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# Investigation of Phytochemical and Pharmacological Evaluation of Smooth Muscle Relaxant Potential of *Diplocyclos Palmatus* Leaves Extract

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## ABSTRACT

The aim of this study was investigation of phytochemical and pharmacological evaluation of smooth muscle relaxant potential *of Diplocyclos Palmatus* leaves extract. *D. palmatus* is medicinal plant commonly known as *Shivlingi*, the different parts of plant have been used by traditional practitioner for curing various ailments. The present study was undertaken for investigation of phytochemical and pharmacological evaluation of smooth muscle relaxant potential of *D.palmatus* leaves extract. The leaves were subjected to aqueous extraction. Extract was screened for their phytochemical constituents and evaluated on isolated chicken ileum. The result showed that test drug (*D. Palmatus)* block the action of ach on smooth muscle and produce relaxation of smooth muscle in chicken ileum.

**KEY WORDS:** *Diplocyclos palmatus*, phytochemicals, smooth muscle relaxant, leaves extract, chickenileum.

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## INTRODUCTION [1,2,3,4,5,6]

The plant *Diplocyclos palmatus* (L.) Jeffry is well known as *Shivalingi*. It is a seasonal climber plant which includes rich medicinal potentials. It is also called as *Lollypop* plant Striped cucumber in English. In Hindi it is referred as *Shivlingi*. It is also known as *Lingini*, *Shivalingi*, *Chitraphalaa* in ayurveda. The plant is a herbaceous climber in nature with smooth stem. Old stem becomes thick and branched, slender, grooved and glabrous. Leaves of the plant are ovate, 3.5-15 x 4-15.5 cm, palmately lobed. Roots are tuberous. Stems are slender, glabrous type. Tendrils present. Flowering/Fruiting season of this plant is from August to December. It is an important member of the family ***Cucurbitaceae*** and has been used traditionally.

Medicinal plants, since times immemorial, have been widely used by the traditional medicinal practitioners for curing various diseases in their primary health care needs and a number of modern drugs have been isolated from natural sources. These isolations were based on the uses of these agents in traditional medicines. Pharmacological basis of its use and Plants contain various phytochemicals that showed muscle relaxant and anti-cholinergic property like alkaloids, flavanoids, saponins, cardiac glycosides and steroids compounds.


**Figure 1*:- Diplocyclso Palmatus leaves***

## *Medicinal uses:*

The plant is found suitable for treating diverse disorders following varied preparations. The plant is registered as their multifold utility among the society. It is potentially applied as several purposes like for Anti inflammation (Leaf) Anti-asthmatic (Root), Analgesic (Stem and Leaf), Antimicrobial (Stem and Leaf), Antidotes (Root, Leaf) etc. The fruits and leaves are used to cure stomach-ache, stems are used as an expectorant, fruits are used as a laxative and seeds are febrifuge.

The aerial plant parts are potentially utilized for several purposes such as for Aphrodisiac and tonic, Constipation, Stomach problem, Diarrhoea, Malaria fever etc.

### *Classical Uses :*

Lingini or Shivalingi has a number of useful medicinal properties and usages. It is considered bitter, aperients and tonic and it is commonly used for relieving bilious attack. The leaves of the plant are applied topically for getting relief from inflammations. The Indian women sometimes take the seeds in combination with other plant drugs for helping conception and prevent miscarriage. The practitioners of Ayurvedic medicine use the plant`s fruit as an aphrodisiac and tonic, while in Siddha; the entire plant is used for getting relief from constipation. Seeds are use in sterility due to blocked tubes in women Snake bite Root Fever  Stomach ache External abscess Fruits are used for Diarrhoea.

