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ORIGINAL ARTICLE

A-Effects of Drugs on Spontaneous Contractions of Rabbit Jejunum

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ABSTRACT

Aim: To find out possible to determine effect of *C. reflexa* extract on spontaneous contractions of rabbit jejunum.
Methods: Animal experimental in vitro study from 1st October 2017 to 30 September 2018 at Pharmacology experimental lab, Wah Medical College Wah Cantt. Study model was Isolated pieces of rabbit jejunum and ileum. Sampling Technique used was Simple Random. Tissue considered fit for experiment after initial equilibration and stabilization.
Result: Three increasing concentrations (0.1mg/ml, 0.2mg/ml and 0.4mg/ml) of C. reflexa extract decreased the response to 3.83±0.40, 2.33±0.33 and 1.33± 0.21mm. Decrease in response was significant with p- value 0.04, 0.008 and 0.003 respectively.
Conclusion: Freshly prepared aqueous solution of crude extract of C. reflexa decreases the amplitude of spontaneous contractions of isolated rabbit jejunum.
Keywords: Rabbit jejunum, effect of drug

INTRODUCTION

Gastrointestinal tract (GIT) is mainly responsible for the intake, digestion, assimilation and absorption of food along with the excretion of waste products. Human GIT comprises of oral cavity, esophagus, stomach, intestines and anus. Major portion of digestion and absorption takes place in intestines. Intestine is divided into small and large intestine. Small intestine is further subdivided into duodenum, jejunum and ileum. Cross section of intestinal wall, includes the following layers from outer to innerside serosa longitudinal smooth muscle circular smooth muscle submucosamucosa and bundles of smooth muscle fibers(mucosal muscles are present in deeper layers of mucosa). Absorption, secretion and peristaltic activity are the regular functions of healthy small intestine. These are under the control of enteric nervous system (ENS), central nervous system (CNS) and gastrointestinal hormones. Endogenous ligands, neurotransmitters and hormonal substances, such as acetylcholine, nor-epinephrine, serotonin, dopamine, cholecystokinin, substance-P, vasoactive peptide, somatostatin, leu-enkaphalin, met-enkephalin, gastrin inhibitory peptide and bombesin play important role in regulation of normal motility of gut .Most of the gut functions are controlled by enteric nervous system (ENS). The ENS is collections of nervous tissue present within the wall of the GIT.It is composed of two connected networks of neurons and nerve fibers: one is myenteric (Auerbach's) plexus, present between circular and longitudinal muscle layers, and the other is submucosal (Meissner's) plexus, present in submucosa. Myenteric plexus is responsible for movement control, where asmeissner's plexus regulates fluid transport, secretion and blood circulation within GIT. It is the only part of the autonomic nervous system (ANS) which can function independently if separated from the (CNS). Both ENS and ANS are also involved in host defense and innervate organs and cells of the immune system (Rhee et al., 2009).

Activation of M1, M3, M5 receptors can also cause release of archidonic acid from membrane phospholipids by phospholipase A2 and production of eicosanoids; also results in activation of adenylyl cyclase and an increase in adenosine monophosphate (cAMP). These effects of M1,M3 and M5 receptors are secondary to elevations of intracellular calcium (Eglen, 2005). Stimulation of M2, M4 receptors leads to interaction with other G proteins (Gi, Go) causing inhibition of adenylyl cyclase, leading to decrease in cAMP, inhibition of voltage gated calcium channels, and activation of K channels (Van Koppen and Kaiser, 2003). These effects cause inhibition of excitable membranes.

MATERIALS AND METHODS

This was animal experimental in vitro study carried out in Post Graduate Medical Institute Lahore and Wah Medical College Pharmacology Experimental Lab. WahCantt District Rawalpindi during one year. Study model was Isolated pieces of rabbit jejunum and ileum. Sample size was fifty four. Sampling technique used was **s**imple random

Sample selection: Tissue considered fit for experiment after initial equilibration and stabilization.

Collection of Plant and preparation of extract: The stems of *C. reflexa* were collected from a garden in Wah Cantt, Pakistan. A specimen was deposited in the Department of Plant Sciences, Quaid-i-Azam University Islamabad Pakistan, for identification. The sample was dried under shade at cool dry place, cleaned off and coarsely grounded. The 1Kg powdered material was soaked in 2 litre 80% aqueous-ethanol for 3 days with occasional shaking. It was filtered through a muslin and then through a Whatman number 3 filter paper. This procedure was repeated thrice and the combined filtrate was evaporated on a rotary evaporator under reduced pressure to a thick, semi-solid mass of dark brown color.Two hundred miligrams were obtained from 1Kg of *C.reflexa*. At the time of experiment solution was freshly prepared by dissolving extract in distilled water.

Animals: Rabbits of either sex weighing 1-1.5Kg were purchased from local market. They were fed on green fodder and grain. They were deprived of food but not water 18 hours prior to experiment.

Details of experiment: A-Effects of drugs on spontaneous contractions of rabbit jejunum.

Effect of C.reflexa extract on spontaneous contractions of rabbit jejunum (n=6): Spontaneous contractions were recorded for one minute. Three doubling concentrations of plant extract were added in organ bath in cumulative way. Following concentrations and time cycle, as suggested by preliminary experiments were used.

Concentrations: E1= 0.1mg/ml
E2= 0.2mg/ml
E3= 0.4mg/ml

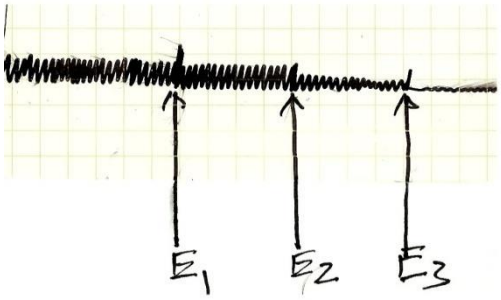
Time cycle:
0 min - Kymograph started and response recorded as under:
1 min - 1st concentration of extract
11 min - 2nd concentration of extract
21 min - 3rd concentration of extract
30 min - Kymograph stopped and tissue washed three times with Tyrode's solution.

