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(54) Title: USE OF A WITHANIA EXTRACT FOR THE TREATMENT OF NEUROMUSCULAR DISEASES

(57) Abstract: The invention relates to the use of a composition from a plant extract of Withania somnifera, to treat or limit development of neuromuscular diseases, including motor neuron diseases like amyotrophic lateral sclerosis.

Use of a withania extract for the treatment of neuromuscular diseases

The invention relates to the use of a composition from a plant extract of *Withania somnifera,* to treat or limit development of neuromuscular diseases, including motor neuron diseases like amyotrophic lateral sclerosis.

Neuromuscular diseases are diseases that affect the muscles and/or their direct nervous system control. A large proportion of these neurological disorders leads to problems with movement. Neuropathies involve dysfunction of the peripheral nerves, called motor neurons, which carry the electrical signals directly from the spinal cord and brain stem to activate muscle movement; the sensory neurons which convey sensory information such as pain, temperature, light touch, vibration and position to the brain; and the autonomic neurons which go to the internal organs and control blood vessel reflexes.

Motor neuron diseases or disorders (MNDs) are characterized by progressive loss of motor neurons of the spinal cord ('lower motor neurons' (MN)) or motor neurons of the brain ('upper motor neurons'), or both, leading to atrophy and/or spasticity of the associated musculature. Spinal muscular atrophy (SMA), amyotrophic lateral sclerosis (ALS) and hereditary spastic paraplegia (HSP) are the most common MNDs.

When there are disruptions in the signals between the upper motor neurons and the lower motor neurons, the limb muscles develop stiffness *{spasticity),* movements become slow and effortful, and tendon reflexes such as knee and ankle jerks become overactive. Over time, the ability to control voluntary movements (such as speaking, walking, breathing, and swallowing) can be lost.

MNDs occur in adults and children. In children, particularly in inherited or familial forms of the disease, symptoms can be present at birth or appear before the child learns to walk. In adults, MNDs occur more commonly in men than in women, with symptoms appearing after age 40.

The causes of most MNDs are not known. In sporadic or non-inherited MNDs, environmental, toxic, viral, or genetic factors may be implicated.

MNDs are classified according to whether they are inherited or sporadic, and to whether degeneration affects upper motor neurons, lower motor neurons, or both.

ALS affects both upper and lower motor neurons. It has inherited and sporadic forms and can affect the arms, legs, or facial muscles. Although the majority of ALS cases are sporadic, up to 10% are inherited (Robberecht & Philips, 2013) and the most common familial forms of ALS in adults are caused by mutations of the superoxide dismutase gene, or SOD1, located on chromosome 21. There are also rare juvenile-onset forms of familial ALS.

Primary lateral sclerosis is a disease of the upper motor neurons, while progressive muscular atrophy affects only lower motor neurons in the spinal cord.

In progressive bulbar palsy, the lowest motor neurons of the brain stem are most affected, causing slurred speech and difficulty chewing and swallowing. There are almost always mildly abnormal signs in the arms and legs.

Spinal muscular atrophy (SMA) is a neurodegenerative disease presented clinically by progressive degeneration of lower motor neurons in the anterior horn of the spinal cord (SC), resulting in hypotonia, muscle atrophy, paralysis and, in severe cases, death (Khaniani *et al.,* 2013). It is a genetically heterogeneous disorder, with most cases displaying a recessive inheritance; however, autosomal dominant and X-linked inheritances have been reported (Jiang *et al.,* 2013).

Hereditary spastic paraplegia (HSP) is the collective term for a group of clinically and genetically heterogeneous neurodegenerative disorders characterized by progressive spasticity and weakness in the lower limbs due to loss of upper motor neurons (Harding, 1983). The clinical heterogeneity of HSP is related to a notable genetic heterogeneity.

There is no cure or standard treatment for the MNDs. Symptomatic and supportive treatment can help people be more comfortable while maintaining their quality of life. Research has provided evidence about the role of excitotoxicity in the pathophysiology of sporadic amyotrophic lateral sclerosis and suggests that glutamate receptors activation contributes greatly in mediating injury to motor neurons.

The drug riluzole (Rilutek®), the only prescribed drug approved by the U.S. Food and Drug Administration to treat ALS, prolongs life by 2-3 months but does not relieve symptoms. The drug reduces the body's natural production of the neurotransmitter glutamate, which carries signals to the motor neurons.

