In- Vitro Spasmolytic Effect of Ethanolic Extract of Calotropis Gigantea Leaves on Isolated Chick Ileum"- A Research Article

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***Abstract*—The Calotropis gigantea (Asclepias gigantea), or giant milk weed, or crown flower is a species of Calotropis, which is native to Bangladesh, Indonesia, Sri Lanka, Malaysia, Thailand, India, China, Pakistan, Nepal, and Cambodia. It belongs to the family Apocynaceae. The plant's bark, roots, latex, leaves, and flowers are all recognized to have a variety of therapeutic uses. The study's objective was to use invitro experimental models to evaluate the ethanolic extract of Calotropis gigantea leaves spasmolytic activity in relation to atropine. The smooth muscle of the chicken ileum is extremely sensitive to pharmacological substances, especially those that interact with serotonergic, histaminergic and muscarinic receptors. It is a great model for researching the effects of agonists and antagonists because of its sensitivity. In this research, we use chicken ileum to evaluate the spasmolytic effect of ethanolic extract of calotropis gigantea leaves. The 100ug/ml of extract inhibited the actions of Acetylcholine due to presence of calotropin like cardiac glycoside.**

***Index Terms*—Spasmolytic effect, Calotropis gigantea, Smooth muscle relaxant, Chicken ileum.**

I. INTRODUCTION

An abrupt, involuntary contraction of a muscle, muscle group, or hollow organ, such the bladder, is called a spasm. Even though they are fleeting, spasms can be excruciating. Muscle spasms, often known as cramps, happen when your muscles tighten uncontrollably and involuntarily, making it impossible for them to relax. Spasms of the muscles are common and typical. Centrally acting muscle relaxants and antispasmodic medications are other names for spasmolytic medications. A type of drug called a spasmolytic agent lessens the body & excessive smooth muscle contraction and is frequently used to treat symptoms like pain and cramping in the abdomen that are linked to diseases like IBS [5]. Spasmolytics are medications that help alleviate painful, involuntary muscle contractions by relaxing skeletal muscles. A wide variety of therapeutic classes with distinct modes of action are included in spasmolytic medications, such as calcium channel blockers (which block the passage of ca 2+ ions into GI smooth muscle) and anticholinergic/antimuscarinic agents (which prevent the contraction of GI smooth muscle) [6]

Calotropis gigantea (Asclepias gigantea), also known as the crown flower or giant milk weed is a species of Calotropis that is indigenous to Bangladesh, Indonesia, Sri Lanka, Malaysia, Thailand, India, China, Pakistan, Nepal, and Cambodia. It is a big shrub that can reach a height of 4 meters (13 ft). It is a member of the Apocynaceae family. It features waxy flower clusters that are either lilac or white. Each flower has five pointed petals and a little "crown" that rises from the middle and contains the stamen. It is a drought-resistant, multibranched, hard, erect, wooly shrub that may be cultivated in any climate. Calotropis has valvate aestivation, meaning that sepals or petals in a whorl only touch at the periphery and do not overlap. The plant has a milky stem and round, light-green leaves. Calcium oxalate, fatty acids, and cardiac glycosides are all present in Calotropis gigantea latex. When the stem and leaves are cut, a thick, milky juice is produced. The ethanolic extract of leaves of calotropis gigantea contains calotropin like

cardiac glycoside, alkaloids, tannins, flavonoids which are determined by phytochemical screening [8]. The calotropin (cardenolide type cardiac glycoside) is responsible for spasmolytic activity.

II. MATERIALS AND METHODS

Collection and authentication of plant materials

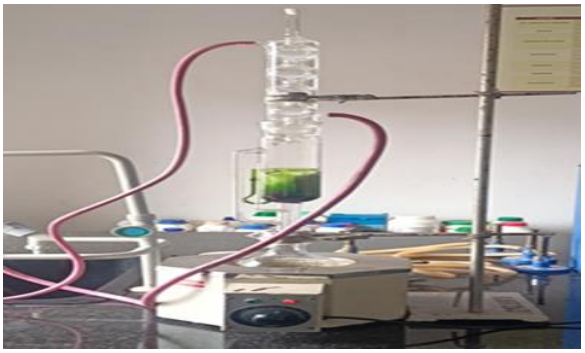
Fresh Calotropis gigantea leaves were gathered from place Ambalamugal, Ernakulam district, Kerala state during the month of November and authenticate by Dr. Justin R. Nayagam, Head, Department of Botany, Union Christian College, Aluva.

