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Phytochemical Screening Andinvitro Antispasmodicactivity of Passiflora Edulis

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ABSTRACT:Passifloraedulis (Passifloraceae) is a widely growing plant which has been used in the traditional medicine for treating many ailments. This study was aimed to investigate the antispasmodic effects on Acetyl choline(Ach) induced contractions on isolated chicken ileum. The antispasmodic activity was assessed by the interpolation method on isolated chicken ileum.Effects of Ach, Ach along with ethanol and petroleum ether extracts were studied on isolated chicken ileum which later compared with atropine as standard antispasmodic agent. The extracts exhibited dose dependant attenuation of contractions induced by Ach on isolated chicken ileum. Preliminary phytochemical studies on both extracts indicated the presence of carbohydrates, alkaloids, glycosides, saponins and flavonoids. Thus, the present study revealed that the extracts of Passifloraedulis shows promising antispasmodic action.

KEYWORDS: Acetyl choline, antispasmodic, Passifloraedulis, Dose response curve, Atropine

I. INTRODUCTION:

Passifloraedulisbelonging to the family Passifloraceaeis a wide spread plant cultivated around all tropical countries of the world. There are two main commercial types, the yellow (P edulisflavicarpaDegenerer) and purple form (P edulis Sims).[1]The principal components of P.edulis include alkaloids, polyphenols, triterpenesand its glycosides, carotenoids, cyanogenic glycosides, polysaccharides, amino acids, essential oils and microelements.[2-3] Various phytochemical constituents have been found and identified from the different plant parts of P.edulis. Its extracts, fruit juice and isolated compounds showed many health effects and biological activities.[4]Studies have revealed various bioactivities of P.edulissuch as antioxidant[5], antimicrobial,anti-inflammatory[6],antihypertensive, hepatoprotective and lung-protective activities, antidiabetic, sedative, antidepressant and anxiolytic-like actions.[4][7][8] Leaves, fruits and roots ofpassiflora species are traditionally used in several countries for the treatment of insomnia, anxiety and irritability.[2]Analgesic activity is also reported.[9]
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Gastrointestinal disorders includes functional abdominal pain, ulcerative colitis, irritable bowel syndrome, infantile colic and constipation, as well as gastroenteritis and acute gastrointestinal disorder which are characterized by recurrent or chronic abdominal pain.[10] Spasm is a sudden involuntary and abnormal muscular contraction. Drugs with antispasmodic effects are normally applied for the symptomatic treatment of contraction and cramping of smooth muscle in GI diseases as well as in other critical clinical situations. The spasm is induced by the stimulation of cholinergic/muscarinic, opioid, and histaminic receptors.The stimulation of the cholinergic receptors (M_1, M_3 and M_5) leads to stimulate the intracellular signaling up to the activation of PKA to PKC. Both of these kinase enzymes stimulate the calcium channel and increases intracellular Ca^{2+} influx. This increased Ca^{2+} level interacts with smooth muscle and causes full contraction which also produces diarrhea.Antispasmodics perform their action (Relaxant effect on SM) in different ways such as; inhibition of Acetyl choline, serotonin, reduction of extracellular Ca^{2+}, blocking of muscarinic receptors, Ca^{2+} channels and activation of potassium ATP channels.[11]

Due to the popularity of the Passifloraedulisin folk medicine, we were prompted to investigate the antispasmodic activities of its aerial part extracts using invitro models.

II. MATERIALS AND METHODS:

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PLANTCOLLECTION AND AUTHENTICATION

Aerial parts of the plant Passifloraeduliswas collected during the month of December 2021 from Kottayam district, Kerala, India. The plant was authenticated by Dr.Rojimon P Thomas, Head of Department of Botany, CMS College, Kottayam. The collected plant was washed for removing dust and dirt and was dried under shade for 14days. The shade dried material was pulverized by means of a mechanical grinder.

EXTRACTION

About 400g of coarsely powdered plant material were placed in stoppered containers separately with Ethyl alcohol and Petroleum ether as solvent (Menstruum). It was allowed to stand at room temperature for a period of 7 days with frequent shaking until the soluble matter has dissolved. The mixture was then strained, the marc (Damp solid material) was pressed, and the combined liquids were clarified by filtration. The solvents were evaporated at room temperature.[12]

PHYTOCHEMICAL SCREENING

Ethanolic and Petroleum ether extracts of Passifloraeduliswere subjected to qualitative analysis for various phytoconstituents like carbohydrates, proteins, amino acids, alkaloids, glycosides, phytosterols, saponins, tannins, phenols and flavonoids.

