ejpmr, 2023, 10(5), 420-423

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

INVESTIGATION OF ANTI-SPASMODIC POTENTIAL OF *CLEOME GYNANDRA* ON ISOLATED CHICKEN INTESTINE

Sathya B.*, Rajesh M., Renuka M. and Rukshana Begum N.

Department of Pharmacology, Surya School of Pharmacy, Surya Group of Institutions, Vikravandi, Villupuram, Tamil Nadu, India.

***Corresponding Author: Sathya B.**

Department of Pharmacology, Surya School of Pharmacy, Surya Group of Institutions, Vikravandi, Villupuram, Tamil Nadu, India.

Article Received on 13/03/2023 **Article Revised on 02/04/2023** **Article Accepted on 23/04/2023**

ABSTRACT

The present study was aimed to investigate the anti-spasmodic effect of *Cleome gynandra* leaves of the family Cleomaceae on isolated chicken ileum by an *in vitro* pharmacological assay. *Cleome gynandra* is richly obtainable throughout India with countless hidden therapeutic values**.** It is used in traditional system for epilepsy, protozoal and worm infections, irritable bowel syndrome and as an antioxidant. The spasm was induced by acetycholine, the spasmogen. The ethanolic extract of *Cleome gynandra* leaves was prepared by simple maceration. Anti-spasmodic activity was assessed by antagonistic method of bioassay on chicken ileum using Tyrode as physiological salt solution. Effects of acetylcholine and acetylcholine followed by ethanolic leaves extract were studied on isolated chicken ileum and compared with atropine, the standard anti-spasmodic agent. The extract revealed marked decrease in acetylcholine induced spasm (the cholinolytic like effect) on chicken ileum induced by acetylcholine. Action of flavanoids and steroids on M_3 receptors in the chicken ileum granted to the effect. In conclusion, the ethanolic extract of leaves of *Cleome gynandra* plant manifested optimistic antispasmodic action on chicken ileum.

KEYWORDS: Acetylcholine, Atropine, Anti-spasmodic, *Cleome gynandra*, Chicken ileum.

INTRODUCTION

Multiple numbers of beneficial medicinal plants are utilized for food, clothing, fuel, shelter, and other essentials of sustainable life.[1] *Cleome gynandra*, from a wide variety of therapeutic plants, has been discovered propagating in great numbers as a weed in common agricultural fields and barren soil.[2,3] Cleome is a significant group of angiosperms, belonging to the family Cleomaceae (formerly known as the Capparaceae) contains many species that can be found in tropical and subtropical regions of the world. The Cleomaceae family of flowering plants from Brassicales (or Cruciales) order, contains more than 764 species in 12 genera, the largest of which is Cleome, which has about 601 species of ecological, ethnobotanical, and medicinal importance.[4] Work on the Cleome genus is incredibly insufficient and dispersed. Particularly, the anatomical and physiological investigations in these species are rare.[5] *Cleome gynandra* typically grows on fertile soils, especially those that had previously been blended with livestock fertilizer or with farmhouse disposed of waste. Temperatures of 25^{o} C, high intensity light, and the right amount of soil moisture are the ideal growing conditions for *Cleome gynandra*. It is used in various traditional culinary systems for its remarkable antioxidant and nutritious properties, as well as in traditional medicine for the treatment of many ailments.[6-8]

Cleome gynandra possess analgesic, rubefacient, vesicant, antimicrobial, anti-inflammatory, anthelminthic, and immunomodulatory activities. The plant extract has antioxidant, antibacterial, antimycotic, and anticancer effects.[9-11] In addition to the blossoms, the tender leaves or young shoots are sometimes cooked and served as a delightful side dish, relish, or potherb. Fresh leaves are utilized as component in other mashed foods, while dried leaves are ground and added in weaning foods.[12] This study sighted on evaluation of spasmolytic effect of *Cleome gynandra* leaves extract on acetylcholine induced muscle contractions of isolated chicken ileum preparation.

MATERIALS AND METHODS

Plant Collection and Authentication

Fresh leaves of *Cleome gynandra* were collected locally from Villupuram District, Tamil Nadu, India and authenticated by DR.M.SIVARAMAN, M.Sc.,M.Ed., M.Phil., Ph.D., Assistant Professor, PG & Research Department of Botany**,** Arignar Anna Govt. Arts College, Villupuram, Tamil Nadu. The leaves were purified of adulterants, dried in the shade, and roughly ground into powder.

