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Bronchodilator activity of ethyl acetate extract of *Nigella sativa*

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Abstract

This study aims to investigate the mechanism(s) included in the bronchodilation effect exerted by *Nigella sativa*. Ethyl acetate extract (NS.EA) was prepared using a maceration method. Adult albino rats were recruited for thoracotomy and removal of the trachea. After cutting into pieces, the tissue was set in organ bath. The influence of cumulative concentrations of ethyl acetate extract was examined on contractile responses of isolated trachea to acetylcholine using different blockers such as Nifedipine (Ca^{2+}channel blocker), Tetraethylammonium (Ca^{2+}-activated K^+ channel blocker), 4-aminopyridine (voltage-dependent K^+ channel blocker), Glibenclamide (ATP-sensitive K^+ channel blocker), $BaCl_2$ (inward rectifier K^+ channel blocker), methylene blue (soluble gaunylate cyclase inhibitor) and indomethacin (non-selective cyclooxygenase inhibitor). Significant inhibition of bronchodilation was observed when tracheal rings were pretreated with indomethacin and $BaCl_2$ with ($P<0.001$), and with methylene blue and nifedipine with ($P<0.05$). The IC_{50}s were (5.635, 6.9, 7.86 and 4.987 mg/ml) respectively. Conversely, 4-AP, GLIB and TEA showed no significant changes in the bronchodilation induced by the extract. Therefore, The E_{max} value for indomethacin significantly reduced from 101.34 to 73.28%, $BaCl_2$ from 53.62 to 30.31%, methylene blue from 55.78 to 38.94% and nifedipine from 101.34 to 80.88%. On the other hand, the E_{max} for 4-AP and GLIB were non-significantly reduced from 53.62 to 40.14 and 40.13% respectively; and TEA more or less unchanged to 54.34%. In general, ethyl acetate extract of *N. sativa* induces bronchodilation through four mechanisms (activation of K_{ir} channel, non-selective cyclooxygenase and to lesser extent the soluble guanylate cyclase, and blockade of Ca^{2+} channel).

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Introduction

The herb *N. sativa* (black cumin) is commonly known as black seed. It is belonging to the Ranunculacaea family (butter container). The indigenous areas are North Africa, South West Asia and Southern Europe, and also grown in many countries around the world like those within the Center Eastern Mediterranean locale, Iran, India, Syria, Pakistan, Turkey and Southern Europe (1). In Arabic it is known as "Habbat Al-Baraka" or "Al-Habba Al-Sawda" and Kurdish name is "Rashk rashk". As an eastern spice, *N. sativa* has long been used as a remedy for many acute as well as chronic diseases (2). Therefore, it has gotten to be a family conventional therapeutic plant within the locale (3). *Nigella sativa* has a long history of traditional folk use in various cultures and has been recognized as a "miracle remedy" for health promotion and disease control (4).

It has been reported that *N. sativa* seeds possess many therapeutic effects like bronchodilation (5), anti-hypertensive (6), anti-histaminic (7), antioxidant (8,9), anti-inflammatory (10), anti-diabetic (11), immunepotentiation (12) and many other effects. Many reports recommend the use of black seeds for the treatment of different respiratory problems such as asthma and chronic obstructive pulmonary

disease (COPD) (13). Several previous studies have shown that *N. sativa* (70% hydromethanol) exerts its bronchodilatory effect through various mechanisms including; Ca^{2+}channel blockade (5,14) and inhibition of histamine release (15).

The present study was conducted to investigate the bronchodilator activity of ethyl acetate extract of *N. sativa* seeds on isolated rat's trachea and to spot out other attainable underlying mechanism(s) included.

Materials and methods

Plant Extract preparation

The seeds of *N. sativa* were obtained from local markets in the Duhok city and were verified by herbalists of Department of Forestry, Agriculture College, Duhok University. The seeds were grounded into powder by an electrical grinder. The powder was extracted by maceration method, in which 1000 g of *N. sativa* powder was soaked in three liters ethyl acetate for 48 hours at room temperature, then Whatman papers were used for filtration. The ethyl acetate yielded a greenish yellow extract coded as NS.EA. The filtrate was concentrated by evaporation under reduced pressure using rotary evaporator (BÜCHI, Switzerland) at a temperature of 40ºC, and the final product was 120 g of NS.EA. Three days of 37ºC and seven days of fresh air are necessary to obtain pure and save extract (without solvent). Ultimately, the extract was entubated and stored at - 20 ºC until use (16).

