

THE CONTRACTION EFFECT OF *ALLIUM PORRUM* SEEDS ON ISOLATED INTESTINE OF RABBITS

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ABSTRACT

Greater attention has been paid to the traditional medical practices in general and herbal medicine in particular even in the developed countries. This study aimed to investigate *Allium porrum* seeds for its potential contraction or relaxation effect on small intestine in rabbits by using organ bath *in vitro*. In order to establish a dose-response curve for the plant, six male rabbits were used in the experiment.

Pharmacological studies of the plant were done by comparing between intestinal contraction after addition of the plant alone as agonist, and intestinal contraction after the addition of both antagonist drugs then the plant extract.

Allium porrum, showed a statistically significant dose-depend increase of intestinal contraction. Atropine, adrenaline, and chlorpheniramine reduce the effect of *A. porrum* on intestinal contraction. Both cholinergic and adrenergic mechanisms may involve the action of the plant with more potent muscarinic action.

INTRODUCTION

There is an increasing interest towards medical plants and their ingredients. Some of the underlying motives can be listed as a followed : Countries lacking developed chemical industries are searching for affordable treatment modalities by using their own plant sources, synthetic drugs have many dangerous side effects, many commonly used drugs (such as steroidal compounds, atropa alkaloids, digitalis glycosides, narcotic alkaloids and ergot alkaloids) can be purified from the plants much more economically as a compared to the synthetic production, many medical plants have a multiple actions whereas synthetic drugs have usually only one (1). Additional drugs like vitamins are some times needed to prevent side effects of the synthetic drugs (Plant – derived drugs do

not necessitate such polypharmacy) (2). At the present time the interest in traditional medicine is increasing (3). One part of this kind of medicine is the herbal medicine for which the world health organization (WHO) has issued a number of publications (4, 5). Generally the pharmacological experiments were designed to assay the plant therapeutic activity which became very important in the assessment of any plant extract that could not be measured by any physical or chemical method.

This study is a part in a series for the evaluation of many traditionally used medicinal plants for their contraction or relaxation effect on isolated rabbit intestine and trying to explore their mechanism of action.

MATERIALS AND METHODS

Plant materials: The seeds of *Allium porrum* were collected from local market in Basrah city. The plants were authenticated at the Collage Science, University of Basrah.

Preparation of aqueous extracts: Fifty grams of *A. porrum* were added to (200ml) of boiled distilled water and boiled for further (10 min), after cooling and filtering, clear supernatants were collected. The aqueous extracts were prepared immediately before the experiment of isolated intestine.

Animals: A total of (24) 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 *ad libidum* on alfa – alfa and a concentration ration (9% ground Soya bean, 60%wheat flour, 30% wheat bran and 1g/kg of minerals and vitamins). 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. (6).

Preparation of isolated jejunum strip for experimentation: 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/min to the Tyroid 's solution in the central vessels. The intestinal contraction was recorded by a pen recorder on a moving drum of the kymograph (7).

At the time of experiment, the fasted animal was scarified by hit 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 worm Tyrod's solution. The piece of intestine was cleaned from its luminal contents by flushing its lumen gently with a stream 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 (8). The preparations allowed to contract and relax for (1h) (9). Under tension (1g) (10). The organ bath was connected to (1000ml) glass aspiration bottle containing Tyrod's solution (11).

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), MgC (0.10g), CaCl (0.02g), NaHPO₄(2H₂O) (0.05g), NaHCO₃(1g), and Glucose (1g) (11).

Experimental design:

1. Effect of different concentrations of aqueous extract of *Allium porrum* on isolated intestine of rabbit: The aqueous extract of the plant was diluted with distilled water with a concentrations of (7.57mg, 15.62mg, 24.19mg, 33.33mg /ml), after allowing for initial rest for about (1h) 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 (7).

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, ie 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 (10). 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 Atropine on intestinal contraction induced by *Allium porrum*: In the first experiment the four concentrations of *Allium porrum* (7.57mg, 15.62mg, 24.19mg, 33.33mg /ml) on isolated rabbit jejunum and the dose response was established, then washing with Tyrode solution for (3) time to ensure that the tissue is free of plant extract. The preparation was allowed to a rest for (30 min) before adding the antagonist drug, then adding (1ml) containing (0.08µg/ml) of atropine sulphate to the isolated intestine and allowed to contract for (2 min), then adding the same four concentrations of the plant on the prepared isolated intestine to observe the effect of atropine on contractions of intestine induced by *A. porrum*. The four concentrations of *A. porrum* were added consequently without washing, this experiment was repeated for six times.

