The relaxant effect of some drugs and aqueous extract of medicinal plants on bronchial smooth muscle in rabbits

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Abstract

Histamine is a biogenic amine that produces bronchospasm when it is used (I V) at a dose of 0.4 mg / kg in rabbits. It significantly reduced the serum levels of calcium (Ca) magnesium (Mg) and selenium (Se). ($p < 0.05$) .

Forty rabbits were involved in this study. The serum Ca, Mg and Se levels were measured before and after giving histamine and following the administration of aqueous extract of medicinal plants Cuminum cyminum (cumin), Thymus vulgaris (thyme) and Anthemis nobilis (chamomile).

The serum Ca, Mg and Se levels were highly elevated after treatment with extract of cumin or chamomile in rabbits pretreated with histamine, the results of Ca were changed from 8.34± 0.40 and 8.78± 0.27 to 33.41± 0.50 and 97.62 ± 7.2 µg /dl respectively while that of Mg from 20.20± 0.37 and 19.47± 0.31 to 74.97± 0.97 and 89.14± 1.41 µg /dl where as the serum level of Se was changed from 137.98± 0.48 and 145.36 ± 0.66 to 302.69 ± 0.68 and 245.41± 0.81 µg /dl respectively .All these results were significant at $p<0.05$.

The aqueous extracts of cumin or chamomile were used orally at a dose of 1.5 gm /kg in rabbits to relieve the bronchospasm and symptoms of dyspnea induced by histamine. These results indicated clearly the possibility of using these extracts for patients with chronic asthma after the adjustment of the effective dose.

تأثير بعض الأدوية والمستخلص المائي للنباتات الطبية على العضلة القصبية الملساء في الأرانب

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المستخلص

يحدث الهستامين وهو الأمين الناشيء بفعل حيوي – تشنجا قصبيا في الأرانب عند استعماله وريديا بجرعة 0.4 ملغم/ كغم كما انه يقلل بصورة معنوية مستويات الكالسيوم والمغنيسيوم والسيلنيوم.في مصل الدم.

شملت هذه الدراسة اربعين ارنبا حيث قيست مستويات الكالسيوم والمغنيسيوم والسيلينيوم لهم قبل وبعد أعطاء الهستامين وكذلك في أعقاب أعطاء المستخلص المائي للنباتات الطبية الكمون والزعتر والبابونج.

لقد سبب المستخلص المائي للكمون والبابونج ارتفاعا عاليا لمستويات الكالسيوم والمغنيسيوم والسيلينيوم في مصل دم الأرنب التي عولجت سابقا بالهستامين والذي احدث انخفاضا فيها حيث تغيرت مستويات الكالسيوم لاحقا من 8.34 ± 0.4 و8.78±0.27 الى 33.41±0.50 و97.62±0.72 مايكروغرام/ دسيلتر على التوالي كذلك تغيرت مستويات المغنيسيوم من 20.20 ±0.37و 19.47±0.31 الى 74.97±0.97 و89.14 ± 1.41مايكروغرام/ دسيلتر على التوالي إما مستويات السيلينيوم فلقد شهدت تغيرا من 137.98±0.48 و145.36±0.66 إلى 302.69±0.68 و 245.41 ±0.81 مايكروغرام/ دسيلتر على التوالي حيث كانت جميع هذه النتائج ذات صفة معنوية($P<0.05$). إن المستخلص المائي للكمون والبابونج والذي أعطي فمويا للأرانب بجرعة 1.5 غم /كغم سبب أراحة لها من التشنج القصبي وأعراض ضيق النفس الذي أحدثه الهستامين وهذا يظهر بوضوح إمكانية استعمال هذا المستخلص للمرضى الذين يعانون من الربو القصبي المزمن ولكن بعد تعديل الجرعة المؤثرة .

Key words: Bronchospasm, Trace elements, Medicinal plants.

Introduction

Bronchial asthma is a syndrome with multiple causes that produces obstruction of the air ways as a result of contraction of bronchial smooth muscle, swelling of the bronchial mucusa and an increased quantity of tenacious sputum [1]. The usual presenting complaints are of chest tightness, wheeze and dyspnea. The release of the mediators e.g. histamine, prostaglandin and leukotrienes from sensitized mast cells lead to prompt bronchocostriction [2] . This study was carried out in vivo and in vitro to investigate the possible activity of aqueous extract of medicinal plants against bronchial asthma in order to develop effective, safe and less expensive therapy.

Materials and Methods

Sixty New Zealand healthy male rabbits were used in this study, weighing 1150-1250 gm. They were supplied by the animal house of medical college – Al-Nahrain University Each animal was kept in a separate cage which was provided with a wire mesh floor to avoid coprophagy. They were fed with a standard diet (Oxoid) and were given water ad libitum

In vivo study

Forty rabbits had been used in this study; they were divided into 4 groups (10 animals for each group). All the drugs were administered at 09:00 a.m.

