Spasmolytic activity of *Ammi visnaga* seeds on isolated rabbit jejunum

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Summary

Ammi visnaga seeds were investigated for their effect on contractibility of small intestine of rabbit by using kymograph. Pharmacological study of the plant compare between intestinal contraction after the addition of an agonist drug alone and intestinal contraction after the addition of both relaxation plant as antagonist followed by the addition of an agonist drug.

Ammi visnaga showed a statistically significant relaxation effect in a dose dependent manner.It is concluded that *A. visnaga* cause reduction of intestinal contraction. Neostigmine and pilocarpine effect was inhibited by the administration of *Ammi visnaga*, neostigmine only cause a parallel shift to the right of the dose response curve, this effect of neostigmine is more potent than pilocarpine probably due to tissue selectively of neostigmine on the muscarnic receptors of the small intestine.

النشاط المرخي لبذور الخلة البلدي على الفائفي المعزول من الأرانب

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الخلاصة

تم التقصي عن التأثير المقلص أو الباسط للأمعاء في الأرانب لمستخلص المائي لنبات الخلة البلدي باستعمال جهاز الكيموكراف. وقد أجريت دراسة دوائية لمعرفة تأثير نبات الخلة باستخدام أدوية كمستضدات متبوعةً بالنبات كمضد أي المقارنة بين تقلص الأمعاء الناتج من إضافة الدواء وحده كمضد وبين تقلص الأمعاء بعد إضافة النبات كمستضد متبوعاً بالدواء كمضد.

أظهرت النتائج أنالخلة البلدي له تأثير باسط للأمعاء المعزولة. أما بالنسبة لتأثير النيوستكمين و البايلوكاربين على الأمعاء ألمعزولة فقد ثبط عند إضافة الخله البلدي, و أظهرت النتائج أن للنيوستكمين تأثير أقوى من البايلوكاربين على العضلات الملساء في الأمعاء الدقيقه بسبب ميكانيكية عمل النيوستكمين على المستقبلات المسكارينيه الموجودة في الأمعاء الدقيقه و هذا التأثير أدى إلى الحصول على منحني مائل نحو اليمين متوازي اعتمادا على ألجرعة.

Introduction

Ammi Visnaga(Arabic name : Khella Beledi, English name: Tooth pick) is annul robust herb, to (100) cm in height. The steam light green has white flower. The fruit is divided into two fruits. The plant is usually found in Mediterranean region, Nile river of Egypt, Labia, Syria, and South America (Hussein, 1988). The main constituent of the plant is khellin, khellol, visnagin, visamin, resin, furochromones,, visnadine, amoidin, ammidin and majudin (Al – Rawi and Chakravarty, 1964). Mature dried fruit which is named commercially as a seeds are the medical part of the plant (Hussein, 1988). The main uses of the plant are as a diuretic, relief of renal colic, urethral pain, and excretion of renal stones in urine (Majeed and Mahmood, 1988. and Rowaiha, 1978). Khellin, visnagin or crude mixture of the *Ammi visnaga* active principles have a direct muscle relaxant (antispasmodic) effect on smooth musculature of bronchi, intestine, uterus and coronary arterioles. Oral preparation is used to dilate the coronary arteries efficiently in angina pectoris. More commonly oral medication with khella is used to relax spasm in bronchial asthma (Al–Rawi and Chakravarty, 1964). The leaves of the plant have a pleasant flavour (Kunkel, 1984). Vasnadine (which is an active constituent of the plant) has highly activity than khellin and visnadine as Ca^{+2} channel blocker (Rauwald *et a*l., 1994). On other hand Durate *et al*., (1995), demonstrate that the visnagin is the active principle of the fruit of *A. visnaga* cause nonspecific inhibition of vascular smooth muscle contraction at a high concentration. Also Durate *et al*., (1997), studied effect of visnadine on the contractile responses in the rat isolated aortic ring and portal vein segment, they found that vasnadine selectively inhibited the contractile response. Plesman (1997) stated that the

administration of khellin cause a coronary artery dilation by Ca^{+2} channel blocking effect. Intravenous administration of visnagin decreased blood pressure with no significant changes on the heart rate as demonstrated by Durate *et al*., (2000). Gunaydin and Erin (2002) identified and determined two active constituents' khellin and visnagin in the extract of *A. visnaga*. Also the aqueous extract of *A. visnaga* has hypoglycemic effect in diabetes mellietis in normal fasting rat (Jouad *et al*., 2002). On the other hand, many researchers had been studied effects of different plants extracts on the isolated intestine (Amose *et al.,* 1998; Aziba *et al.,* 1999; Olumayokun *et al.,* 1999; Vongtau *et al.,* 2000; Gilani *et al.,* 2000; Pohocha and Grampurohit, 2001; Amose *et al.,* 2003 & Naema *et al.,* 2004).

