Medical Journal of The
Islamic Republic of Iran

Volume 2, Number 1
Bahar 1367
Shaaban 1408
Spring 1988

ANTISPASMODIC EFFECTS OF SOME IRANIAN MEDICINAL PLANTS

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ABSTRACT

Alcoholic and water extracts of many medicinal plants are commonly used in German folk medicine and other countries as antispasmodics. However, so far no data on dose relationships or comparisons of the antispasmodic effects with reference substances have been available for most of these plant extracts. Using acetylcholine as the stimulating agent, the antispasmodic effect of preparations of *Mentha piperita* (leaves), *Citrus aurantium* (peels), *Foeniculum vulgare* (fruits) and *Carum carvi* (fruits), consisting of one part of the plant and three and one half parts ethanol (31% w/w) was investigated on isolated guinea pig ileum and the results were compared with that of atropine. All the extracts shifted the dose-response curves of acetylcholine to the right in a dose-dependent manner and also showed a significant increase of the ED_{50} and the dose ratios of acetylcholine-induced contractions, and a significant decrease of the maximal possible contractilty. When the antispasmodic activities of *M. piperita* and *C. aurantium* were compared with the activity of atropine, it was evident that their effects were less than that of the usual therapeutic dose of atropine in man. But when the antispasmodic activities of *F. vulgare* and *C. carvi* were compared with the activity of atropine, it was found that their effects were slightly greater than that of the usual dose of atropine.

MJIRI, Vol.2, No.1,51-55, 1988

INTRODUCTION

It has been known that atropine has an antispasmodic effect both *in vivo* and *in vitro* on most types of smooth muscle. But occurrence of side effects and many contraindications of this drug have limited its use as a safe therapeutic agent. Alcoholic and water extracts of many medicinal plants are commonly used in German folk medicine and other countries as antispasmodics. In experimental animals, chamomile, peppermint, caraway, melissa and fennel have been shown to have antispasmodic activity.[1] Forster and Niklas observed the antispasmodic effects of *Melissa officinalis*, *Rosmarinum officinalis*, matricaria and chamomilla on isolated guinea pig ileum in comparison with atropine.[2-4] However, so far no data on dose relationships and comparisons of the antispasmodic effects with reference substances have been available for most of these plant extracts. Using acetylcholine as the stimulating agent, we investigated the antispasmodic effect of *M. piperita* (leaves), *C. aurantium* (peels), *F. vulgare* (fruits) and *C. carvi* (fruits) on isolated guinea pig ileum and compared their results with the antispasmodic effect of atropine.

MATERIALS AND METHODS

Chemicals

Acetylcholine chloride and atropine sulfate were used as bases.

