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Effect of fresh juice of *Brassica oleracea Var. Capitata* on isolated precontracted rat uterine horns

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Abstract

Plan: The present study was undertaken to investigate the H_2 receptor antagonistic action of fresh juice of *Brassica oleracea* var. *capitata*

Methodology: Two sets of experiments consisted of recording the responses of the uterine horn preparations (precontracted with potassium chloride, KCl) to histamine (4.5; 9; 17.99; 35.99; 71.98 × 10^{-4} and 14.40× 10^{-3} mol/L, 2 minute each concentration) in absence and presence of S- Chlorpheniramine, ranitidine and BOCJ. Relaxant responses were measured as changes in isometric tension and converted into percentage of the reference maximum relaxation induced by standard dose of histamine for each group of experiment.

Outcome: Our results indicate that histamine (EC50 = 19.05 ± 1.76 × 10^{-4} mol/L, $p < 0.001$) produces relaxation of potassium chloride precontracted isolated rat uterus. This effect of histamine is abolished by ranitidine, a selective H2 histamine receptor antagonist and BOCJ in a concentration dependent manner.

Keywords: *Brassica oleracea* (BOCJ) histamine, uterus, relaxation

1. Introduction

Effects of histamine on isolated rat uterine smooth muscle have already been described [1, 2]. Histamine produces relaxation of precontracted rat uterus via H1 and H2 histamine receptors[1]. Although H_2 receptors are present in many tissues, including vascular and bronchial smooth muscle and the right atrium, H_2 receptor antagonists interfere remarkably little with physiological functions other than gastric secretion [3]. Histamine also inhibits spontaneous and electrically stimulated contractions of rat uterus horn [4].Fresh juice of leaves of *Brassica oleracea v*ar. *capitata* Linn. Family- Brassicaceae is one of the hallowed folk remedy for ulcers [5].

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Various studies reported that cabbage juice accelerates healing of peptic ulcers [6-8]. Preliminary studies performed at our laboratory have established the efficacy of fresh juice of *B. oleracea* in treatment of peptic ulcers using various animal experimental models. Further there are no reports with respect to the mechanism of action of fresh juice of *B. oleracea*. Hence, the present study was undertaken to investigate the possible mechanism of action of fresh juice of *Brassica oleracea* var. *capitata* on the isolated uterus motility in rats pretreated with estrogen.

2. MATERIALS AND METHODS

2.1. Collection and identification of Plant Material

Fresh leaves of *Brassica oleracea*, variety: *capitata* (Syn: Cabbage) were obtained from the local market and identified and authenticated by Dr. K. B. Kathiria, Research Scientist, Main Vegetable Research Station, Anand Agricultural University, Anand, Gujarat (APCH-5a)**.**

2.2. Preparation of fresh juice from leaves of B. oleracea

Fresh leaves of *B. oleracea variety capitata* were homogenized in an EIE high speed Teflon coated micro tissue homogenizer at low speed. The pulp was strained through muslin cloth to obtain the fresh juice. Juice obtained through this process was referred to as BOCJ.

2.3. Experimental Animals

Wistar albino female rats weighing, 250-300g, were housed at ambient temperature (21 ± 1^0C) and relative humidity (55±5%) with fixed 12 h light/dark cycles in animal house of Anand Pharmacy College. Animals were fed with a standard pellet diet (Pranav agro Ltd., Baroda) and were provided with water *ad libitum*. The experimental protocol (Project no.4002 dated 29/06/2004) was approved by Institutional Animal Ethical Committee of animal house of Anand Pharmacy College as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The distribution of animals in the groups, the sequence of trials and the treatment allotted in each group were randomized.

2.4. In vitro H_2 receptor antagonism on isolated rat uterus[9]

Young female albino rats of the Wistar strain, weighing between 250 g and 300 g, pretreated with diethylstilbestrol (5 mg/kg i.p., 24 hours before sacrifice), were used in this study. Rats were killed under light chloroform anesthesia as per the guidelines of CPCSEA. Each experiment was conducted on isolated preparations from six different animals.

After laparatomy, both uterine horns were rapidly removed, cut along the longitudinal axis and placed in organ bath and placed in modified de Jalon solution at room temperature and cleaned of mesenteric fat and connective tissue. Two segments, about 2 cm in length, were taken from the ovarian end of the uterus horns.

2.4.1. Experimental design

Each isolated preparation was mounted in the 20 mL organ bath containing De Jalon's solution (NaCl 154 mmol/L, KCl 5.6 mmol/L, $CaCl_2$ 0.4 mmol/L, KH_2PO_4 1.18 mmol/L, $NaHCO_3$ 5.95 and glucose 2.5 mmol/L) maintained at 31±1°C to avoid spontaneous contractions. The bath was aerated continuously and gassed with air. One end of the isolated uterine horn was fixed to the organ bath, and the other was fixed to a force-displacement transducer (IT-1 sensor, EMKA Technologies) coupled with tension amplifier and chart recorder. All preparations were loaded with 1 g weight and allowed to equilibrate for 30 minutes. At the end of the equilibration period, a sub maximal plateau contraction of preparation was obtained by adding potassium chloride (60 mmol/L).

