

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
10 May 2012 (10.05.2012)

PCT

(10) International Publication Number
WO 2012/059942 A2

(51) International Patent Classification:
A61K 31/122 (2006.01)

(21) International Application Number:
PCT/IN2011/000751

(22) International Filing Date:
31 October 2011 (31.10.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
3024/MUM/2010 1 November 2010 (01.11.2010) IN

(71) Applicant (for all designated States except US):
VIRIDIS BIOPHARMA PVT. LTD [IN/IN]; 6/10 Jo-
gani Industrial Complex, V.N. Purav Marg, Chunabhatti,
Mumbai 400022 (IN).

(72) Inventors; and

(75) Inventors/Applicants (for US only): DILIP, Mehta [IN/IN]; 8, Pavlova 10 Little Gibbs Road, Malabar Hill, Mumbai 400006 (IN). RAMA, Vaidya [IN/IN]; 5th Floor, Tanisha Building, L-Raheja Road, Off S.V. Road, Juna Khar, Khar (W), Mumbai (IN). DE SOUZA, Anselm [IN/IN]; A-13, Michael Apartments, P.K Road, Mulund (W), Mumbai 400080 (IN). YOGESH, Dound [IN/IN]; 1/18, Kashmiri Building, Majaswadi, Joheshwari (E), Mumbai 400060 (IN). ASHOK, Vaidya [IN/IN]; 5th Floor, Tanisha Building, L-Raheja Road, Off S.V. Road, Juna Khar, Khar (W), Mumbai (IN).

(74) Agent: SANTHANAM, Kausalya; 203 Quiescent Heights, Mind Space Malad (W), Mumbai 400064 (IN).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))
- of inventorship (Rule 4.17(iv))

Published:

- without international search report and to be republished upon receipt of that report (Rule 48.2(g))

(54) Title: DYNAMIC BALANCING OF AUTONOMIC NERVOUS SYSTEM THROUGH VITAMIN MK-7

(57) Abstract: Sympathovagal balance is essential in maintaining internal homeostasis. Imbalance in this can lead to many disease conditions such as cardiovascular diseases, type 2 diabetes mellitus, high blood pressure, gastroesophageal reflux disease (GERD), IBS, depression, COPD, etc. Sudden cardiac death and Myocardial infarction are result of autonomic dysfunction. Many drugs have been proposed for these conditions but lack in correcting the condition and can themselves produce side effects, such as prolongation of QT interval. Inventors of current invention observed serendipitously that vitamin MK-7 can restore the sympa-thovagal balance and also have cardio protective effect by shortening of QT interval and prolongation of RR interval. Further to this, it was also observed that vitamin MK-7 improves cardiac output, left ventricular work, HDL cholesterol and reduces blood sugar.



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Dynamic Balancing of Autonomic Nervous System through Vitamin MK-7

Related Applications:

This application takes priority from Indian Application No. 3024/MUM/2010 filed 1st
5 November, 2010 and is incorporated herein in its entirety.

Field of the Invention:

The invention provides method of improving sympathovagal balance in mammals by
administering effective amounts of vitamin MK-7 or other vitamin K analogues and their
10 derivatives.

Background of Invention:

Sudden cardiac death and Myocardial Infraction (MI) are major causes of death in both
developed and developing countries. In the US prevalence of MI lies between 300,000 and
15 500,000 deaths every year [1]. The Autonomic Nervous System (ANS) plays an important role in
the pathophysiology of conduction system of heart and thus a major disturbance in ANS can
result in sudden cardiac death. The current available treatment modalities are based on the
correction in the autonomic dysfunction. While doing so most of these drugs themselves produce
serious side effects such as prolongation of QT interval. Such QT change is reason for the recall
20 of several drugs [45]. There is need for safe intervention in these serious life threatening
conditions. It was serendipitously observed that vitamin K2-7, (MK-7), shows promising result
in the correction of sympathovagal balance.

Sympathetic Nervous System (SNS) and Parasympathetic Nervous System (PNS) are tonically
25 active. This means that there is continuous nervous input from both the systems to a given tissue.
In other words, each system may enhance or inhibit tissue activity. This characteristic of the
ANS improves its ability to more precisely regulate a tissue's function.

The balance between these two systems is critical in maintaining the homeostasis in proper state.
Some time the PNS may not always work well like in case of Anxiety Disorders, Aging, Obesity
30 etc [2-4] and to counter this deficiency SNS may be working overtime. This creates an unhealthy

autonomic balance. Research shows that this imbalance in nervous system can be associated with emotional stress as well as cardiac disease.

It is known that the PNS can change faster than the SNS. Thus, as the SNS starts to mediate a stress response the PNS immediately begins to counter it. If the PNS were not faster than the
5 SNS, then any stress response could send the heart into tachycardia and onto ventricular fibrillation before the PNS could act to prevent it.