1.Detection of Alkaloids

The extracts were individually dissolved in Dil.HCl and filtered.

a. Dragendorff's test

To 0.5ml of filtrate, added 1ml of Dragendorff's reagent. Production of an orange-red precipitate confirms the test.

b. Mayer's test

To 1ml of filtrate, added a few drops of Mayer's reagent. Formation of white or pale precipitate.

c. Hager's test

To 1ml of the filtrate, added Hager's reagent. Formation of yellow ppt indicates the presence of alkaloids.

d. Wagner's test

To 1ml of filtrate, added few drops of Wagner's reagent, formation of yellow or brown ppt.

2. Detection of Carbohydrates

The extracts were individually dissolved in distilled water and filtered.

a. Molisch's test

In a test tube containing 2ml of filtrate, 2 drops of freshly prepared 20% alcoholic solution of α-naphthol was added and mixed. To this solution, added Conc.H_2SO_4 through the sides of test tube. Violet ring formation at the junction of the solution.

b. Fehling's test

Filtrates were hydrolyzed with Dil.HCl, neutralized with alkali and heated with Fehling's A&B solutions. Formation of red or brick red ppt.

c. Benedict's test

Extract was treated with benedict's reagent ($CuSO_4$ + sodium citrate + sodium carbonate in water), and heated for 10minutes in waterbath. Formation of orange-red ppt.

3. Detection of Proteins and amino acids

a. Xanthoproteic test

The extracts were treated with few drops of Conc. HNO_3, formation of yellow color

b. Biurette test

To 3ml test solution, added 4% NaOH and few drops of 1% $CuSO_4$ solution, formation of violet or purple color.

c. Ninhydrin test

3ml of the test solution and 3drops of 5% Ninhydrin solution was heated for 10minutes on a boiling water bath. Purple or bluish color.

4.Detection of steroids and triterpenoids

a. Salkowski reaction

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Con. H_2SO_4, shaken and allowed to stand. Appearance of greenish yellow color.

b. Leibermannburchard reaction

To the extract, add 2-3drops of chloroform and few drops of acetic acid and Con.H_2SO_4 is added along the sides of the test tube. Formation of red ring at the junction of two layers.

c. To 2ml of the extract, added 2ml chloroform and 2ml conc H_2SO_4 and shaken well. Red color was produced in the upper layer and a greenish yellow fluorescence in the lower layer.

5.Detection of glycosides

Extracts were hydrolyzed with Dil.HCl, and then subjected to test for glycosides.

a. Extracts were treated with 1ml water and aqueous NaOH. Formation of yellow color.

b. Legal test

Extracts were treated with sodium nitroprusside in pyridine and NaOH. Pink to blood red color.

6. Detection of saponins

a. Foam test

The drug extract or dry powder on vigorous shaking with water for 10 minutes. Foam formation.

7. Detection of flavonoids

a. Alkaline Reagent test

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Extratss were treated with few drops of NaOH solution. Formation of yellow color and disappearance on addition of dil.sulphuric acid

b. Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow color.

8. Detection of Tannins

a. Ferric chloride test

To 1-2ml of extract, a few drops of 5% aqueous $FeCl_3$ solution was added. Formation of blue or green color.

9. Detection of Phenols

a. Ferric chloride test

To 1ml of extract, 2ml of distilled water is added followed by drops of 10% solution of aqueous $Fecl_3$. Formation of blue or green color.

b. Lead acetate test

1ml of extract was diluted to 5ml with distilled water and to this few drops of 1% aqueous solution of lead acetate was added. Formation of yellow ppt.[13-15]

ANTISPASMODIC ACTIVITY

PREPARATION OF TISSUE

The chicken intestine was collected from the slaughter house and immediately transferred it into freshly prepared Modified Tyrode's solution (NaCl-8gm, Glucose-2g, $NaHCO_3$-1g, KCl-0.2g, $CaCl_2$-0.15g, $MgCl_2$-0.1g for 1 litre) maintained at 37℃ and aerated with 95% O2 and 5% CO2. The ileum was dissected out and the lumen of intestine was cleaned and upper end of it was cut into segments of 2-3cm long. The piece of intestine was mounted by tying the bottom end of the muscle to the hook of the oxygen tube while the other end was tied on to the frontal lever (Magnification-3cm). A tension load of 0.5g was applied and the tissue was allowed to stabilize for a period of 45minutes in student's organ bath.

