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TO STUDY & COMPARE THE ANTISPASMODIC EFFECT OF DIFFERENT TURMERIC EXTRACTS

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Abstract:
Turmeric extract has a spasmolytic effect on chicken ileum, which means that it reduces muscle contractions. The spasmolytic effect of turmeric extract is mediated by several mechanisms, including inhibition of cholinergic receptors, inhibition of calcium influx through L-type calcium channels, and down-regulation of acetylcholine-triggered molecular signaling pathways. The research was conducted in vitro, which means it was done in a laboratory using isolated chicken ileum tissue. The research was conducted using three different extracts of turmeric: ethanol, acetone, and water. The research was conducted using methods of stimulating the chicken ileum: acetylcholine and histamine. Acetone extract from turmeric shows the best spasmolytic effects on acetylcholine-induced contraction. The spasmolytic effects of turmeric extract may be beneficial for the treatment of conditions such as irritable bowel syndrome, inflammatory bowel disease, and constipation. The research found that all three extracts of turmeric had a spasmolytic effect on chicken ileum, Acetone extract shows a maximum antagonist effect on chicken ileum as compared to the other extracts of turmeric However, more research is needed to confirm these findings and to determine the optimal dose and duration of turmeric extract therapy.

Keywords: spasmolytic, acetylcholine, histamine, Acetylcholine, Contraction, Antagonism.

Introduction:

Spasm term derived as a condition related to spasm. Briefly, it deals with the problematic condition due to the sudden contraction of the muscle. An antispasmodic(spasmolytic) is a pharmaceutical drug or another agent that suppresses muscle spasms.[1]

The antispasmodic (spasmolytic) effect of drugs is commonly used for the reduction of excessive smooth muscle contractility, responsible for cramping and discomfort in the abdominal area, caused by multiple conditions affecting the gastrointestinal, biliary, or genitourinary tract [2].

A variety of synthetic antispasmodic drugs have been authorized worldwide by regulatory agencies, the most important being anticholinergic agents (butylscopolamine), direct smooth muscle relaxants (papaverine), calcium antagonists (pinaverium) or opioid receptor modulators (trimebutine) [2,3]. Despite their clinical efficacy, the use of these molecules is often limited by the development of unpleasant and sometimes severe side effects which may reduce patient compliance and impair treatment efficiency [2,5].

Historically, a long time before the golden age of medicinal chemistry, several aromatic plants were used in traditional medicine for the treatment of different ailments in some parts of the world. In Europe, aromatic plants like peppermint or thyme have been used for medical purposes since antiquity while in Chinese or Indian traditional medicine other aromatic species like cinnamon or sandalwood were known for centuries [6].

Nowadays, antispasmodic botanical remedies are used by a constantly increasing number of patients for symptomatic treatment of functional dyspepsia, intestinal, colonic, or ureteral spasms, gallbladder hyperactivity, and uterine cramps [7]. Hyoscyamine and dicyclomine are both antispasmodics due to their anticholinergic effects. Herbal remedies such as turmeric, ginger, sandalwood, cinnamon, thyme, and peppermint& their extracts are also used as antispasmodic agents.

TURMERIC

Chemical Name: Curcumin (curcuma Longa) [8]

Synonym: Curcuma, curcumae longae rhizome, curcumin, curmunoid, haldi, haridra, Indian saffron [8]

Family: Zingiberaceae.[8]

Geographical source: - The plant is native to southern Asia & is cultivated extensively in temperate regions. It is grown on a larger scale in India, China, the east indies, Pakistan & Malaysia.[9]

Biological source: Dried rhizome of curcuma longa linn.[9]

Chemical constituents: Curcumin, the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions. Turmeric contains yellow coloring matter called curcuminoids (5%) and essential oil (6%). The chief constituent of the coloring matter is curcumin I (60%) in addition to small quantities of curcumin III, curcumin II, and dihydro curcumin. The volatile oil contains mono- and sesquiterpenes like zingiberene (25%), α-phellandrene, sabinene, turmerone, ar-turmerone, borneol, and cineole. The choleretic action of the essential oil is attributed to β-polymethyl carbinol.

The volatile oil also contains α- and β-pinene, camphene, limonene, terpinene, terpinolene, caryophyllene, linalool, isoborneol, camphor, eugenol, curdione, curzerenone, curlone, AR-curcumenes, β-curcumene, γ-curcumene. α- and β-turmerones, and curzerenone.[10]

Health benefits of turmeric in routine life [13].