3. Effect of Adrenaline on intestinal contraction induced by *Allium porrum*: The same procedure of atropine was carried out. The concentration of adrenaline was (0.03 µg/ml).

4. Effect of Chlorpheniramine on intestinal contraction induced by *Allium porrum*

The same procedure which mentioned above was used. The concentration of chlorpheniramine malcate was (3.33 µg/ml).

Statistical analysis: The SPSS, statistical package of social science, 1998 by using 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 (12).

RESULTS

1. Effect of aqueous seeds extract of *Allium porrum* on isolated rabbit intestine: The effect of various concentrations of *Allium porrum* extract was tested on normal contraction in organ bath in vitro. The height of contractions during (2 min) period was recorded on kymograph paper and was taken as an index of the activity of *A. porrum*. The mean height at normal resting condition was (0.615 ± 0.181 cm). The addition of (4ml) of plant extract containing (62.5mg, 125mg, 250mg, and 500mg /100ml) increase the height of contraction to (0.81 ± 0.161 , 0.923 ± 0.203 , 1.19 ± 0.309 and 1.556 ± 0.1678 cm) respectively (Figure 1). It was noted that there was a positive and linear relation ship between the height of contraction and plant extract concentrations ($r^2 = 0.9834$). The first

concentration of *A. porrum* showed a statistically significant effect ($P < 0.05$). While the other concentrations has a highly statistically significant effect ($P < 0.01$).

2-Effect of atropine on the contraction effect induced by *Allium porrum*: Addition of the aqueous seeds extract of *A. porrum* in a concentration of (62.5mg%, 125mg%, 250mg% and 500mg%) in vitro to the isolated organ bath produced a marked contraction of the isolated intestine in a dose dependent way. Administration of (1ml) of atropine (0.75 μ g) to the central vessel of the organ bath, (3 min) before the addition of the above four concentrations of the plant extracts which resulted in reduction in the tone of spontaneous pendular movement of isolated rabbit jejunum. (Figure 2). Only (62.5mg %) showed statistically significant ($P < 0.05$.) and contraction induced by *A. porrum* extract decrease from (1.41 ± 0.404 , 1.855 ± 0.6846 , 2.235 ± 0.628 and 2.49 ± 0.597 cm). to (1.005 ± 0.3991 , 1.377 ± 0.6 , 1.84 ± 0.706 and 2.075 ± 0.804). The addition of atropine causes a parallel shift to the right of the dose response curve.

3-Effect of adrenaline on the contraction effect induced by *A. porrum*: The administration of the extract of *Allium porrum* at a concentrations of (62.5, 125, 250, 500 mg %) increase the contraction in a dose dependent manner (1.185 ± 0.168 , 1.478 ± 0.205 , 1.753 ± 0.438 and 1.993 ± 0.465 cm) respectively. The addition of (1ml) of adrenaline containing (1000mg) to the (30ml) chamber fluid result in a decrease of the spontaneous movement of the jejunum, this reduce the contraction induced by a concentration to (0.975 ± 0.214 , 1.283 ± 0.2136 , 1.601 ± 0.417 and 1.823 ± 0.425) respectively. The dose response curve of *A. porrum* was shifted to the right (Figure:3) by the addition of adrenaline only (62.5mg%) concentration has statistically significant effect. ($P < 0.05$).

4-Effect of chlorpheniramine on contraction effect induced by *A. porrum* extract: The addition of the plant extract of *A. porrum* at a different concentrations of (62.5, 125, 250, 500mg %) produce a dose dependant increase in the contraction of isolated intestine. The pretreatment of the intestine with (13.3mg) of chlorpheniramine resulted in reduction in the magnitude of contractions induced by the similar extract concentration used above. This resulted shift to the right of the dose response curve. The first three concentrations has statistically significant effect ($P < 0.05$.) only (125mg %) has a slightly significant. (Figure: 4)