## MATERIAL AND METHOD :

### Chemicals :-

### *1) Physiological salt solution :*

**Tyrode solution:** Nacl,KCl,$Mgcl_2$,$Cacl_2$,$NaHCO_3$,$NaH_2PO_4$,Glucose ,water.

***2)Tissue used :*** isolated chicken ileum preparation

**3) *Instrument used:*** Student organ bath, Sherrington rotating drum machine fontal writing lever.

### METHOD:-

### *Collection of  Plant  (D.palmatus) and Preparation of extract:-*

Leaves were collected from karad (Yelgaon), Maharashtra, India and Authentified by  *Sadguru Gadage Maharaj College, Karad in Head dept. of Botany and plant protection*. The location lies between "17.1307°N Latitude and 74.0229°Longitude". Collected plant samples were washed under running tap water and dried in hot air oven at 50 °C for 72 h. Oven dried plant samples were grind into fine powder using grinder. Powdered samples were used for extraction with water using maceration method. About 60 gm of powder was added in a 180ml of solvent (water) and allowed for shaking allow to stand at a room temperature  for a period of seven days with frequent agitation. Then liquid strained off solid residue  pressed. Clarified by filtration evaporation and concentration then  weighed and dissolved in a known volume of respective solvents and used for analysis.

## *Composition of physiological salt solution (PSS)* [7,8]

**Table no :1: Compounds of Tyrode**

| Sr no | Compound | Tyrode (PSS) gm/litter |
|---|---|---|
| 1 | Nacl | 8.0 |
| 2 | KCl | 0.2 |
| 3 | $CaCl_2$ | 0.2 |
| 4 | $Mgcl_2$ | 0.10 |
| 5 | $NaHCO_3$ | 1.0 |
| 6 | $NaH_2PO_4$ | 0.05 |
| 7 | Glucose | 1or2 |

## PHYATOCHEMICAL TEST:[9-15]

**Table no : 2 : Phyatochemical test of Aqueous extract.**

| Sr.No. | Name of the phytochemical | Aqueous extract |
|---|---|---|
| 1. | ALKALOIDS :<br>Mayer's test<br>Wagner test | <br>+ve<br>+ve |
| 2. | FLAVANOIDS | +ve |
| 3. | TANNINS | -ve |
| 4. | SAPONINS | +ve |
| 5 | STEROIDS | +ve |
| 6 | CARDIAC GLYCOSIDES | +ve |

## EXPERIMENTAL WORK [9.10.11.12.13]

***Preparation of physiological salt solution :*** Nacl, KCl, $Mgcl_2$, $Cacl_2$, $NaHco_3$, $NaH_2Po_4$, Glucose , water.(table no:-1) . All values are in gm. Weighed accurate quantity of the ingredients and dissolved in one litre distilled water. The physiological solution prepared should be clear, and if turbid it is advised to prepare fresh solution before the start of the experiment.

## Drug preparation:

### *Preparation of Acetylcholine:*

A stock solution of 100µg/ml was prepared with distilled water. From the stock solution, various doses of the drug was withdrawn at concentrations of 10µg, 20µg, and 30µg and added to the inner organ bath and used as an agonist of M3 receptor.

### *Preparation of Atropine:*

A stock solution of 0.1µg/ml and 1µg/ml was prepared using distilled water and was added to the inner organ bath and used as an antagonist of M3 receptor.

## Animal Experimentation (chicken ileum) :

***Procurement of Chick ileum:-*** Chick ileum was procured from the slaughter house in Yelgoan. The laboratory animals have to be sacrificed just for a piece of tissue. However, *chicken ileum* is a tissue that is available easily, and animals need not be killed additionally for experimental purpose. The advantages of using chick ileum preparation are that it is economical, very easy to mount in organ bath, gives good response, without sacrificing the experimental animal.

### *Ex-vivo experiments on isolated tissues:*

**Experiments for muscle relaxant on isolated chicken ileum preparation :**

1) **For standard :-**

***Procedure:-***

-Fresh entire gastrointestinal tract of healthy cock was obtained from a slaughter house in Yelgoan .Wash out the content present in ileum.

- The ileum was cut into small segments of 2-3 cm long.One small segment of chicken ileum Mounted in organ bath containing PSS (Tyrode solution) maintained at 32-35˚C and bubbled with air.

- Tension adjustments 0.5 gm is applied and tissue allow to relax for 30min and tissue were washed after every 10 min.

***Recording the response-***

-Remove the weight/tension and start the Sherrington rotating drum machine .Record the base line for 30 sec stop the machine. Now administered the 1st dose of Ach and start the drum machine.

- Record the response 90sec then stop the machine and apply the tension to relax the tissue for next 4 min during with four successive washing.

- Record at least 2-3 conc. dependent response produced by Ach .Then administered 0.1ml of given solution of atropine after administered of ATR immediately administered the Ach then observe the response and plot the conc. response curve (CRC ).