ANTI-SPASMODIC ACTIVITY ASSAY

Firstly, concentration dependent responses of Acetylcholine(100µg/ml) were recorded (with dose of 0.1ml, 0.2ml, 0.4ml and 0.8ml) using Sherrington's recording drum with a frontal writing lever.

Time cycle-5minutes
Base line-30 seconds
Contact time-90seconds
Relaxation time-3minutes

The drum was started and run (speed-0.25mm/sec) for 30sec before adding the drug for recording the baseline. An appropriate quantity of working standard of acetylcholine (0.1ml, 10µg) was added to the organ bath and the drum was run for 90sec and the response due to contraction was recorded. The physiological salt solution in the organ bath was then immediately removed after stopping the drum and fresh Tyrode's solution was filled in order to wash the tissue and the inner side of the organ bath. Washing was done for 3min at regular intervals during the relaxation period. The response was measured for increasing concentration of Ach in geometric progression until a supramaximal response was recorded.

Then, same concentration dependent responses of acetylcholine (Ach) using same procedure for a mixture of Tyrode's solution+ Passifloraedulis extract (with a concentration of 100µg/ml) were recorded.

Lastly, the concentration dependent responses of Atropine (100µg/ml)along with acetyl choline, as standard antispasmodic agent were recorded.[16-19]

III. RESULTS AND DISCUSSION

PHYTOCHEMICAL SCREENING

The preliminary phytochemical screening of Ethanolic and Petroleum ether extracts of Passifloraedulishas revealed the presence of phytochemicals as shown in the Table 1.

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Table 1: Phytochemical screening

Extracts of *Passifloraedulis*		
	Inference	
Phytochemical constituents	Ethanolic	Petroleum ether
1.Alkaloids		
Dragendorff's test	+	+
Mayer's test	+	+
Hager's test	+	+
Wagner's test	+	+
2. Carbohydrates		
Molisch's test	+	+
Fehling's test	-	-
Benedict's test	-	-
3. Proteins and Amino acids		
Xanthoproteic test	-	-
Biuret test	-	-
Ninhydrin test	-	-
4.Steroids and Triterpenoids		
Salkowski reaction	+	-
Leibermann-burchard reaction	+	-
5.Glycosides		
Test with NaOH	+	+
Legal test	+	+
6. Saponins		
Foam test	+	+
7. Flavonoids		
Alkaline reagent test	+	+
Lead acetate test	+	+
8.Tannins		
Ferric chloride test	+	-
9.Phenols		
Ferric chloride test	+	-
Lead acetate test	+	-

ANTISPASMODIC ACTIVITY

Effect of Acetylcholine on chicken ileum with an increase in dose is shown in Fig.1

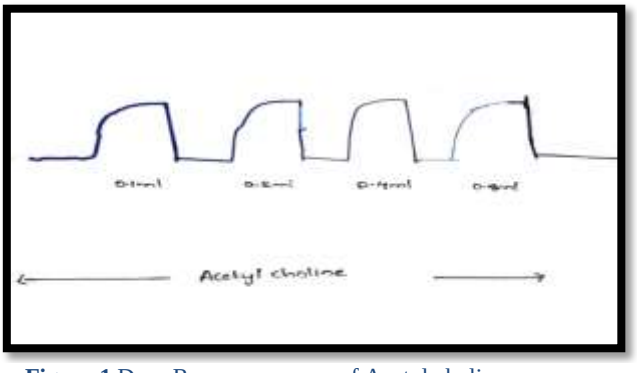

Figure 1 Dose Response curves of Acetyl choline

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Table 2: Dose response relationship observation of Ach

Sl No.	Drug	Dose	Response (cm)
1	Acetyl choline	0.1 ml	1.9 cm
2		0.2 ml	2.1 cm
3		0.4 ml	2.1 cm
4		0.8 ml	2.3 cm