Processing of sample

The plant was collected, cleaned thoroughly with distilled water and the leaves of plant were dried under shade for 14days. The shade dried leaves were ground in a mechanical grinder to obtain coarse powder.

Preparation of ethanolic extract [10]

The powdered leaves were subjected to extraction by the Soxhlet method using ethanol as a solvent. The extraction was done for 8 hours using a Soxhlet unit utilizing about 50 g of the powdered leaves and 400 mL of ethanol. Distillation was used to concentrate the resulting extract and evaporate it into dryness at a 50 ^{0}c temperature.



Soxhlation

Ethanolic extract

III. PHYTOCHEMICAL ANALYSIS [4]

Ethanolic extract of Calotropis gigantea were subjected to phytochemical analysis

3.1 Test for cardiac glycoside

Keller killani test

To the extract 5ml of water and 0.5 ml of strong lead acetate solution was added. Shake well and separate the filter. The filtrate was extracted with equal volume of chloroform. Then chloroform extract was evaporated to dryness and residue was dissolved in 3ml of glacial acetic acid followed by addition of few drops of $FeCl_3$. The resulting solution was transferred to test tube containing 2ml of conc.sulphuric acid. A reddish-brown layer is formed which turns bluish green color after standing indicates the presence of cardiac glycoside.

Legal test

The extract is dissolved in pyridine, sodium nitroprusside solution and 20% NaOH is added to it. Formation of red color indicates the presence of cardiac glycoside.

Baljet test

To the extract sodium picrate solution was added. Formation of yellow to orange color indicates the presence of cardiac glycoside.

3.2 Test for Alkaloids

Mayers test:

3 ml of ethanolic extract was stirred with 3 ml of 1%HCl on steam bath. Mayers reagent (potassium iodide and mercuric chloride) was then added to mixture. Turbidity is formed due to the presence of alkaloid.

Dragendroff test:

To 2ml of the extract solution, Dragendroffs reagent (potassium bismuth iodide solution) was added. Orange brown precipitate is formed due to presence of alkaloid.

Wagners test:

To 2ml of the extract solution, Wagners reagent (iodine potassium iodide solution) is added. Reddish brown precipitate is observed due to presence of alkaloid.

Hager's test:

To 2ml of extract solution, Hager's reagent (saturated solution of picric acid) was added. Yellow precipitate is observed due to the presence of alkaloid.

3.3 Test for Tannins

Ferric chloride test:

About 2ml of the ethanolic extract was stirred with 2ml of distilled water and few drops
of $FeCl_3$ solution were added. Formation of green precipitate indicates the presence of tannins.
Lead acetate test:
Ethanolic extract react with lead acetate ($Pb(C_2H_3O_2)_2$) solution. Formation of white precipitate indicates the presence of tannins.
Gelatin test:
Ethanolic extract mixed with gelatin solution. Formation of cloudy precipitate indicates presence of tannins.
Potassium dichromate Test:
Ethanolic extract reacts with potassium dichromate. Formation of green color indicates presence of tannins.
3.4 Tests for Flavonoids
Shinoda test:
To 2ml of extract solution, few magnesium turnings, 5ml 95% ethanol and few drops of conc. HCl was added. Pink to red color develops due to the presence of flavonoids.
Sodium hydroxide test:
Add aqueous sodium hydroxide to the ethanoic extract. Yellow color develops that gradually disappears on addition of dilute acid (acetic acid) which indicate the presence of flavonoids.
Ferric chloride test:
To the extract add few drops of ferric chloride solution. A green colour indicates the presence of flavonoids.