The PNS through the Vagus have the main controlling influence on respiratory activity. PNS input to the heart is through fibers that synapse deep in the myocardium. SNS influence on the heart is through surface synapses. Due to this arrangement the PNS nerves are more critical to
10 preventing heart damage (i.e., infarct, ischemia, or cardiomyopathies). Since the PNS nerves are faster to respond, it is usually the branch that is first to indicate changes in health status anywhere in the body.

The ANS regulate the conduction system within the heart. It is due to the stimulation of SNS & PNS, there is generation of electrical conduction which record as various waves on log paper.

15

ECG

ECG is a graphical representation of the electrical activity of the heart. P, QRS complex and T are common waves produced during this electrical activity, as shown in Figure 1.

They represent:

- 20 • P wave: represents the depolarization impulse across the atria
- Q, R and S complex: all these three waves represent the ventricular depolarization (the downward stroke followed by an upward stroke is called Q wave and an further upward stroke is called R wave and any downward stroke preceded by an upward stroke is called S wave)
- 25 • T wave: represents the repolarization of the ventricles

Apart from these waves few intervals which are observed during this electrical activity are QT, QTc, PR and RR interval.

- 30 • QT interval: QT- interval provides a measure of ventricular repolarization and is determined by the balance of the repolarizing inward sodium and calcium currents, and the outward potassium and chloride currents. If there is cardiac vagal dysfunction it will

result in prolongation of QT interval and if there is stimulation of cardiac vagal nerve it will result in the shortening of the QT interval. QT interval represents both the dispersion and the lengthening of the action potential duration and correlates directly with the left ventricular mass. Thus in case of cardiac hypertrophy there is increase in ventricular mass and prolongation of ventricular repolarization, so the QT interval is prolonged. During animal toxicity studies if the test product shows prolongation of QT interval then that product is considered as having cardiotoxic properties and may harm the cardiovascular system.

- QTc: In humans and large animals, QT interval varies strongly and inversely with heart rate. For these reasons it is recommended to correct the QT interval which is independent of heart rate. This is called as corrected QT interval (QTc). Conditions such as Coronary artery diseases (CAD), Cardiomyopathy, severe Bradycardia, High-Grade AV Block, Anti-Arrhythmics, Psychotropic Drugs, Hypocalcemia and electrolyte imbalance, Congenital Long QT Syndrome, Hypothyroidism, leads to prolongation of QTc. From toxicological point of view it is important to see whether there is any prolongation of QTc.
- RR interval: RR interval is the interval from the peak of one QRS complex to the peak of next QRS complex. It is used to assess the ventricular rate / Heart Rate. Heart rate may increase by increase in sympathetic activity and decrease in parasympathetic activity and inversely true for decrease in Heart Rate. This sympathovagal balance is essential in maintaining Heart Rate and is believed to be reflected in the beat-to-beat changes of the cardiac cycle.
- PR interval: PR interval represents the time the impulse takes to reach the ventricles from the sinus node. Sympathetic activity increase shall lead to decrease in PR interval and parasympathetic activity increase to prolongation of PR interval.

Sudden Cardiac Death and Myocardial Infraction

Sudden cardiac death could be outcome of increased sympathetic activity and decreased parasympathetic activity. Increase in sympathetic activity leads to the increase in heart rate and tachycardia [6-8]. Reduction in sympathetic over-activation by beta blocker and calcium channel

blocker protect against arrhythmias [9-10]. On the other hand parasympathetic activity is preventive in case of arrhythmias and sudden cardiac death. Early study done by Eckberg *et al* [11] found that there is remarkable change in the parasympathetic activity in most of advanced diseased states. Some population based studies have linked the QT interval on a 12-lead electrocardiogram (ECG) with an increased risk of ventricular arrhythmias and sudden cardiac death in patients with coronary artery disease [12-14] and even in the general population [15-17]. Sudden cardiac death is often the first and only manifestation of coronary artery disease and cardiac arrhythmias. Most common treatment of choice is class III antiarrhythmic drugs. Many of class III antiarrhythmic drugs show adverse effect such as prolongation of QT interval, which leads to life threatening tachyarrhythmia, torsades de pointes (i.e., polymorphic ventricular tachycardia in which the QRS waves seem to “twist” around the baseline), and leads to the increase cardiac mortality [18,19]. This imbalance in sympathovagal tone can be measured by Heart Rate Variability (HRV). HRV primarily reflects tonic vegal activity.

15 **Heart rate variability (HRV)**

HRV is a measure of variation in the heart rate. Decrease in HRV is now considered as a strong predictor of Myocardial Infraction (MI), Arrhythmias and sudden Cardiac Death, which could be a result of insufficient adaptation of the ANS [20,21]. Whereas higher HRV is indicative of good adaptation by ANS. The most widely used methods can be grouped under time-domain ('how much variability') and frequency-domain ('how is variability caused?').

