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(54) Title: USE OF A NUTRITIONAL COMPOSITION FOR TREATING HYPERTENSION

(57) Abstract: The present invention relates to the use of a nutritional composition for the preparation of a pharmaceutical composition for inhibiting smooth muscle cell contraction, and hence lowering hypertension

USE OF A NUTRITIONAL COMPOSITION FOR TREATING HYPERTENSION

This invention relates to the use of a nutritional composition for the preparation of a pharmaceutical composition that inhibits the contraction of smooth muscle cells and, hence, may lower blood pressure in hypertensive patients.

There are many documented pathophysiological and clinical effects of hypertension (elevated blood pressure). These effects include the short-term effects resulting in poor health and bad work performance and the longer-term effects resulting in myocardial infarction, stroke, cardiac arrest, kidney disease, kidney failure and others. Moreover, the effects of hypertension may be exacerbated in conjunction with other diseases such as diabetes. In recent years it is estimated that more than 50% of deaths relating to cardiovascular disease in the United States alone was related to or resulted from hypertension. Hypertension remains the most common cause of cardiac failure or other disease states requiring some amount of hospitalization.

There has been significant and extensive research for treatment for hypertension. However, present treatments for such disorders are treatments such as administration of angiotensin converting enzyme inhibitors (ACE inhibitors). These treatments have serious shortcomings in long-term effectiveness, most notable the cost associated with these treatments and significant adverse effects. There are also a vast number of publications with regard to the mechanisms of pathogenesis of hypertension. Extensive production and activity of angiotensin II is well accepted as one of the major sources in the development of hypertension, since its excess causes abnormally strong contraction of arteries, compromises process of arteries relaxation and lead therefore to elevated blood pressure. Thus, a massive effort is being undertaken to develop pharmaceutical

compounds capable either to reduce formation of angiotensin II (i.e. ACE inhibitors which block a conversion of angiotensin I to angiotensin II by arterial wall cells) or to block a biological activity of angiotensin II (i.e. agonists of angiotensin receptors). Both classes of compounds are being tested in experimental conditions for their capacity to block angiotensin-dependent contraction of arterial wall either using arteries isolated from laboratory animals or a model of cultured smooth muscle cells embedded in collagen gel. A capacity of a tested compound to block a conractile activity of angitensin II in such experimental models unequivocally means that this compound will block angiotensin II activity in *in vivo* conditions and will reduce angiotensin-driven hypertension. Carini *et al.* describe procyanidins from grape seeds that enhance relaxation of human artery (Life Sci. 2003 Oct. 17; 73(22):2883-98). Shen *et al.* describe green tea catechins that evoke a phasic contraction in rat aorta, and Chen *et al.* describe purified green tea epicatechins on contraction. Sanae *et al.* describe the effects of catechins on vascular tone in rat thoracic aorta with endothelium. Huang *et al.* describe the role of endothelium/nitric oxide in vascular response to flavonoids and epicatechin (Acta Pharmacol. Sin. 2000 Dec; 21(12): 1119-24).

In light with the foregoing, the technical problem underlying the present invention might be seen as the provision of means and methods for inhibiting smooth muscle cell contraction and hence for treating the underlying hypertension disease. Moreover, there is a need for preserving and restoring the sensitivity of the arteries to stimuli that would allow for proper contraction and relaxation of smooth muscle cells in the arteries. The technical problem is solved by the embodiments characterized in the claims and hereinafter.

Accordingly, the present invention relates to the use of a nutritional composition comprising a green tea extract, ascorbic acid, lysine, proline, arginine, magnesium, N-acetyl cystein, selenium, copper, and manganese for the preparation of a pharmaceutical composition for treating or preventing hypertension.

The term "nutritional composition" encompasses liquid and solid preparations of the nutritional compounds referred to above as well as gels thereof. The solid preparations may be manufactured in a suitable form including tablets, capsules, powders, granules, tea preparations or the like. Its well known in the art how to manufacture said liquid, gel-like and solid preparations referred to herein. The nutritional composition referred to herein may be provided in accordance with the uses of the invention as mixture of the compounds or by means of a kit including the ingredients separately. The ingredients may be packaged in said kit in separate vials.

The amino acids "proline", "lysine", and "arginine" referred to in accordance with the present invention are preferably the L-amino acids. "Proline" or "Lysine" also encompass its hydroxyl derivatives hydroxyproline and hydroxylysine as well as salts thereof. The term "ascorbic acid" preferably refers to ascorbate, ascorbic acid and salts thereof.

The term "green tea extract" as used in accordance with the present invention, preferably, refers to a preparation of green tea plants comprising the polyphenolic compounds that are present in green tea. Polyphenolic compounds may be present as up to 30% dry weight in green tea. They include bioflavinoids such as flavanols, flavandiols, flavonoids, and phenolic acids. Flavanols represent the most abundant polyphenols in green tea and are commonly known as catechins. Most preferably, said catechins are EGCG, EG, ECG or EC. EGCG refers to (-)-epigallocatechin-3-gallate, EC refers to epicatechin which refers to (-)-epicatechin, ECG refers to eipcatechin-3-gallate which refers to (-)-epicatechin-3-gallate, EGC refers to epigallocatechin which refers to (-)-epigallocatechin. It is well known in the art how such preparations may be obtained.

The nutritional compounds referred to in accordance with the uses of the present invention may be admixed in any suitable ratios or amounts. Whether such a ratio or amount is suitable can be determined by the skilled person by using the assays specified in the accompanied Examples referred to below. Most preferably, the nutritional composition provides a daily dosage of the nutritional compounds as specified in Table 1, below, or is a formulation as indicated in Table 2, below. The specific composition disclosed in Table 1 may be also referred to as "Composition EF" throughout this

specification. The daily dosage may be provided by one or more applications of the nutritional composition to be used in accordance with the present invention.

