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ANALYTICAL EVALUATION OF ANTI-SPASMODIC ACTIVITY OF CEDARWOOD OIL AGAINST ACETYLCHOLINE USNING ISOLATED CHICKEN ILEUM

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ABSTRACT

The cedar tree is a type of coniferous trees that is part of the pinaceae or pine family. The wood and foliage of the cedar tree is very aromatic and contains high amounts of cedarwood oil which is extracted with steam distillation. Cedarwood oil have tremendous properties, as to protect the body against harmful bacteria, also facilitate wound-healing, muscle aches, joint pain or stiffness, soothe coughs as well as spasms. While it's anti-spasmodic activity also helps to soothe spasms associated with digestion. The *In-vitro* method was performed to evaluate the anti-spasmodic activity of Cedarwood oil using chick ileum against acetylcholine. After which the activity was compared with the standard spasmolytic agent, atropine. This study performed to understand the effectiveness of the doses of Cedarwood oil which will inhibit the contraction produced by acetylcholine in isolated chicken ileum was evaluated.

KEYWORDS: Acetylcholine, Atropine, Cedarwood oil, Physiological salt solution (PSS), Spasmolytic.

INTRODUCTION

A spasm is a sudden involuntary contraction of a muscle or group of muscles, in other words it can be called as cramps. It is typically caused by ion imbalance or muscle overload.

Most commonly occurred and it can occur any part of the body. A cramp is usually harmless and ceases after a few minutes, but if tends to show frequent attendants this could be painful and distress. The anti-spasmodic (spasmolytic) agents or drugs are commonly used for the reduction of excessive smooth muscle contractility and they work by slowing the natural movements of the gut and by relaxing the muscles.[1]

Now a days, antispasmodic natural remedies are used by more number of patients for symptomatic treatment of functional dyspepsia, intestinal, colonic, or ureteral spasms. Here one such natural remedy or oil such as Cedarwood oil derived from the leaves, barks, and berries of cedar trees which is also recognized for its anti-spasmodic activity and soothing capacity. So as,it is extracted via several technique including steam distillation and cold pressing to obtain the essential oil and used for various ailments or diseases.[2]

Other beneficial activity of Cedarwood oil is anti-inflammatory activity, anti-microbial activity, anti-spasmodic, antifungal, diuretic and sedative properties. It is also used as an ingredient in products like insect repellent, cologne, shampoo, and deodorant. The cedars are symbolized as a source of protection, wisdom and abundance in the medical world.

Cedarwood oil is generally recognized as safe by the U.S. Environmental Protective Agency when used topically as an insecticide or food additive. Reports of skin irritation caused by cedarwood oil are rare.[3]

This study mainly evaluated that the Cedarwood oil is able to prevent the contractile action induced by acetylcholine in isolated chicken ileum. As the *in-vitro* study result supports the use of Cedarwood oil as an antispasmodic agent.

MATERIALS & METHODS

MATERIALS

Acetylcholine, Atropine, Cedarwood essential oil, Chicken ileum, Physical salt solution(PSS), Equipment's: Sherrington rotating drum, Kymograph, Tension(0.5g), Stopwatch.

METHODS

In-vitro assay method for evaluation of anti-spasmodic activity of Cedarwood oil on acetylcholine induced contraction on isolated chicken ileum.

The *In-vitro* assay method is most commonly used to estimate the action of a drug which show any antagonistic activity on acetylcholine or spasmogen produced contractions. Firstly, fresh ileum of chick was taken from the nearest slaughter house. 10 litre of physiological salt solution was prepared by adding certain compounds or ingredients to their correct quantity

Physiological salt solution (TYRODE SOLUTION)[4]	
Ingredients	**Quantity(1litre)**
NaCl	**8g**
KCl	**0.2g**
MgCl	**0.1-1g**
NaHCO3	**1g**
NaH2PO4	**0.1g**
Glucose	**2g**
CaCl2	**0.2g**
Distilled water	**1 litre**

1. Chick ileum is thoroughly washed with the tyrode solution to remove the mesentery and allowed to stabilize in the prepared PSS.
2. A 1-1.5cm length of ileum was measured and tied with the thread to both side of the ileum alternatively. Upper end and lower loop tied with the tissue holder and mounted in the tissue organ bath with 25ml of tyrode solution at 37°C.
3. Maintained a tension of 0.5g, bubbled the tissue organ bath with air. Stabilized the tissue for 30mins. D
4. Drum speed of 0.25 rpm maintained for recording the responses produced by the ileum, baseline of 30 sec, contact time of 60 sec, with few minutes of washing period and as for one response a 5-minute time cycle were followed.
5. Initially, a concentration response curves were recorded on kymograph for Acetylcholine (100µg/ml) in absence and presence of atropine as a standard drug on Kymograph by using Sherrington's Recording Drum.
6. The same procedure was carried to obtain concentration response curve of Acetylcholine(100µg/ml) in presence of Cedarwood oil (100µg/ml) Kymograph by using Sherrington's Recording Drum.

