
7		0.2+0.2	1.8 cm	21.74
8		0.4+0.4	0.9 cm	72.73
9		0.8+0.8	2.2 cm	42.11
10		1.6+1.6	2.4cm	41.47

**Fig 5:** Comparative dose response relationship of Acetylcholine and methanolic leaves extract of *Lantana camara* on excised rat ileum.

5. Conclusions

From all observations and results obtained for the present study it was concluded that methanolic leaves extract of *Lantana camara* Linn. (Ghaneri) exhibits promising anti-spasmodic activity. Also when compared with a standard anti-spasmodic agent (atropine), it was found that *Lantana camara* has comparatively less potent spasmolytic activity than atropine. As many anti-spasmodic drugs available in market shows side effects such as urinary hesitancy, urinary retention, mydriasis, tachycardia, blurred vision and hypersensitivity reactions; so, *Lantana camara* being a herbal origin drug with high degree of safety and efficacy could be a suitable alternative to existing drugs, as well as could be a new member of antispasmodics family.

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