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Study of Anticholinergic Effect of *Moringa Pterygosperma* Leaves Extract on Intestinal Smooth Muscle in comparison with Atropine

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ABSTRACT

Anticholinergics are the agent that inhibits neurotransmitter acetyl choline in the parasympathetic outflow. Moringa pterygosperma is a commonly available plant that has been used commercially for its anticholinergic property. It also possesses antioxidant, anti-diabetic, anti-inflammatory, anti-ulcer and hypocholesteromic properties. The aim of the study is to assess the Anticholinergic activity of ethanolic extract of Moringa pterygosperma in comparison with Atropine using in vitro experimental models. Ethanolic extract was prepared by soxhlation method. For in vitro study, isolated chick ileum was used. Chick ileum was suspended in an organ bath containing Tyrode solution at 37◦C with adequate oxygen supply. Effect of the Moringa pterygosperma extract was studied on ileum contractions induced by Acetylcholine and compared with that of atropine. Mean percentage response was calculated for ethanolic extract of Moringa pterygosperma and atropine. Ethanolic extract of Moringa pterygosperma and atropine inhibited ileum contraction induced by Acetylcholine. From this study it was concluded that Moringa pterygosperma extract have significant anticholinergic activity compared to atropine. These findings therefore raise hope for the development of a new anticholinergic drug with few side effects that may be useful in the treatment of conditions like diarrhoea, asthma, incontinence, peptic ulcer, and muscle spasms.

Keywords: *Anticholinergic, Moringa pterygosperma leaves*

INTRODUCTION

The autonomic nervous system, a part of peripheral nervous system controls physiological functions that are carried out automatically, such as digestion, blood pressure, respiration, and sexual desire. There are three physically separate divisions in it: enteric, parasympathetic, and sympathetic. The central nervous system receives sensory input and motor output, respectively, from the sympathetic nervous system and the parasympathetic nervous system, which both contain afferent and efferent fibers. The enteric nervous system is a large, web-like structure that may function on its own and

it primarily control digestive functions. The "fight or flight" response is induced when the sympathetic nervous system is activated, which results in a generalized state of increased activity and attention. The parasympathetic nervous system supports "rest and digest" processes by lowering heart rate and blood pressure, restarting gastrointestinal peristalsis /digestion, and soon.[1]

The cholinergic system transmits information regarding peripheral immune status to the central nervous system and vice versa, thus taking part in Neuroimmune communication. The neurotransmitter acetylcholine, cholinergic receptors, choline acetyltransferase, and acetylcholinesterase enzymes are all components of the cholinergic system.

These molecules regulate the immune response and maintain homeostasis. Cholinergic system controls movement, emotions and thinking. Cholinergic system includes muscarinic and nicotinic receptors. Muscarinic receptors are found in many organs throughout the body and act in the peripheral and central nervous systems. Nicotinic receptors are found in the brain and at the neuromuscular junction. Both kinds of receptors are inhibited by anticholinergic.

Anticholinergic agents are substances that competitively inhibit acetyl choline in the parasympathetic outflow. Anticholinergic drugs are helpful in the treatment of illnesses brought on by excessive parasympathetic activity, especially those affecting the gastrointestinal system, by lowering the effects of acetylcholine through competitive inhibition. [2]

An anticholinergic substance called atropine functions as a competitive, reversible antagonist of muscarinic receptors. The medicine gets its name from the plant *Atropa belladonna*, from which it was first synthesized. All visceral smooth muscles receiving parasympathetic motor innervation are relaxed by atropine. Atropine produces bronchodilation and decreases airway resistance, notably in individuals with COPD and asthma. Atropine relaxes the ureter and bladder; urine retention can develop in elderly men with prostatic enlargement. [3]

Moringa pterygosperma [*Moringa oleifera*] belonging to the monogeneric family of shrubs and trees *Moringaceae*. It is also known as the drumstick tree, horseradish tree, ben oil tree, or benzoil tree. This fast- growing, evergreen, perennial tree may reach heights of 10 to 12 meters and a trunk diameter of up to 45 centimeters. There are 13 tropical and subtropical tree species in the Moringa genus, although *Moringa pterygosperma* is the most widely used.