Materials and methods

Seeds of *Ammi visnaga* were purchased from local market in Basrah city. The plant was authenticated at the Science Collage, University of Basrah. Twenty five grams of the plant materials were added to (200ml) of boiled distilled water and boiled for further (10 min). After cooling and filtering, the clear supernatant was taken. These aqueous extracts were prepared immediately before the experiment of isolated intestine.

Animals and Preparation of isolated intestine: A total of (18) male rabbits of about (1500 – 1700g) body weight were purchased from the local market of Basrah city. The animals were housed in the animal house of Collage of Veterinary Medicine. The animals were fed *at libidum* on alfa – alfa and a concentration ration Twelve hours before the experiment, the animals were deprived of food, but allowed free access to water in order to insure that the intestine free from fecal material. (Akah and Oli, 1997).Rabbit small intestine was used for the experiment in an isolated tissue organ bath, temperature was kept constant at (37C˚) and ordinary air was supplied at a rate of (3) bubbles to the Tyrod's solution in the central vessels. The intestinal contraction was recorded by a pen recorder on a moving drum of the kymograph (Naema *et al.,* 2004). At the time of experiment, the fasted animal was scarified by blow on the head. Its viscera were quickly exposed

through an incision in the anterior abdominal wall. The jejunum was located, freed from its mesentery, dissected out and placed quickly in a beaker containing Tyrod's solution. The piece of intestine was cleaned from its luminal contents by flushing it's lumen gently with a steam of Tyrod's solution by using a pipette (10 ml) the jejunum was then cut into small pieces (3.0 cm) in length. Lower and upper ends of the piece of intestine was tied by a thread allowing the lumen open, the thread of the lower end of the intestine was fixed to the free hooked end of the glass oxygen tube, while the thread of the upper end was linked to mobile lever of the recording pen. The piece of intestine was immersed in the central vessel of the organ bath with (30ml) of the Tyrod's solution. (Perry, 1970). The preparations allowed to contract and relax for (1h) (Martinez-Cuesta *et al.,* 1996). Under tension (1g) (Radenkovic *et al.,* 2003). The organ bath was connected to (1000ml) glass aspiration bottle containing Tyrod's solution (Khetabb, 1979).

Preparation of Tyrod's solution for the isolated intestine: Tyrod's solution consists of the following composition in one liter of distilled water: NaCl (8.00g), KCl (0.20g), MgCl (0.10g), CaCl2 (0.02g), $NaHPO_4$ ($2H_2O$) (0.05g), $NaHCO_3$ (1g), and Glucose (1g) (Khetabb, 1979).

Experimental design:

1. Effect of different concentrations of aqueous extract of *Ammi visnaga* on isolated intestine of rabbit:The aqueous extract of the plant was diluted with distilled water in a ratio of (1:3, 2:2, 3:1, 4:0), with a concentrations of (3.78mg, 7.81mg, 12.09mg, 16.66mg/ml) respectively. Initial rest for about (1hr) was allowed to equilibrate the intervals of the spontaneous activity and the tone developed. The volume of aqueous extract of the plant that was added to the intestine was (4ml) which applied away from the piece of intestine (Naema *et al.,* 2004).

The contact time of the study was (3 min), the first minute was neglected because the response of tissue in some cases is not immediate and taken about (2 min), i.e. (2cm) in length after appearing the effect. After recording the effect of first concentration of aqueous extract, the organ was

drained, and washed at least for (3) times and refilled by fresh Tyrod's Solution after each concentration, the tissue should be washed with fresh Tyrod's Solution to ensure that the tissue was free of aqueous extract of plant. The tissue allowed to a rest for about (30 min) before the next concentration of aqueous extract was applied. (Radenkovic *et al.,* 2003). The same procedure was repeated on the same piece of intestinal with using other ascending three concentrations of plant extract. These procedures were repeated for six time using different pieces of rabbit's intestine.

2. Effect of *A. visnaga* on intestinal contraction induced by neostigmine and pilocarpine: *Ammi visnaga* was studied with two agonist drugs that are Neostigmine and Pilocarpine. Neostigmin was the first agonist drug which was used with four concentrations of(0.10µg, 0.20µg, 0.41µg and 0.83µg/ml) respectively, in a volume of (1ml) of each concentration, then washing the preparation for three time to ensure that the tissue is free of drug and allowed to a rest for (30 min) before adding of (4 ml) of one concentration of plant extract (3.78 mg) and allowed to contract for (3 min), then adding the same four concentrations of drug and observe the effect of ammi extract on the contraction induced by neostigmine, repeat this method for six time with the same concentrations and a volume of each plant and drug.