Heart Rate Variability (HRV) is considered as a measure of sympathovagal tone [21, 20]. It represents the most predominant quantitative markers of the autonomic tone. Sympathovagal tone will change with changing physiological and pathological conditions. HRV can be increased with respect to increase in physiological state such as endurance exercise and might be modulated by different body positions, sleep, etc. Pathological conditions like type 2 diabetes mellitus [22], high blood pressure [23], gastroesophageal reflux disease (GERD) [24], IBS [25], depression [26], COPD [27], etc, can alter the Sympathovagal tone and decrease the HRV.

Various studies of HRV, Billman *et al* [28 29], Kleiger *et al* [30], Sztajzel *et al* [31], have concluded that a higher HRV is reflection of compensated heart with good function. Any Autonomic imbalance/ disturbance will show up as lower HRV. Studies of Collins *et al* [32],

Gianaros *et al* [33], strengthen the observations of changes in HRV related to vulnerability to sudden cardiac death.

HRV is commonly used to assess the sympathovagal balance but it has some limitations. It has been found that HRV is of limited use in the certain conditions such as ectopic beats and artifacts, heart transplants, presence of arrhythmias and pacemakers which leads to false interpretation of HRV. HRV combined with ventricular ectopic beats, signal-averaged ECG, or left ventricular function results in 30 % - 50% increase in prediction power in sudden cardiac death and Myocardial Infraction.

Cardiac output:

Cardiac output is the volume of blood being pumped by the ventricle in a minute. An average cardiac output would be 5 L/min for a human male and 4.5 L/min for a female. Cardiac output is a symbol of function of heart. Every cell in the body needs oxygen and nutrients for there functioning. If the cells are working hard, with a high metabolic oxygen demand then the Cardiac Output is raised to increase the supply of oxygen to the cells. Apart from pumping of heart cardiac output is also regulated by the vascular resistance.

Cardiac Output increases when there is an increase in heart rate (HR), Change of posture, increased SNS activity, and decreased PNS activity. Contrary to this Vitamin K2-7 has decreased sympathetic activity and increased cardiac output.

Natural Products:

Thus ANS activity needs to be kept in balanced state, to maintain proper internal homeostasis. It has been seen that ANS activity can be influenced by many natural products such as CoQ10, Green tea, Capsicin, Bezoar, Glycrrhiza, n-3 PUFAs, Curcuminoids, Epigallocatechin gallate, Kava extract etc [34-44] Products with combination's of this ingredients have been used for the control of ANS activity; e.g. CCGC (combined capsicin, green tea, and chichen essence tablets) [35]. ANS activity of these ingredients has been questioned in many trials and several publications [39-44].

There are several patent addressing treatment of autonomic dysfunction through various modalities including receptor inhibition [US 7626015], gangliosides [US5190925], electrical modulation [US7363076], Malto-oligosaccharide [US5965557], Decahydroquinoline-based anti-

cholinergic agents [US5929087] and others [US5965557]. However, these patents & earlier described ingredients, mostly addressed ANS directly through the diseases associated with ANS. Measurement of ANS relates to a disease conditions. Tests to measure ANS dysfunction include Tilt table test where blood pressure is measured after the person, who is lying flat on a pivoting table, is tilted into an upright position. Valsalva maneuver can also be performed to measure the blood pressure. Also Sweat testing can be done either by acetylcholine stimulation or by dye method. Other tests may be done to check for disorders that can cause the autonomic disorder. Present investigation used direct ANS activity through US FD approved instrumentation. Inventors along with the investigator of the clinical and animal study observed that vitamin K2-7 (MK-7) produced marked effect on balancing the sympathovagal tone. This can lead to the use of MK-7 in amelioration and aid to protect treating of cardiovascular diseases, type 2 diabetes mellitus, high blood pressure, gastroesophageal reflux disease (GERD), IBS, depression, COPD, etc, where there is misbalancing of sympathovagal tone.

15 **Summary of the Invention**

In one aspect, the present invention provides a method of improving sympathovagal imbalance in mammals, the method comprising administering therapeutically effective amounts of vitamin MK-7 or other vitamin K analogues and their derivatives.

Vitamin K analogues of the invention include vitamin K1, MK-4, MK-6, MK-8, MK-9 and other molecules having vitamin K activity.

The sympathovagal imbalance as in the above method is altered in conditions selected from a group consisting of cardiovascular diseases, type 2 diabetes mellitus, high blood pressure and depression.

In another aspect, the invention provides a method of promoting cardio protective effect by bringing balance in sympathovagal tone wherein the method comprises administering vitamin MK-7 or other vitamin K analogues and their derivatives.

The cardio protective effect includes shortening of QT interval, shortening of QRS interval or prolongation of RR interval when administered with vitamin MK-7 or other vitamin K analogues and derivatives.

