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**SPASMOLYTIC EFFECT OF ETHANOLIC EXTRACT OF *Calotropis procera* LEAVES ON *In-vitro* GUINEA PIG ILEUM****Jagadeesh K, Shreenivas Revankar\*, Jagadeesh S C***Department of Pharmacology, Shimoga Institute of Medical Sciences, Shimoga, Karnataka, India*

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**ABSTRACT**

*Calotropis procera* is widely distributed flowering plant found in India, the leaves, fruits, root and bark of the plant are known to have various medicinal properties. Guinea pig ileum is a sensitive and standard isolated tissue for evaluating the spasmodic and antispasmodic activity of organic substances. Hence guinea pig ileum was used to evaluate the spasmolytic activity of ethanolic extract of *Calotropis procera*. The 10ug/ml of extract inhibited the actions of histamine due to presence of calotropin like alkaloid. Thus our study showed that ethanol extract of *Calotropis procera* leaves produced an excellent spasmolytic effect on gastrointestinal smooth muscles (guinea pig ileum).

**Keywords:** *Calotropis procera*, Leaves, Ethanolic extract, Guinea pig ileum, Bioassay, Spasmolytic.

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**Introduction**

*Calotropis procera* is a flowering plant in the dogbane family Apocynaceae. It is native of North Africa, tropical Africa, Western Asia, South Asia and Indochina region[1]. The plants is commonly known as Giant swallow wort, Milkweed; called as apple of Sodom, mudar, osher and stabragh in different regions[2]. It is seen throughout India in dry waste places. The whole plant has medicinal value but mainly its leaves flower, fruit, root and bark. The root and bark of plant are largely used for elephantiasis, leprosy and chronic eczema. Leaves are useful in treatment of paralysis, arthralgia, swelling and intermittent fever[3]. Flowers of the plant are used in asthma, cathara, inflammation and tumors. Ethanolic extract is used to obtain lipid soluble substances from organic materials. The solvent used in ethanolic extraction is ethanol. The other solvents that can be used are methanol, butane and acetone[4]. *Cavia Procellus* is the scientific name of the common species of guinea pig; procellus means little pig in Latin. It is called cavy belongs to the species of rodents, it has no association with pig family nor it originated from guinea.

Guinea pigs were first domesticated for food as early as 5000 BC in Andean region (Columbia, Peru, Bolivia) and its use for scientific experimentation dates back to 17th century[5].

Guinea pigs have biological similarity to human being and hence useful in research, they served a major role in development of germ cell theory and currently they constitute approximately 2% of total laboratory animals[6]. Guinea pigs as whole animal have been implemented in studies involving delayed hypersensitivity reactions, local anesthetics, cochlear sensitivity and tuberculosis. Also they are most extensively implemented in the research and diagnosis of infectious diseases like chagas, brucellosis, cholera, diphtheria etc. various isolated tissues of guinea pig like ileum, tracheal chain, vasdeferens etc are utilized for pharmacological studies of different compounds. Terminal ileum is the most common preparation used to screen spasmodic and antispasmodic compound, as it is sensitive and suitable for detection and assay of histamine and related compounds[7].

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Bioassay is defined as comparative assessment of relative potency of the test compound on any living animal or biological tissue[8]. It can be quantitative, for assessment of concentration of the drug or it may be qualitative for assessing the physical effects of the

drug. Substances derived from plants and animal sources are mainly assessed by bioassay[9]. Popularity of bioassay is mainly due to its reliability, specificity, sensitivity and probability.

There are limited studies on *Calotropis procera* leaf and its medicinal properties when compared to other parts of plant, in reference to antispasmodic activity. In the present study the ethanolic extract of *Calotropis procera* leaves was used to study its spasmolytic effect. As guinea pig ileum is sensitive tissue for assay of histamine and related substance, it was used to carry out bioassay.

### Material and methods

- Ethanolic extract of *Calotropis procera* was obtained by using standard procedure[10].
- Animal isolated tissue of guinea pig ileum was used to carry out the bioassay studies
- The fresh leaves were collected from the *C. procera* air dried and powdered material was subjected to soxhlet with ethanol (95%)
- Ethanolic extract was subjected to identification of constituents.
- Preparation of extract: the ethanolic extract was suspended in tween-80(1%) and was preserved in desiccator till further *in-vitro* studies.
- Standard drugs for comparison studies: acetylcholine hydrochloride, histamine.
- Isolated muscle preparations: guinea pig ileum.
- Physiological salt solution: tyrode solution.

### Effect of *C. procera* on isolated GPI

Set up an isolated preparation of guinea pig ileum as per standard protocol[11]. Elicit responses to acetylcholine/histamine so that we can record maximum height of contraction. Add 10 mg of

*C. procera* ethanolic extract of leaves and record its response.

Similarly elicit responses of acetylcholine/histamine in presence of 0.1, 0.2, 0.3 and 0.4 ml of *C. procera* crude extract. Observe the degree of inhibition and calculate percentage of inhibition of acetylcholine and histamine in relation to *C. procera* crude extract.