The same procedure was used, addition of pilocarpine in a four concentrations (2.08µg, 4.16µg, 8.33µg and 16.66µg) respectively by adding only (1ml), each the same four concentrations were used after adding (4 ml) of (31.25 µg) of ammi extract. Statistical analysis Two samples unequal variance t-test was used for data analysis. Linear regression analysis were used to correlate the dose and response, it also allow the comparison between different responses The Statistical analysis were done by the aid of SPSS, statistical package of social science, 1998 (Steel and Torrie, 1980).

Results

1. Effect of aqueous seeds extract of *Ammi visnaga* on isolated rabbit intestine: The normal mean height of contraction of the isolated intestine was (1.513 ± 0.47cm). Administration of the first concentration of plant extract (3.78 mg/ml) resulted in reduction in the tone of isolated intestinal contraction to (1.165 ± 0.4396cm) (Figure 1), however, this dose was failed to reach statistical significance. The other concentration (7.81mg/ml) reduced the height of contraction to (1.046 ± 0.412cm), and the remaining two concentrations (12.09mg, 16.66 mg/ml) produced the maximal reduction of the intestinal activity (0.823 ± 0.442cm), (0.696 ± 0.277cm) respectively. These effects are statistically significant ($P< 0.05$). The effect of *A. visnaga* was a dose dependent and its relation to the concentration is linear and a highly significant within the used doses (r^2=0.8069), (Figure: 1 & Fig: 2).

2. Effect of *Ammi visnaga* on the contraction effect induced by neostigmine on isolated intestine of rabbit: *Ammi visnaga* plant extract produce potent relaxant effect on the isolated intestine of rabbit and therefore further studies was done to elucidate it's mechanism of action.

Neostigmine causes a stimulation of contractions of the isolated rabbit intestine. The used dose (3.125, 6.25, 12.5 25µg/100ml) which result contractions of (1.575±0.474, 1.806±0.468, 2.006±0.41 and 2.263±0.376cm) respectively. This effect is linear and correlated to the dose (r^2= 0.8703) (Figure: 3). The administration of (3.78 mg/ml) was first effective dose of *A. visnaga* extract which result in inhibition of the contraction induced by neostigmine in the above mentioned concentration. The plant extraction caused contraction of (0.973±0.233, 1.248±0.305, 1.473±0.417 and 1.633±0.505cm) respectively. These effects are a statistically significant. ($P<0.05$, Figure: 3).

3.Effect of *Ammi visnaga* on the acontraction effect induced by pilocarpine on isolated intestine of rabbit: The administration of pilocarpine in a concentration of (2.08µg, 4.16µg, 8.33µg and 16.66µg) caused a marked increase in the contraction of the isolated intestine by (2.125 ± 0.862, 2.91 ±

0.846, 3.193 ± 0.7599 and 3.483 ± 0.552cm) respectively, as a compared to the normal contraction of (1.32 ± o.6 cm). The effects is linear and a dose dependent (r^2= 0.7292).The addition of (3.78 mg) a first effective dose of the plant extract result in blockade of the contractile effect of pilocarpine to (1.68 ± 0.6, 1.786 ± 0.678, 2.076 ± 0.843 and 2.115 ± 0.676cm). 125 and 250µg showed a statistically significant effect. (P< 0.05,while 500µg has a highly statistically significant effect. (P< 0.01, Figure: 4).

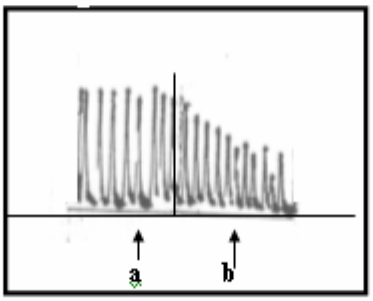

Fig (1): Sample of the effect of *Ammi visnaga* extract on the response of isolated rabbit jejunum

a. normal contraction
b. 7.81 mg of *Ammi visnaga*

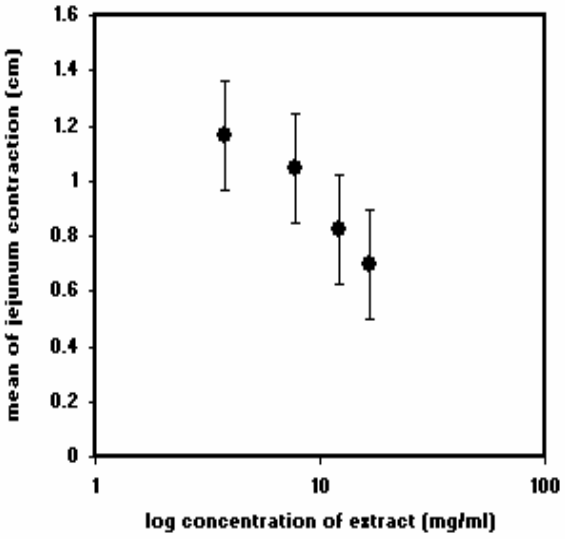

Fig (2) :Effect of *Ammi visnaga* extract on normal jejunum contraction (Logarithmic scale). Mean S.D