Group 1 Normal rabbits were given 1ml normal saline (I.V.) and considered as control group.

Group 2 Rabbits were given histamine (BDH chemicals LTD U.K) 0.4 mg / kg (i.v).

Group 3 Rabbits were given histamine 0.4 mg / kg (I.V.) and then treated with 1.5 gm / kg aqueous extract of cumin orally after 30 minutes.

Group 4 Rabbits were given histamine 0.4 mg / kg (I.V.) and then treated with 1.5 gm / kg aqueous extract of chamomile orally after 30 minutes.

In vitro study

Twenty rabbits had been used in this study. The animals were anesthetized by sodium pentobarbital (May and Baker LTD U.K) at a dose of 30 mg / kg (I.V.), they were killed by blow on the head. The trachea was exposed and removed. Each ring was mounted in 50 ml organ bath containing Krebs-Henseleit solution at 37 C° in an atmosphere of 95% O_2 and 5% CO_2 . Tissues were allowed to equilibrate for one hour under resting tension of 1.25g.

Tension generated by tissue contraction was measured with isometric transducer (Grass transducer FTO3, Grass medical instruments, Quancy mass) connected to a polygraph (Grass model 7). A decrease or an increase in the tone was considered as relaxant or constrictor action of bronchial muscle [3] .

Other drugs which were used in invitro study like: -

Ach (BDH chemical LTD, U.K) $PGF_{2\alpha}$ (Calier Spain).

Diphenhydramine (S.D.I Iraq) Atropine (F.M.S Jordan).

Plants were collected, crushed and grinded. One gram was taken from each and steeped in 50 ml of distilled water by using of electric mixing machine for 15 minutes then the mixture was left for 24 hour. The solution was put in centrifuge of 6000 RPM for 30 minutes. The sediments was discarded and supernatant was taken.This procedure was repeated three times by using the Seitz filter.

Blood samples were collected from the heart of each rabbit before treatment and after giving histamine (I.V.) and administration of aqueous extract orally. Biochemical examination of serum Ca, Mg and Se levels was determined by using atomic absorption spectrophotometer. The obtained results were collected for analysis and assessment.

Results

The results of this study revealed that the aqueous extract of cumin, thyme and chamomile produced relaxation of isolated tracheal rings at concentration of 0.4 mg / ml in trachea pretreated with $2x10^{-3}$ M of Ach, Histamine or $PGF_{2\ \alpha}$, these effects were compared with the action of Atropine (0.006) mg / ml and Diphenhydramine (0.1) mg / ml in trachea pretreated with 2×10^{-3} M Acetylcholine and Histamine respectively. The aqueous extract of cumin, chamomile were used at a dose of 1.5 gm/ kg (In vivo) after histamine injection to protect the animals from the bronchospasm induced by histamine injection group (3,4).

In laboratory findings: the levels of serum Ca, Mg and Se were determined before and after the administration of Histamine and treatment with cumin and chamomile. The serum Ca, Mg and Se levels were significantly reduced after (I.V.) of histamine (Group 3) from 10.84± 0.46 to 8.34± 0.40 µg /dl, 61.57± 0.61 to 20.2± 0.37 µg /dl and 180.2±0.87 to 137.98± 0.40 µg /dl respectively. All these values were significant at $p < 0.05$ table I. All these preparations were used in organ bath (in vitro) and under the same conditions. The serum Ca, Mg and Se levels were highly elevated after treatment with extract of cumin in rabbits pretreated with Histamine, the results were changed from 8.34± 0.40 to 33.41± 0.50 µg /dl, 20.2± 0.37 to 74.97± 0.97 µg /dl and 137.98± 0.48 to 302.69± 0.68 µg /dl respectively. All these values were significant at $p < 0.05$, table I. In the treatment of rabbits (group 4) with chamomile extract, the results showed a significant elevation in serum Ca, Mg and Se levels as compared with results after Histamine administration. They changes were from 8.78± 0.27 to 97.62± 7.21 µg /dl, 19.47± 0.31 to 89.14± 1.41 µg /dl, 145.36± 0.66 to 245.41± 0.81 µg /dl respectively. All values were significant at $p < 0.05$ table (II).

Discussion

The aqueous extract of cumin, thyme and chamomile produced tracheal muscle relaxation (in vitro) and antagonized the contraction induced by Histamine, ACH and $PGF_{2\alpha}$. The relaxant activity of thyme was related to the presence of polymethoxyflavones in the thyme [4] [5] or may be related to phenolic constituents of thymol and carvacrol [6] . The relaxant action of chamomile was related to the presence of azulenes in chamomile that may interfere with action of ACH, $PGF_{2\alpha}$ and Histamine [7] . It may have an anti allergenic and anti-inflammatory properties [8] [9] . The effect of cumin on bronchial muscle was related to phenolic constituent thymol which prevented the contraction induced by Histamine ACH and $PGF_{2\alpha}$ [6] .