Rats

Six male albino rats (*Rattus norvegicus*) weighting 200 - 300 g (from Department of Biology, College of Science, University of Zakho) were recruited for the current study. Prior to start the experiment, the animals were placed under standard laboratory conditions (22±2ºC and free access to water and libitum with a 12 hrs on light/12 hrs off light) (17). Standard pellets comprising 25.6 % soya, 1.5 % lime stone, 4.4 % oil, 0.63 % salt, 0.062 % choline chloride, 0.158 % methionine, 66.6 % wheat and 0.05 % trace elements were given to the animals (18).

Tracheal Preparation

Rats were euthanized and the neck was incised to remove trachea. After washing the trachea was carefully placed in aerated Kreb's solution. After that, 4 pieces (3 - 5 mm width) of trachea were made from its lower part (18).

Drugs and Chemicals

Drugs and chemicals used in this study are: acetylcholine bromide from McRkin and William ltd; 4-aminopyridine, BaCl2, tetraethylammonium, glibenclamide and methylene blue from Fluka AG, Germany; nifedipine and indomethacin from Medo chemie Ltd, Cyprus and ethyl acetate from BDH England.

Experimental Protocol

Cumulative concentrations of the extract 0.25, 0.37, 0.50, 0.63, 0.75, 0.88 mg/ml were applied to the isolated trachea to construct the dose-response curves (DRCs) as follows; Group I: To investigate the role of K^+ channels subtypes in the bronchodilation induced by NS.EA extract, the following blockers: 1 mM of 4-AP, 1 mM of $BaCl_2$, 1 mM of TEA and 10 µM of GLIB were individually pre-treated for 20 minutes on tracheal rings precontracted with ACh (10µM). Group II: To examine the role of Ca^{2+}channel in the bronchodilation effect of NS.EA, the rings were preincubated with nifedipine (30µM) for 10 minutes, and with indomethacin (10 µM) for 20 minutes prior to precontraction with ACh (10 µM) and before cumulative application of NS.EA (19). Group III: To demonstrate the role of cyclic guanosine monophosphate (cGMP) in the bronchodilation mediated by NS.EA, the Ach precontracted tracheal rings were preincubated with M.blue (10 µM) for 20 minutes.

Statistical Analysis

The data were converted into a computerized database format. Two-way ANOVA was used for multiple comparisons between the data (for the cell means within the same row) to detect the statistical significance by the use of Graph Pad Prizm program (version 6). Statistical significant was considered when P-value was ($P<0.05$).

Results

Figure 1 shows the typical dose-response curves (DRCs) of NS.EA in the control tracheal rings and those pretreated with K^+ channel blockers. The DRC of $BaCl_2$ shows a highly significant ($P<0.05$ and $P<0.01$) differences were observed at doses 0.75 and 0.88 mg/ml of NS.EA respectively. This led the curve to shift to right. In contrary, the DRCs of 4-AP, GLIB and TEA showed no significant alterations. Thus, the E_{max} for $BaCl_2$ was significantly decreased from 53.62% (in the control) to 30.31%, and that's for 4-AP and GLIB were non- significantly decreased to 40.14% and 40.13% respectively. However, the E_{max} of TEA was slightly increased to 60.64% (Table 1).

In figure 2, the DRCs of nifedipine and indomethacin were shifted to right. The indomethacin DRC demonstrates a highly significant ($P<0.001$) differences at doses 0.63 and 0.75 mg/ml of NS.EA, and ($P<0.05$ and $P<0.01$) at doses 0.5 and 0.88 mg/ml respectively. Likewise, the DRC of nifedipine showed significant ($P<0.01$) level at 0.63 mg/ml and ($P<0.05$) at doses 0.5 and 0.75 mg/ml of NS.EA respectively. Therefore, the E_{max} for nifedipine and indomethacin were significantly lowered to 80.88% and 73.28% respectively (Table 2).