Preparation of Ethanolic Extract

The powdered material of *Cleome gynandra* leaves (50 g) were macerated with 250ml of ethanol for a week in a closed container to prevent the ethanol from evaporating, with occasional mixing. On the eighth day, the liquid mixture was filtered, and the solid residue, known as the marc, was gently crushed to collect as much solution as possible. The resulting liquid was properly mixed before being dried at 40°C. The extract was dried up to complete evaporation of and stored in an airtight container for future pharmacological studies.[13]

Fig. 1: *Cleome gynandra* leaves

Fig. 2: Simple Maceration

Procurement of Chicken Intestine

Chicken ileum for the study was procured from local market in Vikravandi, Villupuram District.

Drugs and Chemicals

Acetylcholine, Sodium chloride, Potassium chloride, Calcium chloride, Magnesium chloride, Sodium bicarbonate, Sodium hydrogen orthophosphate and glucose were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Atropine sulphate was procured from Hindustan Medicines Pvt. Ltd., Barauni, India.

Anti-Spasmodic Activity

Acetylcholine induced contraction of chicken ileum preparation (*In vitro* assay)

The fresh intestine of a healthy chicken was procured from nearby butcher and washed with Tyrode solution to remove the mesentery. The 2 cm long terminal section of the ileum was prepared and placed in a 20ml Tyrode solution-filled tissue bath. The tissue was aerated using a mixture of 95% oxygen and 5% carbon di-oxide with an aerator maintained at 37°C. The Tyrode solution employed in the study was composed of 2.7 mM KCl, 137 mM NaCl, 1.8 mM $CaCl_2$, 0.1 mM Mg Cl_2, 11.9 mM NaHCO3, 0.4 mM NaH_2PO_4, 5.55 mM Glucose, in mM for 1 liter and pH was maintained at 7.4. For recording each response, a drum speed of 0.25 rpm, contact time of 60 sec, baseline of 30 sec, and a 5-minute time cycle were followed. The dose-dependent responses of acetylcholine were recorded initially on kymograph paper. Followed to which the cumulative concentration-effect curves were recorded for acetylcholine in presence of ethanolic extract of *Cleome gynandra* (1 mg/ml), the test drug and atropine sulphate (1 mg/ml), a standard drug with Sherrington's rotating drum. By comparing the dose with the height of the response from the curve, the percentage decrease in response to the extract and atropine sulphate was calculated and graphed.[14,15]

RESULTS AND DISCUSSION

Extraction of *Erythrina variegata* leaves

Ethanolic extract of *Cleome gynandra* (EECG) was found greenish brown in colour with sticky mass consistency. The percentage yield of the EECG was 17.60 % W/W as shown in table 1.

Table: 1 Physical characteristics of ethanolic extract of *Erythrina variegata* leaves.

S. No.	Name of Extract	Colour	Consistency	%W/W
1	EECG	Greenish brown	Sticky mass	17.60

Anti-spasmodic activity of ethanolic extract of *Erythrina variegata* leaves

Acetylcholine induced contraction of chicken ileum preparation

Acetylcholine produced dose dependant increase in contraction of smooth muscles on chicken ileum preparation at 1 mg/ml concentration. This was significantly inhibited by ethanolic extract of leaves of *Cleome gynandra* at 1 mg/ml. The effect of extract was found nearer to the standard drug atropine at concentration of 1 mg/ml as shown in table 2. This illustrates that there was competitive antagonism between acetylcholine and *Cleome gynandra* extract (Figure 3).

Table 2: Dose response relationship observations of acetylcholine, ethanolic extract of *Cleome gynandra*, and atropine on chicken ileum.

S. No.	Drug	Dose (ml)	Response (cm)
1.	Acetylcholine	0.1	2.2
2.		0.2	2.9
3.		0.4	3.6
4.		0.8	4.3
5.		1.6	5.1
6.	Acetylcholine + *Cleome gynandra*	0.1 + 0.1	0.8
7.		0.2 + 0.2	1.1
8.		0.4 + 0.4	1.4
9.		0.8 + 0.8	1.7
10.		1.6 + 1.6	1.9
11.	Acetylcholine + Atropine	0.1 + 0.1	0.05
12.		0.2 + 0.2	0.06
13.		0.4 + 0.4	0.08
14.		0.8 + 0.8	0.09
15.		1.6 + 1.6	0.1