In yet another aspect, the invention provides a method of increasing cardiac output in a mammal comprising administering vitamin MK-7 or other vitamin K analogues and their derivatives.

The present invention also provides a method of increasing left cardiac work in a mammal, comprising administering vitamin MK-7 or other vitamin K analogues and their derivatives.

5 In another aspect, the invention provides a method of increasing HDL in a mammal, comprising administering vitamin MK-7 or other vitamin K analogues and their derivatives.

The invention also encompasses a method of reducing blood sugar and improving insulin sensitivity in a mammal, comprising administering vitamin MK-7 or other vitamin K analogues and their derivatives.

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Brief Description of Drawings:

Figure 1: ECG with commonly observed wave patterns.

Figure 2: ANS activity indices at day 0 in a healthy volunteer.

Figure 3.1: ANS activity indices at day 0 in volunteer 1.

15 Figure 3.2: ANS activity indices at day 62 in volunteer 1.

Figure 4.1: ANS activity indices at day 0 in volunteer 2.

Figure 4.2: ANS activity indices at day 58 in volunteer 2.

Figure 5: ECG changes in rat model with 500µg of MK-7 (Figure 5.1: in Male Rats; Figure 5.2: in Female Rats)

20 Figure 6: ECG changes in rat model with 1000µg of MK-7 (Figure 6.1: in Male Rats; Figure 6.2: in Female Rats)

Description of Invention:

Effect on ANS:

25 During the course of investigation with MK-7 (PCT/IN2010100014), inventors of current application serendipitously discovered that MK-7 has effect on sympathetic and parasympathetic activity.

The investigator studied the effect of MK-7 on sympathovagal balance on 4 healthy male volunteers of age between 34 to 50 yrs for 2 months. ANS activity was measured and printed out
30 in a graph form by the ANSiscope™, manufactured by DyAnsys a US based company. The

instrument is US FDA approved patented instrument. This instrument assessed the autonomic function by calculating 500 Heart beats / RR intervals. After assessing this, the instrument will analyze the level of the dysfunction and the degree of autonomic neuropathy. For this reason they have classified autonomic dysfunction in 5 stages, namely Healthy (H), Early (E), Late (L),
5 Advanced (A) and Most Advanced (MA). The percentage calculation values demarcating groups are: -11.5% to 11.5%: healthy group, 13.5% to 20%: early group, 23% to 50.99%: late group, 51% to 100%: advanced group and above falls in Most advance group [42].

Fig.2 is an ANS Activity Indices of a Male Healthy Volunteer at the age of 44 yrs. As can be seen, the measured Parasympathetic and Sympathetic activities are within -11.5 to 11.5 % which
10 as classified above is for healthy group. Our human study examples, given later on, show the variabilities that exist in individuals at different stage of health in life.

The study parameters assessed were sympathetic and parasympathetic tone, change in blood pressure (orthostatic intolerance, measured at right. brachial artery), pulse (assessed by measuring the pulse at right. radial artery) and physical and general examination. Study was
15 performed on subjects in relaxed supine position without any external stimulation, usually 2 hrs. after breakfast during morning time for 2 consecutive months.

During the study of the pharmacological activity and tolerability of vitamin MK-7, on ANS in Healthy volunteers, Inventors discovered that dysfunction of ANS if present was getting
harmonized.

20 Subjects who have Blood pressure beyond the range of 140/90 (supine) and 100/70 (standing) mm Hg, and Sugar Fasting more than 250 mg% and with any major illness are ruled out of study. All subjects were checked for baseline parameters on 0 day. Vitamin K2-7 (MK-7) in dose of 350 µg was administered on 3rd day after the baseline reading. MK-7 was continued for 2 months. Readings were taken on 3rd day, 15th day, 28st day, 50th day, 56th day and at 62nd day. It
25 was observed that after 2 months of therapy with MK-7, there was a marked normalization in the sympathetic activity which was initially elevated, with little effect on parasympathetic activity (Figure 3 and 4). Other parameters remained unaffected.

This study provides window for the use of MK-7 in maintaining sympathovagal balance and can also be used as protective and preventive in cardiovascular diseases. Further studies with
30 statistical sample size are needed to define efficacy of MK-7 in balancing ANS activity.

In another study on animal model during chronic toxicity study, it was discovered that MK-7 produces significant changes in ECG of rats. This discovery during basic pharmacology studies on MK-7 was investigated for its effect on Cardio Vascular System. It is during this study the investigator discovered the following findings (Figures 5 and 6).