Plants

M. piperita (leaves), *C. aurantium sp. amara*

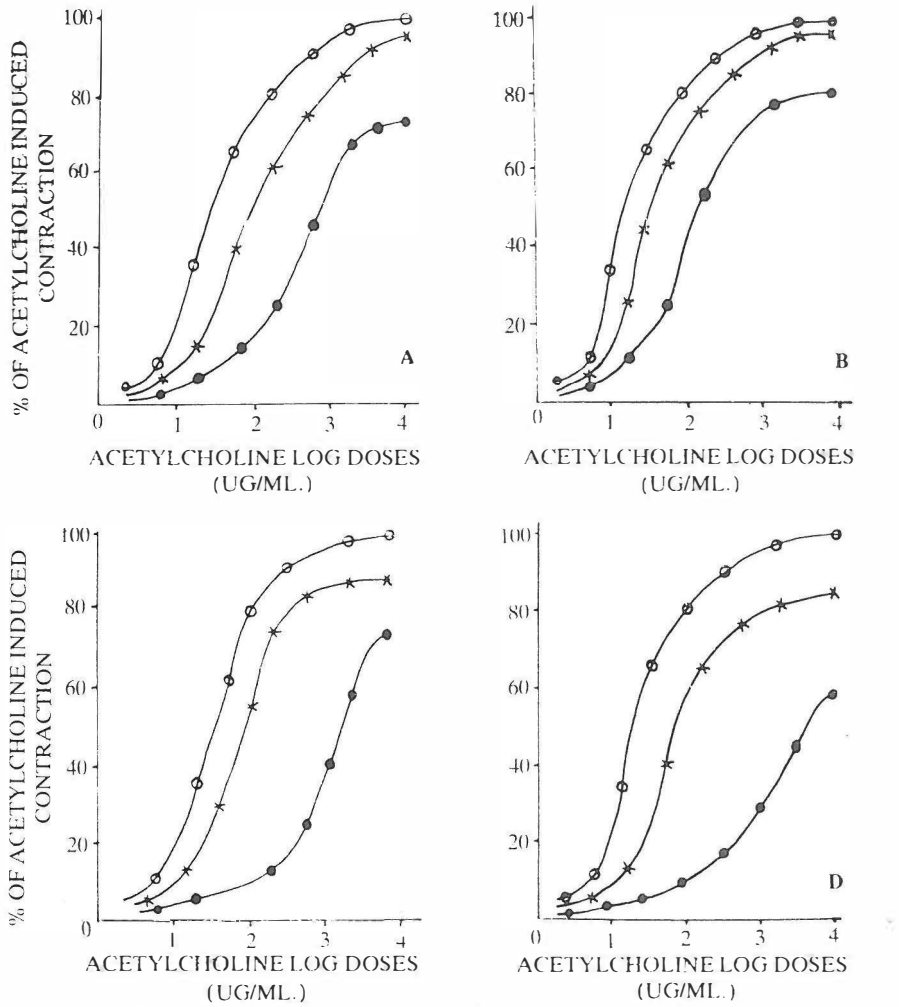

Figure1 Dose-response curves of acetylcholine-induced contractions of the isolated guinea pig ileum in the absence (O) and in the presence of 10 ml/l (X) and 20 ml/l (O) of (A) *Mentha piperita* and (B) *Citrus aurantium*; and 7.5 ml/l (X) and 10 ml/l (O) of (C) *Foeniculum vulgare* are (D) *Carum carvi*.

(peels), *F. vulgare* (fruit) and *C. carvi* (fruit) were used. The quality of the four Iranian plants used was equal to that in the German pharmacopeia, 6th edition. One part of the chopped, dried plant was extracted with 3.5 parts of ethanol (31% w/w) in a percolator according to *Extracta fluida* in the German pharmacopeia, 6th edition. The alcohol concentration of all extracts used in the experiments was 30%.

Animals

Male albino guinea pigs weighing 300 to 400g were used in all experiments. The animals were equally divided into five groups as follows:

1- Atropine-exposed group,
2- *M. piperita*-exposed group,
3- *C. aurantium*-exposed group,
4- *F. vulgare*-exposed group,
5- *C. carvi*-exposed group.

The animals were sacrificed by a blow on the head. The ileum was removed and cleansed, and the preparation was then suspended in a 10ml organ bath containing aerated tyrode solution. The temperature of the bath was maintained at 37° C. Solutions of the drugs were added to the bath and the drug-induced effects on the tissue were recorded by kymograph.

Procedure

The procedure used was similar to that described by Gaddum and Picarelli.[1] The antispasmodic effects of atropine and plant extracts has been measured in terms of dose-ratio.[1] The concentration of the various doses of atropine and plant extracts was maintained in the bath by adding the drug and plant extracts to one of the reservoirs from which the bath could be refilled. In order to avoid errors due to the persistence of effects, a separate ileum preparation was used for each experi-