Dose response curve of Ach with Ethanolic extract of Passifloraedulisis shownin Fig.2

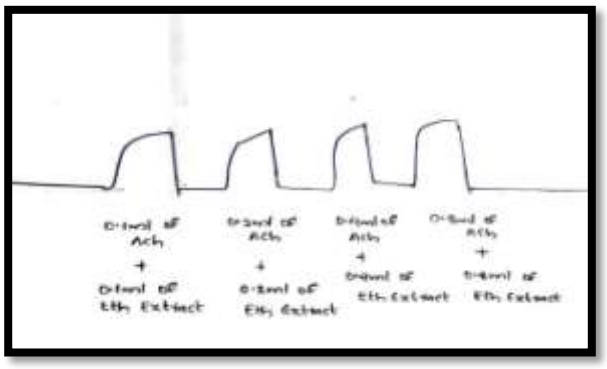

Figure 2 Dose Response curves of Ach + ethanolic extract

Sl No.	Drug	Dose	Response (cm)
1	Acetyl choline + Ethanolic extract	0.1 ml + 0.1 ml	1.5 cm
2		0.2 ml + 0.2 ml	1.5 cm
3		0.4 ml + 0.4 ml	1.6 cm
4		0.8 ml + 0.8 ml	1.8 cm

Table 3: Dose response relationship observations of Ach + Ethanolic extract

Effect of anticholinergic drug Atropine (Standard) on isolated chicken ileum is shown in Fig.3

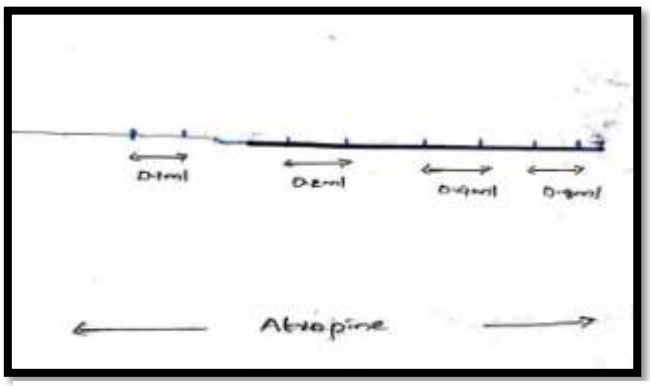

Figure 3 Dose Response curves of Atropine

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Table 4: Dose response relationship observations of Atropine

Sl No.	Drug	Dose	Response (cm)
1	Atropine + Ach	0.1 ml + 0.1ml	0
2		0.2 ml + 0.2ml	0
3		0.4 ml + 0.4ml	0
4		0.8 ml + 0.8ml	0

Effect of Acetylcholine on chicken ileum with an increase in dose is shown in Fig.4

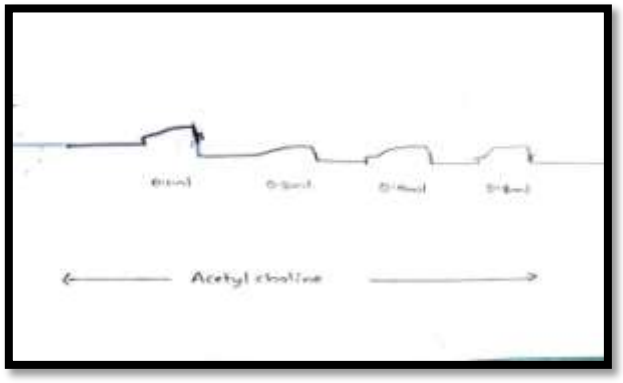

Figure 4 Dose Response curves of Ach

Table 5: Dose response relationship observations of Ach

Sl No.	Drug	Dose	Response (cm)
1	Acetyl choline	0.1 ml	0.4 cm
2		0.2 ml	0.5 cm
3		0.4 ml	0.5 cm
4		0.8 ml	0.6 cm

Dose response curves of Ach with Petroleum ether extract of Passifloraedulisis shown in Fig.5

