ORIGINAL ARTICLE

EFFECT OF *CUSCUTA REFLEXA* EXTRACT ON GASTROINTESTINAL MOTILITY

Sohail Iqbal, Mohsin Ali, Ulfat Sultana, Saddiqa Gul, Irfan Malook, Amjad Ali*

Department of Pharmacology and Therapeutics, *Pathology, Muhammad College of Medicine, Peshawar, Pakistan

Background: Motility disorders of the gut are one of the major challenges to the medical profession. The stem of *Cuscuta reflexa* had been used by traditional practitioners for the treatment of gastrointestinal and bilious disorders. The objective of the current study was to determine the effects of an extract of *C. reflexa* on carbachol-induced contractions of isolated rabbit ileum. **Methods:** The study was conducted from January to July 2020 at Department of Pharmacology, Muhammad College of Medicine, Peshawar after approval from the Ethical Committee of the College. The sample size was calculated using the 'Degree of Freedom in ANOVA' formula. The rabbit's ileum tissues were dissected and divided into two main groups, and each was further divided into three subgroups. Each subgroup consisted of six isolated rabbit's ileum tissues. After equilibration and stabilization of tissues in an organ bath containing Tyrode's solution, the effect of atropine and *C. reflexa* extract on carbachol-induced contractions in isolated rabbit's ileum was recorded. Results were analysed statistically, and $p \leq 0.05$ was considered significant. **Results:** Ethanolic extract of *C. reflexa* of different concentrations significantly decreased the amplitude of carbachol-induced contractions of rabbit ileum ($p<0.05$). The most powerful response of ethanolic extract of *C. reflexa* was observed at a concentration of 0.4 mg/ml. **Conclusion:** The ethanol extract of *C. reflexa* produces antimuscarinic effects on smooth muscles of the rabbit ileum.

Keywords: *Cuscuta reflexa*, ethanol extract, antimuscarinic effect, intestinal motility

Pak J Physiol 2023;19(2):10–4

INTRODUCTION

The gastrointestinal tract (GIT) is responsible for intake, digestion, assimilation and absorption of food, and excretion of waste products. Absorption, secretion and peristaltic activity are under the control of the enteric nervous system (ENS), central nervous system (CNS), and gastrointestinal hormones. Endogenous ligands, various neurotransmitters and hormonal substances play important role in regulation of normal motility of gut.[1] ENS is the only part of the autonomic nervous system (ANS) which can function independently. Peristalsis of the gastrointestinal tract is a necessary physiological function. Contraction of the circular muscle in GIT, is due to the influx of calcium into smooth muscle cells through voltage-sensitive calcium channels and calcium release from intracellular stores.[2] Motor neurons receive input from excitatory and inhibitory interneurons.

Acetylcholine (Ach) is an excitatory neurotransmitter of the motor neurons. The cholinergic receptors are classified as muscarinic receptors (subclasses M_1, M_2, M_3, M_4, M_5) and nicotinic receptors (subclasses N_N, N_M). Muscarinic receptors are found in all organs, tissues, and cell types. M_3 receptors are present in smooth muscles of GIT, bronchi and detrusor muscles of the bladder.[3] Functions of muscarinic receptors are mediated by interaction with G-proteins induced changes in the function of membrane-bound effector molecules.

Carbachol is a muscarinic agonist that causes concentration-dependent increase in amplitude of contractions by acting on muscarinic receptors (M_3).[4] Activation of M_3 receptors produces an inositol triphosphate (IP_3) mediated release of intracellular calcium and release of diacylglycerol (DAG) which activates protein kinase-C causing contraction of smooth muscles, and its effects are antagonized by atropine.[5]

Gastrointestinal motility disorders result in abnormal intestinal contractions.[6] These disorders may be primary or secondary to pathological diseases including irritable bowel syndrome, inflammatory bowel disease, infective diarrhoea, neoplastic diseases, Ogilvie syndrome etc. These disorders may cause severe colicky pain, gastroesophageal reflux disease, recurrent vomiting, abdominal distention and constipation.[7]