Histamine was given in vivo to produce bronchial muscle constriction and that clinically represented by dominant bronchospasm and dyspnea (group 2,3 and 4), the extract of chamomile can protect the animals from the bronchospasm induced by histamine and relief dyspnea (group 4), the action has been attributed to the presence of azulenes in chamomile that may interfere with action of

Bradykinin ACH, Histamine and Serotonin [10] . Also to the presence of quercetin flavonoid which had an inhibitory action on lipooxygenase , an enzyme that contribute to the problems with asthma [11] .

The extract of cumin also can protect the animals from bronchospasm induced by Histamine, this action may be related to phenol constituent of thymol which had been shown to antagonize the action of histamine[6] group (3).

In laboratory findings, the injection of Histamine (I.V.) caused a significant reduction in serum Ca levels group (2,3 and 4). This action may be related to mobilization of Ca into the cell causing arise in intracellular Ca. At the mean time histamine caused a significant reduction in serum Mg and Se levels group (2,3 and 4). The result of reduction in serum Mg levels disagreed with the result of Zervas[12] who confirmed that the serum Mg levels remain unchanged after the histamine challenge.

The treatment of rabbits with extract of cumin group (3) and chamomile group (4) showed a significant elevation in serum Ca, Mg and Se levels because these plants considered an excellent source of these elements [13] .

Administration of Mg (I.V.) has been reported to stop the acute asthmatic attacks within minutes [14] . Ca acts with Mg in a way that may stop acute asthmatic episode by increasing the vital capacity of the lungs and through relaxant action on the bronchial muscle [15] .

Patients with low level of serum Se may have a high risk of asthma [16, 17] and this confirming the important role of Mg and Se in bronchial asthma and the possibility of using medicinal plants in therapy.

Table (1) Serum Ca, Mg& Se levels(µg/dl)in rabbits given histamine (0.4mg/kg) (I.V)and then treated orally by (1.5gm/kg)aqueous extract of Cuminum cyminum (cumin) (Group3)

Animals No.	Ca			Mg			Se		
	Pre-Histamine	Post-Histamine	Post-cumin	Pre-Histamine	Post-Histamine	Post-cumin	Pre-Histamine	Post-Histamine	Post-cumin
1	11.4	8.2	34.8	60.2	20.2	77.3	180	140.9	303.6
2	10.3	8.4	33	60.12	18.9	78	183.3	135.7	303.4
3	10.1	9.6	31.2	59.8	20	78.2	181.2	137.3	304.2
4	12	10.3	32.9	61.4	19.7	74.2	182.6	138.9	301.4
5	13	7.6	33.1	65	19.4	72	184.1	139.9	301.2
6	11.4	9.3	35	58.9	18.8	71.2	179.8	140.8	301.2
7	9.2	9.3	35.8	63	20	74.2	179.3	137.1	304.9
8	9.8	7.58	31	62	21.3	72.7	175	138.2	303.3
9	12.5	6.61	33.1	61.4	21.2	79.7	177.8	136.3	303.3
10	8.7	6.55	34.2	63.8	22.5	72.2	178.9	134.77	300.4
Mean	10.84	8.34	33.41	61.57	20.2	74.97	180.2	137.98	302.69
SE±	±0.46	±0.40	±0.50	±0.61	±0.37	±0.97	±0.87	±0.48	±0.68

All these values were significant at **P<0.05**

Table (2) Serum Ca,Mg& Se levels(µg/dl)in rabbits given Histamine (0.4mg/kg)(I.V) and then treated orally by (1.5gm/kg)aqueous extract of *Anthemis nobilis* (chamomile) (Group4)

Animals No.	Ca			Mg			Se		
	Pre-Histamine	Post-Histamine	Post-Chamo.	Pre-Histamine	Post-Histamine	Post-Chamo.	Pre-Histamine	Post-Histamine	Post-Chamo.
1	11.4	8.2	34.8	60.2	20.2	77.3	180	140.9	303.6
2	10.3	8.4	33	60.12	18.9	78	183.3	135.7	303.4
3	10.1	9.6	31.2	59.8	20	78.2	181.2	137.3	304.2
4	12	10.3	32.9	61.4	19.7	74.2	182.6	138.9	301.4
5	13	7.6	33.1	65	19.4	72	184.1	139.9	301.2
6	11.4	9.3	35	58.9	18.8	71.2	179.8	140.8	301.2
7	9.2	9.3	35.8	63	20	74.2	179.3	137.1	304.9
8	9.8	7.58	31	62	21.3	72.7	175	138.2	303.3
9	12.5	6.61	33.1	61.4	21.2	79.7	177.8	136.3	303.3
10	8.7	6.55	34.2	63.8	22.5	72.2	178.9	134.77	300.4
Mean	10.84	8.34	33.41	61.57	20.2	74.97	180.2	137.98	302.69
SE±	±0.46	±0.40	±0.50	±0.61	±0.37	±0.97	±0.87	±0.48	±0.68

All these values were significant at **P<0.05**

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