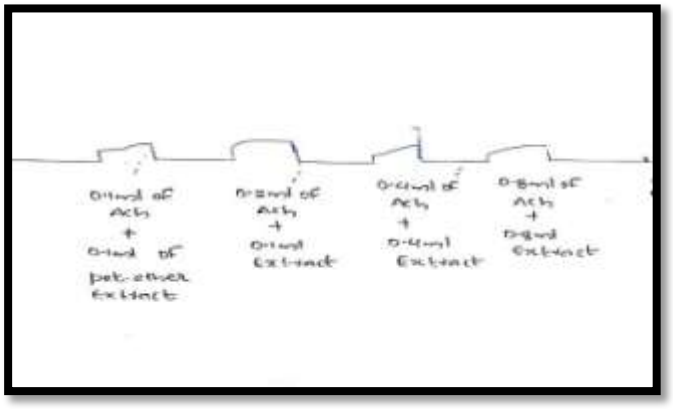

Figure 5 Dose Response curves of Ach + Petroleum ether extract

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Table 6: Dose response relationship observations of Ach + Pet.ether extracts

Sl No.	Drug	Dose	Response (cm)
1	Acetyl choline + Petroleum ether extract	0.1 ml + 0.1 ml	0.3cm
2		0.2 ml + 0.2 ml	0.4 cm
3		0.4 ml + 0.4 ml	0.4 cm
4		0.8 ml + 0.8 ml	0.5 cm

Effect of anticholinergic drug Atropine (Standard) on isolated chicken ileum is shown in Fig.6

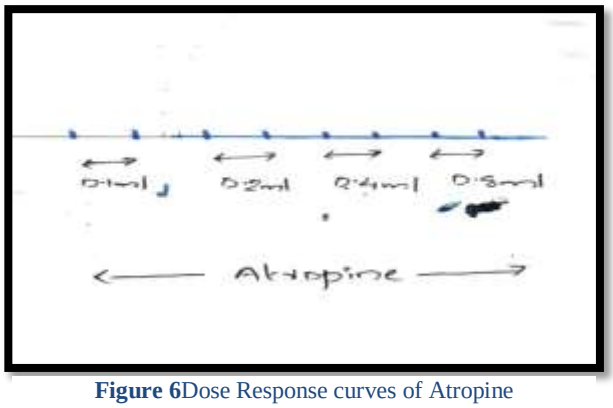

Figure 6 Dose Response curves of Atropine

Table 7: Dose response relationship observations of Atropine

Sl No.	Drug	Dose	Response (cm)
1	Atropine + Ach	0.1 ml + 0.1ml	0
2		0.2 ml + 0.2ml	0
3		0.4 ml + 0.4ml	0
4		0.8 ml + 0.8ml	0

As stated in Table 1**,** the preliminary phytochemical screening of ethanolic extract of Passifloraedulishas revealed the presence of alkaloids, carbohydrates, steroids and triterpenoids, glycosides, saponins, tannins, flavonoids & phenols. The petroleum ether extract has showed the presence of alkaloids, carbohydrates, glycosides, saponins and flavonoids. The ethanolic extract gave positive results for more phytoconstituents than the petroleum ether extract, thus indicates the presence of more phytoconstituents in ethanolic extract. This may be due to the higher polarity of ethanol.

The effect of Ach on isolated chicken ileum showed an increase in contraction(response) with an increase in dose as depicted in Table 2 and Figure 1. Ach induced spasm followed by treatment of Ethanolic extract of Passiflora edulis showed a decrease in response as shown in Table 3 and Figure 2. This indicates the antispasmodic potential of the extract. Treatment of anticholinergic drug Atropine with Ach showed expected nicotinic receptor(N_M) blocking action(antispasmodic) on isolated chicken ileum as shown in Table 4 and Figure 3.

Table 5 and Figure 4 summarized that Ach produces increase in contraction(response) with an increase in dose on isolated chicken ileum. Ach induced spasm followed by treatment of Petroleum ether extract of Passifloraedulisshowed a decrease in response as shown in Table 6 and Figure 5. This indicates the antispasmodic potential of the extract. Treatment of anticholinergic drug Atropine showed expected nicotinic receptor(N_M) blocking action(antispasmodic) on isolated chicken ileum as shown in Table 7 and Figure 6.