IV. PROCUREMENT OF CHICKEN ILEUM

Fresh chicken ileum was gathered from the slaughterhouse in order to execute the experiment. It was stored in newly made Tyrode solution at room temperature with adequate aeration. The study was conducted in the pharmacology laboratory of the Department of Pharmacology at the Chemists College of Pharmaceutical Sciences and Research in Ernakulam, Kerala, India.
Drugs and Chemical reagents
Acetylcholine (Loba chemie), Atropine (Suvidhinath laboratories), Ethanol (Spectrum chemicals)

V. EXPERIMENTAL DESIGN

Drug Prepration and Serial Dilutions
A solution of $1x10^{-1}$ g/ml was prepared by dissolving 100 mg of the Calotropis extract in 100 ml of distilled water. From this, 10 ml was transferred and diluted to 100 ml with distilled water. Acetylcholine (100 mg/ml) was prepared using the same dilution procedure. Atropine (10mg/ml) was prepared by dissolving 100mg in 100 ml of distilled water. From this, 1 ml was transferred and diluted to 100 ml with distilled water. Tyrode solution was made by dissolving the following components in one liter of water: 8 g NaCl, 0.2 g KCl, 0.2 g $CaCl_2$, 1 g NaHCO3, 1 g NaH2PO4, 0.1 g MgCl2, and 2 g glucose.

Evaluation of spasmolytic activity on isolated chicken ileum [11]
The experiment was performed by using Sherrington rotating drum. Fresh chicken ileum was collected, placed in a beaker with a tyrode solution at 37 °C and then aerated. A portion of the ileum measuring 2-4 cm was excised, mounted, and kept at 37 ° C in an organ bath containing tyrode solution with oxygen supply. The kymograph and its attachments were set up so that the tissue would be properly tensioned. Before starting the medication infusions, the tissue was given 15 minutes to acclimatise.
Acetylcholine dose responses were established in the following order

1. Acetylcholine alone
2. Acetylcholine in presence of atropine
3. Acetylcholine in presence of ethanolic extract of Calotropis gigantea leaves

Statistical Analysis
The mean standard error of mean values was used to represent all of the collected data.

VI. RESULTS

Phytochemical analysis
The chemical screening tests confirmed the presence of Cardiac glycoside, alkaloids, flavonoids, and tannins in the ethanolic extract of Calotropis gigantea. (Table 1)

VII. ASSESSMENT OF SPASMOLYTIC ACTIVITY

DRC of Acetylcholine alone
Dose of 0.1 ml, 0.2 ml, 0.4ml, 0.8m, 1.6ml, 3.2ml was administered to the isolated chicken ileum and the DRC was recorded. The contraction produced in the

smooth muscles of chicken ileum cause an increase in height of the response. (Fig 1)

DRC of Acetylcholine in presence of atropine

Dose of 0.1 ml, 0.2 ml, 0.4ml, 0.8m, 1.6ml, 3.2 ml was administered to the isolated chicken ileum by keeping the dose of Atropine (0.1ml) as constant, Atropine inhibits the activity of the Acetylcholine causes decrease in height of the response. (Fig 2)

DRC of Acetylcholine in presence of ethanolic extract of Calotropis gigantea leaves

Dose of 0.1ml, 0.2 ml, 0.4ml, 0.8m, 1.6ml, 3.2ml was administered to the isolated chicken ileum by keeping the dose of Atropine (0.1ml) as constant, Calotropis gigantea extract inhibits the activity of the acetylcholine which cause a decrease in height of the response. (Fig3).

Table 1: Phytochemical analysis of leaves of Calotropis gigantea.

Phytochemicals	Ethanolic extract
Cardiac glycoside	+
Alkaloids	+
Flavonoids	+
Tannins	+

"+" denotes the presence of the phytochemical constituents of Calotropis gigantea.

