Antihistaminic and Anticholinergic Activity of Methanolic Extract of Barberry Fruit (*Berberis vulgaris*) in the Guinea- Pig Ileum

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Abstract

Background: **Barberry (*Berberris vulgaris*) is a well known medicinal plant in Iran and has also been used as food.**

Objective: **This study was conducted to evaluate antihistaminic and anticholinergic activity of methanolic extract of barberry fruit.**

Methods: **Methanolic extract was prepared and pharmacologically studied on isolated guinea-pig ileum, dose- response curves of histamine and acetylcholine with and without methanolic extract were plotted.**

Results: **The pA_2 values for antihistaminic activity of methanolic extract and dexchlorpheniramine were calculated (extract; $pA_2\pm$ S.E.M = 3.53 ± 0.16 [-log$C$(g/l)]; dexchlorpheniramine; pA_2 ± S.E.M.= 9.36 ± 0.14 ([-log$C$ (M)]) and compared with each other. The pA_2 values of anticholinergic activity of methanolic extract and atropine were also calculated (extract; $pA_2\pm$ S.E.M = 4.18 ± 0.17 [-log$C$(g/ι)]; atropine, PA_2 +S.E.M = 8.99 ± 0.13 [-log$C$(M)]) and compared.**

Conclusion: **The results indicated antihistaminic and anticholinergic activity of methanolic extract.**

Keywords: **Barberry, Antihistaminc, Anticholinergic, Guinea-Pig ileum, Methanolic extract**

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Introduction

Barberry (*Berberis vulgaris* L., Var. asperma Don., family Berberidaceae) grows in Asia and Europe; It is well known medicinal plants in traditional medicine, the fruits has also been used as food [1, 2]

Medicinal properties for all parts of the plant have been reported, including tonic, antimicrobial, antiemetic, antipyretic, antipruritic, antioxidant, anti-inflammatory, hypotensive, antiarrhythmic, sedative, antinociceptive, anticholinergic and cholagogue actions, and it has been used in some cases like cholecystitis, cholelithiasis, jaundice, dysentery, leishmaniasis, malaria and gall stones [1-10].

In spite of extensive applications and numerous properties, the mechanism of action in most of its effects is not exactly clear. Some of these properties may occure due to antihistaminic and anticholinergic effects. According to Shamsa et al studies results, this work was designed to evaluate antihistaminic and anticholinergic activity of methanolic extract of barberry fruit (*Berberis vulgaris*) in guinea- pig ileum as a valuable and accurate method, in order to isolate and identify components are responsible for pharmacological properties of barberry.

Materials and Methods

Sample preparation

Plant materials (barberry fruit) obtained from Bazar and authenticated at the Herbarium of Faculty of Pharmacy of Tehran Medical Science University, where the voucher specimen is deposited under No. 6507. Barberry fruits (280 g) were extracted by continuously refluxing of methanol in Soxhlet extractor 8-12 hours. The obtained extract was concentrated in a rotary vacuum evaporator. The thick obtained syrup was dried (evaporated completely) by freeze drying to yield 34.88 g adhesive powder. The desired concentration (w/v) were prepared from this powder.

Pharmacological test

Male albino fasted (24 h) guinea pigs weighing 250- 500 g were killed by a blow to the head and exsanguinated. Terminal segments of ileum about 1 – 1.5 cm in length were prepared and placed in 30 ml baths filled with Tyrode solution (NaCl, 136.7; KCl, 2.68; $MgCl_2$ 1.05; NaH_2PO_4 0.42; $CaCl_2$, 1.80; $NaHCO_3$, 11.90 ; glucose, 5.55 mM).

The solution was kept at 37ºC and oxygenated continuously. Initial tension was 1g and stabilization time was 45- 60 min. Isometric contractions were recorded on NARCO F-60 transducer connected to a NARCO trace 80 recorder.

Increasing concentration of histamine and acetylcholine (10^{-9} to 10^{-4} M) were added to the bath and the control cumulative concentration- response curve for each one (histamine or acetylcholine) was constructed. Methanolic extract, dexchlorpheniramine or atropine was then added to the bath ~1 min before the corresponding concentration-response curve was recorded [7].

Drugs and Solvents

Dexchlorpheniramine maleate (Schering), atropine sulfate (Merck), histamine dihydrochloride (Sigma), acetylcholine chloride (Sigma) and methanol 99.8% (Merck). All drugs were dissolved in distilled water and desired concentration were prepared.