7. The percentage inhibition of Cedarwood oil and standard drug were compared and calculated.
8. Graph was plotted by taking log dose on x-axis verses percentage response curve y-axis.

RESULT

Evaluation of spasmolytic activity of cedarwood oil

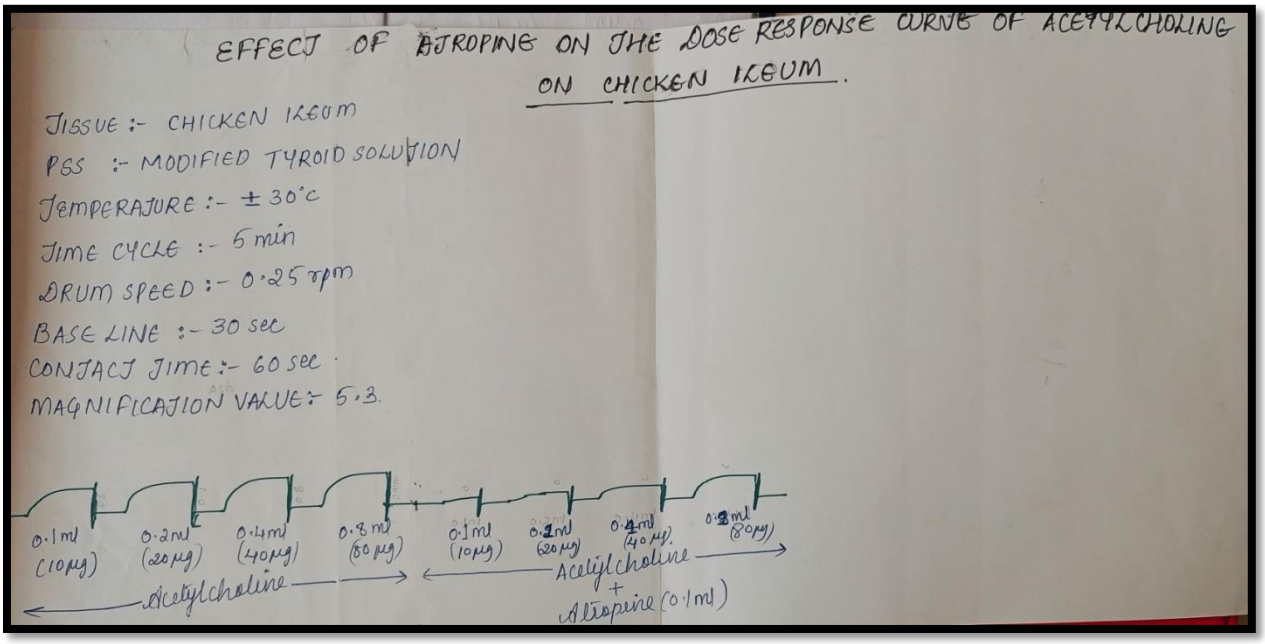


Fig.1: Dose response curve of Ach and Atropine

Effect of Ach on chicken ileum shows an increase in spasmodic activity (response) with an increase in the dose. DRC of Ach in presence of atropine was taken as shown in figure 1. which shows decrease in the spasmodic activity.

