Pharmacological effects of extracts from Mentha Iongifolia L. Huds

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ABSTRACT

The pharmacological effects of volatile oil fraction, butanol and the residual water extracts of *Mentha longifolia* (L. Huds, family Lamiaceae) on intestinal smooth muscle were studied *in vitro*. Volatile oil fraction (40-100 µg/ml) exhibited an inhibitory effect on the acctylcholine-induced contraction of the rabbit ileum. This action was transient and reversible after washing the tissue. In addition, volatile oil fraction (40-100 µg/ml) inhibited the histamine-induced contraction of the ileum in guinea pig. Butanolic extract (100 µg/ml) exhibited weaker inhibitory effect on both acetylcholine- and histamine-induced contractions on' rabbit and guinea pig ileums. Residual water extract (40-200 µg/ml) showed no effect. However, further investigations are still recommended.

ملخص

التأثيرات الدوائية لمستخلصات نبتة النعنع البري

أجريت دراسة التأثير الدوائي لخلاصة نبات النعناع *Mentha Iongifolia* على العضلات الملساء للأمعاء في الزجاج *In Vitro*. أوضحت الدراسة بأن الزيت الطيار *40-100* ميكروغرام/مل للنبات له تأثير تثبيطي على تقلص اللفائفي للأرنب الناتج من فعل الاسيتيل كولين. إن هذا التأثير هو تأثير مؤقت وينتهي فعله بعد غسل النسيج وإزالة الزيت الطيار. إن خلاصة الزيت الطيار *40-100* ميكروغرام/مل قد ثبطت أيضا تقلص اللفائفي لخنزير غينيا الناتج من فعل الهستامين. وقد أعطيت خلاصة النبات *100* ميكروغرام/مل المستخلصة بواسطة البيوتانول نفس التأثير ولكن بشدة أقل على التقلص الناتج من كل من الاسيتيل كولين والهستامين في لفائفي الأرنب وخنزير غينيا. لم يظهر المستخلص المائي للنبات *40-200* ميكروغرام/مل أي تأثير. أن هذه النتائج تدعم الاستعمال الطبي الشعبي لنبات النعناع لعلاج المغص البطني والربو القصبي.

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Introduction

Mentha longifolia is a wild growing herb in Jordan. It is used in folkloric medicine for the treatment of intestinal and respiratory ailments like abdominal colic and asthma. Three main chemotypes of *M. longifolia* were reported in the literatures [1-4]. Gas chromatography and mass spectrormetry analyses have indicated that the main chemical constituents in the aerial parts of the locally grown *M. longifolia* are piperitenone oxide and piperitone oxide in addition to discrete amounts of terpineol, limonene, 1 ,8-cineole, p-cymen-8-ol and isopiperitenone [5]. Extracts of *M. longifolia* were reported to be pharmacologically active as anticonvulsant, hypothermic [6] and central nervous system depressant in mice and rats [7]. In addition, extracts of *M. longifolia* exhibited antimicrobial activity [8, 9]. The aim of this work was to detect the pharmacological effects of extracts from the aerial parts of *M. longifolia* on smooth muscle *in vitro*.

Materials and methods:

Plant material:

The aerial parts of *M. longifolia* (L.) Huds, Lamiaceae, were collected, at the beginning of flowering, from Om Al-Basateen area in Amman, at an altitude of approximately 790-830 m. The plant was identified by Prof. D. Al-Esawi (University of Jordan).

The plant is a long-leafed type of mint, which attains a height of more than 70 cm, and may exceed 1 m in height. It has an erect grooved square stem. The stem gives branches from its upper part. Leaves are sessile (auriculate) with an opposite decussate arrangement. Other macroscopic characters are typical of the genus *Mentha*. The microscopic characters are typical to peppermint species, i.e. uniseriate covering trichomes up to 8 cells. The glandular trichomes are of 2 types, labiaceous and unicellular head unicellular stalk glandular trichomes. Stomata are of the diacytic type. However,

both covering trichomes and unicellular head glandular trichomes are very abundant.

Plant extracts:

The volatile oil fraction and butanolic extract of *M. longifolia* were prepared according to the following methods.

Volatile oil fraction:

The aerial parts of the fresh plant were boiled in water (hydrodistillation) for 90 minutes by using Clevenger type glass apparatus to obtain the volatile oil. Then the oil subsequently was extracted from traces of water with diethyl ether and dried over anhydrous sodium sulphate. The oil was diluted to the required concentration (see Figures) by emulsification with distilled water using 1% tween 80.