Analysis of results

Contractions were expressed as a percentage of the maximal contraction obtained from the corresponding control curve, each point represents the mean ± S.E.M. of four experiments. The histamine and acetylcholine dose- response curves, in the absence or presence of antagonists, were plotted using the SPSS computer program. The EC_{50}, potency (pD_2= -log (EC_{50}) and affinity (ı/EC ˉ) of histamine and acetylcholine were determined separately. The antagonist potencies (pA_2) of methanolic extract, dexchlorpheniramine and atropine were also calculated [7, 11, 13].

Results

Methanolic extracts displaced the cumulative histamine dose-response curve towards higher concentration, i.e. it increased the EC_{50} of histamine (Fig. 1A) as did dexchlorpheniramine (Fig. 1B). This displacement increased with increasing both extract and dexchlorpheniramine dose, histamine affinity and potency decreased with increasing dose of extract and dexchlorpheniramine (Table 1).

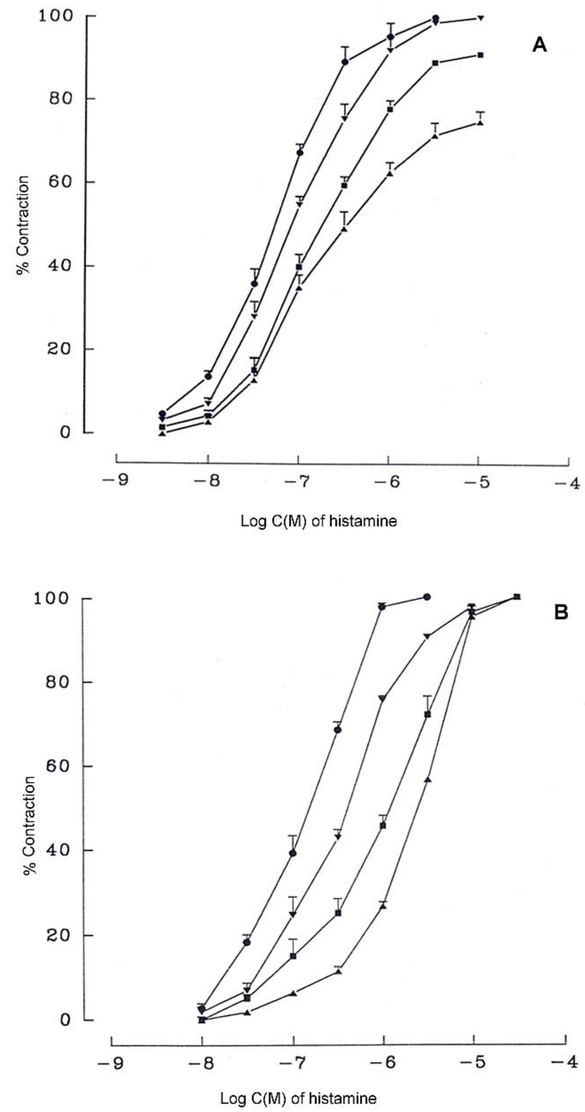

Fig. 1- Effect of increasing concentrations of methanolic extract (A) and dexchlorpheniramine (B) on the cumulative dose- response curves to histamine in the guinea-pig ileum. Methanolic extract: Control (●), 3*10^{-4} g/l (▼), 6*10^{-4} g/l (■), 9*10^{-4} g/l (▲). Dexchlopheniramine: Control (●), 1.3*10^{-9} M (▼), 2.6*10^{-9} M (■), 3.9*10^{-9} M (▲)

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Table 1- The affinity and pD_2 values of histamine in the presence of different dose of dexchlorpheniramine and methanolic extract

Dose (M)	pD_2	Affinity	Dose (g/l)	pD_2	Affinity
Dexchlorpheniramine			Methanolic extract		
0	6.82	$6.60*10^6$	0	7.27	$1.86*10^7$
$1.30*10^{-9}$	6.39	$2.45*10^6$	$3.00*10^{-4}$	7.09	$1.20*10^6$
$2.60*10^{-9}$	5.92	$8.24*10^5$	$6.00*10^{-4}$	6.75	$5.62*10^6$
$3.90*10^{-9}$	5.61	$4.07*10^5$	$9.00*10^{-4}$	6.47	$2.95*10^6$
	pD_2 ± S.E.M. = 6.18 ± 0.26			pD_2 ± S.E.M. = 6.90 ± 0.18	

The cumulative acetylcholine dose-response curve shifted to the right in the presence of extract (Fig. 2A). A similar rightward shift was observed in the acetylcholine dose-response curve by adding atropine to the bath (Fig. 2B); rightward shifts were dose dependent, the affinity and potency of acetylcholine decreased with increasing dose of extract and atropine (Table 2).