1. It is a natural antiseptic and antibacterial agent, useful in disinfecting cuts and burns.
2. When combined with cauliflower, it has been shown to prevent prostate cancer and stop the growth of existing prostate cancer.
3. Prevented breast cancer from spreading to the lungs in mice.
4. May prevent melanoma and cause existing melanoma cells to commit suicide.
5. Reduces the risk of childhood leukemia.
6. Is a natural liver detoxifier.
7. May prevent and slow the progression of Alzheimer's disease by removing amyloid plaque Build-up in the brain.
8. May prevent metastases from occurring in many different forms of cancer.
9. It is a potent natural anti-inflammatory that works as well as many anti-inflammatory drugs but without side effects.
10. Has shown promise in slowing the progression of multiple sclerosis in mice.

Material and Methods:

1.) COLLECTION OF CRUDE DRUGS OF TURMERIC:

- Rhizomes part of the Turmeric plant was collected from the market.
- Later on, rhizomes were washed with water to remove dirt and dried by keeping them in direct sunlight.
- Whereas 200 gm rhizomes were ground to convert them into powder form and the remaining Crude drug was used for morphology & microscopical study.

2.) ORGANOLEPTIC PARAMETERS:

Color:

- External -Yellowish to yellowish-brown
- Internal - Yellowish-orange to orange

Odor: Characteristic

Taste: Slightly bitter
Size: 2 to 5 cm in length & 1 to 2 cm in thickness
Shape: Ovate or oblong

3.) AUTHENTICATION THROUGH MICROSCOPY:

The following slides are prepared for microscopical study.

Unstained section

- Take & cut the sanction of turmeric rhizomes with the help of a blade.
- Take the section in the test tube & add chloral hydrate & boil it for 1 minute.
- Put the section on the slide transfer the section to be mounted with the help of a brush & add glycerine.
- Cover it with a coverslip to prepare an unstained slide & observe it in the microscope.

Stained section

- Take a section in a watch glass.
- Add concentrated HCl + phloroglucinol & keep for 1 minute.
- prepare a stained slide & observe it in the microscope.

Unstained powder

- Take powder of turmeric rhizomes with the help of a blade.
- Take powder in a test tube & add chloral hydrate & boil it for 1 minute.
- Put the powder on the slide transfer the section to be mounted with the help of a brush & add glycerine.
- Cover it with a coverslip to prepare an unstained slide & observe it in the microscope.

Stained powder

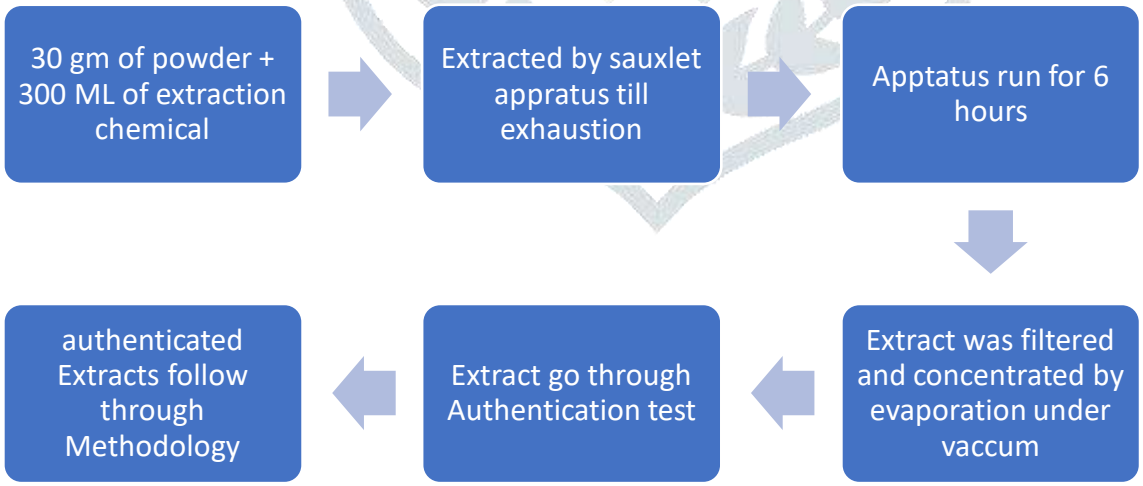
- Take powder in a watch glass.
- Add concentrated HCl + phloroglucinol & keep for 1 minute.
- prepare a stained slide & observe it in the microscope.