- 5 The main parameter which was observed during chronic toxicity studies in rat ECG was the prolongation of QT interval. During toxicity studies if the test product shows prolongation of QT interval then that product is consider as having cardiotoxic properties and may harm the cardiovascular system. In this particular study the QT interval remained unaffected at all the three dose level i.e. at 100, 500, 1000 $\mu\text{g}/\text{kg}$.
- 10 In humans and large animals, QT interval varies strongly and inversely with heart rate. So it is advisable to have QTc. In this particular toxicity study conducted on rats, it did not cause any prolongation of QTc, whereas on another side the test drug causes moderate non-significant shortening of QTc.

QRS interval is indicative of ventricular depolarization and intra ventricular conduction time. In this particular toxicity study conducted on rats there was no increase in heart rate, which rules out supraventricular causes. There was significant shortening of QRS interval observed at 500, 1000 $\mu\text{g}/\text{kg}$ dose level.

Also there were significant prolongation of RR interval complex was observed at 500, 1000 $\mu\text{g}/\text{kg}$ dose level. These indicate that MK-7 causes prolongation of RR interval either by inhibition of sympathetic tone or stimulation of fast acting parasympathetic tone.

20 The PR interval was not affected to significant extent hence there is no influence of the drug on impulse transmission from atria to ventricle.

Thus the effects of MK-7 seem to be limited only to the ventricle since it is not reflected in changes in the PR interval.

25

Effect on Cardiac Output parameters:

During the yet another study conducted to assess the cardioprotective effect of vitamin K2-7, inventors of the current invention observe that the vitamin K2-7 improves the cardiac output parameters. Isoproterenol (ISO) induced cardiac injury model was selected for the study. It was observed that ISO induced cardiac output decrease in all the dose levels i.e. at 90 $\mu\text{g}/\text{kg}$, 450

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$\mu\text{g}/\text{kg}$ and $900 \mu\text{g}/\text{kg}$. This ISO induced cardiac output decrease was found to be reversed in $90 \mu\text{g}/\text{kg}$ and $450 \mu\text{g}/\text{kg}$ dose test drug administered ISO injected rats. This effects was non-linear and was enhanced in $900 \mu\text{g}/\text{kg}$ dose.

Left cardiac work:

5 Effect of vitamin K2-7 on the left cardiac work has been evaluated in the same study. It was observed that administration of ISO leads to marked decrease in left cardiac work when measured 48h after ISO injection in comparison to pre ISO level i.e. control. This ISO induced left cardiac work decrease was found to be reversed to a great extent at all the dose levels i.e. at $90 \mu\text{g}/\text{kg}$, $450 \mu\text{g}/\text{kg}$ and $900 \mu\text{g}/\text{kg}$ with ISO injected rats in comparison to ISO control. It was
10 found that at the dose level of $900 \mu\text{g}/\text{kg}$ decrease in reversal was higher than other treatment groups.

HDL (High Density Lipoprotein) cholesterol level:

In the same study it was also observed that vitamin K2-7 enhances the High Density Lipoprotein (HDL) level in all the dose levels studied i.e. at $90 \mu\text{g}/\text{kg}$, $450 \mu\text{g}/\text{kg}$ and $900 \mu\text{g}/\text{kg}$.
15 Isoproterenol (ISO) induced cardiac injury model was selected for the study. In Isoproterenol control group significant decrease in HDL cholesterol level was observed in comparison to normal control group. HDL cholesterol level for the treated group increased in comparison to the ISO controlled group. The observed increase was greater with respect to the dose level increased.

20 **Blood Sugar level:**

In our previous patent PCT/IN2010100014, we have described that vitamin K2, helps in reducing insulin resistances and bringing down blood sugar. Inventors, while conducting animal study have found that vitamin K2-7 helps in reducing blood sugar within 12 days of time at the dose level of $90 \mu\text{g}/\text{kg}$, $450 \mu\text{g}/\text{kg}$ and $900 \mu\text{g}/\text{kg}$. These findings are in consistent with our earlier
25 findings reported in the PCT/IN2010100014.

From the above novel, serendipitously observed finding it can be even proposed that the our cardioprotection claim is based upon several beneficial effects observed of Vitamin MK including deferent body system including ANS, Cardiac Output Parameters, Left cardiac work, HDL and Blood sugar level implying improved insulin sensitivity and enhance energy
30 utilization.