**Standard conc. of Ach : 100µg/ml**

**Standard conc. of ATR: 1µg/ml and 10µg/ml**

**2)Effect of Test drug in DRC of Ach :**

***For Test :***

***Procedure :*** Isolation of chicken ileum ref in standard page no -7

***Recording the response*** :

- Remove the weight/tension and start the Sherrington rotating drum machine .Record the base line for 30 sec stop the machine. Now administered the 1st dose of Ach and start the drum machine .

-Record the response 90sec then stop the machine and apply the tension to relax the tissue for next 4 min during with four successive washing

- Record at least 2-3 conc. dependent response produced by Ach .Then administered 2ml of given test solution after administered of test solution immediately administered the Ach then observe the response.

**Standard conc. of Ach :- 100µm/ml**

**Conc. of Test drug :- 2gm/10ml=200mg/ml**

## RESULT AND DISCUSSION:-

### *1) For standard :-*

**Standard Conc. of Ach :- 100µg/ml**

**Standard Conc. of ATR:-1µg /ml or 10µg/ml.**

**Length of tissue :- 2cm**

**Load :- 0.5gm**

**Fig : 1 : Effect of standard drug.**

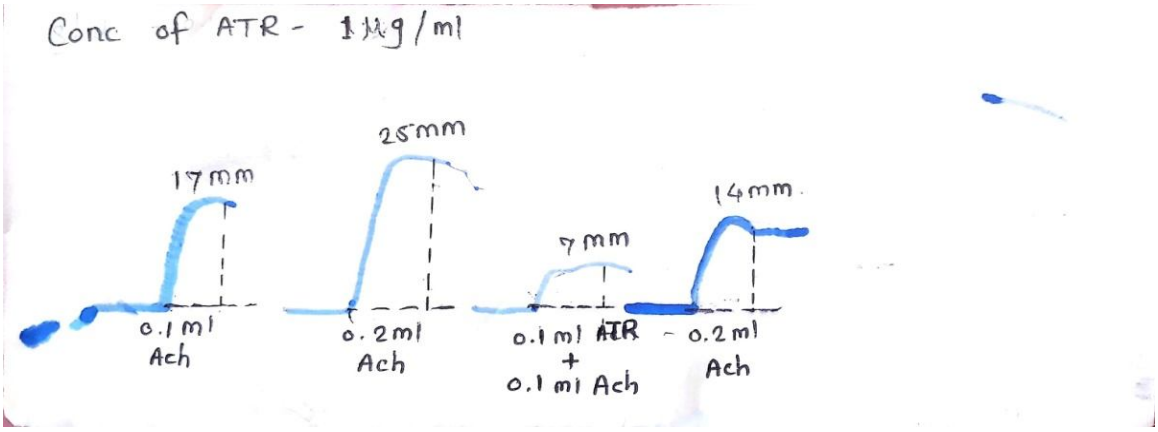


**Figure 1:- Effect of ATROPIN(1µg/ml) on DRC of Ach by using isolated chicken ileum preparation.**

**Table no: 1 : Result of standard drug in mm**

| Sr. No. | Drug administered | Dose administered | Conc. (µg) | Response (mm) |
|---|---|---|---|---|
| 1 | Ach | 0.1ml | 10 | 17 |
| 2 | Ach | 0.2ml | 20 | 25 |
| 3 | ATR + Ach | 0.1ml+0.1 ml | 0.1+10 | 7 |
| 4 | Ach | 0.2 ml | 20 | 14 |