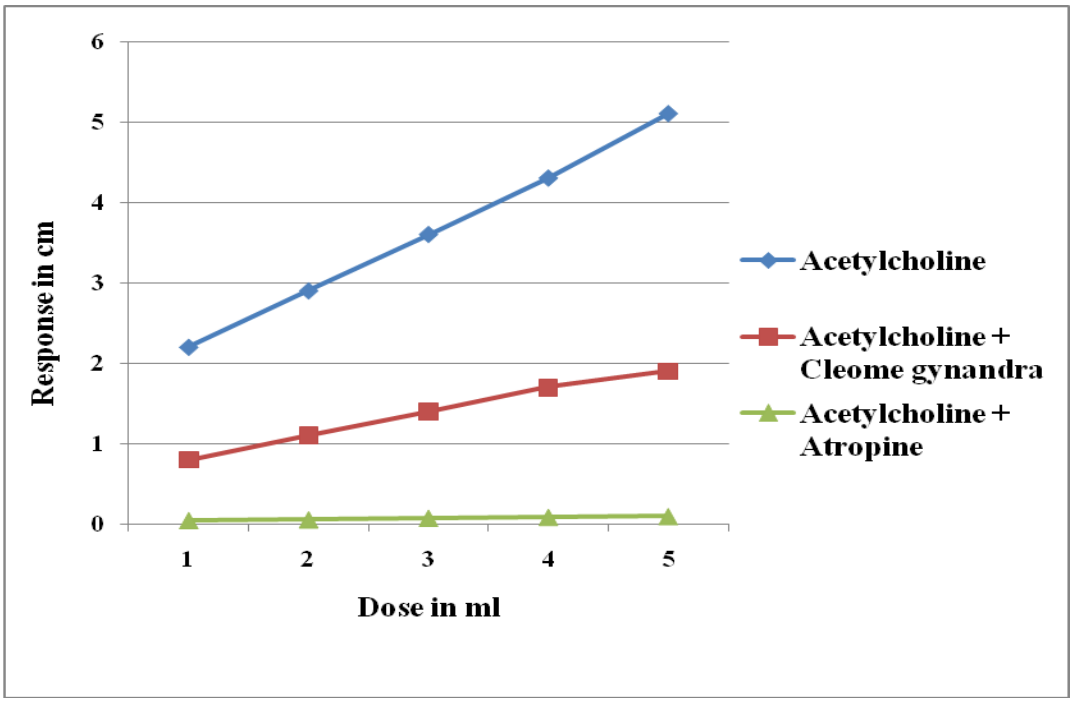

Fig. 3: Comparative dose response relationship of acetylcholine, ethanolic extract of *Cleome gynandra*, and atropine on chicken ileum.

DISCUSSION

For screening the activity of a drug on intestinal smooth muscles, chick ileum preparations can be used. Chicken intestine is easier to acquire, handle and easier to dissect and has the same reactions to spasmogenic and spasmolytic drugs.[16] Cholinergic agonists like acetylcholine elicit a contractile response in isolated chicken ileum.[17] M_3 receptor, a subtype of cholinergic (muscarnic) receptor activation causes contraction of intestinal smooth muscle. The M_3 receptor function through Gq protein and trigger membrane bound phospholipase C (PLc) provoking inositol triphosphate (IP_3) and diacylglycerol (DAG) which in succession release Ca^{2+} intracellularly leads to actin-myosin phosphorylation causing increased smooth muscle tone.[18] Thus, the contraction of intestinal smooth muscle *in vitro* has often been utilized for the study of contractile/dilator responses of agonists as well as antagonist. In current investigation, acetylcholine showed greater contraction while *Cleome gynandra* leaves extract significantly inhibited the acetylcholine induced contraction on isolated chicken ileum preparation. The parallel shift of graph towards right side in acetylcholine dose-response curves in the presence of increasing concentrations of *Cleome gynandra* leaves extract indicating that there was competitive antagonism between acetylcholine and *Cleome gynandra* extract for M_3 receptors present on the smooth muscle. This effect may be due to its antimuscarinic or antispasmodic activity. Acetylcholine elicited contractions in ileum are

believed to be mediated through M_3 receptors present on ileum ref. It was found that *Cleome gynandra* produced dose dependent inhibition of ileum contractions induced by acetylcholine. The parallel rightward shift in agonist concentration response curves of acetylcholine in presence of increasing concentrations of *Cleome gynandra* leaves extract was indicating antispasmodic activity (anti-muscarnic). The inhibition may be due to the antagonism of cholinergic-muscarnic receptors or nonspecific spasmolytic action of *Cleome gynandra* leaves.

CONCLUSION

It can be concluded from the study that the ethanolic extract of *Cleome gynandra* leaves possesses significant anti-spasmodic activity that may be credited due to the M_3-antagonism (cholinergic-muscarnic-antagonism) on smooth muscles of chicken ileum. This may substantially benefit in the treatment of spasm and other intestinal muscular disorders.

