Research Article

In-vitro Spasmomimetic effect of *Annona squamosa* L on Isolated Chicken Ileum

Lakshmi Kanta Kanthal*, Suman Pattanayak, Sudipta Rani Bera, Payel Jana, Kunal Roy, Monish Ghanta, MD Anowar Hossain
Haldia institute of Pharmacy, Haldia, Purba Medinipur, West Bengal- 721657, India.
***Corresponding author's E-mail:** lkkhaldia@gmail.com

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ABSTRACT

The current study aims to assess the spasmodic property of methanolic extract of aerial parts of *Annona Squamosa* L. on segments of chicken ileum preparation. The aerial parts extract was prepared by employing simple maceration method using methanol as menstrum. The four different graded doses (0.1 ml., 0.2 ml., 0.4ml., 0.8ml.) of acetylcholine and plant extracts were utilized to trigger the contractile responses which are recorded on kymograph paper. The methanolic extract of *Annona Squamosa* L. (0.8 ml.) and Acetylcholine (0.8 ml.) showed maximum Spasmomimetic effect as 2.1 cm. &1.7 cm. responses on kymograph paper. The results thus revealed the promising Spasmomimetic potential of *Annona Squamosa* L. aerial parts. *Annona Squamosa* L. is therefore recommended as a spasmodic medicine in the pharmacy research field and can be established as a novel medicinal property-containing substance, as shown by the results.

Keywords: Spasmomimetic, Acetylcholine, Chicken ileum, *Annona squamosa* L.

INTRODUCTION

Annona squamosa L. or sweetsop is a small tropical deciduous tree of about 3-6 m tall with slender branches. It has cone-shaped fruit. Fruit color is recorded as light green, green, yellowish green, yellowish red and reddish as per descriptor[1]. The leaves consist of two colors, namely brilliant green on the top and bluish green on the underside of the leaves also has petiole that can reach 0.7 - 1.5 cm, while the leaf shape can be oval or elliptical[2,3]. The flower greenish, fleshy, drooping, extra-axillary, more on leafy shoot than on the older wood and tending to open as the shoot elongates. The seeds 1.3–1.6 cm long, oblong, smooth, shiny, blackish or dark brown. Stem is irregular branches with thin gray bark[4,5].

A. squamosa is widely distributed in tropical and subtropical countries in Asia such as Malaysia, Laos, Thailand and Vietnam. In India, the plant can be observed in W.B., Madhya Pradesh, Tamil Nadu, Assam, Bihar, Telangana[6].leaves of *A. squamosa* are used as a vermicide, for treating cancerous, tumors and are applied to abscesses, insect bites and other skin complaints[7].The plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problem, warm infection, constipation, hemorrhage, antibacterial infection, dysuria, fever and ulcers. It also has anti fertility, antitumor properties[8]. Leaves of custard apple are widely used in many places as a popular dietary supplement for the treatment of diabetes[9].

MATERIALS AND METHODS

Collection and Authentication of plant material

Based on the literature survey, one plant named *Annona Squamosa* L. was selected for study. The aerial of *Annona Squamosa* L. was collected in the month of March, 2023 from various areas of Haldia, Purba Medinipur District, West Bengal and Authenticated by Scientist-in-charge, Central National Herbarium, Botanical Survey of India, Kolkata, West Bengal, India. Herbarium was prepared and preserved in the Department of Pharmacognosy, Haldia Institute of pharmacy, Haldia, Purba Medinipur, West Bengal, India.

Preparation of extract

The selected plant products were extracted by cold maceration method. 71.6 g of dried (aerial parts) powder of *Annona Squamosa* L. was extracted with 350 ml methanol, then the product was filtered. And after that the extract was dried and kept for further use[9].

Preparation of Tyrode solution

Weighed accurate quantity of all the ingredients mentioned in below table. Mix all the ingredients in a small amount of water until it dissolves and make up the volume to 1000ml[10].

Tyrode's solution is prepared by dissolving 8.0 grams of sodium chloride (NaCl), 0.2 grams of potassium chloride (KCl), 0.2 grams of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), 0.1 grams of magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), 1.0 grams of sodium bicarbonate ($NaHCO_3$), and 1.0 grams of glucose in distilled water. The pH of the solution is then adjusted to around 7.4 using hydrochloric acid (HCl) or sodium hydroxide (NaOH). The volume is brought to 1 liter with distilled water, and the solution is sterilized by filtering through a 0.22 µm filter or autoclaving. Tyrode's solution is commonly used in physiological experiments and mimics the ionic composition of extracellular fluid.

Experimental procedure

The fresh entire gastrointestinal tract of a healthy cock was obtained from Haldia for the experiment. The caecum was

carefully lifted forwards to identify the ileocecal junction. A few centimeters of the ileac portion were then cut and immediately placed in a watch glass containing physiological salt solution. The mesentery and adhering tissues were gently removed to avoid any damage to the gut muscle.