Herbal remedies have played a significant role in treating and preventing a variety of diseases, and 25 to 50% of all medicines currently available are derived from plants.[8] There is a lot of scientific information on phytoconstituents and therapeutic uses of plants. Medicinal plants have formed the basis for traditional medicinal systems for thousands of years, with the first records dating from about 2,600 BC in Mesopotamia. Isolation of active principles from herbs and plants began in early 1800s.[9]

The plant *Cuscuta reflexa* is a perennial herb of Convolvulaceae family, commonly known as '*Amarbel,* امربیل' or '*Akaashbel,* آکاش بیل'. This plant contains flavonoids, glycosides, alkaloids, cuscutin, cuscutalin, amarvel, amarbelin, betasterol, stigmasterol, kaempferol, dulcitol, qurecetin, astragallin, myrecetin, benzopyrones, glucopyranosides, Violaxanthin, lutein, lycopene, carotene, α-cryptoxanthin, and bergenin.[10] The plant is

used to treat the headache, paralysis, diphtheria, and fever. Extract are used externally to relieve itches and internally for relief of liver disorder, fruits are used to treat cough, and paste of the plant is applied to promote healing of tongue ulcers.[11] The aim of the current study was to determine the anti-muscarinic effect of *C. reflexa* ethanol extract on isolated tissues of rabbit jejunum and compare it with anti-muscarinic effects of atropine on isolated rabbit ileum tissues.

MATERIAL AND METHODS

Animal experimental *in vitro* study was designed to achieve our goal. The study was carried out at Department of Pharmacology, Muhammad College of Medicine, Peshawar after ethical approval. The sample size was calculated using the 'Degree of Freedom in ANOVA' formula. A random sampling technique was used. Isolated pieces of rabbit ileum were isolated. The isolated tissue samples were divided into two main groups, and each was further sub-divided into three subgroups. Each subgroup consisted of six isolated tissues, making the total as 36 tissues. 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, for identification. The sample was dried under shade at a cool dry place, cleaned off and coarsely grounded. A semi-solid mass of dark brown colour ethanolic extract of the powdered plant material was obtained. At the time of the experiment solution was freshly prepared by dissolving extract in distilled water.

Rabbits of either sex weighing 1–1.5 Kg were purchased from the local market. They were deprived of food but not water 18 hours before the experiment. The rabbit was sacrificed and a length of ileum proximal to Peyer's patch was removed and placed in a dish containing Tyrode's solution at 37 °C. Thirty minutes were allowed for equilibration before the addition of drugs. During this period preparation was washed with fresh Tyrode's solution every ten minutes. The tissue was stabilized with sub maximum concentration of acetylcholine (8 µg) at 5 minutes intervals with washing in between until constant responses were recorded. Isotonic contractions were recorded on graph paper by Harvard kymograph. Responses were measured in mm and concentration-response curves were plotted.

Increasing concentrations of carbachol, as selected from preliminary experiments were added in organ bath in cumulative way. Responses were recorded and measured in mm displacement. Carbachol induced contractions were recorded for one minute. After this, three doubling concentrations of plant extract (0.1 mg/ml, 0.2 mg/ml and 0.4 mg/ml) and atropine (0.002 µM, 0.005 µM and 0.011 µM) were added in organ bath in cumulative way, following concentrations and time cycle, as suggested by preliminary experiments.

Data were analysed on SPSS-20 and described as Mean±SEM. ANOVA was applied to determine the significant differences between means. Post hoc Scheffe test was applied where applicable, and $p \leq 0.05$ was considered significant.

RESULTS

Three increasing concentrations of carbachol 0.22 µM, 0.44 µM, and 0.88 µM produced a response of 15.75±2.83 mm, 22.33±3.42 mm and 29.66±4.27 mm, respectively. The effect of the same concentrations of carbachol after the addition of three increasing concentrations of *C. reflexa* showed that 0.1 mg/ml of *C. reflexa* extract decreased these responses to 11±2.38, 14.66±2.65 and 20.83±4.87 mm. Decrease in response was significant with *p*-values 0.02, 0.004 and 0.006, respectively. *C. reflexa* extract 0.2 mg/ml decreased these responses to 8.83±2.06, 10.58±2.19, and 13.25±2.33 mm. The decrease in response was significant with *p*-values 0.02, 0.001 and 0.001, respectively. *C. reflexa* extract 0.4 mg/ml further decreased these responses to 5.91±1.74, 7.16±1.74, and 8.66±1.67 mm. The decrease in response was significant with *p*-values 0.002, 0.001 and 0.001, respectively.