The pA_2 values which had been obtained from histamine dose-response curve for methanolic extract and dexchlorpheniramine were:

Methanolic extract: pA_2 ± S.E.M= 3.53 ± 0.16 [-log$C$(g/l)];

Dexchlorpheniramine: pA_2±S.E.M= 9.36 ± 0.14 [-log$C$(M)].

Calculated pA_2 values for methanolic extract and atropine from acetylcholine dose-response curves were:

Methanolic extract: pA_2±S.E.M.= 4.18 ± 0.17 [-log$C$(g/l)];

Atropine: pA_2±S.E.M.= 8.99 ± 0.13 [-log$C$(M)].

Discussion

The parallel rightward shift in agonist concentration-response curves in the presence of increasing concentrations of antagonist is observed with competitive antagonists. The occurred inhibition with competitive antagonists can be overcome by increasing the concentration of agonist. Finally, a maximal effect can be achieved by using sufficient agonist [7, 13]. The results of this study indicate a similar rightward shift in dose-response curves of histamine and acetylcholine in the presence of methanolic extract (Fig. 1A and Fig 2A), dexchlorpheniramine (Fig. 1B) and atropine (Fig 2B). By increasing the dose of methanolic extract, the EC_{50} increased and pD_2 and the affinity of histamine and acetylcholine decreased, similar to dexchlorpheniramine and atropine (Tables 1 and 2). The maximal effects of histamine and acetylcholine that were depressed in the presence of extract were not achieved by increasing concentrations of histamine and acetylcholine (Fig. 1A anf Fig. 2A), but in presence of dexchlorpheniramine and atropine, the maximal effect of histamine and acetylcholine in control curves was obtained again. This decrease in maximal effect (contraction) in the presence of methanolic extract might perhaps reflect a partly non-competitive or irreversible competitive type of antagonism or the impurity of methanolic extract possibly being responsible for this decrease.

From the cumulative dose-response curves, the pD_2 values for histamine were calculated: pD_2 ± S.E.M.= 6.90 ± 0.18 (Fig. 1A), pD_2 ± S.E.M. = 6.18 ± 0.26 (Fig. 1B), which are in the same order of magnitude as the values found in the literatures [7, 13]. Also, the pD_2 for acetylcholine, pD_2 ± S.E.M. = 6.01 ± 0.31 (Fig. 2A), pD_2 ± S.E.M.= 6.64 ± 0.33 (Fig. 2B),

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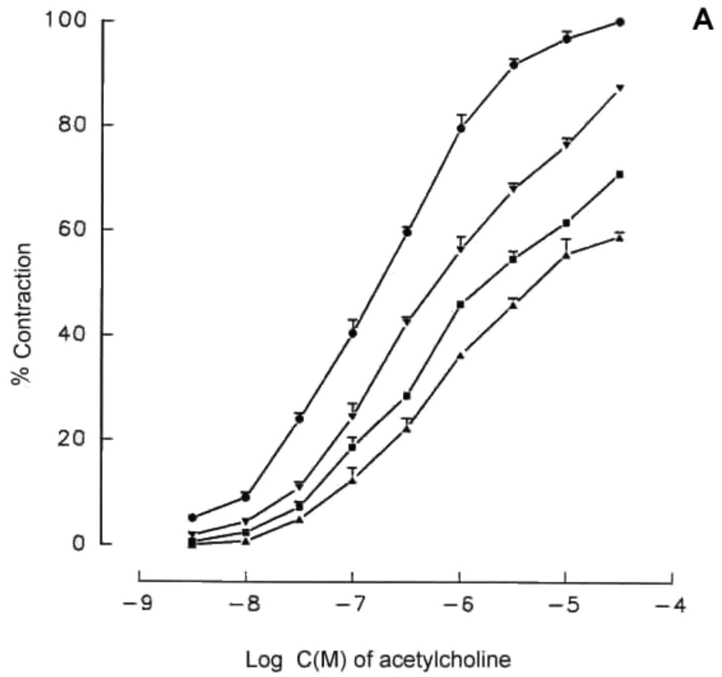