Definitions:

1. As used here in the term “ANS” refers to the Autonomic Nervous system.
2. As used here in the term “SNS” refers to the Sympathetic Nervous system.
- 5 3. As used herein the term “PNS” refers to the Parasympathetic Nervous system.
4. As used herein the term “SA Node” refers to the Sino Atrial Node.
5. As used herein the term “AV Node” refers to the Atrio Ventricular Node.
6. As used herein the term “HRV” refers to the Heart Rate Variability.
7. As used herein the term “BRS” refers to the baroreflex sensitivity.
- 10 8. As used herein the term “PUFAs” refers to the Poly unsaturated fatty acid.
9. As used herein the term “GERD” refers to the gastroesophageal reflux disease
10. As used herein the term “IBS” refers to the Inflammatory bowel syndrome.
11. As used herein the term “COPD” refers to the Chronic obstructive pulmonary diseases.
12. As used herein the term “CVD” refers to the Cardiovascular diseases.
- 15 13. As used herein the term “AP” refers to the Action Potential.
14. As used herein the term “MK-7” refers to the Vitamin K2-7.
15. As used herein, Vitamin K analogues of the invention include but not restricted to
vitamin K1, MK-4, MK-6, MK-8, MK-9 and other molecules having vitamin K activity.
16. As used here in the term “dromotropic effect” refers to increase in AP conduction
20 velocity
17. As used herein, ‘improving sympathovagal imbalance’ encompasses improvement in the
sympathetic & parasympathetic activity.
18. As used herein, ‘promoting cardio protective effect’ refers to the protective action of
MK-7 on the cardiovascular system.
- 25 19. As used herein, ‘increasing cardiac output’ refers to the action of MK-7 on the
cardiovascular system by enhancing cardiac output.
20. As used herein, ‘increasing left cardiac work’ refers to the action of MK-7 on the
cardiovascular system by enhancing the left cardiac work.
21. Mammals as used in the disclosure refers to but not limited to human beings.

30

Case Studies:**Example 1:**

Male, 50 yrs, otherwise healthy was accessed for the ANS activity by the ANSiscope™, manufactured by DyAnsys a US based company on 28th of April 2009. His percentage of dysfunction was 61% which fall under the 'Advance stage'. General and systemic examination including Blood Pressure (Supine) and Pulse was measured, which was in normal range. This was considered as a Baseline readings. On 3rd day from his baseline reading he was then put on 1 cap per day of 350 µg, vitamin K2-7 for 2 months after breakfast at around 8 am in morning. He was accesses regularly initially at 1 weeks of interval in first month and at interval of 15 days in next month for ANS activity, B.P. and pulse. At the end of 2 months his percentage of ANS dysfunction changed from 61 % to 31%, which falls under 'Early stage'. Other parameters such as general and systemic examination including Blood Pressure (Supine), pulse were in normal range without any change from base line records. He was also feeling very energetic and can even work for longer time without any tiredness. (FIG. 3.1 and 3.2)

Example 2:

Male, 32 yrs, otherwise healthy was accessed for the ANS activity by the ANSiscope™, manufactured by DyAnsys a US based company on 28th of April 2009. His percentage of dysfunction was 25% which fall under the 'Late stage'. General and systemic examination including Blood Pressure (Supine) and Pulse was measured, which was in normal range. This was considered as a Baseline readings. On 3rd day from his baseline reading he was then put on 1 cap per day of 350 µg, vitamin K2-7 for 2 months after breakfast at around 8 to 8.30 am in morning. He was accesses regularly for two months at interval of 15 days for ANS activity, B.P. and pulse. At the end of 2 months his percentage of ANS dysfunction changed from to 21%, which falls under 'Early stage' of dysfunction. Other parameters such as general and systemic examination including Blood Pressure (Supine), pulse were in normal range without any change from base line records. (FIG. 4.1 and 4.2).

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Claims

We claim:

1. A method of improving sympathovagal imbalance in mammals, the method comprising administering therapeutically effective amounts of vitamin MK-7 or other vitamin K analogues and their derivatives.
5
2. The method of improving sympathovagal imbalance by administering vitamin MK-7 as in claim 1 wherein the sympathovagal imbalance is altered in conditions selected from a group consisting of cardiovascular diseases, type 2 diabetes mellitus, high blood pressure and depression.
- 10 3. The method of improving sympathetic activity as in claim 1, wherein the said method comprises administering vitamin MK-7 or other vitamin K analogues and their derivatives.
4. A method of promoting cardio protective effect by bringing balance in sympathovagal tone wherein the method comprises administering vitamin MK-7 or other vitamin K analogues and their derivatives.
15
5. The method of promoting cardio protective effect as in claim 4, wherein the shortening of QT interval comprises administering vitamin MK-7 or other vitamin K analogues and their derivatives.
6. The method of promoting cardio protective effect as in claim 4, wherein the shortening of QRS interval comprises administering vitamin MK-7 or other vitamin K analogues and derivatives.
20
7. The method of promoting cardio protective effect as in claim 4, wherein the prolongation of RR interval comprises administering vitamin MK-7 or other vitamin K analogues and their derivatives.
- 25 8. A method of increasing cardiac output in a mammal, comprising administering vitamin MK-7 or other vitamin K analogues and their derivatives.
9. A method of increasing left cardiac work in a mammal, comprising administering vitamin MK-7 or other vitamin K analogues and their derivatives.

10. A method of increasing HDL in a mammal, comprising administering vitamin MK-7 or other vitamin K analogues and their derivatives.