Butanolic extract:

The aqueous residue left after hydrodistillation of the volatile oil was filtered. The filtrate was extracted with butanol. The butanolic extract was dried under vacuum at 50° C. The dry butanolic extract was reconstituted with distilled water to give the required concentration (see Figures). This fraction is referred to as butanolic extract.

Exhausted water extract:

The residual water left after butanol extraction was dried under vacuum at 50° C. The solid substance obtained was reconstituted in water to the concentrations used in the work. This fraction is referred to as exhausted water extract.

Effects of extracts on smooth muscles:

Experiments were conducted *in vitro* on isolated ileum. Rabbit (white. about 1 kg in weight) or guinea pig (400-500 g in weight) were used in the study respectively. For each individual experiment for acetylcholine and histamine, a segment of 2-3 cm long of the ileum was flushed by tyrode solution (NaCl 0.8%, KCl 0.02%, NaH2PO4 0.005%, NaHCO3 3.1% and glucose 0.1%). The segment was immediately transferred into oxygenated tyrode-containing organ bath at 37° C. The ileal segment was fixed at one end and connected to isotonic transducer, by a thread, at the other end. The isotonic transducer is connected to a physiogram (Harvard). The changes in the length of the ileal segment (due to contraction and relaxaton) in response to the test extracts were transferred to the transducer and then recorded in the physiogram. The ileal segment was stabilized in the organ bath, and tested to give similar contractions in response to repeated similar doses of the agonist (acetylcholine or histamine) before starting the experiment.

Statistics:

Results are presented as mean ±S.D. Analysis was done by using two tailed student's test.

Results and discussion:

The effect of M. longifolia extracts on rabbit ileum:

Acetylcholine (55-7040 nmol) showed a sigmoid shape dose-response curve, wllich is typical for an agonist [10]. It is in correspondence with what has been reported [11]. Volatile oil fraction (40 and 100 μg/ml) caused a shifting of the acetylcholine dose-response curve to the right. A control of 1% solution of tween 80 was used. It exhibited no effect on the measured parameters. The effect of the volatile oil indicates that it contains antagonistic activity to acetylcholine. Acetylcholine could not achieve the same maximal

effect in the presence of the volatile oil extract. This type of the antagonistic effect is either due to the presence of irreversible antagonist to the muscarinic receptors or physiological antagonist to acetylcholine [10] (see Figure 1).

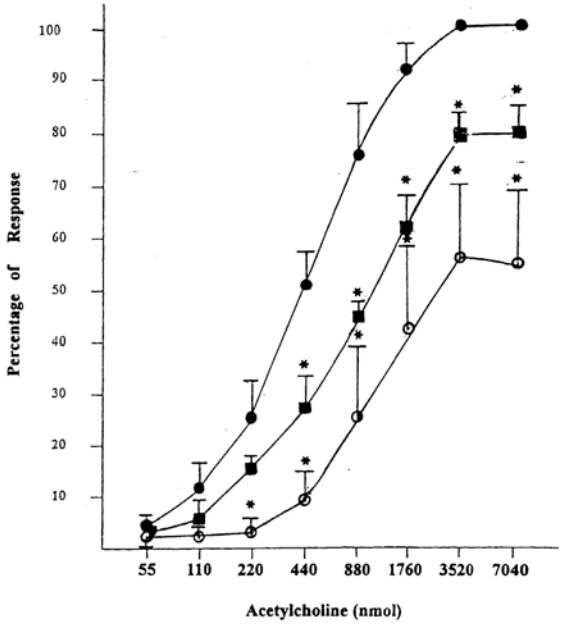

Figure 1. Dose-dependent effect of acetylcholine (●) with 40 (■) and 100 µg/ml (O) of volatile oil extract of *Mentha longifolia* on rabbit ileum *in vitro*. Acetylcholine has been added 30 seconds after the extract. Each point represents the mean ± S.D.
* : Significant difference from acetylcholine alone at $P< 0.05$.

Butanolic extract (100 µg/ml) induced, qualitatively, the same effect of the volatile oil fraction (100 µg/ml) while the butanolic extract was found to be less potent (inhibition of about 25% only of the maximal effect of acetylcholine). Exhausted water extract had no effect on the rabbit ileum in the tested concentrations of 40-200 µg/ml. It has been found that the main chemical constituent of the volatile oil is piperitenone oxide (83%) [5]. Chromatography has showed that butanolic extract contains mainly phenolic com-

pounds and saponins in addition to piperitenone oxide. Piperitenone oxide has not been detected in the exhausted water extract [12]. Piperitenone oxide has been reported to be active as smooth muscle relaxant [13]. The differential effect of volatile oil, butanolic and exhausted water extracts in our study could be attributed to the differences in the concentrations of piperitenone oxide in each extract.