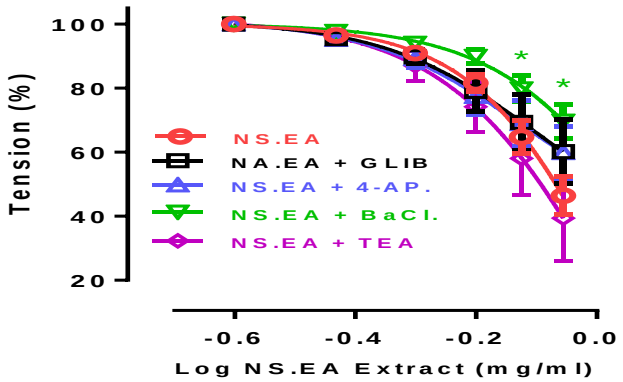

Figure 1: Dose-response curves for the bronchodilatory effect of NS.EA on tracheal rings in absence and presence of 4-AP, GLIB, $BaCl_2$ and TEA, and pre-contracted with ACh. *= P<0.05.

Table 1: Log IC_{50} (Log IC_{50} of CI 95%) and E_{max} for the bronchodilatory effect of NS.EA on rat's isolated trachea preincubated with GLIB, 4-AP, $BaCl_2$ and TEA

Blockers	Log IC_{50}± SEM (mg/ml)	Log IC_{50} of CI 95%	E_{max} (%)
Control	0.01015±0.3378	-0.6711 to 0.6914	53.62
$BaCl_2$	0.1156±1.153	-2.210 to 2.442	30.31
GLIB	-0.1064±0.3835	-0.8949 to 0.6821	40.14
4-AP	-0.1287±0.2785	-0.6903 to 0.4330	40.13
TEA	0.03463±0.9393	-1.925 to 1.994	60.64

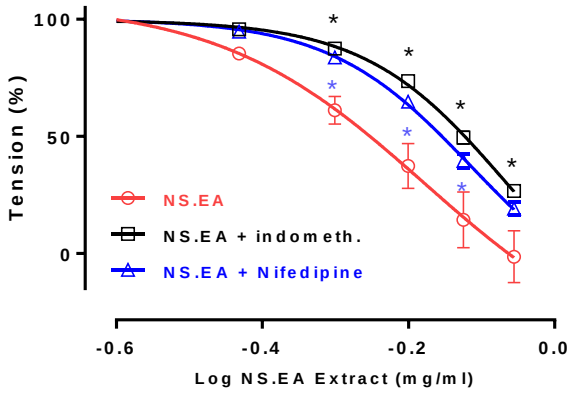

Figure 2: Dose-response curves for the bronchodilatory effect of NS.EA on tracheal rings in absence and presence of nifedipine and indomethacin, pre-contracted with ACh. *= P<0.05.

It is revealed in figure 3 that the percentage of relaxation was non-significantly decreased from 55.77% (in the control) to 42.73% (Table 3). Therefore, there is no significant difference between the dose-response curve of the control and that of m. blue.

Table 2: Log IC_{50} (Log IC_{50} of 95%) and E_{max} for the bronchodilatory effect of NS.EA on rat's isolated trachea preincubated with nifedipine and indomethacin

Blockers	Log IC_{50}± SEM (mg/ml)	Log IC_{50} of CI 95%	E_{max} (%)
Control	-0.1776±0.1534	-0.4870 to 0.1317	101.34
Indomethacin	-0.06710±0.05995	-0.1880 to 0.05381	73.28
Nifedipine	-0.1147±0.03777	-0.1908 to -0.03848	80.88