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Table 8: Comparative dose response relationship of Ach and Ach followed by Ethanolic extracts of Passifloraedulis

Sl no.	Treatment given	Dose (ml)	Response (cm)	% decrease in Response
1	Acetyl choline	0.1	1.9	-
2		0.2	2.1	
3		0.4	2.1	
4		0.8	2.3	
5	Acetyl choline + Ethanolic extract	0.1 + 0.1	1.5	21.10%
6		0.2 + 0.2	1.5	28.57%
7		0.4 + 0.4	1.6	23.81%
8		0.8 + 0.8	1.8	21.74%

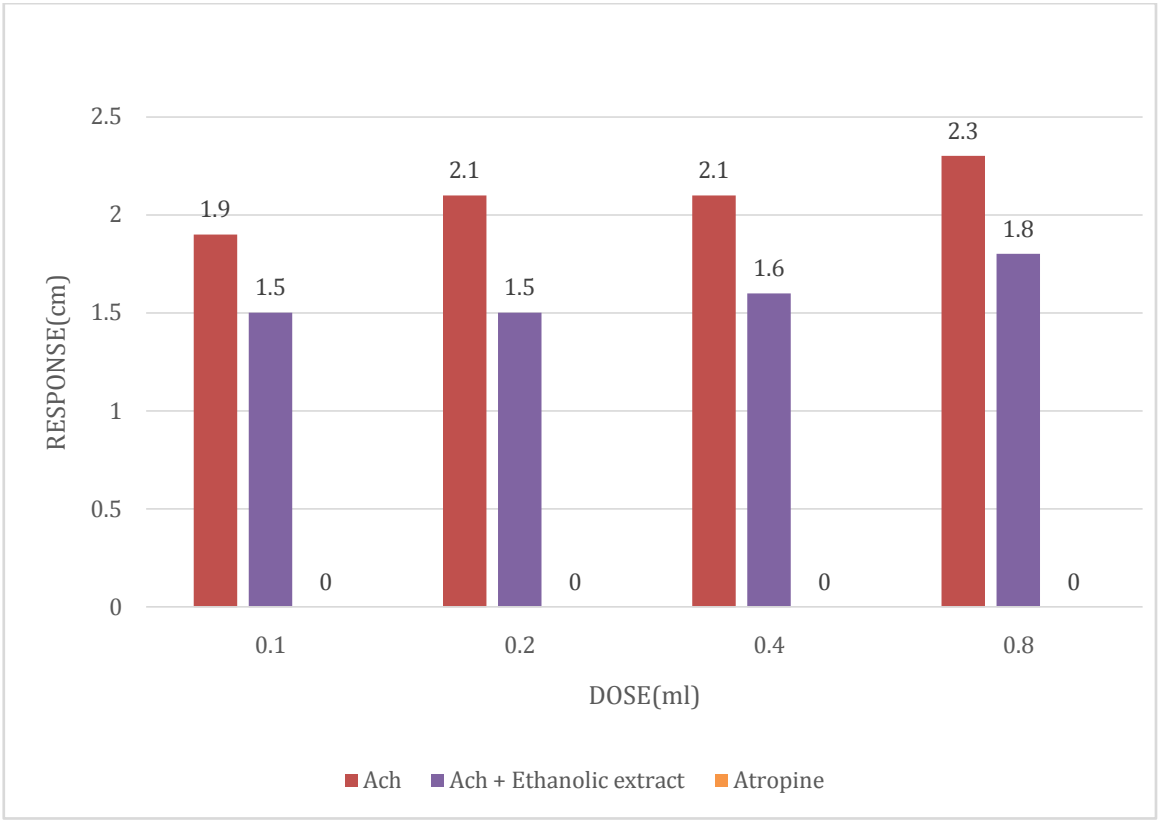

Figure 7 Comparative dose response relationship of Ach and Ethanolic extract of Passifloraedulis on isolated chicken ileum

Table 9: Comparative dose response relationship of Ach and Ach followed by Pet.ether extracts of Passiflora edulis

Sl no.	Treatment given	Dose (ml)	Response (cm)	% decrease in Response
1	Acetyl choline	0.1	0.4	-
2		0.2	0.5	
3		0.4	0.5	
4		0.8	0.6	
5	Acetyl choline	0.1 + 0.1	0.3	25%

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6	+ Petroleum ether extract	0.2 + 0.2	0.4	20%
7		0.4 + 0.4	0.4	20%
8		0.8 + 0.8	0.5	16%