4.) EXTRACTION:

Extraction of Turmeric Extract from turmeric powder can be done by using the following method.

Soxhlet apparatus method

- It is a method used to extract herbal plant drug extract with different solvents such as Acetone, ethanol, methanol, benzene & its derivatives.



5.) PHYTOCHEMICAL SCREENING:

Preliminary phytochemical screening of TE was carried out according to the standard procedures of qualitative chemical examination for the detection of alkaloids, carbohydrates, glycosides, flavonoids, amino acids, volatile oils, fixed oils & fats [11].

6.) PHARMACOLOGICAL SCREENING

Material Preparation

The chicken intestine was procured from the local market from Chicken Shop in Maktampur village, District Bharuch.

SAMPLE PREPARATIONS OF TURMERIC

1. Plant Material Curcuma longa officinale rhizomes powder was obtained from drying and Authenticated by the Department of Pharmacognosy.

   A specimen was deposited for the same for future reference.

   The powdered material (60 gm) was extracted with ethanol for 6 hours and the solvent was evaporated under a vacuum to obtain the crude ethanolic extract of Curcuma longa officinale extract (TE).

   The extraction was performed using the Soxhlet apparatus.

   The extract was concentrated.

2. **Physiological salt solution: [For 10 liters of water]**

   Ringer lock's solution (10 Litre)

   Composition (g/L):

   - Sodium Chloride (NaCl) = [90.0g],
   - Potassium Chloride (KCI) = [4.2g],
   - Calcium Dichloride (CaCl2) = [2.4g],
   - Sodium Bicarbonate (NaHCO3) = [5.0g],
   - Glucose powder (C6H12O6) = [10.0g]

3. **Standard drugs used were:**
   - Acetylcholine chloride,
   - Atropine Sulphate,
   - Histamine.
4. **Instrument & Accessories:**
   - Organ bath,
   - Kymograph paper,
   - Dissection box with scissors, forceps, knives, probes & scalps.

METHODOLOGY

1. **Isolated Chicken Ileum Preparation:** About 2 cm length ileum segment was cut, mounted in an organ bath containing Tyrode solution, and continuously aerated and maintained at 37±0.5◦C. One end of the ileum was attached to an S-shaped aeration tube and the other end to an isotonic frontal writing lever attached to a Sherrington recording drum with a kymograph. The tissue was allowed to equilibrate for 30 min under an optimum load. A Contact time of 60 seconds with three items of washing at an interval of 50 seconds was followed by recording the responses.

2. **Dose-response curve of acetylcholine (Ach):** Acetylcholine (0.1gm/ml) of dose 0.4ml was given to the tissue in the organ tube to obtain a contractile response. The contractile responses were measured from the baseline of the response. Wash was given to the tissue after recording the contractile response.
3. **Dose response curve of acetylcholine+ atropine:** In similar way to acetylcholine, responses of acetylcholine(0.1gm/ml) + atropine (0.1gm/ml) of dose 0.4 ml were recorded. Washings were given to the tissues after the response have been recorded.
4. **Dose-response curve of histamine:** After the response has been recorded, the histamine (0.1gm/ml) of dose 0.4 ml was given to the tissue, and the contractile responses were recorded. Wash was done after the responses were recorded.
5. **Dose-response curve of turmeric ethanol extract in the presence of acetylcholine:** In a similar way to Acetylcholine, responses of the turmeric ethanol extracts of dose 0.4 ml were taken in the presence of acetylcholine of dose 0.4 ml were recorded.
6. **Dose-response curve of turmeric ethanol extract in the presence of histamine:** After the washing period, the response of the turmeric ethanol extract of dose 0.4ml was taken in the presence of histamine of dose 0.4ml were recorded.
7. **Dose-response curve of turmeric acetone extract in the presence of acetylcholine:** Response of the turmeric acetone extracts of dose 0.4ml was taken in the presence of acetylcholine of dose 0.4ml were recorded.
8. **Dose-response curve of turmeric acetone extract in the presence of histamine:**
   Responses of the turmeric acetone extract of dose 0.4ml taken in the presence of histamine of dose 0.4ml were recorded.
9. **Dose-response curve of turmeric water extract in the presence of acetylcholine:**
   In a similar way to Acetylcholine, responses of the turmeric water extracts of dose 0.4ml were taken in the presence of acetylcholine of dose 0.4ml were recorded.
10. **Dose-response curve of turmeric water extract in the presence of histamine:**
    after the washing period, the response of the turmeric ethanol extract of dose 0.4ml was taken in the presence of histamine of dose 0.4ml was recorded.