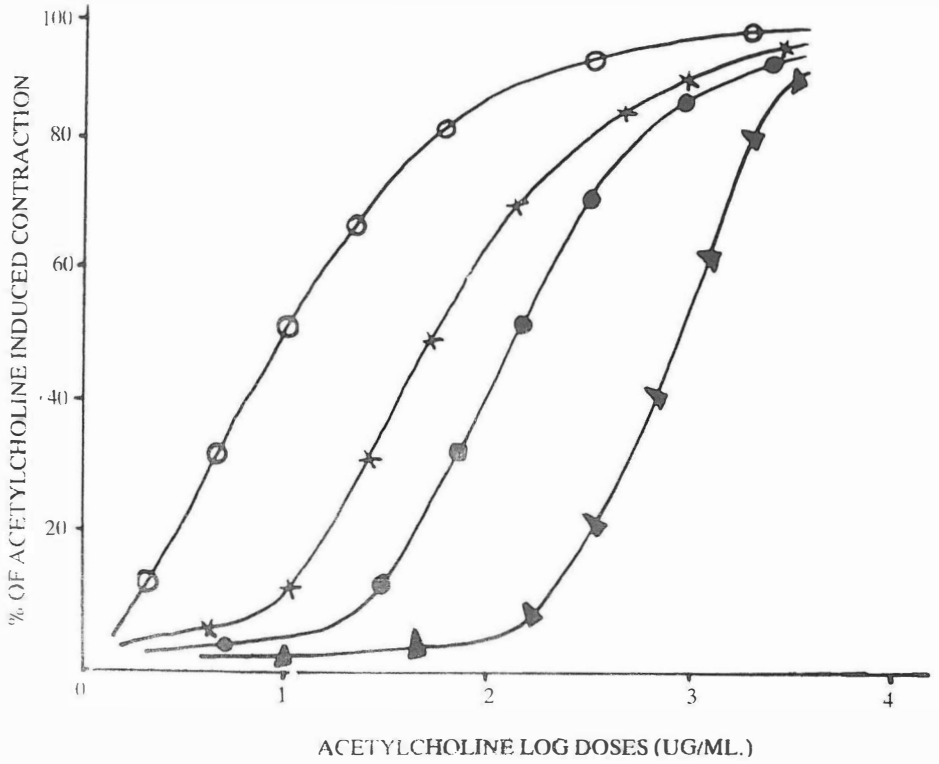

Figure 2. Dose-response curves of acetylcholine-induced contractions of the isolated guinea pig ileum in the absence (O) and in the presence of 1 ug/l (X), 5 ug/l (O) and 10 ug/l (A) of atropine.

Table 1. Effect of medicinal plant extracts on acetylcholine-induced contractions of the isolated guinea pig ileum. Results are expressed as ED_{50} and dose ratios of acetylcholine (ug/ml) in the presence and absence of plant extracts, and as maximal possible contraction in response to acetylcholine.

Extracts	ml/l	No *	Aver. ED_{50} of acetylcholine in ug with extracts	Dose ratios**	Maximal possible contraction (%).***
M. piperita	10.0	8	451.5 ± 12.7	2.8 ± 1.01	95.1 ± 2.6
	20.0.	8	3466.6 ± 20.7	18.3 ± 3.5	72.7 ± 4.3
C. aurantium	10.0	8	97.6 ± 4.6	2.2 ± 1.1	97.1 ± 1.5
	20.0	8	338.6 ± 12.5	10.2 ± 2.8	80.7 ± 2.0
F. vulgare	7.5	8	65.1 ± 6.9	3.1 ± 1.1	86.2 ± 3.8
	10.0	8	1285 ± 30.0	61.1 ± 5.6	74.7 ± 3.5
C. carvi	7.5	8	130.6 ± 15.5	4.5 ± 1.1	83.5 ± 3.9
	10.0	8	1876.6 ± 38.8	64.7 ± 5.9	58.8 ± 4.9

* No. = number of experiments

** *Dose ratios* were calculated by ratios of doses of acetylcholine causing equal contractions in the absence and presence of plant extracts

*** *Maximal possible contraction* was calculated by comparing the maximal possible contraction in the presence and absence of plant extracts.

ment.

The dose-response curve of acetylcholine was plotted by increasing the concentration of acetylcholine from minimum to maximum response in a non-cumulative manner. After obtaining the dose-response tracing with acetylcholine, the tissue was then exposed to either atropine or one of the plant extracts for 30 minutes. The dose-response tracing with acetylcholine was again plotted in the presence of the same concentration of atropine or the plant extract. The concentration of atropine or the extract was kept constant in the bath throughout the experiment. This procedure was repeated for all different concentrations of atropine and the plant extracts.

The antispasmodic effects of atropine and the plant extracts were then measured in terms of dose-ratio. The results given in the figures and tables are expressed as the percent of maximal possible contraction due to acetylcholine. The ED_{50} given in the tables were calculated by parallel line assay method in the presence of the antispasmodics and dose ratios were calculated in their presence and absence (acetylcholine alone). The percents of maximal possible contractions in the experiments were also compared with 100% maximal effects of acetylcholine (controls).

RESULTS

As shown in Figure 1 and Table I, ethanolic extracts of *M. piperita*, *C. aurantium*, *F. vulgare* and *C. carvi* significantly shifted the dose-response curve of acetylcholine to the right and therefore increased the median effective dose (ED_{50}) and the dose ratios of acetylcholine in a dose-related manner. A significant increase of the ED_{50} and the dose ratios of acetylcholine were observed at a dose of 20ml/l for *M. piperita*, *C. aurantium*, and *F. vulgare*; and 10ml/l for *C. carvi*. Atropine sulfate also shifted the dose-response curve of acetylcholine to the right in a dose-related manner (Fig. 2).

We also observed the effect of ethanol on acetylcholine-induced contractions of isolated guinea pig ileum. Ethanol did not significantly shift the dose-response curve of acetylcholine to the right and therefore did not increase the median effective dose of acetylcholine in a dose-related manner.