Other medicines may help with symptoms. Muscle relaxants and the benzodiazepines may reduce spasticity. Anticonvulsants and nonsteroidal anti-inflammatory drugs may help relieve pain, and antidepressants may be helpful in treating depression. Some individuals may eventually require stronger medicines such as morphine to cope with musculoskeletal abnormalities or pain, and opiates are used to provide comfort care in terminal stages of the disease.

Thus, there is a clear need for alternative therapies that prevent progression and hopefully reverse the motor neuron loss that occurs in this devastating condition.

It has been reported that extracts of *Withania somnifera*, *Emblica officinalis* and *Bacopa monnieri* show anti-angiogenic activity. However, the extracts of these plants were not used because of the high toxicity related to the extracts obtained, and, in particular, the extract from *Withania somnifera.*

Surprisingly, the applicant has found that, by combining an extraction step and a fermentation step using filamentous fungi on the extracts of the plant *Withania somnifera*, it is possible to use the detoxified extract to treat MNDs and other pathologies of progressive neurological disorders.

The purpose of the invention is therefore to use a non-toxic composition based on extracts of *Withania somnifera*, having a

protective effect against motor neuron diseases to treat or limit development of ALS and related neuron disorders.

Other objects, features, aspects and advantages of the invention will appear more clearly on reading the description and examples that follow:

Figure 1: Effect of WEB-2 on Motor neurons survival. Data were expressed as percentage of control as mean ± SEM (100% = control no glutamate). Statistical analyses were performed using the Graph pad prism for one-way ANOVA followed by Dunnett's test. $p < 0.05$ was considered significant. * $p < 0.05$ vs glutamate condition.

Figure 2: Effect of WEB-2 or riluzole on mean size (2a) or on number (2b) of NMJ after glutamate injury. Data were expressed as percentage of control as mean ± SEM (100% = control no glutamate). * $p < 0.05$ vs glutamate condition.

Figure 3: Effect of WEB-2 or riluzole (5 µM) on the total neurite network after glutamate injury. Data were expressed as percentage of control as mean ± SEM (100% = no glutamate). * $p < 0.05$ vs glutamate group.

Figure 4: Effect of WEB-2 on Motor neurons survival (4a) and neurite network (4b) after 3 days of culture. Data were expressed as percentage of control as mean ± SEM (100% = control). Statistical analyses were performed using the Graph pad prism for one-way ANOVA followed by Dunnett's test. $p < 0.05$ was considered significant. * $p < 0.05$ vs control.

Figure 5: Effect of WEB-2 on Motor neurons survival (5a) and neurite network (5b) after 5 days of culture. Data were expressed as percentage of control as mean ± SEM (100% = control). Statistical analyses were performed using the Graph pad prism for one-way ANOVA followed by Dunnett's test. $p < 0.05$ was considered significant. * $p < 0.05$ vs control.

Figure 6: Effect of WEB-2 or riluzole on Motor neurons survival. Data were expressed as percentage of control as mean ± SEM (100% = control no glutamate). Statistical analyses were performed using the Graph pad prism for one-way ANOVA followed by Dunnett's

test. $p < 0.05$ was considered significant. * $p < 0.05$ vs glutamate condition.

Figure 7: Effect of WEB-2 or riluzole on Motor neurons survival at day 1 and after 5 days of culture. Data were expressed as percentage of control as mean ± SEM (100% = control). Statistical analyses were performed using the Graph pad prism for one-way ANOVA followed by Dunnett's test. $p < 0.05$ was considered significant. * $p < 0.05$ vs control.

The invention is directed to a composition containing a *Withania somnifera* extract for its use to treat or limit development of motor neuron diseases in a mammal. Preferably, the mammal is a human.

Preferably, the *Withania somnifera* extract has been fermented by its incubation with a filamentous fungus in a suitable environment.

The *Withania somnifera* plant is obtained from India. The root of this plant is marketed by Alp Erbo (Marseille).

The process of production of extracts according to the invention can be found in WO 2014/202469. Briefly, the plants are fermented in presence of a filamentous fungus of the family *Cordycipitaceae,* preferably the genus Beauveria. More preferably, the filamentous fungus is derived from the strain *Beauveria bassiana*, more particularly the strain having reference ATCC 7159. Preferably, the roots of the plant are used.

The controlled fermentation detoxifies the *Withania Somnifera* extract by a series of biocatalysis of various molecules contained in this extract and, more particularly, the chemical family of withanolide aglycones, the substances mainly responsible for the toxicity of the extract.