The leaves are compound tripinnate, fluffy, pale green, and 30 to 60 cm long with many tiny leaflets. The zygomorphic, bisexual bloom is 12 mm tall, white or cream in color, and scented. Fruit is a removable cover capsule with a length of 20–60 cm.

The leaves of the moringa tree are rich in minerals like calcium, potassium, zinc, magnesium, iron, and copper. The leaves are a source of complete protein that includes all the necessary amino acids preferably methionine and cysteine.
Moringa pterygosperma leaves have been shown to improve milk production in lactating women due to their high iron content, which makes them an excellent treatment for anaemia.

Moringa pterygosperma is being utilized commercially for its anticholinergic properties. And it have antioxidant, anti-inflammatory, anti-diabetic, antimicrobial, anti-ulcer and antipyretic, anti diarrhoeal and hypocholesteromic properties. [4]

MATERIALS AND METHODS

Plant Materials

Fresh *Moringa pterygosperma* leaves were gathered from Ernakulum district, Kerala state.

Animals

For the investigation, fresh chicken ileum was collected from slaughter house. It was kept at room temperature with adequate aeration in freshly prepared Tyrode solution.

Preparation of Plant Extract

Soxhlet extraction was used to create the *Moringa pterygosperma* extract. In this procedure, fresh *Moringa pterygosperma* leaves were air dried at room temperature for 7 days before being manually blended into a coarse powder. The soxhlet extraction chamber was filled with 35 g of the powdered substance, and ethanol was used as the extraction solvent. The extraction was carried out for 48 hours; 78ºC was kept as the extraction temperature. An electric oven was used to evaporate the ethanol at a low temperature at the conclusion of the experiment to produce a crude extract that weighed 10.50g and had a yield of 30%. [10]

IDENTIFICATION TEST

Test for Flavonoids [5]

- **Shinoda test:** To dried powder or extract, added 5 ml 95% ethanol, few drops of concentrated hydrochloric acid and 0.5 g magnesium turnings. Pink color indicates the presence of flavonoids.

- When lead acetate solution was added to a little amount of residue, a yellow colored precipitate emerged , indicating the presence of flavonoids.

- Addition of increasing amount of sodium hydroxide to the residue, yellow coloration, which decolorizes after addition of acid.

- **Alkaline reagent test:** To the test solution add few drops of sodium hydroxide solution, Intense yellow colour is formed, which turns to colorless on addition of few drops of dilute acid.

- **Zinc hydrochloride test:** Zinc dust and strong hydrochloric acid are added to the test solution, and after a short while, it becomes red.

Test for Tannins and Phenolic Compounds [6]

- 2-3 ml of aqueous or alcoholic extract, 5% ferric chloride solution, deep blue-black color indicates the presence of tannins and phenolic compounds.
- The presence of tannins and phenolic compounds was determined using 2-3 ml of an aqueous or alcoholic extract, added lead acetate solution, and white precipitate.
- Gelatin solution is added to the test solution, white precipitate confirmed the presence of tannins and phenolic compounds.
- Acetic acid solution is added to the test solution, red color confirmed the presence of tannins and phenolic compounds.
- When potassium dichromate was added to the test solution, the presence of tannins and phenolic compounds was confirmed by the solution's red hue.
- When bromine water is introduced to the test solution, the presence of tannins and phenolic compounds is shown by the bromine water's discoloration.
- When diluted iodine solution is added to the test solution, a brief red color shows that tannins and phenolic chemicals are present.
- Addition of Dilute nitric acid gives Reddish to yellow color which indicates

the presence of tannins and phenolic compounds.