Results and Discussion:

1) **ORGANOLEPTIC PARAMETERS**

Color:

- External -Yellowish to yellowish-brown
- Internal - Yellowish-orange to orange

Odor: Characteristic

Taste: Slightly bitter

Size: 2 to 5 cm in length & 1 to 2 cm in thickness

Shape: Ovate or oblong

2) AUTHENTICATION THROUGH MICROSCOPY [14]

- The transverse section of the rhizome is characterized by the presence of mostly thin-walled rounded parenchyma cells, scattered vascular bundles, definite endodermis, few layers of cork developed under the epidermis, and scattered oleoresin cells with brownish contents.
- The epidermis consists of thick-walled cells, cubical in shape, of various dimensions.
- The cork cambium is developed from the sub-epidermal layers and even after the development of the cork, the epidermis is retained.
- Cork is generally composed of four to six layers of thin-walled brick-shaped parenchymatous cells.
- The parenchyma of the pith and cortex contains grains altered to a paste, in which sometimes long lens-shaped unaltered starch grains of 4–15 μm diameter are found.
- Oil cells have supersized walls and contain either orange-yellow globules of a volatile oil or amorphous resinous masses.
- Cortical vascular bundles are scattered and are of a collateral type.
- The vascular bundles in the pith region are mostly scattered and they form discontinuous rings just under the endodermis.
- The vessels have mainly spiral thickenings and only a few have reticulated and annular structures.

Figure 8 Microscopic Observations

3) Extraction of Turmeric Extract from Turmeric powder:

Three Extracts have been authenticated as given below

1. Turmeric + Water extract
2. Turmeric + Acetone Extract
3. Turmeric + Methanol Extract

4. PHYTOCHEMICAL SCREENING:

Table 1 Phytochemical Screening

Sr. No.	CONSTITUENT	IDENTIFICATION TEST	ETHANOLIC EXTRACT OF TURMERIC	ACETONE EXTRACT FROM TURMERIC	METHANOLIC EXTRACT FROM TURMERIC	WATER EXTRACT FROM TURMERIC
1.	CARBOHYDRATES	• BENEDICT TEST • FEHLING'S TEST	+ +	+ -	+ -	+ -
2.	PROTIEN'S TEST	• MILLION'S TEST • BUSET TEST	- -	- -	- -	- -
3.	AMINO ACIDS	• NINHYDRIN TEST • TYROSINE	+/- +	+/- +	+ +	+/- +
4.	STEROID TEST	• SALKOWSHI TEST • LIBERMAN BURCHARD TEST	- +/-	+ +/-	+ -	+ +/-
5.	GLYCOSIDES	• BUFFET'S TEST • KELLER-KILLIANI'S TEST	+ +	+ +	+ +	+ +
6.	TANNINS TEST	• LEAD ACETATE TEST • DILUTE HNO3 TEST	- -	- -	+ +	- -
7.	ALKALOIDS TEST	• DRAGENDROFF'S TEST • MAYER'S TEST	+ +	+ -	+ +	+ -