Experiment was carried out on isolated pieces of rabbit jejunum from six animals. Response to each concentration of extract was measured in mm. Data was expressed as mean±S.E.M and it was taken to construct a concentration response curve. Two tailed paired samples t-test was applied to calculate the significance of difference.

RESULTS

Effect of *C.reflexa*extract on spontaneous contractions of rabbit jejunum (n=6): Spontaneous contractions were recorded for one minute. The mean of amplitude of spontaneous contractions (control) was 5.5±0.992mm. Three increasing concentrations (0.1mg/ml, 0.2mg/ml and 0.4mg/ml) of *C.reflexa* extract decreased the response to 3.83±0.40, 2.33± 0.33 and 1.33± 0.21mm. Decrease in response was significant with *p*- value 0.04, 0.008 and 0.003 respectively. Table 1 gives results of six experiments with mean±SEM, mean decrease in response±SEM, % decrease in response and *p*-values. These values were used to construct a dose response curve and EC_{50} was calculated which was 0.18mg/ml.

Fig.1: Effect of *C.reflexa* extract on spontaneous contractions of rabbit jejunum.



E1= *C.reflexa*extract 0.1mg/ml
E2= *C.reflexa*extract 0.2mg/ml
E3= *C.reflexa*extract 0.4mg/ml

Table 1: Effect of *C.reflexa*on rabbit jejunum.Results of six experiments with mean±SEM, mean decrease in response±SEM, % decrease in response as well as level of significance as calculated by two tailed paired samples t-test.

Conc. of *C.reflexa* extract	**Control**	***C.reflexa* extract (0.1mg/ml)**	***C.reflexa* extract (0.2mg/ml)**	***C.reflexa* extract (0.4mg/ml)**
Log Concentration	_	-1	-0.698	-0.397
Responses of Experiments (Height of Cotractions in mm)	8	5	3	2
1-				
2-	9	5	3	2
3-	4	3	2	1
4-	4	3	2	1
5-	5	4	3	1
6-	3	3	1	1
Mean response± SEM(mm)	5.5± 0.992	3.83± 0.40	2.33± 0.333	1.33± 0.211
Mean decrease in response±SEM(mm)		1.67±0.61	3.17± 0.74	4.17± 0.79
%age decrease				
in response	_	30.37	57.64	75.82
p- value	_	0.04*	0.008**	0.003**

*p ≤0.05, ** p ≤ 0.01

DISCUSSION

This study was designed to observe the effect of crude extract of *C.reflexa*on amplitude of contractions of isolated preparations of rabbit jejunum and its interaction with known agonists on rabbit ileum. Comparison was made with known antagonists,(which decrease the amplitude of contractions of smooth muscles of isolated rabbit jejunum and ileum) atropine and verapamil to find out possible mechanism of action. The study was carried out in three main groups with division of each group into three subgroups so total number of groups was nine. Each group contained six isolated rabbit jejunum or ileum (n=6).

Motility disorders are used to describe a variety of conditions in which the gut has lost its ability to co-ordinate muscular activity because of endogeneous and exogeneous causes (Keller and Layer, 2009; Di Lorenzo and Youssef, 2010).

Nature has been a source of medicinal treatments for thousands of years, and medicines derived from plants play an important role in the primary health care.*C.reflexa* plant is traditionally used as carminative, to control vomiting, in bilious disorders, flatulence and stomach ache (Patel *et al*., 2012).

Rabbit jejunum was selected to study the inhibitory effects of different drugs because spontaneous contractions are better in jejunum than ileum however for induction of contractions ileum was selected as it is considered more suitable for studying the responses of agonists in presence of inhibitor/antagonists.

Intestinal motility is controlled by multiple physiological mediators, mainly acetylcholine, histamine, serotonin, bradykinins, prostaglandins, substance-p, and cholecystokinin which achieve their contractile effects through an increase in cytosolic calcium. Antagonists of all the above mentioned mediators inhibit responses by their respective agonists but calcium channel blockers will inhibit responses of all agonists(Gilani*et al*., 2008).

Carbachol was selected as muscarinic agonist because it is resistant to hydrolysis by cholinesterase enzyme so cumulative dose effect may be recorded (Brown and Laiken, 2011).

KCl (Potassium Chloride) was used because high K^+ is known to cause smooth muscle contractions through opening of L-type Ca^{2+} channels, thus allowing influx of extracellular Ca^{2+}. The agents blocking L-type Ca^{2+} channels, like verapamil can antagonize effects of KCl (Godfraind*et al*., 2008). So the effects of *C.reflexa extract on* Carbachol& Potassium Chloride induced contractions were compared to atropine & verapamil.

In the first set of experiments the effect of three doubling concentrations of *C.reflexa* extract were studied on spontaneous contractions of rabbit jejunum. It was observed that extract decreased the magnitude of spontaneous contractions in a dose dependant manner. Inhibitory effect of *C.reflexa* extract was compared to known antagonist drug atropine (antimuscarinic drug) and verapamil (standard Ca^{+2} channel blocker).. Difference between groups was insignificant statistically. The effects of *C.reflexa* on jejunum by other workers is not available in literature.

CONCLUSION

From the results of the study it is concluded that:Freshly prepared aqueous solution of crude extract of C.reflexa decreases the amplitude of spontaneous contractions of isolated rabbit jejunum.

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