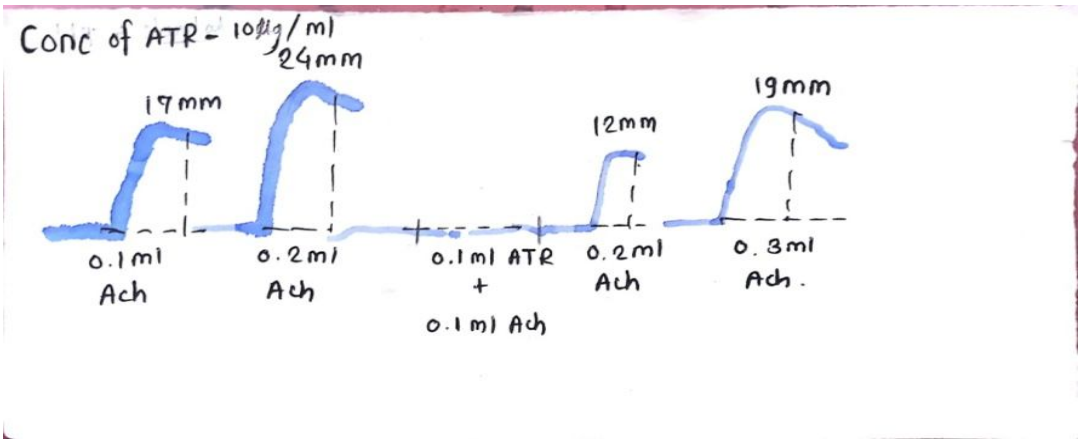


**Figure 2 :- Effect of ATROPIN (10µg/ml) on DRC of Ach by using isolated chicken ileum preparation.**

**Table no : 2: Result of standard drug in mm**

| Sr no | Drug administered | Dose administered | Conc. (µg) | Response (mm) |
|---|---|---|---|---|
| 1 | Ach | 0.1ml | 10 | 17 |
| 2 | Ach | 0.2ml | 20 | 24 |
| 3 | ATR + Ach | 0.1ml+o.1ml | 10+1 | 0 |
| 4 | Ach | 0.2 ml | 20 | 12 |
| 5 | Ach | 0.3ml | 30 | 19 |

Atropine is an anti-cholinergic drug ,atropine produce relaxation of all visceral smooth muscle in chicken ileum by acting on muscarnic $M_3$ Receptor. Atropine is an anti-cholinergic drug block the $M_3$ receptor in smooth muscle and produce relaxation. The action of ach are inhibited in presence of atropine. In presence of Atropine the (CRC) concentration response curve indicated in ( fig A and B).

## *2) For test Drug (D.Palmatus) :-*

**Standard conc. of Ach:-100µg/ml**

**Conc. of test drug :- 2gm/10ml : 200mg/ml**

**Length of tissue :-2cm**

**Load : -0.5gm**

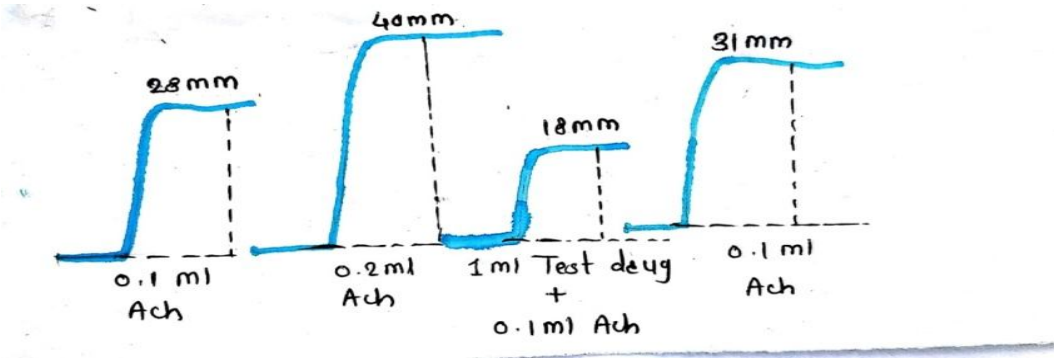


**Figure 3 :- Effect of Test drug 9200mg/ml) on DRC of Ach by using isolated chicken ileum preparation.**

**Table no:-3 : Result of test drug in mm.**

| Sr no | Drug administered | Dose administered | Conc. (µg) | Response (mm) |
|---|---|---|---|---|
| 1 | Ach | 0.1ml | 10 | 28 |
| 2 | Ach | 0.2ml | 20 | 40 |
| 3 | Test drug +Ach | 1ml+o.1ml | 200mg+10 | 18 |
| 4 | Ach | 0.1 ml | 10 | 31 |