### The isolated guinea pig ileum

Guinea pig ileum is particularly suitable for histamine bioassay. The responses are generally quite consistent and the relation to doses fairly predictable. Discrimination between doses is good enough for purpose of bioassay. Acetylcholine can also be assayed by this method, but this again has the advantage of promoting the development of spontaneous contractions. The sensitivity of the tissue is not marked. The guinea pig is fasted for 18-24 hrs and then sacrificed with blow ion head or by cutting the throat. Open abdomen portion and identify ileum and cut. A piece of 3 cm length is selected; it is freed from mesenteric attachments and washed gently. With a pipette one end of the gut is placed over the tip of the pipette and warmed tyrode solution is allowed to flow through the gut lumen from the pipette with minimum pressure. A thread is tied at one end of the lever and to the other end made aloof and fixed to tip of the tissue holder. Confirm that the threads do not close the lumen. After mounted the tissue in the organ bath is supplied tyrode solution with carbogen gas through the oxygen tube. Temperature of the organ bath is maintained at 37°C and uniform temperature is maintained by stirrer. The tissue is stabilized for 30 minutes to minimize the spontaneous movements.

### Results:

Results are shown in Figure 1, 2 and Table 1, 2.

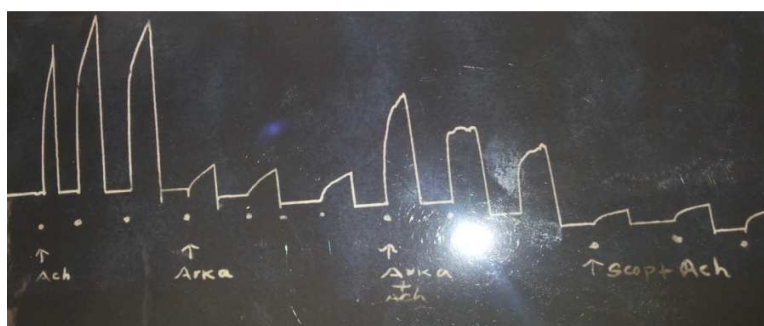


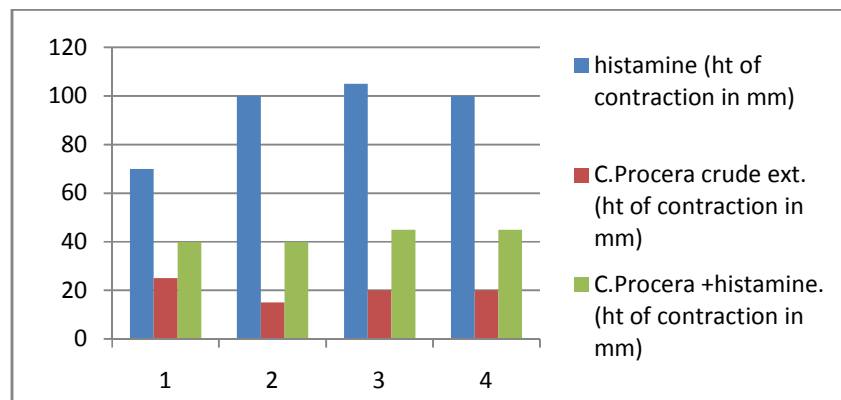
Figure 1: Shows graph of Scopolamine and Ach

**Table 1: Showing the inhibition of Scopolamine+acetyl choline induced contraction by *C.procera* leaves ethnolic extract in GPI**

Ach (ht of contraction in mm)	<i>C.procera</i> ethnolic ext. (ht of contraction in mm)	% of inhibition	Ach + <i>C. procera</i> ethnolic extract	% of inhibition	Scopolamine+acetyl choline	% of inhibition
50	13	74	34	32	07	86
58	13	77.58	28	51.72	10	82.75
58	13	77.58	25	54.54	10	82.75

**Table2: Showing the inhibition of histamine induced contraction by *C.procera* leaves ethnolic extract in GPI**

Histamine (ht of contraction in mm)	<i>C.procera</i> crude ext. (ht of contraction in mm)	% of inhibition	<i>C.procera</i> +histamine. (ht of contraction in mm)	% of inhibition
70	25	64.28	40	30
100	15	85	40	30
105	20	85	45	55
100	20	80	45	55



**Figure 2: Showing the inhibition of histamine induced contraction by *C.procera* leaves ethnolic extract in GPI**

**Discussion**

The present study was carried out to evaluate the direct effects of ethnolic extract of *Calotropis procera* leaves on guinea pig ileum as an *in vitro* experimental model.

The smooth muscle in contrast to skeletal muscle lacks visible cross striations because the contractile proteins actin and myosin are not arranged in a regular way. Troponin is absent and sarcoplasmic reticulum is poorly developed[12]. There are dense bodies to which actin filaments are attached. These bodies are present

in the cytoplasm and also in the cell membranes. Some of these are bound to adjacent cell dense bodies which transmit force of contraction from one cell to the next. Smooth muscle cells can generally be divided into two types, multi unit smooth muscle cell and unitary smooth muscle cell. In multiunit type each muscle fiber is often innervated by a single nerve fiber. The unitary smooth muscle consists of mass of muscle fibers and contract together as a single unit[13].

These effects are correlated with release of biogenic amines such as histamine and serotonin from the mast cells. The ethnolic extract also produced auto desensitization as observed with serotonin (5-HT) further it is interesting to note that the ethanol extract of *Calotropis procera* leaves abolished the pendular movements of guinea pig ileum. This mechanism is mediated through histamine receptors located outside the myentric plexus.

### Conclusion

*Calotropis procera* leaves contain important active constituents like calotoxin, calotropin, triglyceride etc. This evidence was confirmed by photochemical securing of respective extract. The 10ugm/ml of concentration of extract which inhibits the actions of histamine and serotonin probably due to calotropin like alkaloids of crude extract. Thus our study shows that ethanol extract of *Calotropis procera* leaves produces an excellent antispasmodic effect on gastrointestinal smooth muscles.

**Source of Support: NIL**

**Conflict of Interest: None**

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