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EVALUATION OF ANTI SPASMODIC ACTIVITY OF NEEM SEED OIL IN ISOLATED CHICKEN ILEUM

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ABSTRACT

Background:

This study is to evaluate spasmolytic activity of neem seed oil in isolated chicken ileum. Effect of neem seed oil in presence of acetylcholine were studied in isolated chicken ileum and compared with atropine(anti spasmodic agent).

Method:

The evaluation of spasmolytic activity was performed by using Concentration response curve in isolated chicken ileum .The effect of atropine in the dose response curve of acetylcholine using chicken ileum was studied and a graph was plotted between percentage response and log dose.

Conclusion: The study demonstrated that inhibitory effect of NSO against acetylcholine a spasmogen is dosedependent.The action is due to the blockade of Muscaric action of Ach on smooth muscles.

Result: The study concluded that Neem seed oil(NSO) posses significant anti-spasmodic activity due to its M3 antagonist activiy on smooth muscles.

Words: Acetylcholine, Atropine, Anti-spasmodic, Chicken ileum, Neem seed oil(NSO)

INTRODUCTION :

Neem is a natural herb that comes from the neem tree, other names for which include Azadirachta indica and Indian lilac. The extract comes from the seeds of the tree and has many different traditional uses. Neem has been used from past for various diseases. All parts of the neem tree-leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. The medicinal utilities have been described especially for neem leaf. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, anti-spasmodic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties. Neem is a popular plant with its description and uses known for thousands of years. Its medicinal benefits have been exploited by many of the world's traditional medical systems including Ayurvedic, traditional Greek medicine, and homeopathy. Many parts of the plant including seeds, roots, bark, leaves, flowers and fruit are known for their medicinal benefits and used to treat various conditions. The various studies on the neem plant are antioxidant, anti-inflammatory, antibacterial, antianxiety, hepatoprotective, and anticancer properties of neem. Azadirachta indica has complex of various constituents including nimbin, nimbidin, nimbolide, and limonoids. Quercetin and ß-sitosterol were first polyphenolic flavonoids purified from fresh leaves of neem and were known to have antifungal and antibacterial activities.

Neem oil is a golden to dark-brown liquid with a strongly unpleasant and offensive odour and is obtained from fruits, seeds, and flowers of the neem tree. It contains fatty acids, limonoids, vitamin E, triglycerides, antioxidants, and calcium. This plant is very important in many sectors including agriculture and medicine. Neem oil can also be used as a green solvent because it is eco-friendly. The neem seed contains up to 40% oil content.

Nimbidin, a major crude bitter principle extracted from the oil of seed kernels of Azardirchta indica demonstrated several biological activities such as dose dependent anti -inflammatory and antipyretic activity. The oil also contains some tetranortriterpenes, including nimbin , nimbinin, nimbidinin, nimbolide and nimbidic acid . Neem oil also contains steroids (campesterol, beta-sitosterol, stigmasterol).

MATERIALS AND METHODS:

Drugs and Chemicals

Atropine sulphate (ATR), Acetylcholine (Ach), Sodium chloride, Potassium chloride, Magnesium sulphate, Calcium chloride, Sodium bicarbonate, Dextrose, Neem Seed oil was purchased. way solutions for atropine were prepared in distilled water and diluted to appropriate concentration with physiological salt solution. Only freshly prepared Tyrode solution (pH -7.3 to 7.4) was used for smooth muscle preparation. The other chemicals and reagents used were of analytical grade.

Anti spasmodic activity

EXPERIMENTAL PROTOCOL:

Fresh entire gastrointestinal tract of healthy ileum was obtained from a local slaughterhouse and was transferred Tyrode solution. Aeration was provided immediately in the laboratory. The caecum was lifted forward and the ileocaecal junction was identified. The ileum and duodenum were cut and transferred to a beaker containing Tyrode solution. A thread was tied at each end taking care that tissue is left open and the thread does not close the lumen. A segment of 2cm long was mounted in an organ bath containing Tyrode solution and maintained at 37°C. The temperature was maintained and allowed to equilibrate for 30 minutes. Load was adjusted to 0.5 -g, the magnification from 5-7 folds and bath volume of about 20 -ml was maintained. Contact time 60 sec,base line 30 sec and washing time for 90 seconds .Dose response curve of std (Acetyl choline) was recorded first. Record atleast 4 response to increased doses of Acetylcholine or till maximum responses obtained. Record responses with 0.1,0.2,0.4,and 1.8 of Acetylcholine. The cumulative concentration curve were recorded for acetylcholine in presence of neem seed oil.The same procedure was carried for concentration effect curve of acetylcholine in presence of Atropine sulphate.The percentage inhibition of extract and standard drug were calculated and graph was plotted by taking log dose verses height of the response.