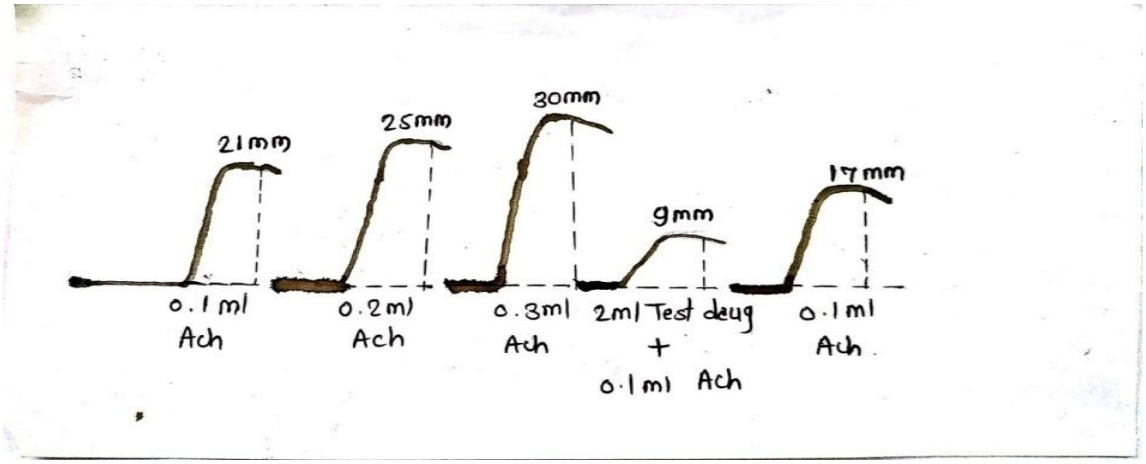


**Figure 4 :- Effect of test drug 400mg/ml on DRC of Ach by using isolated chicken ileum preparation.**

**Table no :4 : Result test drug in mm.**

| Sr no | Drug administered | Dose administered | Conc. (µg) | Response (mm) |
|---|---|---|---|---|
| 1 | Ach | 0.1ml | 10 | 21 |
| 2 | Ach | 0.2ml | 20 | 25 |
| 3 | Ach | 0.3ml | 30 | 30 |
| 4 | Test drug +Ach | 2ml+o.1ml | 400mg+10 | 9 |
| 5 | Ach | 0.1 ml | 10 | 17 |

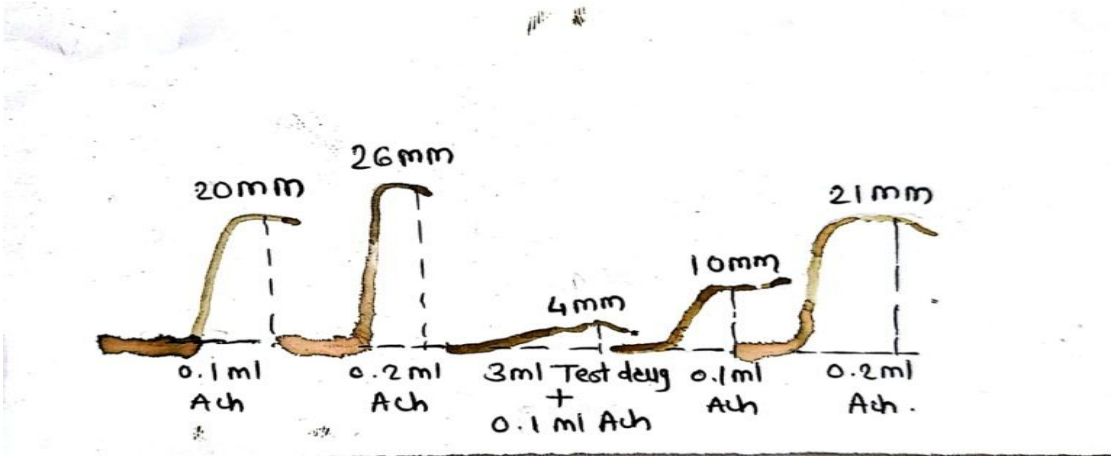


**Figure 5:- Effect of test drug (600mg/ml) on DRC of Ach by using isolated chicken ileum preparation .**

**Table no: 5:-Result of test drug in mm.**

| Sr no :- | Drug administered | Dose administered | Conc. (µg) | Response (mm) |
|---|---|---|---|---|
| 1 | Ach | 0.1ml | 10 | 20 |
| 2 | Ach | 0.2ml | 20 | 26 |
| 3 | Test drug +Ach | 3ml+o.1ml | 600mg+10 | 4 |
| 4 | Ach | 0.1 ml | 10 | 10 |