As indicated in Table I, 10ml/l and 20ml/l extracts of *M. piperita* and *C. aurantium*, and 7.5ml/l and 10ml/l extracts of *F. vulgare* and *C. carvi* respectively, employed in this study, significantly decreased the maximal possible contraction in response to acetylcholine, whereas there was no such effect with either of these concentrations of atropine.

DISCUSSION

Alcoholic extracts of *M. piperita*, *C. aurantium sp. amara*, *F. vulgare* and *C. carvi* which have been prepared from crude dried parts of plants under similar conditions exhibited different qualitative and quantitative effects on the contraction of isolated guinea pig ileum induced by acetylcholine (Fig. 1 and Table I). As far as the shift of the dose-response curve of acetylcholine to the right is concerned, with concentrations of 10ml/l *M. piperita*, 20ml/l *C. aurantium*, 7.5ml/l *F. vulgare* and 10ml/l *C. carvi*, a significant antispasmodic effect was observed. The plant extracts not only produced a shift of the dose-response curve of acetylcholine to the right, but also decreased the maximal contractile effect in response to acetylcholine in the following order: using 7.5 ml/l alcoholic extracts of *C. carvi* and *F. vulgare*, the former was more active than the latter; with a concentration of 10ml/l the results in decreasing order of activity were *C. carvi*, *F. vulgare*, *M. piperita* and *C. aurantium*. With 20ml/l, *M. piperita* was more active than *C. aurantium*. All these effects of the alcoholic extracts have been shown to be reversible.

Therefore, it appears that when the effect of the four plant extracts are compared on the basis of their dose ratios by 10ml/l in our study, both *C. carvi* and *F. vulgare* have the most pronounced activity, whereas *M. piperita* and *C. aurantium* were only of little effect. The dose ratios of the four plant extracts by 10ml/l were in the following order: 64.7, 61.1, 2.8 and 2.2 for *C. carvi*, *F. vulgare*, *M. piperita* and *C. aurantium* respectively. From our data, no conclusion about the mechanism of action of the plant extracts can be made. The antispasmodic activity of these extracts may possibly be, at least in part, due to their contents of essential oils.

In the treatment of abdominal spasms, 0.5-1.0mg of atropine is generally used. This corresponds to approximately 7ug/l atropine in the experimental medium.[3] As far as the dose ratios are concerned, in our study, the effect of 10ml/l *C. aurantium* and *M. piperita* is equivalent to 0.5-0.6ug/l of atropine, whereas the effect of 20ml/l corresponds to 3-5.3ug/l atropine. This indicates that the antispasmodic effect of *C. aurantium* and *M. piperita* extracts is by far less than that of the usual therapeutic dose of atropine. The effect of 7.5ml/l *F. vulgare* and *C. carvi* is equivalent to 0.7-1ug/l atropine, whereas the effect of 10ml/l corresponds to 11.5-12ug/l atropine. This indicates that the antispasmodic effect of *F. vulgare* and *C. carvi* in concentrations of 10ml/l is slightly greater than that of the usual dose of atropine. These results indicate that the Iranian plant extracts of *F. vulgare* and *C. carvi* may be used in severe abdominal spasms in place of atropine, and extracts of *C. aurantium* and *M. piperita* should not be used in severe spasms. Rather, they may be used in subjective abdominal discomfort and meteorism. It is conceivable that the insignificant antispasmodic effect of the above two extracts may be sufficient for the treatment of minor spasms, while *F. vulgare* and

Table II Effect of atropine on acetylcholine-induced contractions of the guinea pig ileum. Results are expressed as ED_{50} and dose ratios of acetylcholine (ug/ml) in the presence and absence of atropine, and as maximal possible contraction in response to acetylcholine.

Drug	ug/l	No.	Aver. ED_{50} of acetylcholine in ug with extracts	Dose ratios	Maximal possible contraction (%).
Atropine	1	8	204 ± 12.4	4.4 ± 1.1	98.2 ± 1.6
	5	8	444.1 ± 22.3	17 ± 3.2	97.5 ± 4.9
	10	8	2231.8 ± 63.3	52.8 ± 5.3	97 ± 2.7

Maximal possible contraction was calculated by comparing the maximal possible contraction in the presence of atropine, with experiments without addition of atropine.
Dose ratios were calculated by ratios of doses of acetylcholine, causing equal contractions in the absence of atropine.

C. carvi extracts may be used in severe abdominal spasms. However, further evidence is necessary to support this view.

ACKNOWLEDGMENTS

The authors would like to thank Mrs. Khazoni, Mr. Mollaye, Mr. Memer, Mr. Arhami and Mr. Hashemi for their valuable help in the experiments. This investigation was supported by a research grant from the Ferdowsi University, Mashad.

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