5. PHARMACOLOGICAL SCREENING:

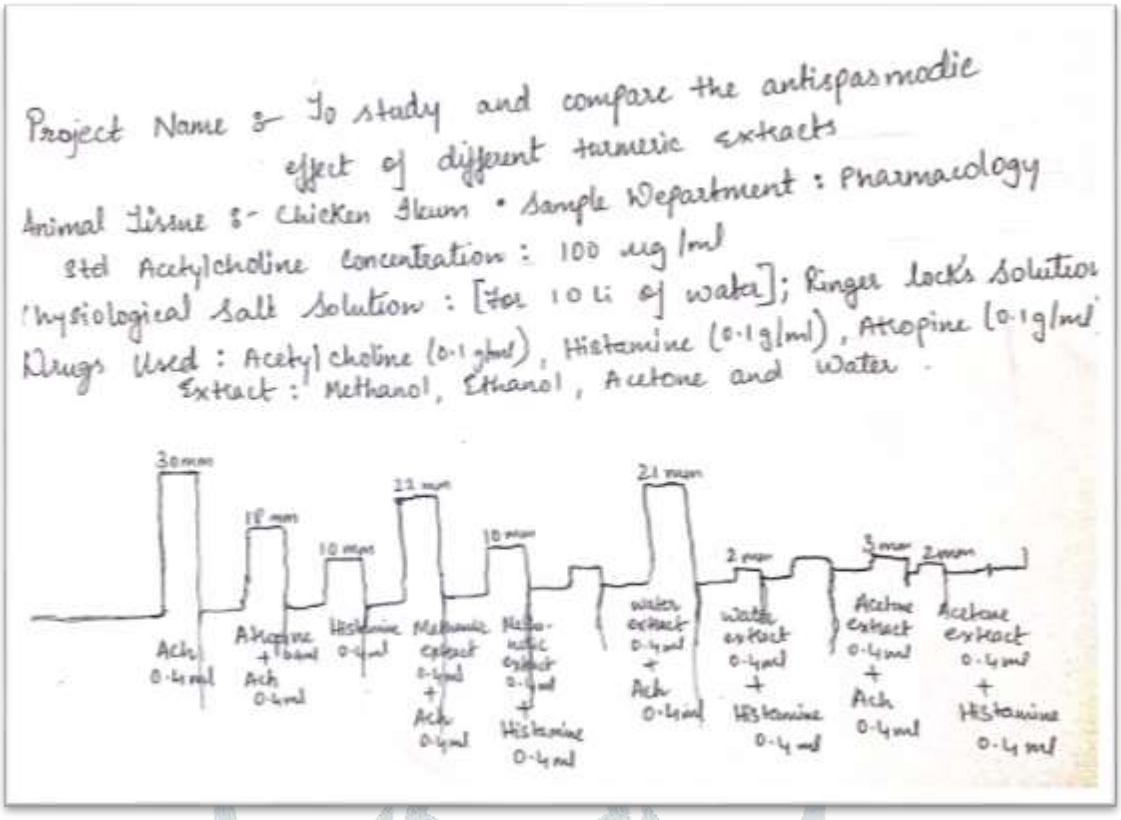


Figure 21 Kymograph readout

Table 2 Pharmacological screening

SAMPLE	DOSE (ML)	HEIGHT IN MM	SAMPLE	DOSE (ML)	HEIGHT IN MM
Acetyl Choline	0.4 ml	30 mm	Methanol extract + Acetylcholine	0.4 ml	22 mm
Atropine + Acetyl Choline	0.4 ml	18 mm	Methanol extract + Histamine	0.4 ml	14 mm
Histamine	0.4 ml	10 mm	Acetone extract + Acetylcholine	0.4 ml	3 mm
			Acetone extract + Histamine	0.4 ml	2 mm
			Water + Acetylcholine	0.4 ml	21 mm
			Water + Histamine	0.4 ml	20 mm

Table 3 Observation table

SAMPLE	HEIGHT (MM) OF STD	HEIGHT (MM) OF EXTRACT
Ach & Ach + Atropine	30	18
Ach & Methanolic ext + Ach	30	22
Ach & Acetone ext + Ach	30	3
Ach & Water ext + Ach	30	21
Histamine & Methanol ext + Histamine	10	11
Histamine & Acetone ext + Histamine	10	2
Histamine & Water ext + Histamine	10	2