ACKNOWLEDGEMENT

The authors are grateful to the Chairman, Dr. P. Gauthama Sigamani and Secretary, Dr. P. Ashok Sigamani, Surya Educational Trust, Villupuram, Tamil Nadu, India, for providing essential infrastructure and resources to accomplish this work in success.

REFERENCES

1. Adhikari PP, Talukdar S, Borah A. Ethnomedicobotanical study of indigenous knowledge on medicinal plants used for the treatment of reproductive problems in Nalbari district, Assam, India. J Ethnopharmacol, 2017; 210: 386-407.
2. Adhikari PP, Paul SB, Choudhury MD, Choudhury S. GC-MS studies on the steam-distillate of the medicinally important plant *Cleome gynandra* L. Int J Appl Res Stud, 2014; 3: 1-4.
3. Aparadh VT, Mahamuni RJ, Karadge BA. Taxonomy and physiological studies in spider flower (Cleome species): A critical review. Plant Sci Feed, 2012; 2: 25-46.
4. Ravichandra B, Ram PS, Saritha C, Shankaraiah P. Anti-diabetic and anti-dyslipidemia activities of *Cleome gynandra* in alloxan induced diabetic rats. J Pharm Toxicol, 2014; 9: 55-61.
5. Kori ML, Gaur K, Dixit VK. Investigation of immunomodulatory potential of *Cleome gynandra* Linn. Asian J Pharm Clin Res, 2009; 2: 35-9.
6. Albarello N, Simões C, Rosas PF, Castro TC, Gianfaldoni MG, Callado CH, *et al*. *In vitro* propagation of *Cleome spinosa* (Capparaceae) using explants from nursery-grown seedlings and axenic plants. In Vitro Cell Dev Biol Plant, 2006; 42: 601-6.
7. Mishra SS, Moharana SK, Dash MR. Review on *Cleome gynandra*. Int J Res Pharm Chem, 2011; 1: 681-9.
8. Chweya JA, Mnzava NA. Cat's Whiskers, *Cleome gynandra* L. Vol. 11. Rome, Italy: Bioversity International, 1997.
9. Arts ICW, Hollman PCH. Polyphenols and disease risk in epidemiologic studies. Am J Chin Nutr, 2005; 81: 317-325.
10. Bala A, Kar B, Haldar PK, Mazumder UK, Bera S. Evaluation of anticancer activity of *Cleome gynandra* on Ehrlich's Ascites Carcinoma treated mice. J Ethnopharmacol, 2010; 129(1): 131-134.
11. Rastogi RP, Mehortra BN. Compendium of Indian Medicinal Plants, Publications & Information Directorate CSIR, New Delhi, India, 1996, 5, 224.
12. Adhikari PP, Paul SB. Medicinally Important Plant *Cleome Gynandra*: A Phytochemical and Pharmacological Explanation. Asian J Pharm Clin Res, 2018; 11(1): 21-29.
13. Santhiya N, Priyanga S, Hemmalakshmi S, Devaki K. Phytochemical analysis, Anti inflammatory activity, *in vitro* antidiabetic activity and GC-MS profile of *Erythrina variegata* L. bark. J Appl Pharm Sci, 2016; 6(07): 147-155.
14. Ghodake PP, Kulkarni AS, Aloorkar NH, Osmani RA. *In-vitro* Antispasmodic Activity Analysis of Methanolic Leaves Extract of *Lantana camara* Linn. on Excised Rat Ileum. J Pharmacogn Phytochem, 2013; 2(3): 66-71.
15. Aswathy C, Haridas H, Asna KA, Irshad M, Raihana P, Priyanka P. Evaluation of in vitro Anti Spasmodic Effect of *Michelia Champaca* Stem Bark. World J Pharm Res, 2020; 9(12): 1345-1351.
16. Undale VR, Jagtap PN, Yadav AV, Sangamnerkar SK, Upasani CD, Bhosale AV. An isolated chicken ileum: Alternative to laboratory animals for isolated tissue experimentation. IOSR J Pharm, 2012; 2(5): 39-45.
17. Chinnappan S, Mogana R, Qin TX. In vitro Antimotility and Antispasmodic effects of *Nephelium lappaceum* on isolated chicken ileum. Res J Pharm Technol, 2020; 13(9): 4346-4350.
18. Tripathi KD. Cholinergic System and Medicine. Essential of Medical Pharmacology. 7th ed. New Delhi: Jaypee Brothers Medical Publishers Private Ltd, 2013; pp. 102.