The ileum was then cut into small segments of 2-3 cm long. One piece of the ileum had a thread tied to its top and bottom ends without closing the ileum. This piece was mounted in an organ bath containing Tyrode's solution maintained at 32-35°C and bubbled with air. A magnification of 5-7 folds and a bath volume of about 25 ml were maintained, and the tissue was allowed to equilibrate for 30 minutes before adding acetylcholine to the organ bath. Acetylcholine induced contractions in the ileac smooth muscles, which were recorded on a kymograph using a frontal writing lever.

A contact time of 30 seconds and a 5-minute time cycle were kept for proper recording of the responses. The contraction response curve (CRC) was recorded until a ceiling effect to acetylcholine was obtained, and the height of the response was measured. Various parameters such as magnification value 3 & 5, load/tension 0.5, 1.0 & 1.5 gm, and tissue length 1.5, 2.0 cm were changed, and responses were recorded.

The experiment was repeated in the presence of 100µg/ml methanolic extract of *Annona squamosa*. The log doses and response height in mm were calculated, and the concentration-response curves of acetylcholine in the absence and presence of the test drugs were plotted with log doses on the X-axis and response height on the Y-axis, respectively. The effect of acetylcholine and the methanolic extract of *Annona squamosa L*. was observed on chicken ileum, and the dose-response curve (DRC) or CRC (contraction response curve) was prepared based on the experimental data[11, 12].

RESULT AND DISCUSSION

The four different doses i.e., 0.1 ml (10µg), 0.2 ml (20 µg), 0.4 ml (40µg) & 0.8ml (80 µg) of Acetylcholine showed contraction responses on chicken ileum i.e.,0.1cm,0.2 cm, 0.8 cm & 1.7 cm, respectively. Four doses of A.S.L. (*Annona Squamosa* L.) extracts i.e., 0.1 ml (10 µg),0.2 ml (20 µg),0.4 ml (40 µg),0.8 ml (80 µg) showed significant contraction responses on chicken ileum i.e., 0 cm, 0.3 cm, 1.1 cm & 2.1 cm, respectively.

Table 1: Doses & Responses of Acetylcholine and *Annona Squamosa* L. on chicken ileum preparation:

SL NO.	Drug/Sample	Dose **(Concentration)**	Height of Response **(cm)**	Log Dose
1	Acetylcholine	0.1ml (10µg)	0.1	1.0
2	Acetylcholine	0.2ml (20µg)	0.2	1.3
3	Acetylcholine	0.4ml (40µg)	0.8	1.6
4	Acetylcholine	0.8ml (80µg)	1.7	1.9
5	Extract	0.1ml (10µg)	0	1.0
6	Extract	0.2ml (20µg)	0.3	1.3
7	Extract	0.4ml (40µg)	1.1	1.6
8	Extract	0.8ml (80µg)	2.1	1.9

The response of Acetylcholine (Ach) on chicken ileum observed that an increase in response (spasmodic activity) with an increase in dose. DRC (Dose Response Curve) of Ach shown in figure 1.

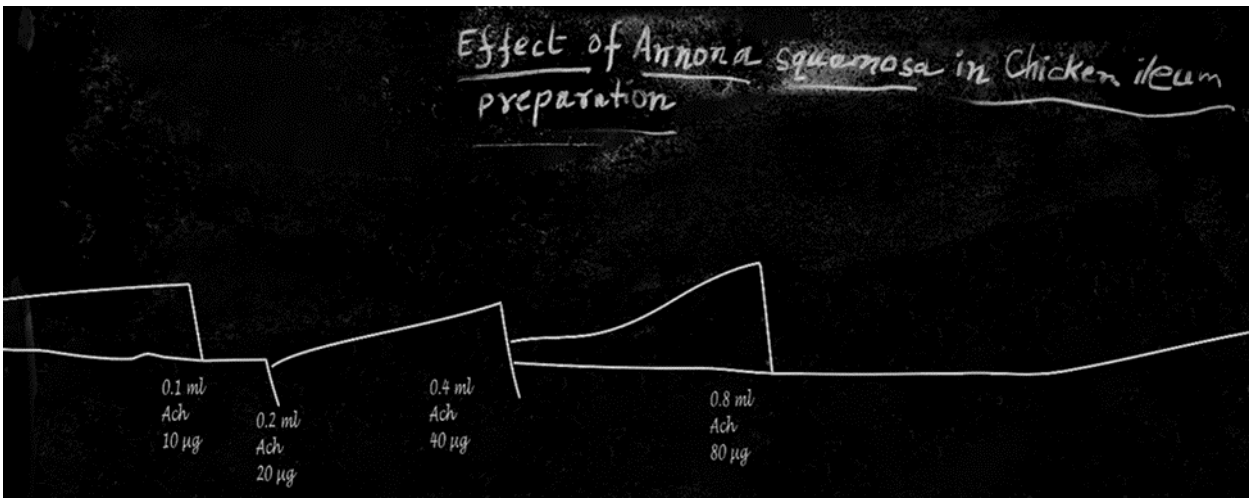

Figure 1: Dose of Acetylcholine on chicken ileum.