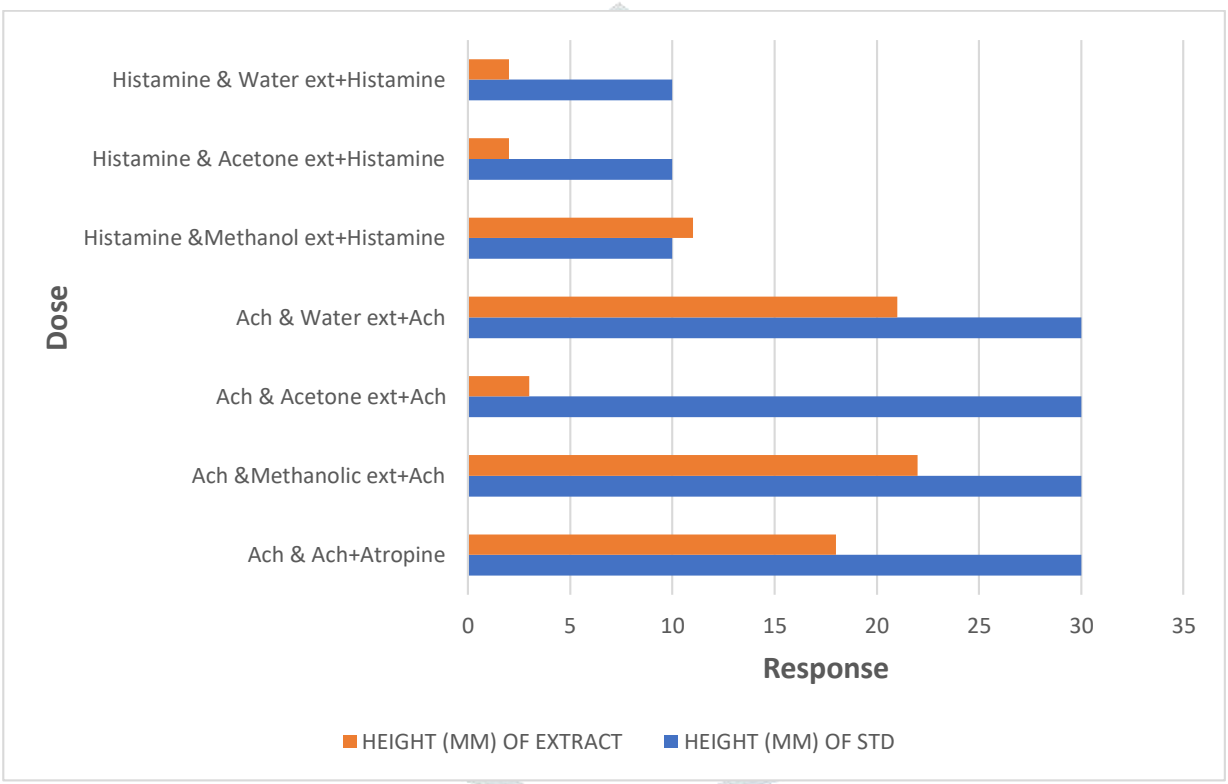


Figure 2 Response of std and various turmeric extracts

Discussion

Ach and histamine produce a contractile effect. These drugs are spasmogenic. Atropine and different extracts of Turmeric do not produce any effect of their own but inhibit the responses to Ach, and histamine respectively. The antagonism by atropine and extracts to Ach and histamine respectively is specific, while that produced by curcumin is non-specific. Ach and histamine act through specific receptors muscarinic and H_1 receptors while atropine and curcumin especially block them. Antagonism is a type of drug interaction in which one drug inhibits the response of another. Antagonism may be (1) Physiological (e.g., antagonism between sympathetic and parasympathetic systems). (2) Chemical (e.g., calcium and EDTA) or (3) Pharmacological (e.g., Ach and atropine).

Pharmacological antagonism may be competitive or non-competitive.As per the results, Turmeric rhizomes extracts show an antagonist effect. however, these extracts produce relaxation of smooth muscles. Acetone extract shows a maximum antagonist effect on chicken ileum as compared to the other extracts of turmeric.

Summary

Turmeric extract has a spasmolytic effect on chicken ileum, which means that it reduces muscle contractions. The spasmolytic effect of turmeric extract is mediated by several mechanisms, including inhibition of cholinergic receptors, inhibition of calcium influx through L-type calcium channels, and down-regulation of acetylcholine-triggered molecular signaling pathways. Acetone extract from turmeric shows the best spasmolytic effects on acetylcholine-induced contraction. The spasmolytic effects of turmeric extract may be beneficial for the treatment of conditions such as irritable bowel syndrome, inflammatory bowel disease, and constipation. However, more research is needed to confirm these findings and to determine the optimal dose and duration of turmeric extract therapy.

Conclusion

The research was conducted in vitro, which means it was done in a laboratory using isolated chicken ileum tissue. The research was conducted using three different extracts of turmeric: ethanol, acetone, and water. The research was conducted using methods of stimulating the chicken ileum: acetylcholine and histamine. The research found that all three extracts of turmeric had a spasmolytic effect on chicken ileum, Acetone extract shows a maximum antagonist effect on chicken ileum as compared to the other extracts of turmeric.

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