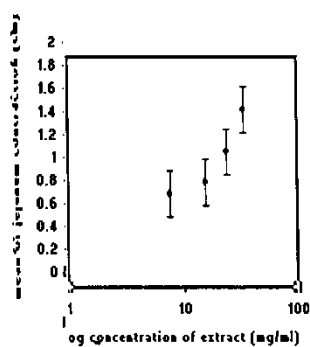


Fig (1): Effect of *Allium porrum* extract on normal jejunum contraction (Logarithmic scale) mean-S.D

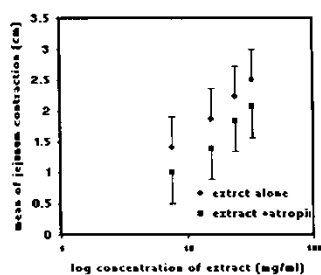


Fig (2): Effect of atropine (0.08 Mg) on jejunum contraction induced by *Allium porrum* extract (Logarithmic scale)

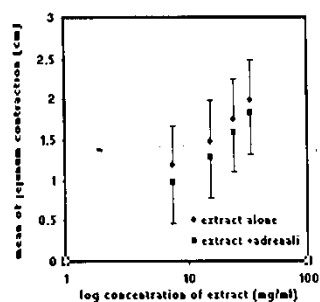


Fig (3): Effect of adrenaline(0.03 Mg) on jejunum contraction induced by *Allium porrum* extract (Logarithmic scale)

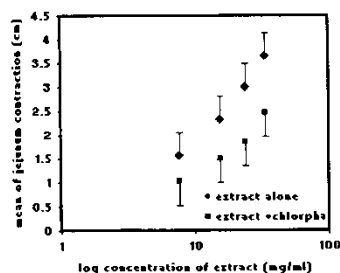


Fig (4): Effect of chlorpheniramine (3.33 Mg) on jejunum coontraction induced by *Allium porrum* extract (Logarithmic scale). Mean S.D

DISCUSSION

The present study indicates that the administration of four concentrations of the aqueous seeds extract of *Allium porrum* produce a statistically significant increase in a contraction of isolated rabbit intestine in vitro, also there is a direct correlation between the doses and the amplitude of contraction of isolated intestine. This contraction effect of *A. porrum* was shown to be antagonized by the addition of atropine, adrenaline and chlorpheniramine prior to the plant extract. Both atropine and adrenaline produce parallel shift to the right of the dose response curve respectively, the result demonstrate that the effect of the plant extract could be mediated at least in a part by cholinergic and adrenergic mechanism, While the effect of chlorpheniramine was more potent antagonist than either atropine or adrenaline, but the shift dose response curve was not parallel, physiological antagonist may explain this effect. (Although the two an anticholinergic and adrenergic effect of chlorpheniramine).

The main active ingredients of the plant are essential oil (allyl sulphide), sulphur, iron, phosphorus, and calcium (13). Others compound vitamin A and B (14), the plant also consist of alliinase enzyme (15), also other compound were isolated from *A. porrum* sapogenins, porrigenins A and B (16).

التأثير المقلص لبذور نبات الكراث على الأمعاء المعزولة من الأرنب

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الخلاصة

أعطي موضوع الممارسات الطبية الشعبية التقليدية عموماً وطب الأعشاب خصوصاً اهتماماً كبيراً حتى في الدول المتقدمة. تهدف هذه الدراسة لتقصي التأثير المحتمل لنبات الكراث كمقلص أو مرخي للقناة المعوية في الأرانب باستعمال جهاز الأعضاء المعزولة خارج الجسم، وللحصول على منحنى الاستجابة الدوائية المعتمد على الجرعة، أجريت للنبات ستة اختبارات. إضافة إلى ذلك تمت المقارنة بين تقلص الأمعاء بعد إضافة الدواء (الأترابين، الأدرينالين، الكلورفينيرامين) كمستضد متبوعاً بالنبات كضد.

أظهرت النتائج بأن الكراث له تأثير مقلص معنوي إحصائياً على الأمعاء المعزولة. وأن إضافة كلاً من الأتروبين ، الأدرينالين، والكلورفينيرامين أدى إلى تثبيط الفعل المقلص للكراث على الأمعاء المعزولة، وتم الحصول على منحنى متوازي مع المنحنى المسبب بإضافة الكراث مائل إلى اليمين بإضافة كلاً من الأتروبين والأدرينالين.

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