Figure-1 shows the effect of carbachol in the absence and presence of three increasing concentrations of *C. reflexa* extract in one of the experiments.

Table-1 gives results of 6 experiments with Mean±SEM, mean decrease in response, % decrease in response as compared to control (100%). These values were used to construct a dose-response curve. The extract caused a rightward shift of the concentration response curve of carbachol (Figure-2).

Three increasing concentrations of carbachol 0.22 µM, 0.44 µM and 0.88 µM produced a response of 11.83±1.62 mm, 23.33±4.50 mm and 32.83±4.61 mm, respectively. Atropine 0.002 µM decreased these responses to 8.83±1.25 mm, 12.66±1.56 mm and 20.5±1.28 mm, respectively. Decrease in response was significant (*p*-values 0.11, 0.04 and 0.03, respectively). Atropine 0.005 µM decreased these responses to 7.83±1.30 mm, 11±1.24 mm and 17±1.44 mm, respectively. The decrease in response was significant (*p*-values 0.01, 0.05, and 0.02 respectively). Atropine 0.011 µM decreased these responses to 5.33±0.76 mm, 8.16±1.25 mm and 13.33±1.52 mm, respectively. The decrease in response was significant (*p* 0.004, 0.02 and 0.01, respectively). Figure-3 shows the effect of carbachol in the absence and presence of three increasing concentrations of atropine in one experiment.

Table-2 gives results of six experiments with Mean±SEM, mean decrease in response, % decrease in response as compared to control (100%) and *p*-values. These values were used to construct a dose-response curve. Atropine caused a rightward shift in the concentration response curve of carbachol (Figure-4).

Table-1: Effect of *C. reflexa* extract on carbachol induced contractions of rabbit ileum, (2-tailed paired samples *t*-test)

	Carbachol (control)		Carbachol & *C. reflexa* extract (0.1 mg/ml)				Carbachol & *C. reflexa* extract (0.2 mg/ml)			Carbachol & *C. reflexa* extract (0.4 mg/ml)		
Concentration	0.22	0.44	0.88	0.22	0.44	0.88	0.22	0.44	0.88	0.22	0.44	0.88
Log concentration	-0.66	-0.35	-0.05	-	-	-	-	-	-	-	-	-
Responses (Height of Contractions)												
1 mm	18	22	26	14	16	24	8	10	13	6	8	10
2 mm	7.5	11	20	5	10	13	4.5	6	8	4.5	6	7
3 mm	15	17	24	5	7	9	3	4	9.5	2	3	7
4 mm	14	28	36	13	20	23	13.5	13.5	14	6	7	8
5 mm	12	21	24	9	11	14	8	11	11	3	4	4
6 mm	28	35	48	20	24	42	16	19	24	14	15	16
Response (mm) Mean±SEM	15.75± 2.83	22.33± 3.42	29.66± 4.27	11± 2.38	14.66± 2.65	20.83± 4.87	8.83± 2.06	10.58± 2.19	13.25± 2.33	5.91± 1.74	7.16± 1.74	8.66± 1.67
Decrease in response (mm) Mean±SEM	-	-	-	4.75± 2.38	7.67± 2.65	8.83± 4.87	6.92± 2.06	11.75± 2.33	16.41± 2.33	9.84± 1.74	15.17± 1,74	21± 3.03
% decrease in response	-	-	-	30.16	29.05	29.78	43.94	48.79	55.33	62.48	65.35	70.81
p	-	-	-	0.02	0.004	0.006	0.02	0.001	0.001	0.002	0.001	0.001

Table-2: Effect of atropine on carbachol induced contractions of rabbit ileum, (2-tailed paired samples *t*-test)