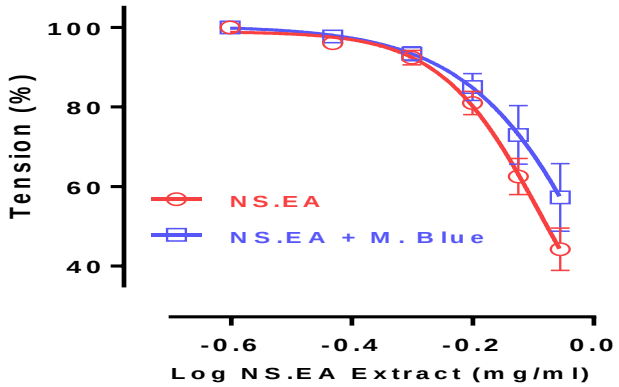

Figure 3: Dose-response curves for the bronchodilatory effect of NS.EA on tracheal rings in absence and presence of methylene blue, and pre-contracted with ACh**.** *= P<0.05.

Table 3: Log IC_{50} (Log IC_{50} of CI 95%) and E_{max} for the bronchodilatory effect of NS.EA on rat's isolated trachea preincubated with methylene blue

Blockers	Log IC_{50}± SEM (mg/ml)	Log IC_{50} of CI 95%	E_{max} (%)
Control	-0.08769±0.09805	-0.2854 to 0.1101	55.77
M. Blue	0.05485±0.8456	-1.684 to 1.793	42.73

Discussion

The data obtained from this study revealed that inhibition of COX enzyme produced a potent inhibition of bronchodilation induced by NS.EA extract. This refers to the crucial role of PGI_2 in NS.EA-induced bronchodilation (1) by some active compounds in this plant such as thymoquinone, thymol and carvacrol (20,21). The results of the current study are not comparable due to absence of a corresponding work. However, some earlier *in vivo* studies revealed that intraperitonial injection of thymoquinone (a *N. sativa* active compound) in a mouse model had inhibited the protein expression of COX_2, but slightly inhibited COX_1 protein expression (20). Furthermore, thymoquinone component of the essential oil of *N. sativa* showed an inhibitory effect on both COX and 5-Lipoxygenase of arachidonic acid metabolism in rat's peritoneal leukocytes (22).

On the other side, the results of the current study showed that the soluble guanylate cyclase enzyme has no role in the dilatory effect of the extract. It is impossible to compare the results of the present study, since no similar data on the effect of NS.EA on rat's tracheal soluble guanylate cyclase enzyme are available so far.

In the present work, blocking of L-type Ca^{2+} channels significantly inhibited the extract-induced bronchodilation. This effect suggests presence of sufficient amounts of Ca^{2+} channel blocker components in the NS.EA extract, may be mainly the thymoquinone. Ghayur *et al.* (23) revealed similar findings when he used thymoquinone. He found that thymoquinone inhibits muscle contraction by interacting with calcium signaling pathways in bronchial smooth muscle of mouse preincubated with verapamil. Another study illustrated that aqueous extract of *N. sativa* caused relaxation in guinea pig trachea precontracted with $CaCl_2$ through calcium blocking effect (24).

The effects of NS.EA extract on K^+ channels revealed that, the extract caused bronchdilation via increasing channel conductance of the K_{ir} channel. Furthermore, the exploration for the role of K_{Ca} channels, K_v channels and K_{ATP} channels in the NS.EA bronchodilation effects were also performed by the current work. It was shown that such K^+ channels subtypes play no role in this effect.

These results are supported by Keyhanmanesh *et al.* (25), they suggested the opening effect of aqueous extract of *N. sativa* on K^+ channel in guinea pig tracheal rings. However, the channel subtype in trachea in this study was not specified (26). Recently, it was found in a study that flavonoid components (compferol diglucoside) of 20% methanolic fraction of *N. sativa* induces a bronchdilation effect, although it was lower than that of theophylline. However, the exact mechanism has not been reported, but opening of K^+ channel (25) and inhibition of muscarinic receptor (27) may be implicated.

We conclude that NS.EA extract causes bronchodilation through the soluble guanylate cyclase, cyclooxygenase, K_{ir} channel and blockade of Ca^{2+} channel. The various mechanisms of action of bronchodilation indicates presence of a variety of active compounds in NS.EA extract which makes *N. sativa* versatile in its therapeutic success. *N. sativa* may be beneficial for controlling asthma. Therefore, in future the plant may be considered as the object of clinical studies, pharmacological applications and as adjuvants in medicine.