As used herein, the term "treating" is used to mean reducing, inhibiting, attenuating or treating the syndromes accompanied with the pathological conditions referred to in accordance with the present invention. Treating becomes apparent for the clinician by monitoring the symptoms accompanied with the said pathological conditions. The symptoms are described in detail in standard text books such as Stedman or Pschyrembel. Treatment preferably refers to significant reduction, inhibition, attenuation or treatment. The significance can be determined by standard methods of statistics, e.g., Student's t-test, chi square test an others.

The term "hypertension" includes all hypertension diseases and disorders. The symptoms accompanied with said diseases or disorders are well known in the art and described in detail in medical text books such as Stedman or Pschyrembel. Accordingly, the clinician can determine without further ado whether a patient suffers from hypertension. The term also includes pre-hypertension, i.e. a systolic blood pressure in the range of 120-139 mmHg and a diastolic blood pressure of 80-90 mmHg.

The term "prevention" means said the nutritional composition may also be administered in order to avoid the development of hypertension.

Surprisingly, it has been found that the nutritional composition referred to in accordance with the present invention is useful in lowering blood pressure. Moreover, the nutritional composition is effective in retarding adverse effects of stimuli, which lead to contraction of smooth muscle cells, which in turn increase blood pressure and results in hypertension. Advantageously, the nutritional compounds of the composition are derived from a natural source that is safe. Together, the results of the studies made in accordance with the present invention show that a nutritional composition comprising a green tea extract (including ECGC as a bioflavonoid), ascorbic acid, lysine, proline, arginine, magnesium, N-acetyl cystein, selenium, copper, and manganese, has a synergistic effect in regulation of smooth muscle cell (SMC)-mediated contraction. The nutritional composition has a strong potential in counteracting pathophysiological effects of agonists such as thrombin and angiotensin II. The components present in the nutritional composition act

synergistically in inhibiting SMC contraction and hence, reverse and minimize the lack of sensitivity of arteries that lead to hypertension. Furthermore, the present invention provides a therapy based on the nutritional composition that may retard adverse effects of stimuli, which lead to contraction of smooth muscle cells, which increase blood pressure and results in chronic hypertension. The present invention relates to the selection of compounds and extracts from nature, which are more effective without undue side-effects of artificial pharmaceutical compounds, not to mention its further advantages of economic cost.

The definitions and explanations of the terms made hereinabove apply mutatis mutandis for the other embodiments of the present invention disclosed hereinafter.

The present invention also relates to the use of a nutritional composition comprising a green tea extract, ascorbic acid, lysine, proline, arginine, magnesium, N-acetyl cystein, selenium, copper, and manganese for the preparation of a pharmaceutical composition for preserving and restoring the sensitivity of the arteries to stimuli that would allow for proper contraction and relaxation of smooth muscle cells in the arteries.

The term "stimuli" as used herein preferably relates to stimulating factors which modulate the vascular tone in vivo such as angiotensine or nitric oxide.

In a preferred embodiment of the uses of the invention said green tea extract comprises at least one compound selected from the group consisting of epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate.

In another more preferred embodiment of the uses of the present invention the ascorbic acid is calcium ascorbate, magnesium ascorbate or ascorbyl palmitate.

In a further more preferred embodiment of the uses of the present invention the nutritional composition provides a daily dosage of 1,000 mg green tea extract, 710 mg

ascorbic acid, 1,000 mg lysine, 750 mg proline, 500 mg arginine, 1 mg magnesium, 30 mg N-acetyl cystein, 30 μg selenium, 2 mg copper, and 1 mg manganese.

Moreover, in a further preferred embodiment of the uses of the invention said nutritional composition further comprises resveratrol, genistein or a combination thereof.

The term "resveratrol" includes resveratrol as well as its derivatives having the same biological activity. Whether a resveratrol derivative has the same biological activity as meant in accordance with the present invention can be tested by using an assay as described in the accompanied Examples.

The term "genistein" encompasses genistein and its derivatives having the same biological activity. Whether a genistein derivative has the same biological activity as meant in accordance with the present invention can be tested by using an assay as described in the accompanied Examples.

The present invention also relates to a nutritional composition comprising a green tea extract, ascorbic acid, lysine, proline, arginine, magnesium, N-acetyl cystein, selenium, copper, and manganese wherein said composition further comprises resveratrol, genistein or a combination thereof.

Finally, also more preferably, said pharmaceutical composition is to be administered to a human subject.

The figures show:

Figure 1 depicts the effects of 0.1 IU/ml thrombin on smooth muscle cells in SMC gels containing composition EF ("composition 1") and a control without composition EF. Control SMC gel is without thrombin.

Figure 2 depicts the effects of 1.0 μM angiotensin II on smooth muscle cells in SMC gels containing composition EF ("composition 1") and a control without composition EF. Control SMC gel is without angiotensin II.

Figure 3 depicts SMC gel contraction by 1 μM angiotensin II and in the presence of increasing concentrations of composition EF.

Figure 4 depicts SMC gel contraction by increasing concentrations of 110 nM, 330 nM, and 1,000 nM angiotensin II and in the presence of 100 μg/ml of composition EF.

Figure 5 depicts SMC gel contraction by angiotensin II and in the presence of aomposition EF, ascorbic Acid, EGCG, and ascorbic Acid-EGCG combination.

Figure 6 depicts SMC gel contraction by angiotensin II and in the presence of arginine at various concentrations.

Figure 7 depicts SMC gel contraction by angiotensin II and in the presence of calcium chloride, magnesium chloride, and calcium chloride-magnesium chloride combination.