The first set of experiments consisted of recording the responses of the uterine horn preparations (precontracted with potassium chloride, KCl) to histamine (4.5; 9; 17.99; 35.99; 71.98 × 10–4 and 14.40× 10–3 mol/L, 2 minute each concentration) in animals pretreated with diethylstilbestrol. Logarithmic dose-response curve for histamine were constructed from mean effects of single doses.

In the second set of experiment consisted of recording the responses of uterine horn preparations (precontracted with potassium chloride, KCl) logarithmic dose-response curve for histamine (4.5; 9; 17.99; 35.99; 71.98 × 10^{-4} and 14.40× 10^{-3} mol/L, 2 minute each concentration) in animals pretreated with diethylstilbestrol in the presence of S(+)-Chlorpheniramine (permanent perfusion for 5 minutes before agonist use and during agonist's action, with final concentration of 5 × 10–7 mol/L) or ranitidine (permanent perfusion for 5 minutes before agonist use and during agonist's action, with final concentration of 3.18; 6.36; 9.54, 12.72 1 × 10^{-4} mol/L), or BOCJ (permanent perfusion for 5 minutes before agonist use and during agonist's action, with final concentration of 0.2; 0.4; 0.6; 0.8 ml in 20 ml organ bath).

Next concentration of ranitidine, S(+)-Chlorpheniramine, BOCJ on the same preparation was applied only after a period of 15 min. Relaxant responses were measured as changes in isometric tension and converted into a percentage of the reference maximum relaxations induced by standard dose of histamine for each group of experiments.

2.5. Statistical analysis

Each concentration was assayed from six different animals. Concentration-response curves were constructed using linear regression according to least-squares analysis [10]. Effective concentration of agonists that produced 50% of maximal response and response duration (EC50) was calculated together with its confidence limits (1.96 × standard error). The Student *t* test was used for the comparison of maximal responses (expressed as mean ± standard error mean). The results were considered statistically significant when $P \leq 0.05$.

3. Result

The effects of histamine on the isolated uterus horns from oestrogenized rats:

Histamine (4.5×10^{-4} mol/L to 14.40×10^{-3} mol/L) produced concentration-dependent relaxation of isolated uterus horns (precontracted with KCl) from oestrogenized rats (EC50 = 19.05 ± 1.76 × 10^{-4} mol/L $p < 0.001$). The maximal relaxation (85.85 ± 3.03% of KCl induced contraction) in this experimental group was reached with the highest concentration of histamine (14.40× 10–3 mol/L) *(Figure 1*). This maximal relaxation was inhibited in the presence of the highest concentration of BOCJ (0.8 ml) and ranitidine (12.72×10^{-4} mol/L) by 44.09 % *(Figure 1).*

S (+)-Chlorpheniramine (5 × 10–7 mol/L), H_1 receptor antagonist, did not affect the response of isolated uterus to histamine (EC50 = 17.82 ± 1.1 × 10^{-4} mol/L).

Ranitidine (3.18; 6.36; 9.54, 12.72 1 × 10^{-4} mol/L), H_2 receptor antagonist, shifted concentration-dependent relaxation curve of histamine to the right (EC50 = 18.19 ± 0.56; 41.69 ± 1.23; 66.07 ± 2.45; 93.33 ± 2.15 × 10^{-4}mol/L) in a concentration dependent manner. (R^2=0.9864*) (Figure 2).* BOCJ (0.2; 0.4; 0.6; and 0.8 ml), also, shifted concentration-dependent relaxation curve of histamine to the right (EC50 = 26.30 ± 0.56; 43.65 ± 1.23; 64.57 ± 2.45× 10^{-4}mol/L; 10.0 ± 2.15 × 10^{-3}mol/L, $p < 0.001$) in a concentration dependent manner. (R^2=0.9859) *(Figure 3).*

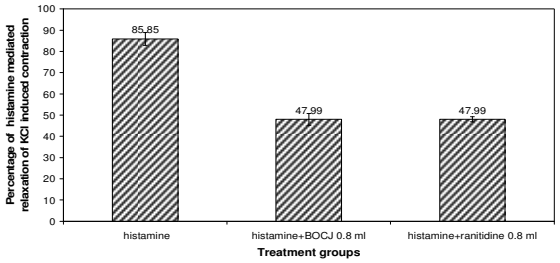

Figure 1: Maximal relaxation of isolated uterus horns (KCl-induced contraction)