RESULT AND DISCUSSION:

DRUG	DOSE	PERCENTAGE RESPONSE
	0.1	46.1
	0.2	69.23

Acetylcholine	0.4	92.3
	0.8	100
Atropine+ Acetylcholine	0.1+0.1	28.57
	0.1+0.2	57.14
	0.1+0.4	71.42
	0.1+0.8	85.7
NSO+ Acetylcholine	0.1+0.1	30.76
	0.1+0.2	53.84
	0.1+0.4	76.92
	0.1+0.8	84.61

Table1: Evaluation of antispasmodic activity

The above table explains the dose response relationship of Acetyl choline , Neem seed oil and Atropine on isolated chicken ileum.

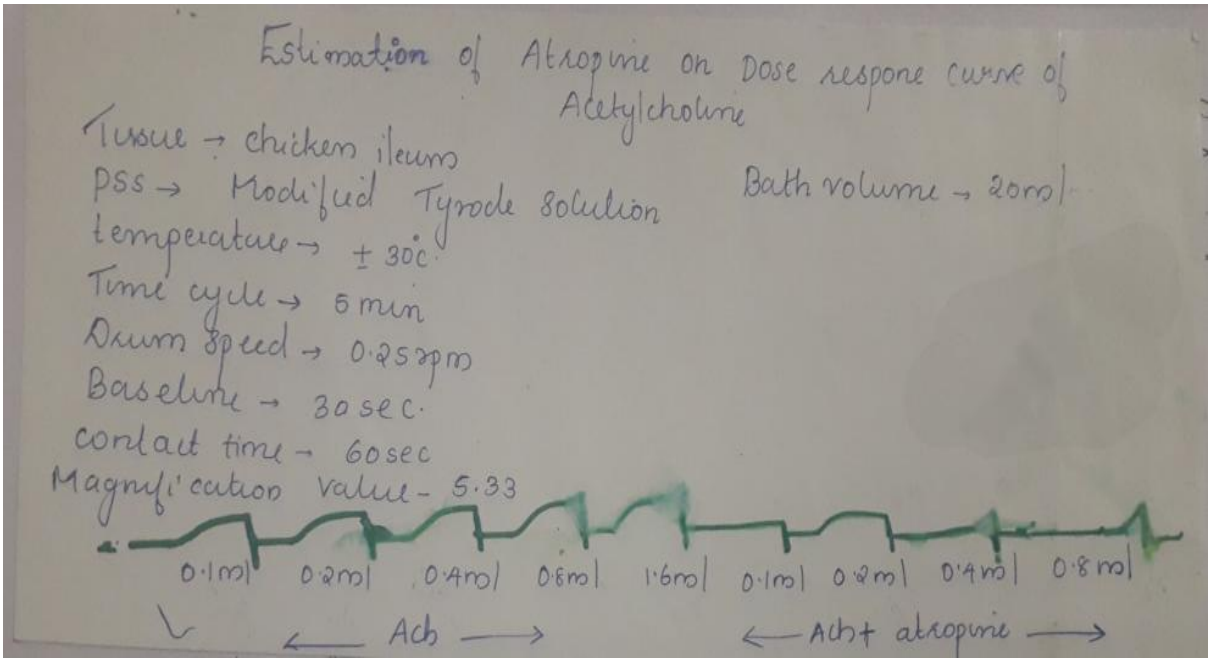

Fig 1: Effect of Acetylcholine on chicken ileum shows the increase in activity with graded concentration. The DRC graph represent the effect of atropine on DRC of Ach in isolated chicken ileum