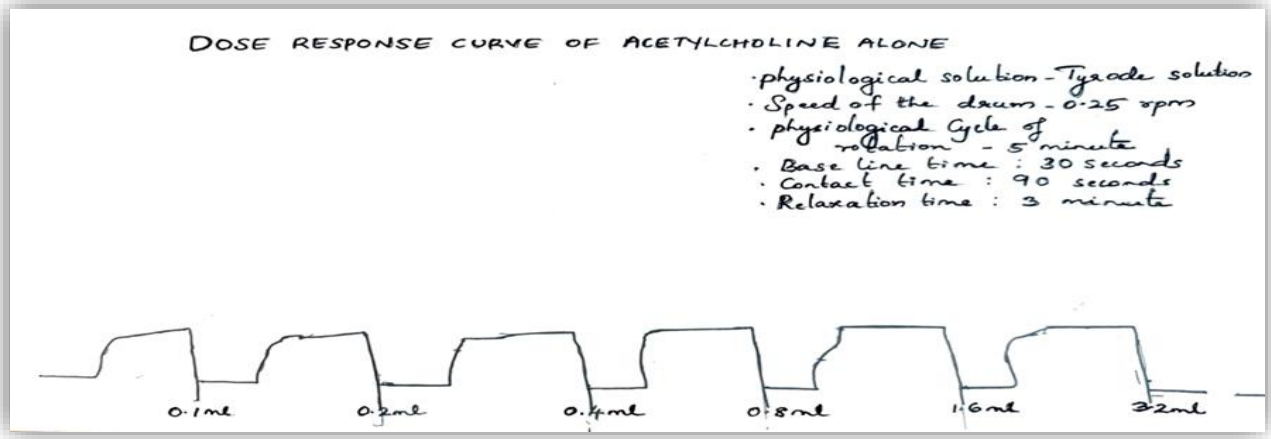


Fig1: Dose Response Curve of Acetylcholine

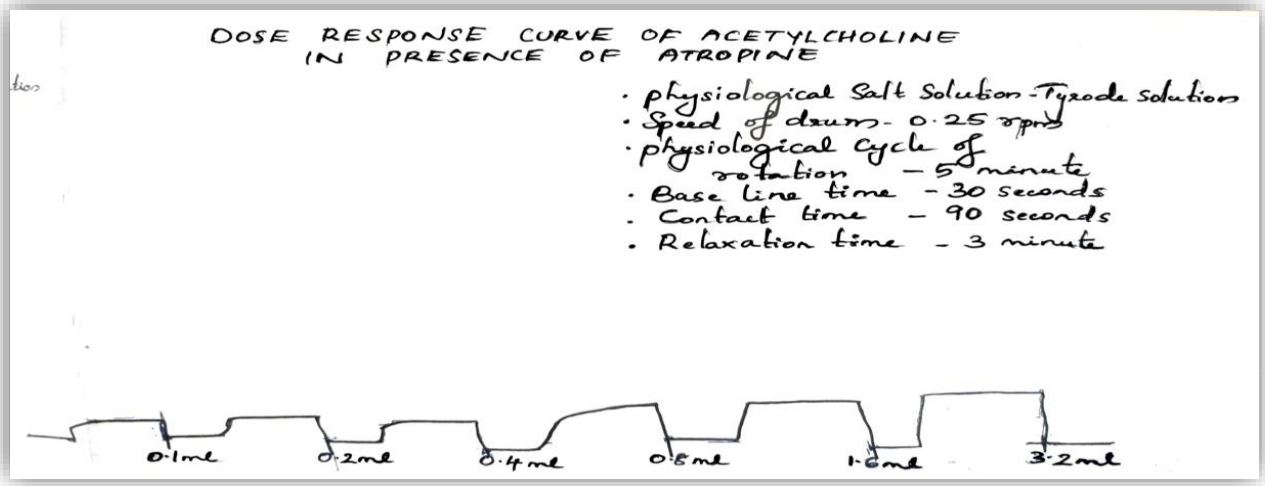


Fig2: Dose Response Curve of Acetylcholine in presence of Atropine