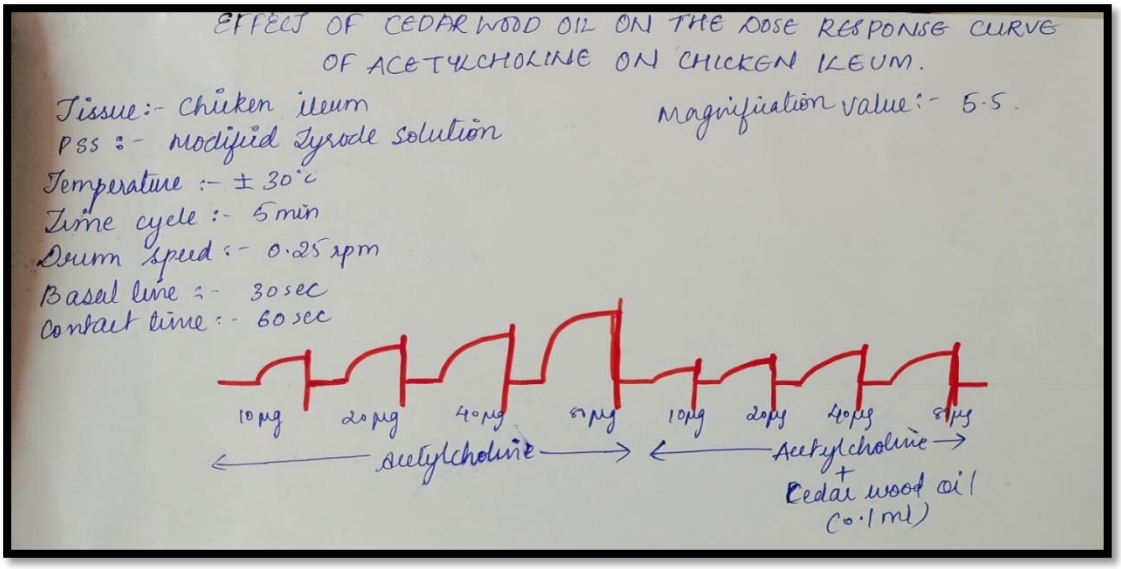


Fig. 2: Response curve of Ach + Cedar wood oil

Ach induced spasm followed by the treatment with cedarwood oil which showed prominent antispasmodic activity as shown in the figure 2.

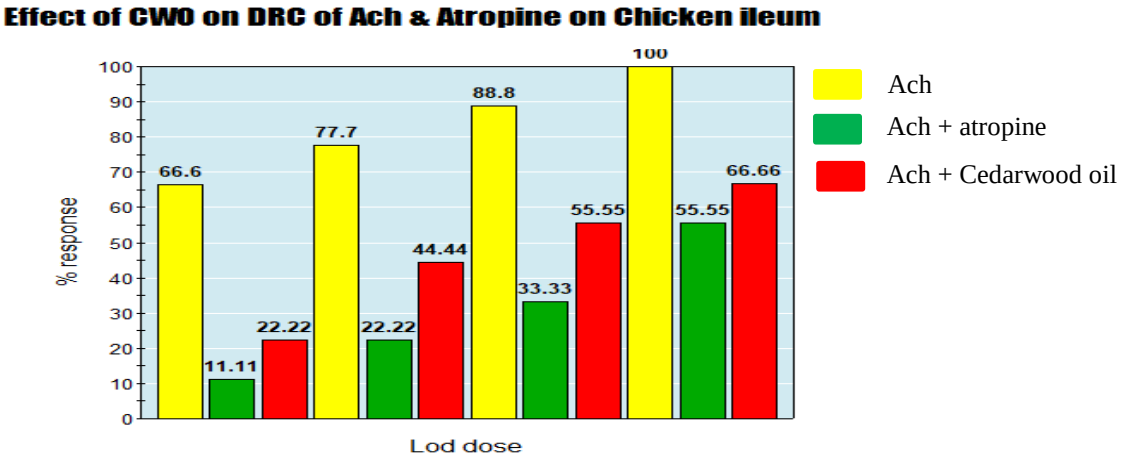
Table no.1: Dose response relationship of acetylcholine and atropine on chicken ileum.

DRUG	DOSE (ml)	% RESPONSE
Acetylcholine	0.1	66.6%
	0.2	77.7%
	0.4	88.8%
	0.8	100%
Acetylcholine + atropine	0.1 +0.1	11.11%
	0.2+0.1	22.22%
	0.4+0.1	33.33%
	0.8+0.1	55.55%

Table no.2 : Dose response relationship of acetylcholine and Cedarwood oil on chicken ileum.

DRUG	DOSE (ml)	% RESPONSE
Acetylcholine	0.1	66.6%
	0.2	77.7%
	0.4	88.8%
	0.8	100%
Acetylcholine + cedar wood essential oil	0.1+0.1	22.22%
	0.2+0.1	44.44%
	0.4+0.1	55.55%
	0.8+0.1	66.66%

Graphical representation of effect of Cedarwood oil on the dose response curve of acetylcholine



DISCUSSION

In-vitro chicken ileum method which is a non-humane procedure is one of the most commonly used methods . Where the chicken intestine is easier to acquire, handle and easier to dissect and has the same reactions to spasmogenic and spasmolytic drugs. As cholinergic agonists like acetylcholine produces a contractile response in isolated chicken ileum. The M_3 receptor function which in activates the release Ca^{2+} intracellularly leads to actin-myosin phosphorylation causing increased smooth muscle tone or contraction. In current investigational study, acetylcholine showed greater contraction while Cedarwood oil significantly inhibited the acetylcholine induced contraction on isolated chicken ileum preparation. The decrease in the height of the acetylcholine dose-response curve in the presence of cedarwood oil indicating that there was a competitive antagonism between acetylcholine and cedarwood oil. This effect may be due to its antispasmodic activity. The effect of atropine (standard drug) response and cedarwood oil response were compared. Cedarwood oil showed almost same action as atropine on the acetylcholine contractile response. This proves that Cedarwood oil possess anti-spasmodic action on acetylcholine produced contraction.

CONCLUSION

From this study it can be concluded that the cedar wood essential oil possesses significant anti-spasmodic activity on smooth muscles of chicken ileum. This may substantially benefit in the treatment of spasm and other intestinal muscular disorders.

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