Figure 8 depicts SMC gel contraction by angiotensin II and the effects of resveratrol and genistein, and in the presence of 100 μg/ml of composition EF.

Figure 9 depicts SMC gel contraction by angiotensin II in presence of various concentrations of N-acetyl cystein.

Figure 10 depicts SMC gel contraction by angiotensin II at 1μM in the presence of various concentrations of lysine and proline.

The following examples are presented to further illustrate the present invention. It is not intended that the invention be limited in scope by reason of any of the following examples. It will be understood that there is no intent to limit the present invention to the preferred embodiment disclosed, but rather it is intended to cover all modifications and alternate constructions falling within the spirit and scope of the invention.

Example 1:

Experimental Protocol

Materials:

The following starting material and equipment were used.

1. Cultured vascular smooth muscle cells (SMC) isolated from human aorta. Cells are used from 4th to 8th passages.
2. Human collagen type I.
3. Angiotensin II.
4. Thrombin.
5. Composition EF (lysine, proline, arginine, vitamin C (as ascorbic acid, calcium ascorbate, magnesium ascorbate, or ascorbyl palmitate), magnesium, N-acetyl cystein, selenium, copper, and manganese. 6 capsules of composition EF contain 1,000 mg of lysine, 750 mg proline, 500 mg L-Arginine, 710 mg of vitamin C, 50 mg magnesium, 1000 mg standardized green tea extract (80% polyphenols – 800 mg (decaffeinated)) 30 mg N-acetyl cystein, 30 μg selenium, 2 mg copper, 1 mg manganese. (all ingredients commercially available)
6. Epigallocatechin gallate (EGCG)
7. Resveratrol
8. Cell culture medium (DMEM)

9. 24 well plastic cell culture plate pre-incubated with 2 mg/ml bovine serum albumin.
10. Digital camera.
11. Digital image analyzing software (Scion Corporation).

Table 1: "Composition EF"

Compound	**Dosage per day**
L-Lysine	1,000 mg
L-Proline	750 mg
L-Arginine	500 mg
Vitamin C, as ascorbic acid, Calcium Ascorbate, magnesium Ascorbate or Ascorbyl Palmitate	710 mg
Magnesium	50 mg
Standardized Green Tea Extract, 80% polyphenols – 800 mg (decaffeinated)	1,000 mg
N-Acetyl –Cystein	200 mg
Selenium	30 mcg
Copper	2 mg
Manganese	1 mg

The "Composition EF" may be formulated as follows:

Table 2: Epican Forte (EF) composition (6 capsules weight 4.46 g) at 100 mcg/ml:

Ascorbic Acid	100 mcM
Green tea extract	22 mcg/ml
Including EGCG	15 mcM
Lysine	110 mcM
Proline	100 mcM
Arginine	50 mcM
N-acetyl cystein	20 mcM
Selenium	8.5 nM
Calcium	12 mcM
Magnesium	50 mcM

Methods:

We tested the ability of green tea extracts (i.e., bioflavonoids) and various ingredients on inhibiting the contractile activity of smooth muscle cells. Cultured human aortic smooth muscle cells (SMC) (Navab, J Clin Invest 1988, 82(6):183-93) were used and embedded in a three-dimensional type I collagen (1 mg/mL) matrix. Gel contraction was stimulated by adding 1 μmolar angiotensin II (Ang II) in serum-free media and the gel area was assessed by digital image analysis after 24 hours.

Culture of Smooth Muscle Cells

Confluent cultures of SMC were removed from culture flask by trypsinization and washed with phosphate-buffered saline (PBS) from serum-containing medium. Cell concentration in suspension was brought to 500,000 cell/mL in serum-free DMEM. Cell suspension was then mixed 1:1 with ice-cold 2 mg/ml collagen type I solution in phosphate buffered solution (PBS). Final concentration of collagen type I was 1 mg/mL, final cell concentration was 250,000/mL.

Collagen-SMC suspensions were distributed by 300 μl to 24 well plates in such a manner to cover the entire bottom surface of the wells. The plates were then incubated for one

hour at 37°C to allow gel to polymerize. 0.5 mL of experimental serum-free medium contaning no additions (control), or 1 micromol/L angiotensin II with or without tested compound was added to polymerized gel. Plates were then gently tapped on the side to detach gel from the bottom of plastic well, and plates were then placed to incubator with the controlled atmosphere containing 5%CO_2 at 37°C for incubation. After 24-hour incubation plates were taken from the incubator and plate image with floating gels were taken using digital camera. Gel flat surface area is measured with digital image analyzing software. Sample of cell culture media was taken for analysis of matrix metalloproteinases activity by zymography (Novorex Corp). Experiments were performed in triplicates and results are presented as a mean +/- SD.

Studies were carried out to observe the effects of various components in composition EF and to determine the synergistic effect of the ingredients in composition EF, if any, in inhibiting smooth muscle cell contraction. These studies may shed light on the treatment and/or prevention of hypertension. Various ingredients including epigallocatechin gallate (EGCG) was studied. Epigallocatechin gallate and other ingredients were first studied by evaluating the single effect of epigallocatechin gallate and respective ingredients. Synergistic effects between epigallocatechin gallate with other ingredients were then studied.

Results:

Both angiotensin II and thrombin (used as agonists) caused contraction of the smooth muscle cells in the SMC gel. These agonists further caused contraction of the entire gel. Addition of angiotensin II or thrombin caused a reduced gel surface area. The differential between the gel surface area at 24 hours after pouring of the SMC gel that does not contain a contracting agent, and the gel surface area at 24 hours after pouring of an SMC gel that does contain a contracting agent is attributed to the effect of the contracting agent.