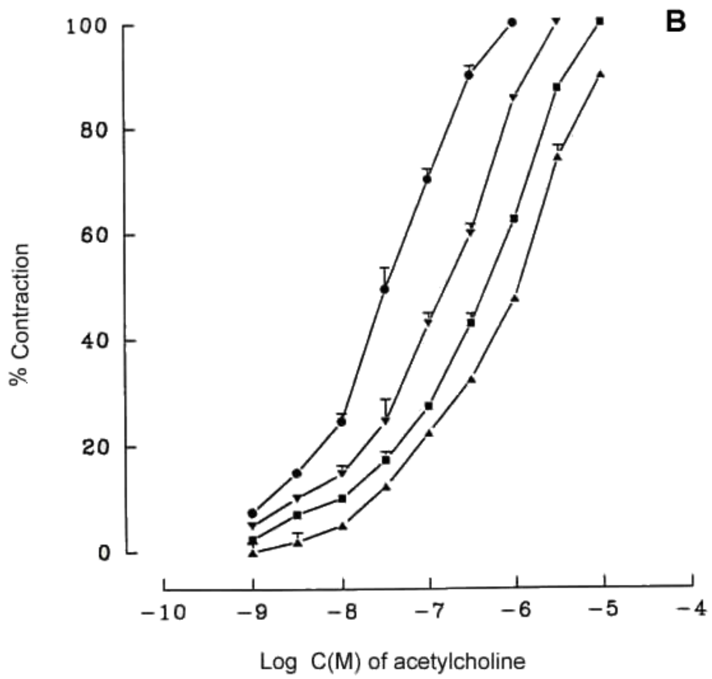

Fig. 2- Effect of increasing concentrations of methanolic extract (A) and dexchlorpheniramine (B) on the cumulative dose- response curves to acetylcholine in the guinea-pig ileum. Methanolic extract: Control (●), $3*10^{-4}$ g/l (▼), $6*10^{-4}$ g/l (■), $9*10^{-4}$ g/l (▲). Atropine: Control (●), $6.66*10^{-9}$ M (▼), $1.33*10^{-8}$M (■), $2*10^{-8}$M (▲)

Table 2- The affinity and pD_2 values of acetylcholine in the presence of different dose of atropine and methanolic extract

Dose (M)	pD_2	Affinity	Dose (g/l)	pD_2	Affinity
Atropine			Methanolic extract		
0	7.49	$3.09*10^7$	0	6.75	$5.62*10^6$
$6.66*10^{-9}$	6.80	$6.24*10^6$	$3.00*10^{-4}$	6.23	$1.70*10^6$
$1.33*10^{-8}$	6.32	$2.10*10^6$	$6.00*10^{-4}$	5.77	$5.89*10^5$
$2.00*10^{-8}$	5.96	$9.02*10^5$	$9.00*10^{-4}$	5.28	$1.90*10^5$
	pD_2 ± S.E.M. = 6.64 ± 0.33			pD_2 ± S.E.M. = 6.01 ± 0.31	

were comparable with those of other authors [7, 13]. Results showed by increasing the dose of antagonist, the inhibitory effect is increased in the same way. Due to the complexity of methanolic extract, concentrations of extract were calculated in a different way (g/l) from dexchlorpheniramine and atropine (M), thus the pA_2 values of extract obtained from this study (antihistaminic, pA_2 ± S.E.M. = 3.53 ± 0.16
[-logC (g/l)]; antocholinergic, pA_2± S.E.M.= 4.18 ± 0.17 [-logC (g/l)]) were not exactly comparable with pA_2 values of dexchlorpheniramine (pA_2 ± S.E.M.= 9.36 ± 0.14 [-logC(M)]) and atropine (pA_2 ± S.E.M.= 8.99 ± 0.13 [-logC (M)]). However, the relative comparison of calculated pA_2 values indicated that methanolic extract has antihistaminic and anticholinergic activity. Also, the rightward shift in a parallel manner that occurred in dose-response curves of histamine and acetylcholine in the presence of increasing concentrations of methanolic extract and the similar inhibitory effect of extract with dexchlorfeniramine and atropine, confirmed the ability of methanolic extract to inhibit histamine and acetylcholine on the guinea-pig ileum.

As many of the H_1 antagonists tend to inhibit responses to acetylcholine (anticholinergic activity) that are mediated by muscarinic receptors, maybe one component from the extract is responsible for both antihistaminic and anticholinergic effects of extract. Also, the number of potent components are not distinguished and perhaps more than one component from the extract is able to inhibit histamine and acetylcholine. Also the presence of alkaloids in the methanolic extract may account for the anticholinergic and antihistaminic activity. This group of compounds widely occurring in the medicinal plants have been shown to display a remarkable array of biochemical and pharmacological actions, including relaxing effects on intestinal smooth muscle [5, 7, 8].

Conclusion

In conclusion, barberry (*B. valguris*) fruit methanolic extract seems to have antihistaminic and anticholinergic activity on the guinea-pig ileum, similar to that of H_1-antihistamine (dexchlorpheniramine) and anticholinergic (atropine). Analysis of methanolic extract, isolation, purification and identification of the structure of the components like alkaloids and investigation of antihistaminic and anticholinergic activity of each one in future studies is necessary to confirm these properties definitely.

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