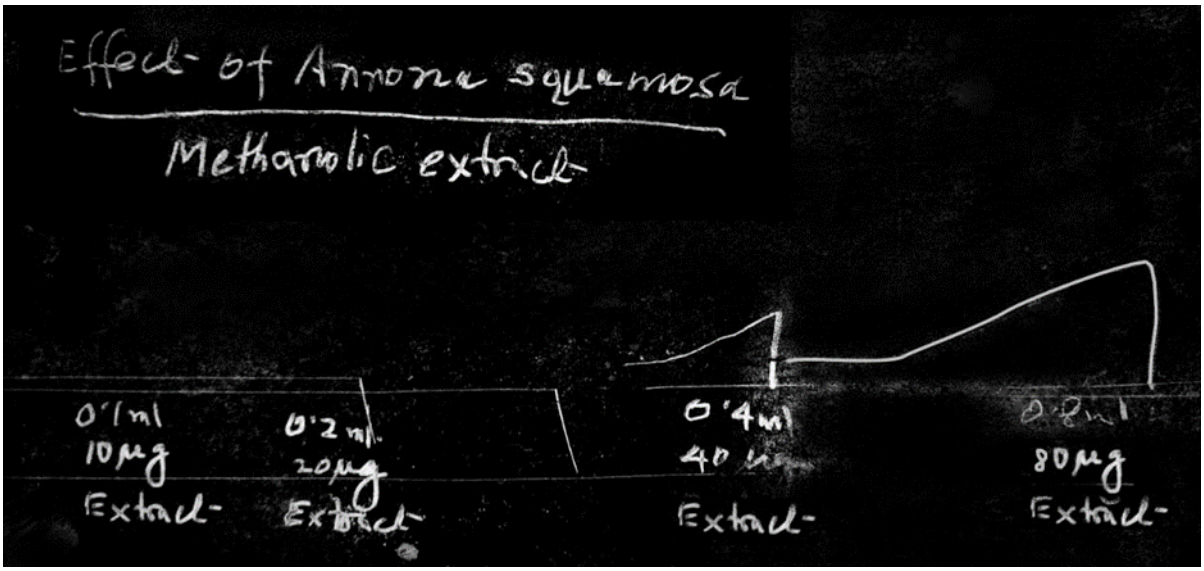

Figure 2: Effect of *Annona Squamosa* L. extract on chicken ileum.

The response of methanolic extract of *Annona Squamosa* L on chicken ileum preparation shows a marked increase in spasm. It is compared with spasmodic effect produced by the standard drug Acetylcholine

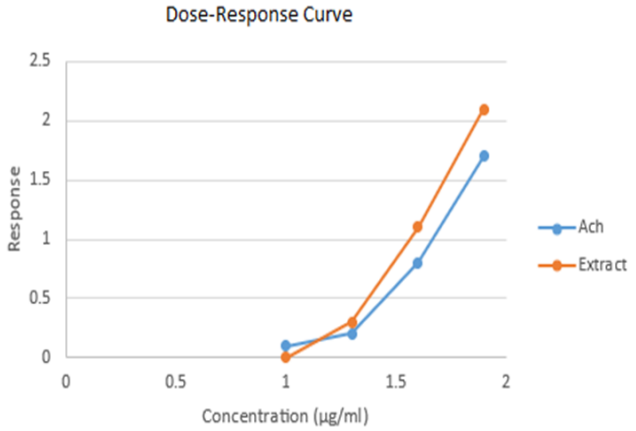

Figure 3: Log dose vs. Response curve of Ach & A.S.L extract

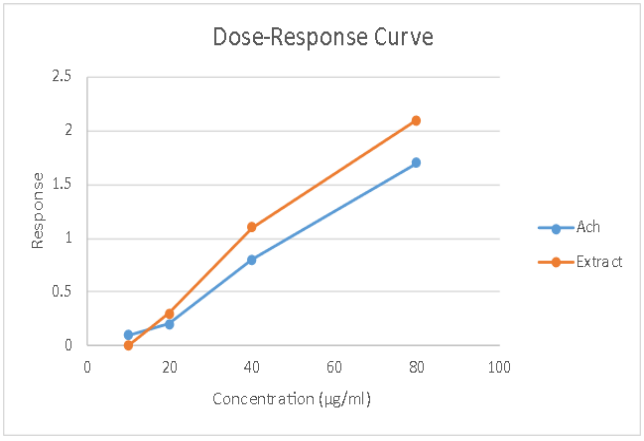

Figure 4: Dose-Response curve of Ach & A.S.L extract.

CONCLUSION

From all observations & results obtained for the present study it was concluded that the methanolic aerial parts extract of A.S.L. (*Annona Squamosa* L.) exhibits promising spasmodic activity. Also, when compared with a standard spasmodic agent (Acetylcholine), it was found that A.S.L has comparatively more potent Spasmomimetic or spasmodic activity than Acetylcholine. Annona Squamosa L. showed significant spasmodic property in the present research work but which one or more than one phytoconstituents are responsible for this pharmacological activity should be performed in future research work.

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