Using this SMC gel contraction assay, we evaluated various compounds for their ability to inhibit the smooth muscle cell contraction. Among the ingredients in the green tea

extracts, epigallocatechin gallate is noted to be the most active inhibitor of gel contraction tested. When added at the concentration of 30 μmole/L. In comparative analysis of structure-related activity the presence of gallic acid residues in the catechin molecule was shown to enhance its activity. Inhibition of gel contraction by bioflavonoids (including EGCG) did not depend on antioxidant activity, since ascorbic acid did not have any activity in this assay.

Example 2:

Fig. 1 shows the ability of composition EF in inhibiting smooth muscle cell contraction as induced by thrombin. In this study, a SMC gel without the contracting agent (control) and a gel with the contracting agent (thrombin at 0.1 IU/ml) were compared to a gel with thrombin at 0.1 IU/ml treated with 100 μg/ml of composition EF. Control SMC gels without a contracting agent and treating agent showed some contraction. Thus, smooth muscle cells have a tendency to contract, even without the presence of a contracting agent.

SMC gel with contracting agent thrombin showed greater contraction of the SMC gel. However, when an SMC gel was treated with thrombin at 0.1 IU/ml and composition EF, the SMC gel did not contract as much as an SMC gel with or without the contracting agent by themselves. Thus, composition EF showed significant effect in inhibiting the contraction of the SMC gel and in acting as an anti-hypertensive.

Example 3:

Fig. 2 shows the ability of composition EF in inhibiting smooth muscle cell contraction as induced by angiotensin II (as an contracting agent). SMC gel without the contracting agent angiotensin II and a gel with the contracting agent angiotensin II at 1.0 μM were compared to a gel with angiotensin II at 1.0 μM treated with 100 μg/ml of composition EF.

SMC gel with contracting agent angiotensin II showed greater contraction of the SMC gel. When a SMC gel was treated with angiotensin II at 1.0 μM and composition EF, the SMC gel did not contract as much as an SMC gel with or without the contracting agent by themselves. Both of these experiments at least tested the premise that composition EF was effective as an anti-hypertensive agent. Accordingly, these data together (Fig. 1 and 2) clearly show that composition EF is effective in inhibiting the contraction of smooth muscle cells, and thereby may be useful in anti-hypertensive purposes.

Example 4:

Fig. 3 shows a dose-dependent effect of composition EF on inhibiting smooth muscle cell contraction as induced by angiotensin II. SMC gel containing angiotensin II at 1.0 μM was treated with increasing concentrations of composition EF at 11, 33, and 100 μg/ml, and compared to a control of angiotensin II without composition EF. This produced a dose response curve, showing less contraction (greater reduction in SMC gel surface area loss) with increased concentrations of composition EF.

Example 5:

The respective constituent of the composition EF in inhibiting smooth cell contraction was tested next. We also tested if various constituents of composition EF might act in a synergistic manner. To test this, various constituents of composition EF were tested either alone or in combination with other ingredients in their ability to inhibit smooth muscle cell contraction.

Fig. 4 shows the effects of ascorbic acid, EGCG, and ascorbic acid + EGCG on their ability to inhibit smooth muscle cell contraction. SMC gels were induced to contract by angiotensin II (1.0 μM). Control SMC gel contained only angiotensin II. Composition EF at 100 μg/ml greatly inhibit smooth muscle cell contraction. Ascorbic acid at 100 μM alone did not affect angiotensin II induced smooth muscle cell contraction. EGCG at 15 μM alone did not have an appreciable inhibitory effect. The combination of ascorbic acid and EGCG also did not have any appreciable inhibitory effect. These data show that

there is a synergistic effect among the various components of composition EF that inhibiting smooth muscle cell contraction. Note that ascorbic acid and EGCG were used at equivalent concentrations found in composition EF.

Example 6:

Fig. 5 shows the single effect of arginine on inhibiting smooth muscle cell contraction. Arginine (0.50 mM and 1.0 mM) was applied to SMC gels containing 1.0 μM of angiotensin II and 0.5 mM of ascorbic acid. Equivalent concentrations of arginine were applied to SMC gels containing 1.0 μM of angiotensin II but with no ascorbic acid.

The concentration of arginine in 100 μg/ml of composition EF is 50 μM. Therefore the concentrations of arginine applied singly to the SMC gels were respectively 10 times and 20 times greater than the concentration of arginine in the composition EF. The concentration of ascorbic acid in SMC gels containing ascorbic acid was 0.5 mM, which is 5 times greater than the concentration of ascorbic acid in EF. Despite these higher concentrations, ascorbic Acid and arginine, either alone or in combination did not produce a detectable effect in inhibiting smooth muscle cell contraction.

Example 7:

Fig. 7 shows the single and combined effect of calcium and magnesium (in the form of calcium chloride and magnesium chloride) on inhibiting smooth muscle cell contraction. The concentration of calcium in 100 μg/ml of composition EF is 12 μM. The concentration of magnesium in composition EF is 50 μM. The concentration of calcium and magnesium used in this study for SMC gel contraction was 2.0 mM. Therefore, the concentrations of calcium and magnesium applied to the SMC gels were respectively approximately 160 times and 40 times greater than the concentration of calcium and magnesium in composition EF. Angiotensin II was added at 1 μM as contracting agent to all SMC gels. Despite these higher concentrations, calcium chloride and magnesium chloride, either alone or in combination, did not produce a detectable inhibition on smooth muscle cell contraction induced by angiotension II.