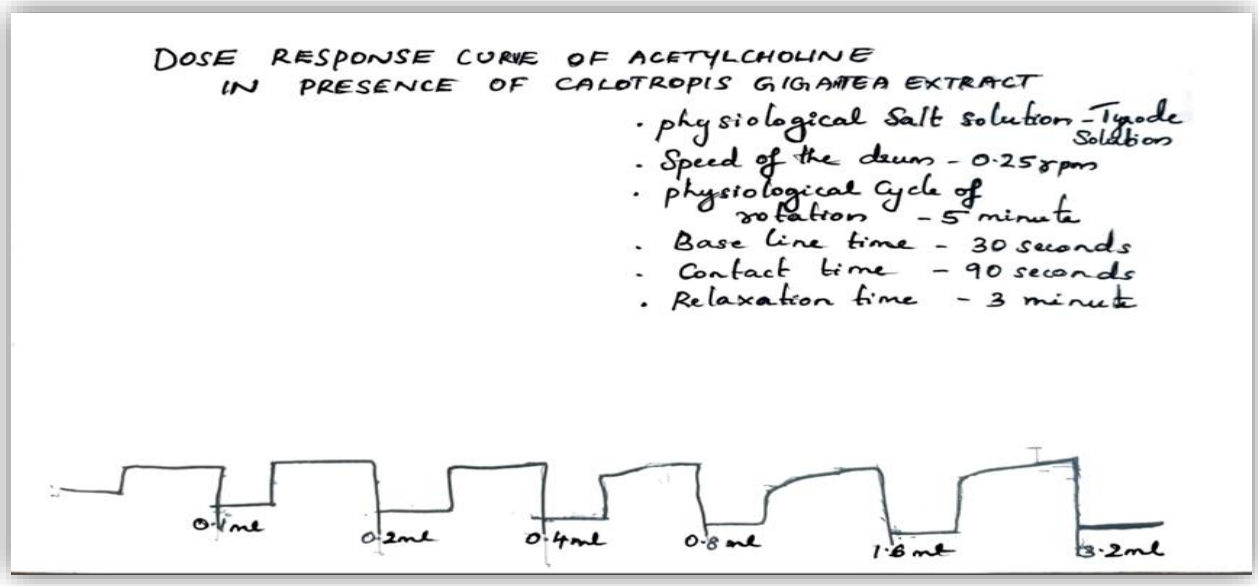


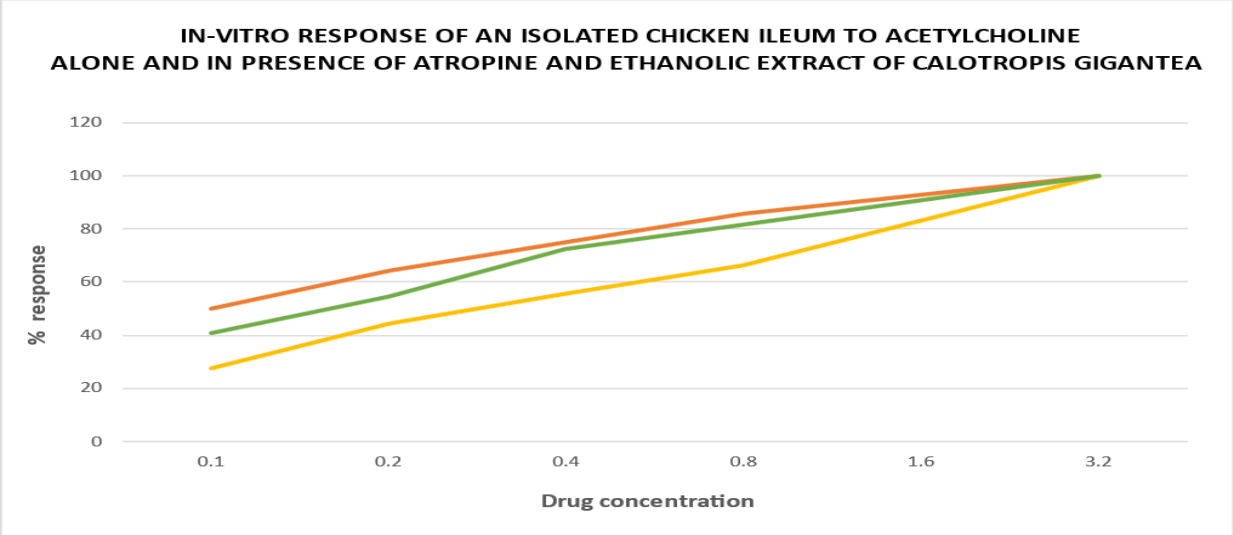
Fig3: Dose Response Curve of Acetylcholine in presence of Calotropis gigantea