11. A method of reducing blood sugar and improving insulin sensitivity in a mammal, comprising administering vitamin MK-7 or other vitamin K analogues and their derivatives.

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Figures

Figure 1: Common waves patterns of ECG

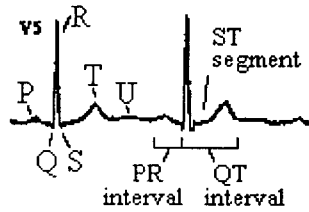


Figure 2: Healthy Volunteer

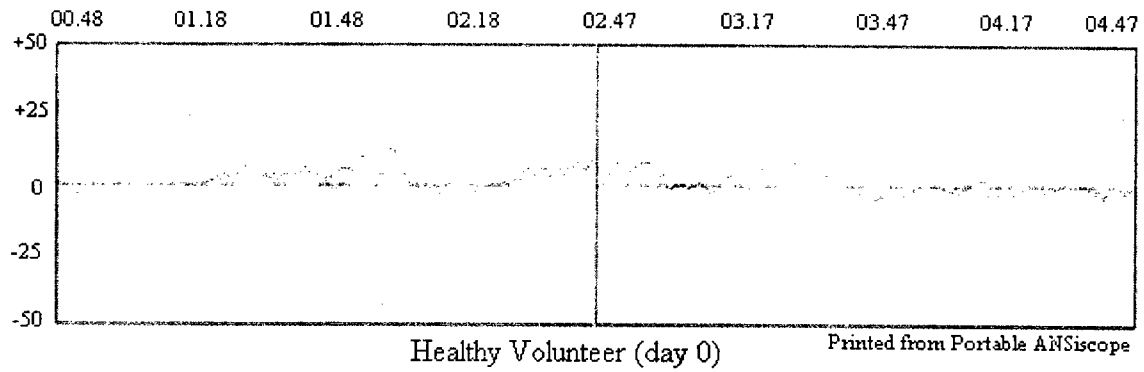


FIG. 2. ANS Activity Indices at day 0
Volunteer, M/44
Result: 12 % **Healthy** Autonomic Dysfunction Level 7 % + 5 % age related

Figure 3: Volunteer 1

FIG. 3.1: Mr; SJ_0

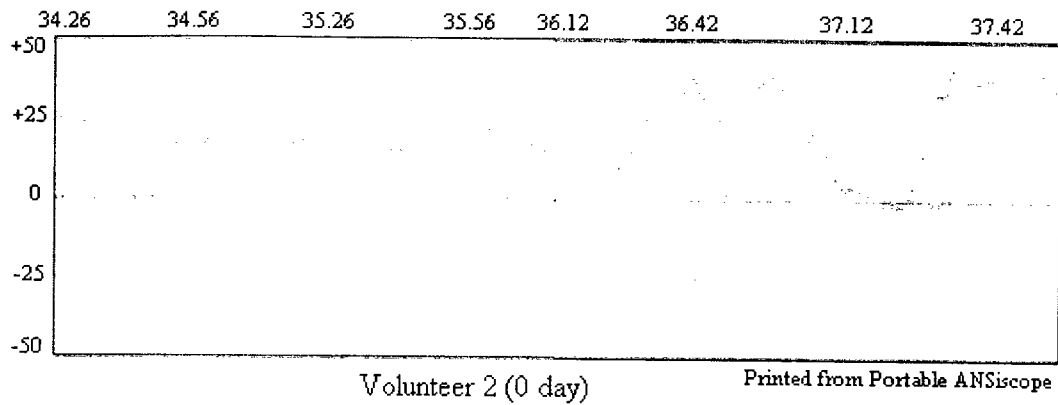


FIG. 3.1. ANS Activity Indices at day 0
Volunteer 1, M/50, 72 kg
Result: 61 % **Advanced** Autonomic Dysfunction Level 54 % + 7 % age related