Although composition EF did not contain any resveratrol or genistein, we tested their combined effect with composition EF. Fig. 8 shows the effects of genistein and resveratrol to either individually or in combination with each other, in inhibiting smooth muscle cell contraction. Resveratrol was applied to SMC gels and compared with SMC gels that did not contain resveratrol. The concentration of resveratrol applied to the SMC gel was 15 μM and 30 μM. In one SMC gel genistein was added at a concentration of 30 μM to test the effect, if any, of genistein by itself. The concentration of resveratrol applied to the SMC gels was 15 μM and 30 μM. Genistein and resveratrol in combination were applied to the SMC gel both at 15 μM. Angiotensin II was added at 1 μM as contracting agent to all SMC gels. Two groups of experiments were carried out, one set of SMC gels without composition EF, and the other set SMC gels containing composition EF at 100 μM.

While resveratrol, genistein, and their combination tended to show some inhibiting effect, this effect was more pronounced when composition EF was present. There was a clear detectable additive anti-hypertensive effect in all SMC gels, whether containing only resveratrol, only ginestein, or both. A dose response curve was evident in the groups containing 15 μM and 30 μM of resveratrol in groups with or without composition EF, however the dose response curve in the resveratrol groups containing Composition EF was more pronounced.

Example 8:

Fig. 9 shows the effectiveness of N-acetyl cystein for inhibiting smooth muscle cell contraction. The concentration of N-acetyl cystein in 100 μg/ml of composition EF is 20 μM. The concentration of N-Acetyl Cystein applied to the SMC gels was 2.2, 6.7, 20 and 60 μM respectively. Angiotensin II was added at 1 μM as contracting agent to all SMC gels. Despite these higher concentrations, N-acetyl cystein did not produce a detectable anti-contracting effect.

Example 9:

Fig. 10 shows the effects of lysine and proline, either individually or in combination with each other, to inhibit smooth muscle cell contraction. The concentration of lysine in 100 μg/ml of composition EF is 110 μM. The concentration of lysine applied to the SMC gels was 0.25, 0.50, and 1 mM. Therefore, the concentrations applied to the SMC gel were respectively approximately 2 times, 4.5 times, and 9 times greater than the concentration of lysine in composition EF. The concentration of proline in 100 μg/ml of composition EF is 100 μM. The concentration of proline applied to the SMC gels was 0.25, 0.50, and 1 mM. Therefore, the concentrations applied to the SMC gel were respectively 2.5 times, 5 times and 10 times greater than the concentration of proline in composition EF. Lysine and proline were added as a combination to an SMC gel at a concentration of 0.50 mM. Angiotensin II was added at 1 μM as contracting agent to all SMC gels. Despite these higher concentrations, proline and lysine, either alone or in combination did not produce a detectable anti-contracting effect.

It will be understood that there is no intent to limit the present invention to the preferred embodiment disclosed, but rather it is intended to cover all modifications and alternate constructions falling within the spirit and scope of the invention. All publications and other references mentioned herein are incorporated by reference in their entirety.

Claims

1. Use of a nutritional composition comprising a green tea extract, ascorbic acid, lysine, proline, arginine, magnesium, N-acetyl cystein, selenium, copper, and manganese for the preparation of a pharmaceutical composition for treating or preventing hypertension.

2. Use of a nutritional composition comprising a green tea extract, ascorbic acid, lysine, proline, arginine, magnesium, N-acetyl cystein, selenium, copper, and manganese for the preparation of a pharmaceutical composition for restoring the sensitivity of the arteries to stimuli that would allow for proper contraction and relaxation of smooth muscle cells in the arteries.

3. The use of claim 1 or 2, wherein said green tea extract comprises at least one compound selected from the group consisting of epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate.

4. The use of any one of claims 1 to 3, wherein the ascorbic acid is calcium ascorbate, magnesium ascorbate or ascorbyl palmitate.

5. The use of any one of claims 1 to 4, wherein the nutritional composition provides a daily dosage of 1,000 mg green tea extract, 710 mg ascorbic acid, 1,000 mg lysine, 750 mg proline, 500 mg arginine, 1 mg magnesium, 30 mg N-acetyl cystein, 30 µg selenium, 2 mg copper, and 1 mg manganese.

6. The use of any one of claims 1 to 5, wherein said nutritional composition further comprises resveratrol, genistein or a combination thereof.

7. The use of any one of claims 1 to 6, wherein said pharmaceutical composition is to be administered to a human subject.