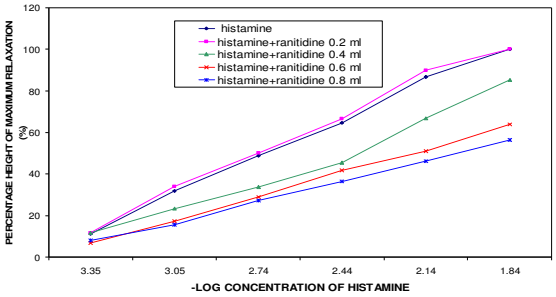

Figure 2: Histamine (4.5 × 10–4 mol/L to 14.40× 10–3 mol/L) caused concentration-dependent relaxation of isolated uterus horns (precontracted with KCl) from oestrogenized rats. Ranitidine (3.18; 6.36; 9.54, 12.72 × 10^{-4} mol/L) shifted concentration-dependent relaxation curve of histamine to the right.

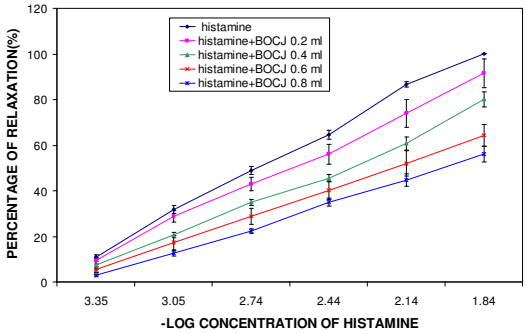

Figure 3: Histamine (4.5 × 10–4 mol/L to 14.40× 10–3 mol/L) caused concentration-dependent relaxation of isolated uterus horns (precontracted with KCl) from oestrogenized rats. BOCJ (0.2; 0.4; 0.6; and 0.8 ml) shifted concentration-dependent relaxation curve of histamine to the right.

4. Discussion

It is documented that histamine receptor (H_2) antagonist inhibit competitively the interaction of histamine with H_2 receptors responsible for acid secretion in the stomach. In the group of pylorus ligated rats that received ranitidine, the mean titratable acidity, acid output and pepsin concentration were significantly reduced (probably by blocking the histamine H2-receptors and allowing the muscarinic-receptors to predominate) despite the stability in gastric juice volume. Although H_2 receptors are present in many tissues, including vascular and bronchial smooth muscle and the right atrium, H_2 receptor antagonists interfere remarkably little with physiological functions other than gastric secretion[2]. Effects of histamine on isolated rat uterus have already been described[1, 3.] Namely, histamine produces relaxation of precontracted rat uterus [1]. Histamine also inhibits spontaneous and electrically stimulated contractions of rat uterus horn[4]. These effects can be antagonized by H_2, but not by H_1 antagonist.

Our results indicate that histamine (EC50 = 19.05 ± 1.76 × 10^{-4} mol/L $p < 0.001$) produces relaxation of potassium chloride- precontracted isolated rat uterus which is in accordance with similar published results [2, 11]. S (+)-Chlorpheniramine (5 × 10–7 mol/L), H_1 receptor antagonist, did not affect response of isolated uterus to histamine (EC50 = 17.82 ± 1.1 × 10^{-4} mol/L).

Ranitidine (3.18; 6.36; 9.54, 12.72 1 × 10^{-4} mol/L), H_2 receptors antagonist, shifted concentration-dependent relaxation curve of histamine to the right (EC50 = 18.19 ± 0.56; 41.69 ± 1.23; 66.07 ± 2.45; 93.33 ± 2.15 × 10^{-4}mol/L) in a concentration dependent manner. BOCJ (0.2; 0.4; 0.6; and 0.8 ml), also, shifted concentration-dependent relaxation curve of histamine to the right (EC50 = 26.30 ± 0.56; 43.65 ± 1.23; 64.57 ± 2.45× 10^{-4}mol/L; 10.0 ± 2.15 × 10^{-3}mol/L, $p <$ 0.001) in a concentration dependent manner, suggesting its role as H_2 receptor antagonist.

Ranitidine, H_2-receptor antagonist, inhibits acid production by reversibly competing with histamine for binding to H_2 receptors on the basolateral membrane of parietal cells. The H_2-receptor antagonists predominantly inhibit basal acid secretion, which accounts for their efficacy in suppressing nocturnal acid secretion. Because the most important determinant of duodenal ulcer healing is the level of nocturnal acidity, evening dosing of H_2-receptor antagonists is adequate therapy in most instances. In context to this, our findings of BOCJ suggest that fresh juice of leaves of *B. oleracea* inhibits acid secretion by acting as H_2 receptor antagonist similar to ranitidine, proving its use beneficial in the acute and chronic treatment of duodenal ulcer, benign gastric ulcer, and hypersecretory conditions.

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