	Carbachol (control)		Carbachol & atropine (0.002 µM)				Carbachol & atropine (0.005 µM)			Carbachol & atropine (0.011 µM)		
Concentration	0.22	0.44	0.88	0.22	0.44	0.88	0.22	0.44	0.88	0.22	0.44	0.88
Log concentration	-0.66	-0.35	-0.05	-	-	-	-	-	-	-	-	-
Responses (Height of cotractions)												
1 mm	9	15	25	9	9	23	7	8	22	5	7	19
2 mm	9	13	20	4	8	16	3	9	13	3	4	9
3 mm	8	20	32	9	12	17	10	13	17	4	8	16
4 mm	18	34	45	12	17	22	9	11	19	7	10	11
5 mm	15	40	48	7	13	23	6	9	13	5	7	11
6 mm	12	18	27	12	17	22	12	16	18	8	13	14
Mean response±SEM (mm)	11.83 ±1.62	23.33 ±4.50	32.83 ±4.61	8.83 ±1.25	12.66 ±1.56	20.5 ±1.28	7.83 ±1.30	11 ±1.24	17 ±1.44	5.33 ±0.76	8.16 ±1.25	13.33 ±1.52
Mean decrease in response ±SEM (mm)	-	-	-	3 ±1.54	10.67 ±3.92	12.33 ±4.12	4 ±1.91	12.33 ±4.81	15.83 ±5.03	6.5 ±1.31	15.17 ±4.46	19.5 ±5.24
% decrease in response	-	-	-	25.36	45.74	37.56	33.82	52.86	48.22	54.95	65.03	59.4
p	-	-	-	0.11	0.04	0.03	0.01	0.05	0.02	0.004	0.02	0.01

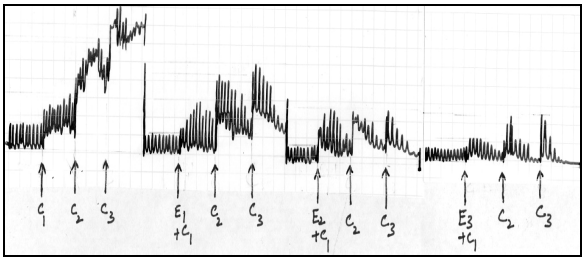
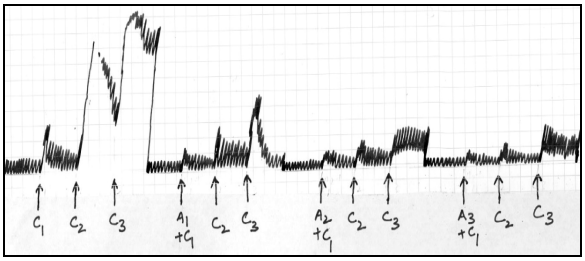


Figure-1: Effect of *C. reflexa* extract on carbachol induced contractions of rabbit ileum (n=6)

C1=Carbachol: 0.22 µM, C2=Carbachol: 0.44 µM, C3=Carbachol: 0.88 µM, E1=*C. reflexa* extract: 0.1 mg/ml, E2=*C. reflexa* extract: 0.2 mg/ml, E3=*C. reflexa* extract: 0.4 mg/ml



Figure=3: Effect of atropine on carbachol induced contractions of rabbit ileum

C1= Carbachol: 0.22 µM, C2= Carbachol: 0.44 µM, C3= Carbachol: 0.88 µM, A1= Atropine 0.002 µM, A2= Atropine: 0.005 µM, A3= Atropine:0.011 µM

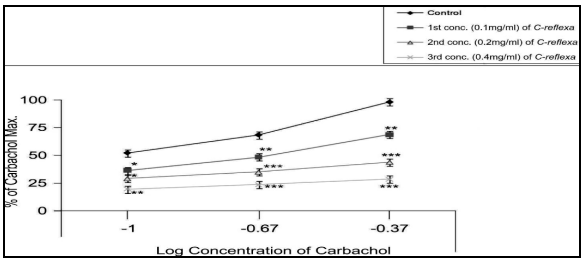


Figure-2: Response curves for carbachol in absence and presence of *C. reflexa* extract on rabbit ileum