Figure 1

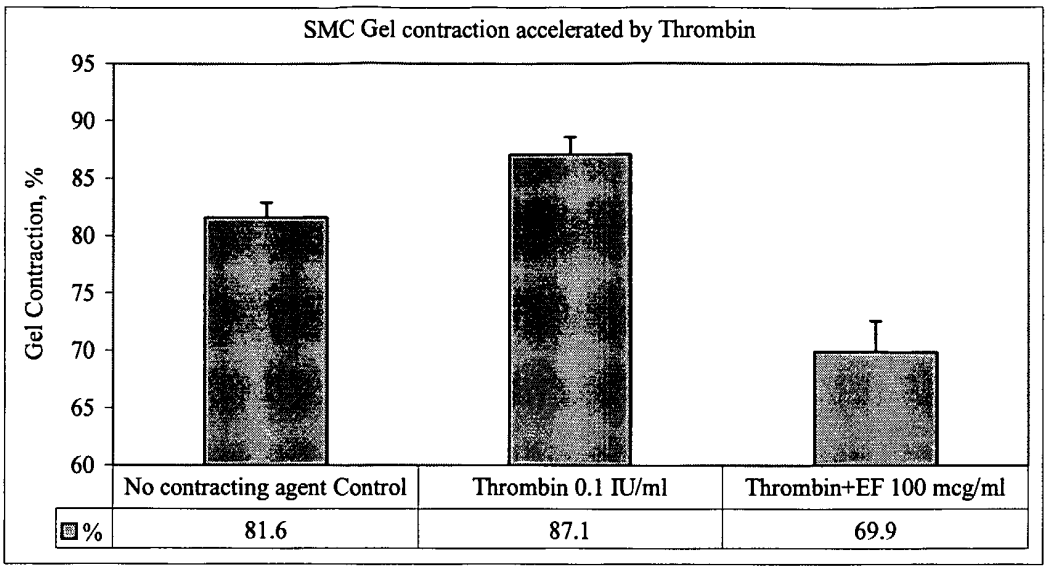

Figure 2

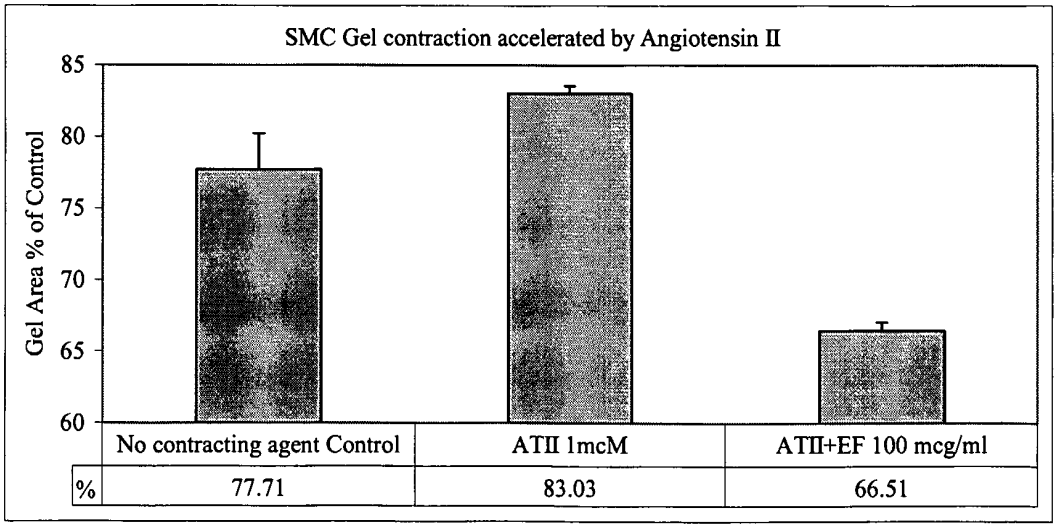

Figure 3

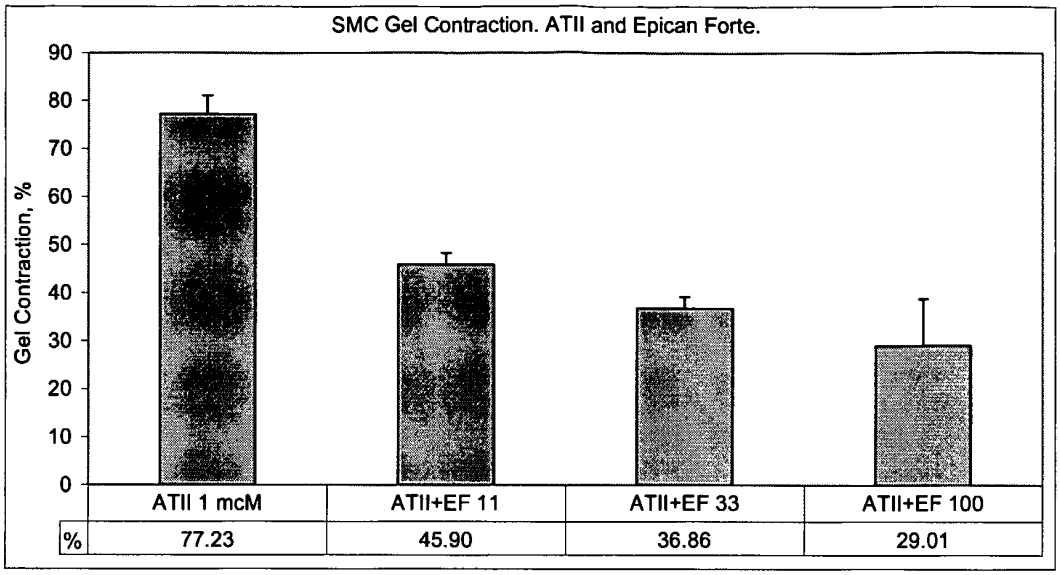

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Figure 4