FIG. 3.2: Mr; SJ_62

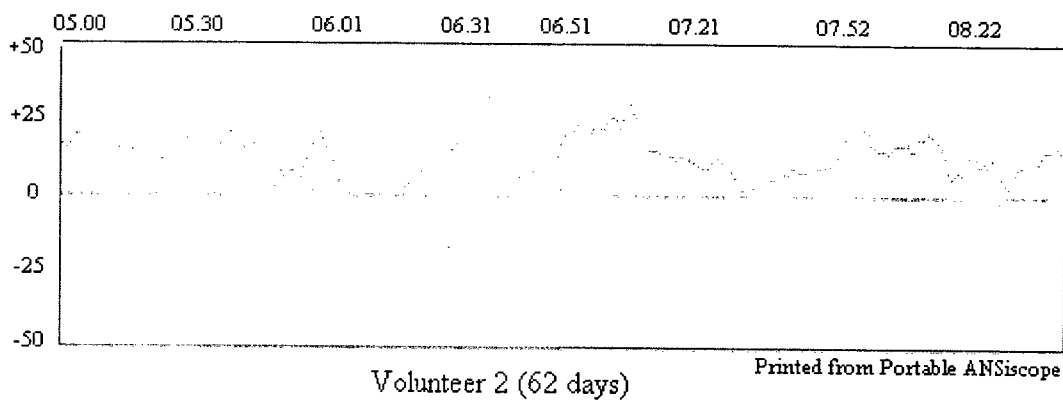


FIG. 3.2. ANS Activity Indices at day 62
Volunteer 1, M/50, 72 kg
Result: 31 % **Late** Autonomic Dvsfunction Level 28 % + 7 % age related

Figure 4: Volunteer 2

FIG. 4.1: Mr; SN_0

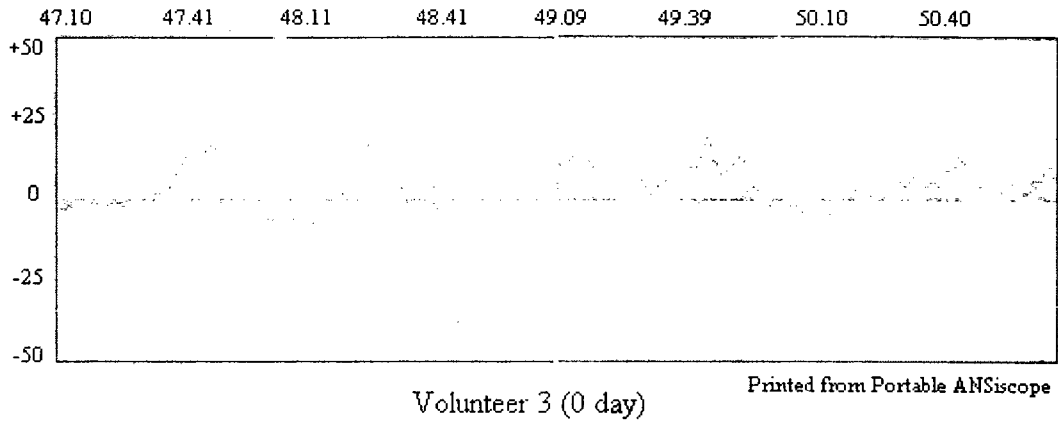


FIG. 4.1. ANS Activity Indices at day 0
Volunteer 2, M/32, 77 kg
Result: 25 % **Late** Autonomic Dvsfunction Level 25 % + 0 % age related

FIG. 4.2: Mr; SN_58

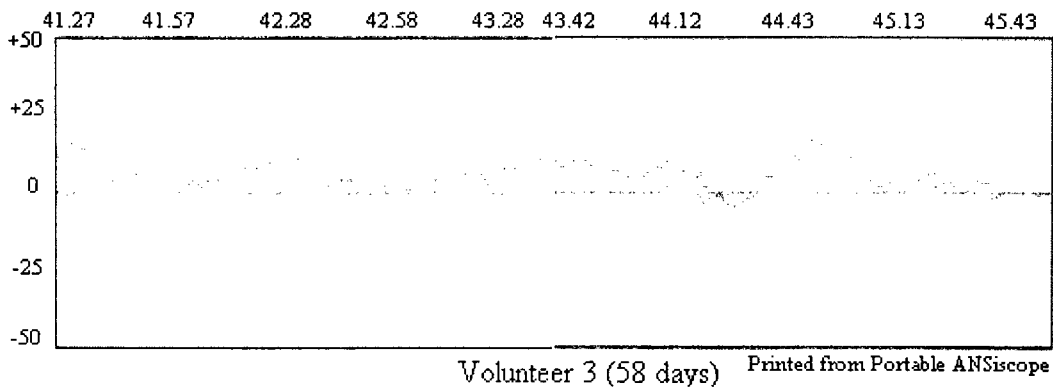


FIG. 4.2. ANS Activity Indices at day 58
Volunteer 2, M/32, 77 kg
Result: 21 % **Early** Autonomic Dysfunction Level 25 % + 0 % age related

⊗ Para

■ Sym

Figure 5: ECG changes in rat model with 500 µg of MK-7

5.1. ECG changes in male rats



Male Rat - 1



Male Rat - 2

5.2. ECG changes in female rats



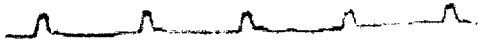
Female Rat - 1



Female Rat - 2

Figure 6: ECG changes in rat model with 1000µg of MK-7

6.1. ECG changes in male rats



Male Rat - 1

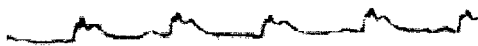


Male Rat - 2

6.2. ECG changes in female rats



Female Rat - 1



Female Rat - 2