Table: 2 In-Vitro response of an isolated chick ileum to Acetylcholine alone and in the presence of Atropine and Calotropis gigantea

Sl. No:	Dose of Acetylcholine (ml)	Response of Ach alone		Response of Ach in presence of Atropine		Response of Ach in presence of Calotropis gigantea	
		Height in mm± SEM	% Response	Height in mm± SEM	% Response	Height in mm ± SEM	% Response
1	0.1	14±0.202	50.00	5± 0.088	27.7	9 ±0.088	40.9
2	0.2	18±0.088	64.20	8± 0.120	44.4	12±0.066	54.5
3	0.4	21±0.088	75.00	10± 0.120	55.5	16± 0.88	72.7
4	0.8	24±0.088	85.70	12±0.115	66.6	18± 0.115	81.8
5	1.6	26±0.100	92.80	15±0.088	83.3	20±0.115	90.9
6	3.2	28±0.133	100.00	18±0.115	100	22±0.115	100
Mean			77.95		62.91		73.45

VIII. DISCUSSION

Dose response curve of Acetylcholine alone, Dose response curve of Acetylcholine in presence of Atropine and Dose response curve of Acetylcholine in presence of Calotropis gigantea was compared. The height of dose response curve of Atropine and Calotropis gigantea was showed as decreased compared to that of the height of Acetylcholine, because Acetylcholine causes smooth muscle contraction whereas Atropine and Calotropis gigantea shows smooth muscle relaxation. It indicates that Calotropis gigantea is having Spasmolytic activity.



Percentage response of Acetylcholine alone
Percentage response of Acetylcholine in presence of Calotropis gigantea
Percentage response of Acetylcholine in presence of Atropine

The Spasmolytic activity was evaluated by plotting dose response curve. Using the mean height obtained from the DRC, corresponding percentage response was calculated. The mean percentage response of Acetylcholine, Atropine, Calotropis gigantea was found to be 77.95%,62.91%, 73.45% respectively.

IX. CONCLUSION

Spasmolytic properties of an ethanolic extract of Calotropis gigantea was studied in comparison with Atropine. Acetyl choline is a neurotransmitter which produce contraction in smooth muscles. Spasmolytics inhibit acetylcholine and relaxes all smooth muscles. Botanist authenticated the collected plant specimen, and using the soxhlation process, an ethanolic extract was made from the dried, powdered leaves of Calotropis gigantea. Standard methodology was used to conduct the preliminary phytochemical analysis, which identified the presence of cardiac glycoside, alkaloids, tannins, and flavonoids. Then spasmolytic activity of ethanolic extract of Calotropis gigantea leaves was studied in-vitro using isolated chick ileum. Isolated chick ileum is an intestinal smooth muscle and it contains number of receptors such as muscarinic, histaminic, adrenergic, serotonergic and GABAergic receptors. Contractions induced by acetyl choline were recorded as dose response curve (DRC) which suggest that acetyl choline increases contraction in dose dependent manner. Spasmolytics relaxes all visceral smooth muscles. Spasmolytic activity of the ethanolic extract of Calotropis gigantea was compared with standard atropine. Comparison of mean percentage response of Calotropis gigantea with that of atropine proved significant spasmolytic activity of the extract. The 100ug/ml of extract inhibited the actions of Acetylcholine due to presence of calotropin like cardiac glycoside.

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