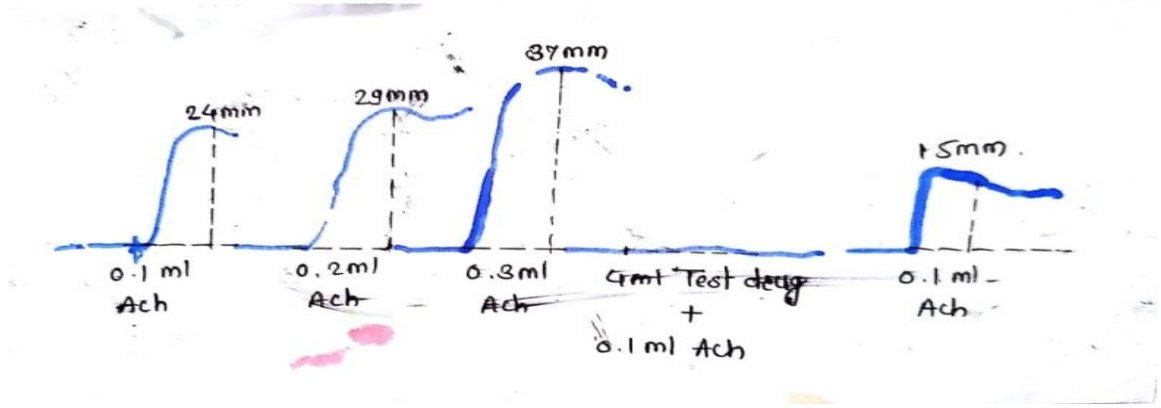


**Figure 6:- Effect of test drug 800mg/ml on DRC of Ach by using isolated chicken ileum preparation .**

**Table no :-6: Result of test drug in mm.**

| Sr no : | Drug administered | Dose administered | Conc. (µg) | Response (mm) |
|---|---|---|---|---|
| 1 | Ach | 0.1ml | 10 | 24 |
| 2 | Ach | 0.2ml | 20 | 29 |
| 3 | Test drug +Ach | 4ml+o.1ml | 800mg+10 | 0 |
| 4 | Ach | 0.1 ml | 10 | 15 |

Pharmacological and medicinal use of *Diplocyclos Palmatus* leaves extract Anti inflammation (Leaf) Anti-asthmatic (Root), Analgesic (Stem and Leaf), Antimicrobial (Stem and Leaf), Antidotes (Root, Leaf) The fruits and leaves are used to cure stomach ache, stems are used as an expectorant. Leaf are used Aphrodisiac and tonic, Constipation, Stomach problem, Diarrhoea, Malaria fever etc. Test drug (D.Palmatus) produce relaxation of smooth muscle in ileum of chicken. smooth muscle are present in ileum by acting on muscarnic $M_3$ Receptor. Test drug is block the $M_3$ receptor in smooth muscle and produce relaxation as a result the action of ach are inhibited. In presence of test drug the (CRC) concentration response curve (fig 1,2,3,4).

*D.palmatus* has been used traditionally by different communities throughout the world for the treatment of various ailments. Therefore current research work was planned with the intention to explore its medicinal uses for the treatment of abnormalities predominated by smooth muscle containing tissues such as gastrointestinal modulation on pharmacological basis. As a result pharmacological basis of its use *D.palmatus* shows the muscle relaxant property in isolated chicken ileum.

## CONCLUSION:

According to results, Test drug *Diplocyclos palmatus* has anti cholinergic effect, drug produce relaxation of all visceral smooth muscle of ileum. Muscarinic ($M_3$) Receptor are present on these smooth muscles of ileum. It is seen that the *Diplocyclos Palmatus* extract block the $M_3$ receptor in smooth muscle and produce relaxation. (fig no. C,D,E,F) and table no(5,6,7,8). Acetylcholine is natural neurotransmitter, act on cholinergic receptor and shows muscle contraction. The *Diplocyclos Palmatus* extract inhibit the effect of Ach in dose dependant manner, so it is concluded that *Diplocyclos Palmatus* extract has muscarinic blocking property as atropine show.

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