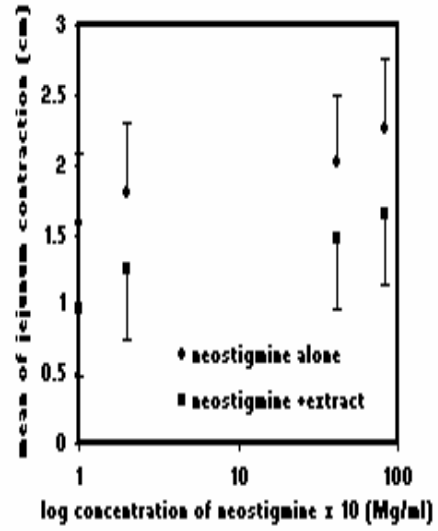

Fig (3): Effect of Ammi visnaga extract (3.78 mg) c jejunum contraction induced by neostigmine (Logarethmic scale). Mean S.D

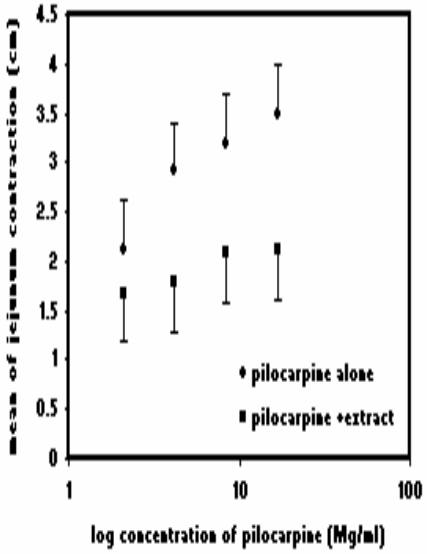

Fig (4) :Effect of Ammi visnaga (3.78 mg) extract on jejunum contraction induced by pilocarpine (Logarethmic scale). Mean S.D

Discussion

The addition of aqueous extract of *A. visnaga* caused a relaxant effect on isolated rabbit jejunum, this relaxation effect is statistically significant and dose dependent. Two agonist drugs acting at cholinergic receptors were used to illucidate it's mechanism of action, neostigmine is an indirectly acting cholinomimetic which act by inhibiting cholinesterase enzyme, and pilocarpine which is also cholinomimetic drug acting directly on cholinoreceptors. Neostigmine with increasing concentration cause a dose depended stimulation of rabbit jejunum contraction. This effect is attributed to the muscarinic effect of the drug (Rang *et al*., 1999). The addition of (4ml) of (3.78 mg/ml) of the aqueous extract of *A. visnaga* blocks the contraction effect of neostigmine and caused a parallel shift to the right of a dose response curve of neostgmine, this demonstrate the important of the muscarinic antagonist effect of the plant extract, in addition, the plant extract was reported to have Ca^{+2} channel blocking effect (Rauwald et *al.*,

1994). Pilocarpine also produces a dose dependent stimulating effect on the rabbit isolated intestine. This effect is due to its muscarinic action on the post-ganglionic nerve ending (Rang et *al*., 1999). The addition of (4ml) of (3.78 mg) of the aqueous extract of *Ammi visnaga* caused antagonism of this effect and shift to the right of a dose-response curve, the effect of the extract on pilocarpine contraction was less than neostigmine, probably due to the tissue selectively of pilocarpine which acts more on the muscarinic receptors of the eyes than the gastrointestinal tract (Laurence *et al.,* 1997). Recent studies revealed that the plant has inhibition effect of isolated rat vascular smooth muscle (Durate *et al.,* 1995. and Durate *et al.,* 1997), and coronary arteries which reduce angina pectoris risk (Plesman, 1997). *A. visnaga* fruits consist of khellin, visnagin and visnadine which are the active ingredient of the plant (El-Domiaty, 1992. And Durate *et al.,* 1997). The relaxant effect of the plant is due to this component. In folk medicine *A. visnaga* used as spasmolytic, especially on the gastrointestinal tract, bronchi, bilary tract, urogenital system and coronary vessels (Al-Maiyah, 2001). The ability of *A. visnga* to produce relaxation of the isolated rabbit intestine is due to khellin (an active ingredient of plant) also has similar to Ca^{+2} channel blocking effect (Plesman, 1997).

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