The inhibitory effect of volatile oil fraction on the rabbit ileum was directly proportional with the incubation period (Figure 2). However, even after the maximal tested incubation period of 120 seconds complete recovery of the ileum from the inhibitory effect was achieved after washing the tissue. This indicates that the effect of the extract is reversible and not due to permanent damage or toxicity to the tissue.

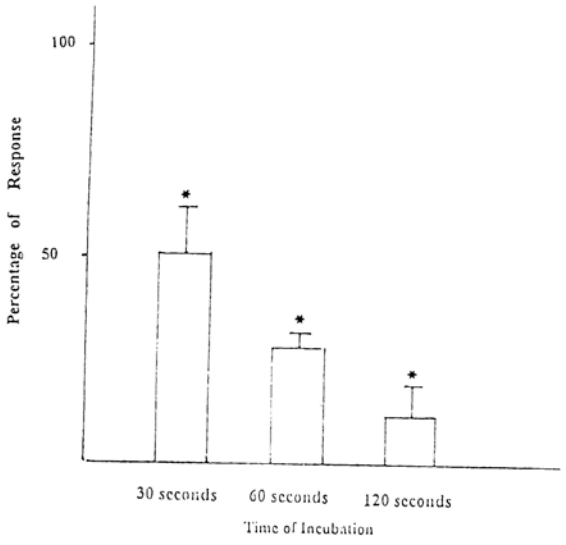

Figure 2. *Mentha* volatile oil extract incubation time-dependent effect on the inhibition of acetylcholine (1100 nmol) induced contraction of rabbit ileum.
*: Significant difference from acetylcholine alone at $P < 0.05$.

The effect of M. longifolia extracts on guinea pig ileum:

Histamine (55-7040 nmol) produced dose-dependent contraction of the

guinea pig ileum. The dose-response relationship was a sigmoid curve, typical for an agonist [10]. A similar effect of histamine, with comparable doses, has been reported by others [14, 15, 16]. The effect of volatile oil fraction on the histamine-induced contraction of smooth muscle was similar to, but more potent than, that on acetylcholine-induced contraction (Figure 3).

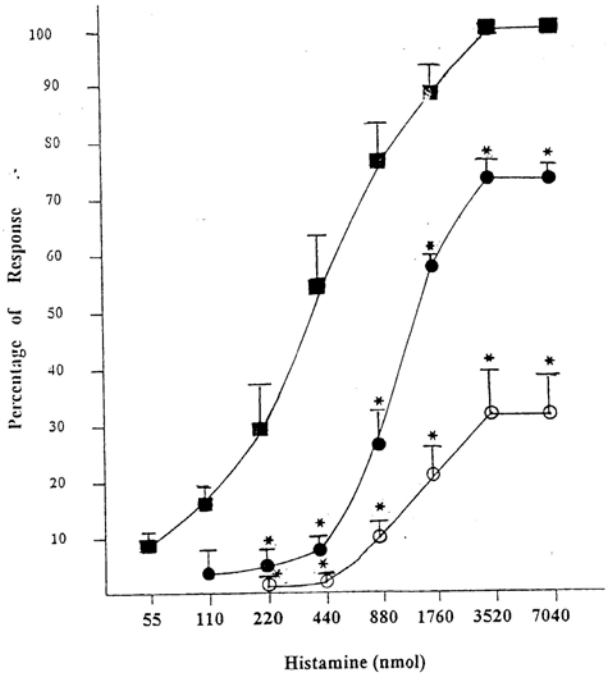

Figure 3. Dose-dependent effect of histamine (■) with 40 µg/ml (●) and 100 µg/ml (O) of volatile oil extract of *Mentha longifolia* on guinea pig ileurn *in vitro*. Histamine has been added 30 seconds after the extract. Each point represents the mean ± S.D.
* : Significant difference from histamine alone at $P < 0.05$.

The results indicated that the extracts of *M. longifolia* exhibited smooth muscle relaxation. The mechanism of action could not be explored during this study. The effects are due to physiological antagonism to both acetyl-

choline and histamine, which could be attributed to the effect of piperitenone oxide.

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