Conclusion

NS.EA extract caused bronchodilation through cyclooxygenase, Kir channel and blockade of Ca_2^+ channel. The various mechanisms of action of bronchodilation is an indication of presence of a variety of active ingredients in N. sativa which showed the potential of N. sativa in the prevention and/or treatment of respiratory problems.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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النشاط الموسع للقصبات الهوائية لمستخلص خلات الاثيل لحبة البركة

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الخلاصة

استخدمت بذور حبة البركة على نطاق واسع في الطب التقليدي لأمراض الجهاز التنفسي. ومع ذلك لا يُعرف الكثير عن الآليات الدقيقة لنشاط موادها الفعالة. دراسة هذه الآليات ستكون ذات قيمة في إنتاج الأدوية العشبية. للتحقيق في الآليات المشاركة في توسيع القصبات الهوائية بخلاصة حبة البركة. تم تحضير مستخلص خلات الأثيل باستخدام طريقة النقع. تم تجنيد الجرذان البيضاء البالغين من أجل فتح الصدر والعنق وإزالة القصبة الهوائية. بعد التقطيع إلى حلقات، تم وضع الأنسجة في حمام الأعضاء. تم فحص تأثير التركيزات التراكمية لمستخلص خلات الأثيل على الاستجابات التقلصية للقصبة الهوائية المعزولة لكلوريد البوتاسيوم أو أستيل كولين، باستخدام حاصرات مختلفة مثل نيفيديبين، رباعي ايثيل الأمونيوم (حاصرة قناة بوتاسيوم المفتعلة بأيون الكالسيوم)، ٤-بيريدين أميني (حاصرة قناة بوتاسيوم المعتمدة على الفولتية)، جليبينكلاميد (حاصرة قناة بوتاسيوم الحساسة للاندينوز ثلاثي الفوسفات)، كلوريد الباريوم (حاصرة قناة بوتاسيوم المقوم للداخل)، الأزرق الميثيلين (مثبط انزيم الجوانيليل سايكليز المذاب) والاندوميثاسين (مثبط أنزيم السايكلواوكسيجينيز). لوحظ تثبيط معنوي كبير من توسع القصبات عندما تم معالجة حلقات القصبة الهوائية بالإندوميتاسين وكلوريد الباريوم مع (P <٠,٠٠١)، وبالأزرق الميثيلين ونيفيديبين مع (P <٠,٠٥). أما تركيز المادة الموافق للتثبيط النصفي أن ٦,٩،٧,٨٦ و ٤,٩٨٧ ملغ / مل) على التوالي. على العكس، لم تظهر ٤-بيريدين أميني، جليبينكلاميد ورباعي الأثيل الأمونيوم أي تغييرات كبيرة في توسع القصبات الناجم عن المستخلص. لذلك، انخفضت قيمة معدل اقصى تأثير للإندوميتاسين بشكل معنوي من ١٠١,٣٤ إلى ٧٣,٢٨٪، وكلوريد الباريوم من ٥٣,٦٢ إلى ٣٠,٣١٪، والأزرق الميثيلين من ٥٥,٧٨ إلى ٣٨,٩٤٪ و نيفيديبين من ١٠١,٣٤ إلى ٨٠,٨٨٪. من ناحية أخرى، انخفض معدل اقصى تأثير لي ٤-بيريدين أميني وجليبينكلاميد بشكل غير معنوي من ٥٣,٦٢ إلى ٤٠,١٣ و ٤٠,١٤٪ على التوالي؛ ورباعي الأثيل الأمونيوم ازداد قليلا إلى ٦٠,٦٤ ٪. بشكل عام، مستخلص خلات الأثيل لحبة البركة تسبب توسع القصبات من خلال أربع آليات (تنشيط قناة بوتاسيوم المقوم للداخل، تنشيط إنزيمات السايكلواوكسيجينيز وبدرجة اقل الجوانيليل سايكليز المذاب، ومحاصرة قناة كالسيوم).

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