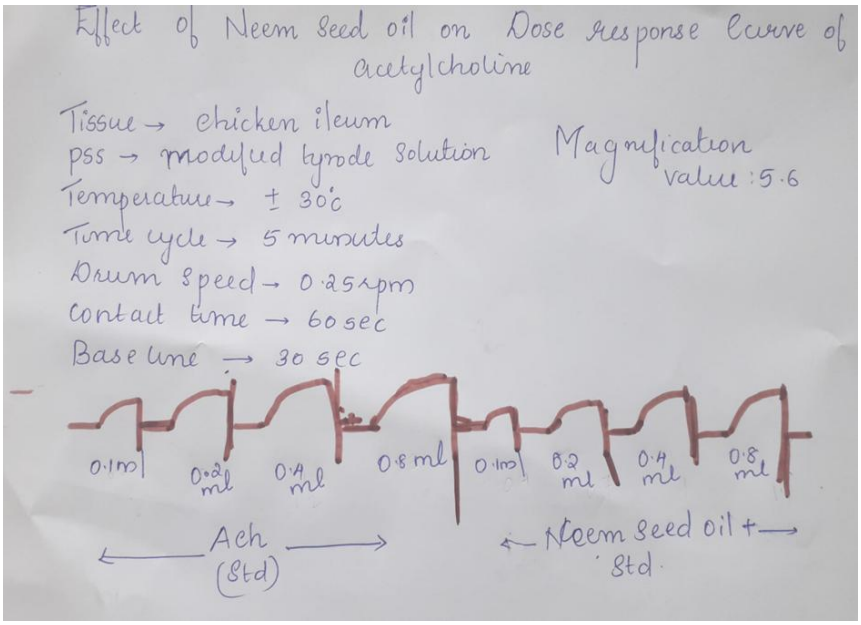

Fig 2: Effect of NSO on dose response curve of acetylcholine in isolated chicken ileum

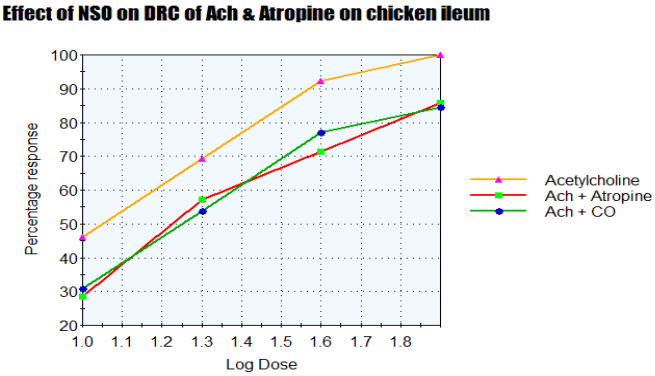

Fig 3: Comparative dose response relationship of acetylcholine, ethanolic Neem Seed oil , and atropine on isolated chicken ileum.

DISCUSSION:

For evaluating screening the activity of drugs on intestinal smooth muscles, chick ileum preparations can be used. Chicken ileum preparation are used to find spasmolytic or spasmogenic activity .Cholinergic agonists like acetylcholine elicit a contractile response in isolated chicken ileum. M3 receptor, a subtype of cholinergic (muscarnic) receptor activation causes contraction of intestinal smooth muscle. Thus, the contraction of intestinal smooth muscle in vitro has often been utilized for the study of contractile/dilator responses of agonists as well as antagonist. In current investigation, acetylcholine showed greater contraction while NSO significantly inhibited the acetylcholine induced contraction on isolated chicken ileum preparation. This effect may be due to its antimuscarinic or antispasmodic activity.

The results depicted in table 1 indicates that effect of Ach,a good spasmogen ,on chicken ileum dose dependently increases the contractile response of the mooth muscles.The response of chicken ileum was compared with Atropine and along with NSO. .The response of acetylcholine were found decreased in presence of NSO and also in response to atropine. The overall outcome of the study revealed that Ach alone cause spasm but when given in presence of NSO and atropine there is marked decrease in muscle contraction.

CONCLUSION:

NSO have shown the reduction in acetyl choline induced contractile response in smooth muscle.It can be concluded that from the present study that presence of NSO have specific anti-spasmodic activity due to M_3 antagonist action in smooth muscle.

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