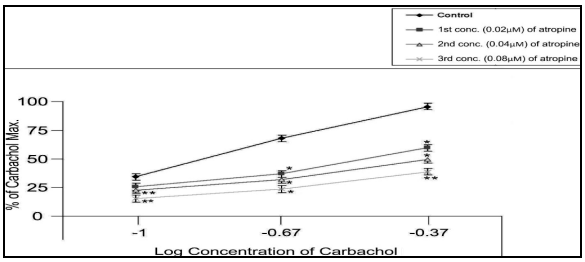


Figure-4: Response curves for carbachol in absence and presence of atropine on rabbit's ileum

DISCUSSION

Peristalsis of gastrointestinal tract is an essential physiological function. Contraction of the circular muscle in GIT is due to influx of calcium into smooth muscle cells through voltage-sensitive calcium channels and calcium release from intracellular stores. In experiments on isolated tissues as well as in studies on intact animals, GIT muscle contraction is mainly because of inward movement of calcium (blocked by L-type calcium channel blockers). Depolarization of muscle causes opening of calcium channels. Calcium present inside cell is involved in depolarization of smooth muscles of gastrointestinal tract by bringing the muscle cells close to threshold for calcium influx.[12] Motility disorders describe a variety of conditions in which the gut has lost its ability to coordinate muscular activity because of endogenous and exogenous causes.[13]

Traditional medicinal plants had been a source of medicinal treatments for thousands of years, and medicines derived from plants play an important role in primary health care. *C. reflexa* plant is traditionally used as a carminative, to control vomiting, in bilious disorders, flatulence and stomachache.[14] 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.[15] Antagonists of all the above-mentioned mediators inhibit responses by their respective agonists but calcium channel blockers will inhibit responses of all agonists.[16]

In our study, carbachol was selected as a muscarinic agonist because it is resistant to hydrolysis by cholinesterase enzyme hence cumulative dose effect could be recorded.[17] In our experiment, both atropine and *C. reflexa* extract reduced the amplitude of contractions, i.e., *C. reflexa* extract significantly decreased the magnitude of spontaneous contractions in a dose-dependent manner. Our finding is related to findings of Prasad *et al*[18], who showed that a freshly prepared aqueous solution of crude extract of *C. reflexa* possesses antispasmodic activity on guinea pig and rabbit isolated ileum against acetylcholine. However, according to Kayath *et al*[19], the extract of *C. reflexa* showed a cholinomimetic effect on rabbit ileum, frog rectus and heart muscles which were blocked by atropine on ileum and heart and by pancuronium on rectus abdominis muscle of frog. This contradiction may be because in the present study, the effects of freshly prepared solution of extract were studied while earlier Kayath *et al*[19] used 5–6 days older solution. This fact is supported by the study of Paudel *et al*[20] who observed that 4–5 days old solution of crude extract of *C. reflexa* produced marked contractions of isolated ileum of guinea pig and rabbit in a concentration which had earlier exhibited relaxant and spasmolytic effects. Given these findings, freshly prepared *C. reflexa* extract has a relaxant effect on intestinal smooth muscles of rabbit.

CONCLUSION

The freshly prepared *C. reflexa* extract has an inhibitory effect on the amplitude of carbachol-induced contractions of isolated rabbit ileum through antimuscarinic activity. It may be a valuable antispasmodic therapy. Further studies are recommended to explore the exact mechanism of action and to rule out the involvement of other receptors.