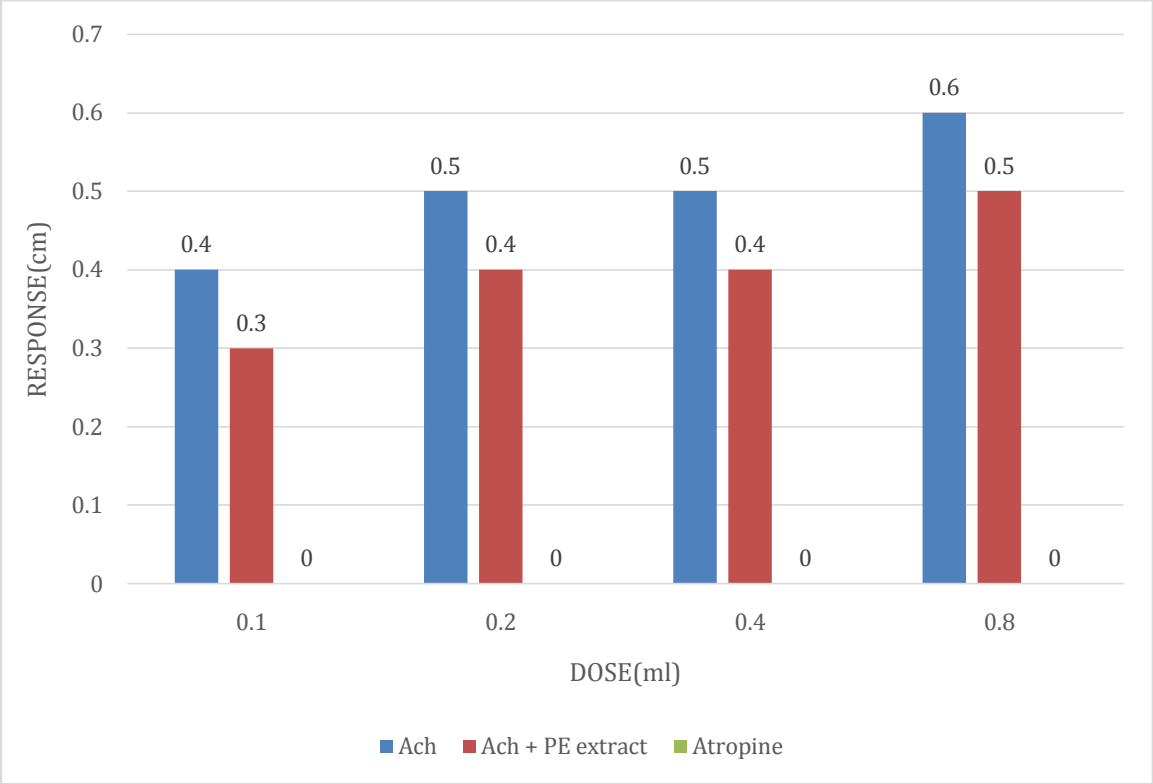

Figure 8 Comparative dose response relationship of Ach and Petroleum ether extracts of Passifloraedulis on isolated chicken ileum

Comparative dose responses of Ach and Ach followed by Ethanolic and Petroleum ether extracts of Passifloraedulisare shown in Table 8,Figure 7 and Table 9,Figure 8 respectively. The mean percentage decrease in response was 23.81% for ethanolic extract and 20.25% for petroleum ether extract compared to 100% receptor blockade of Atropine.

Thus, from the antispasmodic screenings, it was observed that Ach alone causes contraction of isolated chicken ileum, but when Ach was given in the presence of plant extracts of Passifloraedulis there was a marked decrease in contraction of ileum. This revealed that ethanolic and petroleum ether extracts of Passifloraedulis possess spasmolytic activity by blocking cholinergic receptors.

IV. CONCLUSION

On the basis of obtained results, it might be concluded that the ethanolic and petroleum ether extracts of Passifloraedulisexhibits promising antispasmodic activity. When compared with a standard antispasmodic agent (Atropine), it was found that Passifloraedulis has comparatively less potent spasmolytic activity than atropine. The ethanolic extract of the plant is more active than the petroleum ether extract. As many antispasmodicdrugs available in the market shows many side effects, Passifloraedulisbeing a herbal origin could be a suitable alternative to the existing drugs, as well as could be a new member of the antispasmodic family.

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