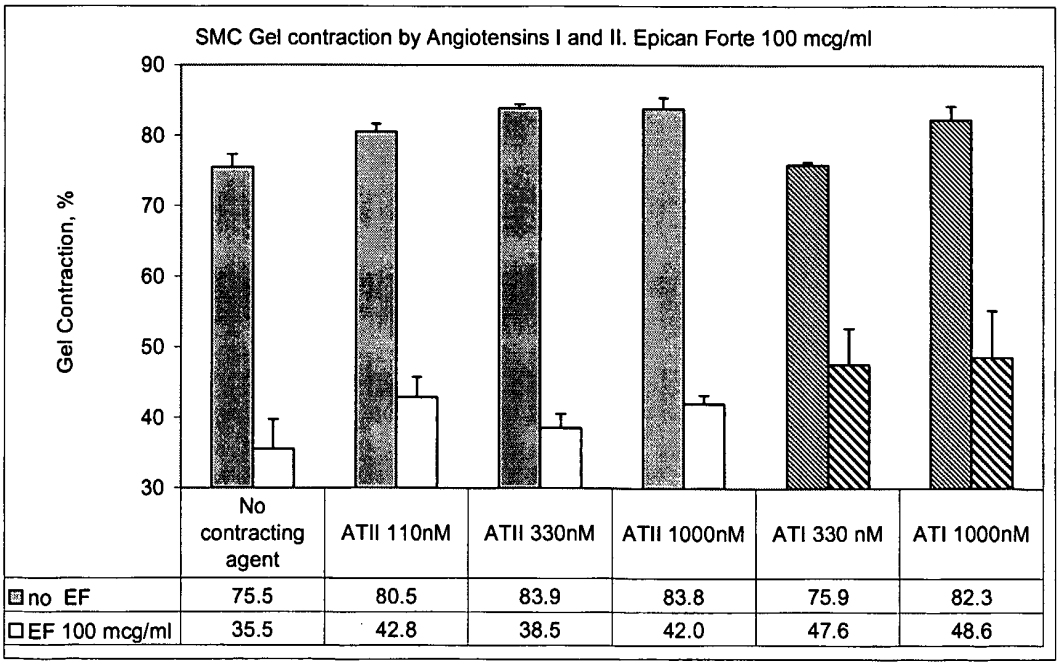

Figure 5

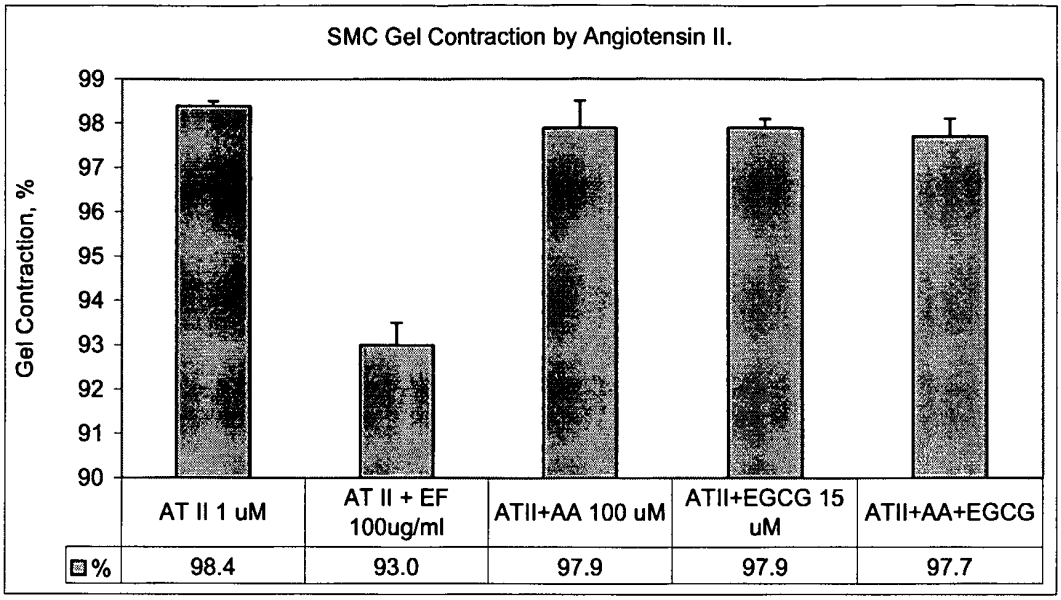

Figure 6

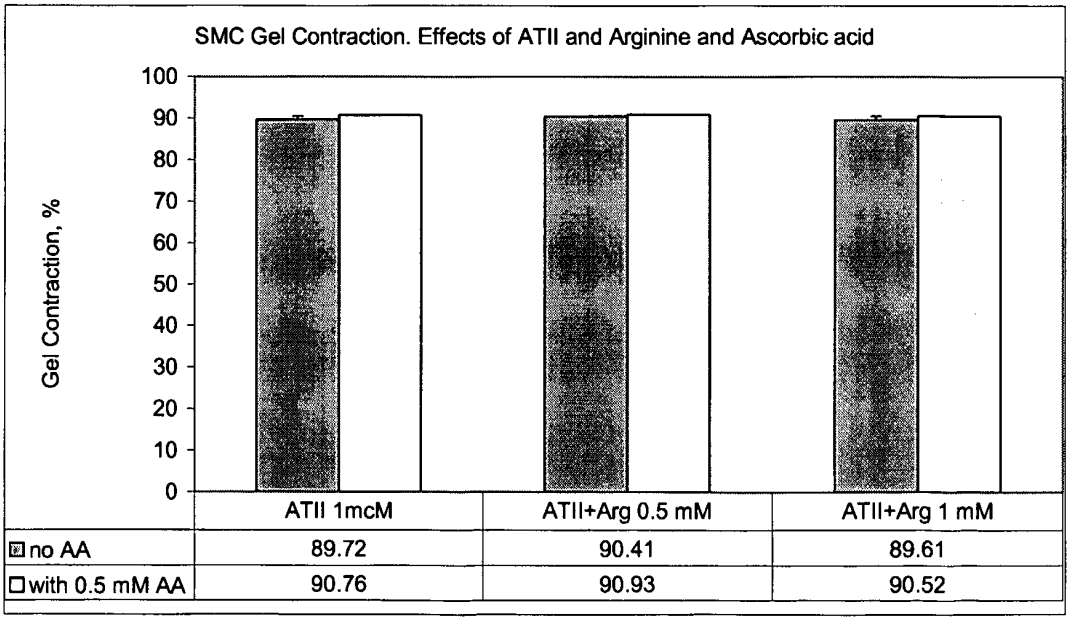

Figure 7