REFERENCES

1. Spencer NJ, Hu H. Enteric nervous system: sensory transduction, neural circuits and gastrointestinal motility. Nature Rev Gastroenterol Hepatol 2020;17(6):338–51.
2. Fung C, Vanden Berghe P. Functional circuits and signal processing in the enteric nervous system. Cell Mol Life Sci 2020;77:4505–22.
3. Konishi M, Hayakawa Y, Koike K. Role of muscarinic acetylcholine signaling in gastrointestinal cancers. Biomedicines. 2019;7(3):58.
4. Yang CG, Wang WG, Yan J, Fei J, Wang ZG, Zheng Q. Gastric motility in ghrelin receptor knockout mice. Mol Med Rep 2013;7(1):83–8.
5. Mergenthal A, Bouteiller JC, Yu GJ, Berger TW. A Computational Model of the Cholinergic Modulation of CA1 Pyramidal Cell Activity. Front Comput Neurosci 2020;14:75.
6. Ehlert FJ, Thomas EA, Gerstin EH, Griffin MT. Muscarinic receptors and gastrointestinal smooth muscle. In: Eglen RM. (Ed). Muscarinic receptor subtypes in smooth muscle. CRC Press; 1997.p. 87–147.
7. Morotti R, Jain D. Motility Disorders of the Gastrointestinal Tract. In: wang HL, Chen ZE. (Eds). Practical Gastrointestinal Pathology: Cham: Springer; 2021.p. 313–37.
8. Radha, Kumar M, Puri S, Pundir A, Bangar SP, Changan S, *et al.* Evaluation of nutritional, phytochemical, and mineral composition of selected medicinal plants for therapeutic uses from cold desert of Western Himalaya. Plants 2021;10:1429.
9. Aziz MA, Adnan M, Khan AH, Shahat AA, Al-Said MS, Ullah R. Traditional uses of medicinal plants practiced by the indigenous communities at Mohmand Agency, FATA, Pakistan. J Ethnobiol Ethnomed 2018;14(1):2.
10. Aung TTT, Xia MY, Hein PP, Tang R, Zhang DD, Yang J, *et al.* Chemical constituents from the whole plant of *Cuscuta reflexa*. Nat Prod Bioprospect 2020;10(5):337–44.
11. Noureen S, Noreen S, Ghumman SA, Batool F, Arshad M, Noreen F, *et al*. Seeds of giant dodder (*Cuscuta reflexa*) as a function of extract procedure and solvent nature. Not Bot Horti Agrobo 2018;46(2):653–62.
12. Huizinga JD, Lammers WJ. Gut peristalsis is governed by a multitude of cooperating mechanisms. Am J Physiol Gastrointest Liver Physiol 2009;296(1):G1–8.
13. Keller J, Bassotti G, Clarke J, Dinning P, Fox M, Grover M, *et al.* Advances in the diagnosis and classification of gastric and intestinal motility disorders. Nat Rev Gastroenterol Hepatol 2018;15(5):291–308.
14. Khan A, Siddiqui A, Jamal A. Traditional uses, Chemistry and Pharmacological activities of *Cuscuta reflexa Roxb*: A Compendious Review. Int J Sci Res Rev 2018;7(10):685–93.
15. Gribble FM, Reimann F, Roberts GP. Gastrointestinal hormones. In: Said H. (Ed). Physiology of the gastrointestinal tract. Cambridge, MA: Else; 2018.p. 31–70.
16. Neiger R, Salavati S. Motility Disorders of the Alimentary Tract. In: Bruyette DS, Bexfield N, Chretin JD, Kidd L, Kube S,

Langston C, *et al*. (Eds). Clinical Small Animal Internal Medicine. 1st ed. Wiley; 2020.p. 563–81.
17. Javid FA, Bulmer DC, Broad J, Aziz Q, Dukes GE, Sanger GJ. Anti-emetic and emetic effects of erythromycin in *Suncus murinus*: role of vagal nerve activation, gastric motility stimulation and motilin receptors. Eur J Pharmacol 2013;699(1–3):48–54.
18. Prasad DN. Preliminary pharmacological investigations on *Cuscuta reflexa Roxb*. Indian J Med Res 1965;53:465–70.
19. Kayath H, Goel N. Effects of cuscuta stem extract on various animal tissues. Indian J Pharmacol 1995;27(4):227–9.
20. Paudel P, Satyal P, Maharjan S, Shrestha N, Setzer WN. Volatile analysis and antimicrobial screening of the parasitic plant *Cuscuta reflexa Roxb* from Nepal. Nat Prod Res 2014;28(2):106–10.

Address for Correspondence:

Dr Mohsin Ali, Senior Lecturer, Department of Pharmacology, Muhammad College of Medicine, Peshawar, Pakistan.
Cell: +92-321-5275212
Email: mohsin.ibms86@gmail.com

Received: 16 Jun 2021 **Reviewed:** 15 Mar 2023 **Accepted:** 20 Apr 2023

Contribution of Authors:

SI: Lab work and manuscript writing
MA: Manuscript writing
US: Lab work and data collection
SG: Animal handling and lab work
IM: Lab work and animal handling
AA: Statistical analysis and discussion

Conflict of Interest: None to declare
Funding: None received