Test for Saponins [7]
Two ml of alcohol diluted with water is added to the 2 ml of the plant extract, formation of foam indicates the presence of Saponins.

Test for Alkaloids [8]
To the small amount of extract, few drops of dilute HCL were added and then filtered. The filtrate is treated with Dragendroffs reagent; formation of orange brown precipitate confirmed the presence of alkaloids.

Test for Glycosides [9]
To 5 ml of extract, add 25 ml of dilute sulphuric acid and boil it for 15 minutes. Cool and neutralize with 10% sodium hydroxide, then 5 ml Fehling solution was added to it, brick red precipitate is obtained.

DRUG PREPARATIONS AND DILUTIONS
A concentration of 1 x 10 -1g/ml was created by dissolving 1 g of the extract in 10 ml of distilled water. The conventional medications, acetylcholine and atropine, were administered using the same method.

Tyrode solution was prepared per liter of water by the dissolution of the following substances:

NaCl –8g, KCl – 0.2g, CaCl2 – 0.2g, NaHCO3 – 1g, NaH2PO4 – 1g, MgCl2 – 0.1g, and Glucose – 2g.

***In Vitro* Study of the Effect of *Moringa Pterygosperma* on Isolated Chicken Ileum**
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 a 35 cc 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:

- Acetylcholine alone
- Acetylcholine in presence of atropine
- Acetylcholine in presence of Ethanolic extract of *Moringa pterygosperma* leaves. [10]

RESULT AND DISCUSSION

Sl No	Dose of Ach (ml)	Response of Acetyl choline alone		Response of Acetyl choline in presence of Moringa		Response of Acetyl choline in presence of Atropine	
		Height in mm±SEM	% Response	Height in mm±SEM	% Response	Height in mm±SEM	% Response
1	0.1	17.00±4.50	68.00	1.66±0.33	55.33	1.66±0.33	41.50
2	0.2	18.00±5.00	72.00	2.00±0.57	66.66	2.33±0.66	58.25
3	0.4	21.00±5.13	84.00	2.33±0.33	77.66	3.00±1.00	75.00
4	0.8	23.00±5.68	92.00	2.66±0.33	88.66	3.33±1.20	83.25
5	1.6	24.00±6.24	96.00	2.66±0.66	88.66	3.66±1.33	91.50
6	3.2	25.00±7.09	100.00	3.00±1.00	100.00	4.00±1.52	100.00
Mean			85.33		79.49		74.91

Table.1: *In vitro responses of an isolated chick ileum to Ach in presence of atropine and Moringa extract*

The Anti-cholinergic activity was evaluated by plotting dose response curve. Using the mean heights obtained from DRC, corresponding percentage response was calculated. The mean percentage response of Acetyl choline, *Moringa pterygosperma*, Atropine was found to be 85.33%, 79.49%, 74.91% respectively. From the observed data, the mean percentage response of *Moringa pterygosperma* was found to be 4.58% more than that of atropine.

CONCLUSION

Anti-cholinergic properties of an ethanolic extract of *Moringa pterygosperma* leaves was studied in comparison with Atropine. Acetyl choline is a neurotransmitter which produces contraction in smooth muscles. Anti-cholinergics inhibit Acetyl choline 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 *Moringa pterygosperma*. Standard methodology was used to conduct the preliminary phytochemical analysis, which identified the presence of flavonoids, phenols and tannins, alkaloids, saponins, and glycosides.Anti-cholinergic activity was studied *in vitro* using isolated chick ileum. Isolated chick ileum is an intestinal smooth muscle and it contains of a 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 suggests that acetylcholine increase contraction in a dose dependent manner. Anti-cholinergic relaxes all visceral smooth muscle. Anti-cholinergic activity of the ethanolic extract of *Moringa pterygosperma* was compared with standard atropine. Comparison of mean percentage response of *Moringa pterygosperma* with that of atropine proved significant anti-cholinergic activity of the extract.

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