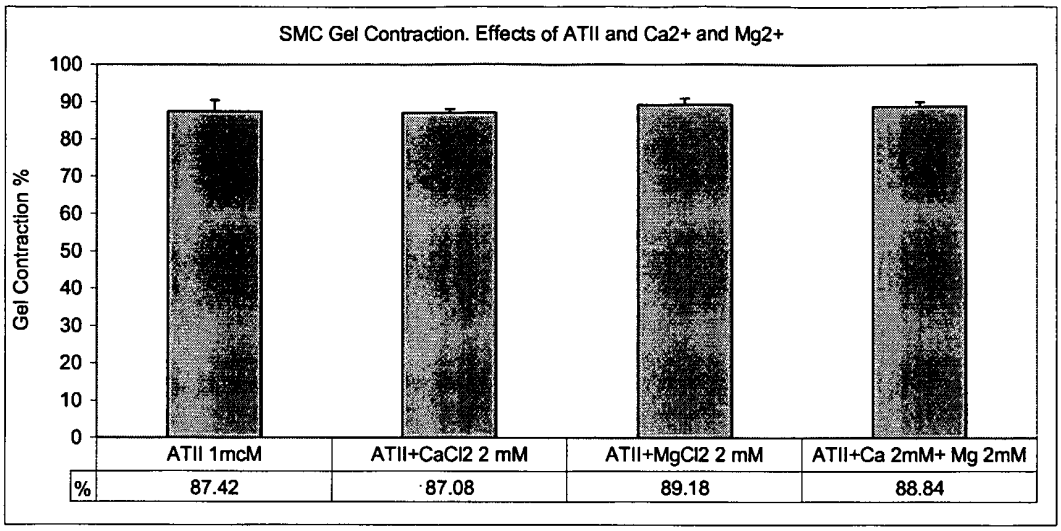

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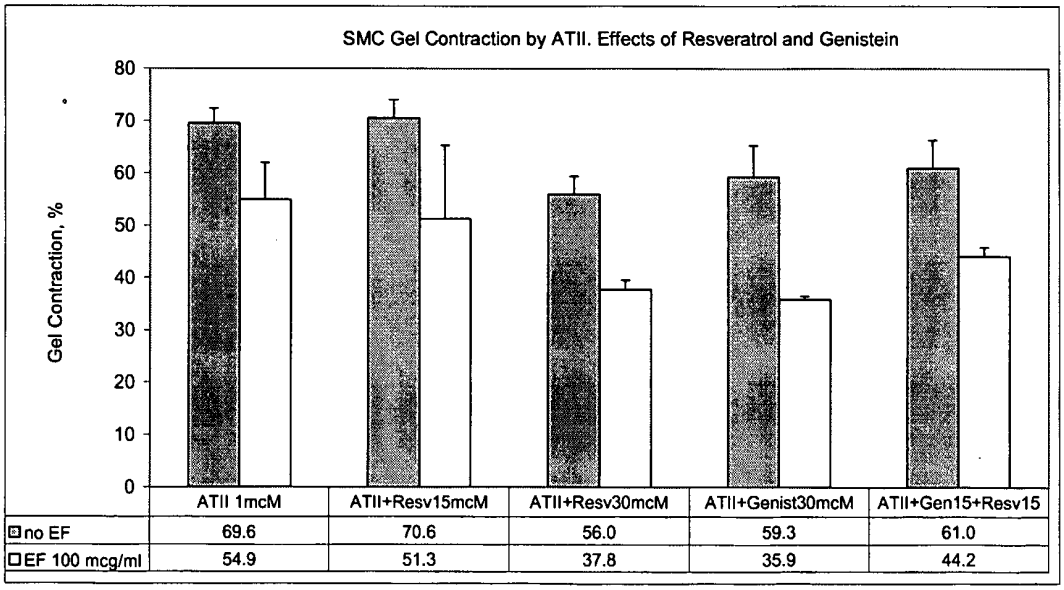
Figure 8



10

Figure 9

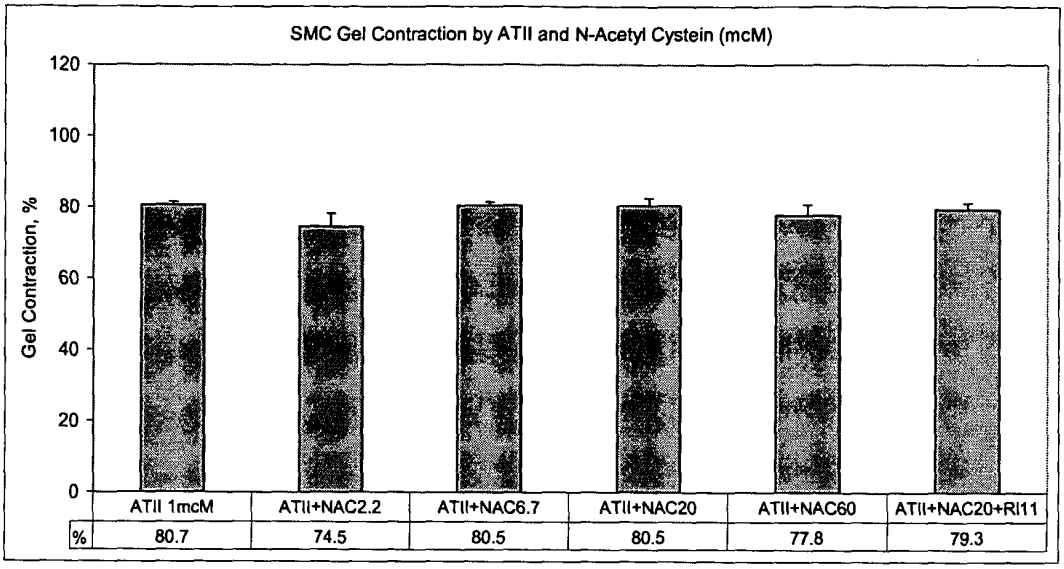


Figure 10