The term "detoxification" is used to mean elimination by the microorganism of potentially toxic molecules in the medium.

Preferably, after the fermentation, filtration, the medium is then subjected to sterilization steps, preferably by ultrafiltration, in order to obtain the solution which constitutes the plant extract.

The plant extract of the invention contains *Withania somnifera* but may also contain at least one of the following extracts: *Emblica*

officinalis, originating from India and marketed by Infrag, Bengalore), *Bacopa monnieri* (India) marketed by Alp Erbo (Marseille), *Punica granatum* (China) (Shanghai Brightol International Co, Ltd (Shanghai), *Curcuma longa* (India) (Omnipharm, Chambery), *Piper longum* (Thailand) (Omnipharm, Chambery), or *Calendula officinalis* (China) (Shanghai Brightol International Co, Ltd (Shanghai), using the same procedure), by independent extraction steps for each plant extract used in the realization of the said preparation.

Advantageously, the composition used in this invention includes between 5 and 100 g/L of *Withania somnifera,* preferably 20 g/L. Preferentially, this composition also includes one of the following extracts:

- between 5 and 100 g/L of *Emblica officinalis,* preferably 15 g/L,
- between 5 and 100 g/L of *Bacopa monnieri,* preferably 15 g/L,
- between 5 and 50 g/L of *Punica granatum,* preferably 10 g/L,
- between 5 and 250 g/L of *Curcuma longa,* preferably 20 g/L,
- between 20 and 50 mg/L of *Piper longum,* preferably 30 mg/L,
- between 5 and 50 g/L of *Calendula officinalis,* preferably 10 g/L,

Preferably, the composition used in this invention comprises an extract of the plants *Withania somnifera, Emblica officinalis* and *Bacopa monnieri.* More preferably, the composition according to the invention comprises *Withania somnifera* at a concentration of 20 g/L, of *Emblica officinalis* at a concentration of 15 g/L and of *Bacopa monnieri* at a concentration of 15 g/L.

The compositions according to the invention are used to treat or limit development of MN diseases like ALS (amyotrophic lateral sclerosis), PBP (progressive bulbar palsy), PMA (progressive muscular atrophy), PLS(primary lateral sclerosis), SMA (spinal muscular atrophy), Kennedy's disease, PPS (Post-polio syndrome), PPMA (Post-Polio Muscular Atrophy), MMN (Multifocal motor neuropathy), MMA (Monomelic amyotrophy), paraneoplastic motor neuron disease, LEMS

(Lambert-Eaton Myasthenic Syndrome), MG (Myasthenia gravis) and botulism, in particular by limiting degeneration of motor neurons.

Amyotrophic lateral sclerosis (ALS), also called Lou Gehrig's disease or classical motor neuron disease, is the most common form of MND, with both upper and lower motor neuron involvement. This form of the disease is characterized by weakness and wasting in the limbs. Muscle weakness and atrophy occur on both sides of the body. Affected individuals lose strength and the ability to move their arms and legs, and to hold the body upright. Other symptoms include spasticity, spasms, muscle cramps, and fasciculations. Speech can become slurred or nasal. When muscles of the diaphragm and chest wall fail to function properly, individuals lose the ability to breathe without mechanical support. Although the disease does not usually impair a person's mind or personality, several recent studies suggest that some people with ALS may develop cognitive problems involving word fluency, decision-making, and memory. Most individuals with ALS die from respiratory failure, usually within 3 to 5 years from the onset of symptoms.

Progressive bulbar palsy (PBP) involves both the upper and lower motor neurons. Symptoms include pharyngeal muscle weakness (involved with swallowing), weak jaw and facial muscles, progressive loss of speech, and tongue muscle atrophy. Limb weakness with both lower and upper motor neuron signs is almost always evident but less prominent. Affected persons have outbursts of laughing or crying (called *emotional lability).* In about 25 % of individuals with ALS, early symptoms begin with bulbar involvement. Some 75 % of individuals with classic ALS eventually show some bulbar involvement. Life expectancy is between six months and three years from onset of symptoms.

Progressive muscular atrophy (PMA) affects only a small proportion of people, mainly causing damage to the lower motor neurons. Early symptoms may be noticed as weakness or clumsiness of the hand. Most people live for more than five years.

Primary lateral sclerosis (PLS) is a rare form of MND involving the upper motor neurons only, causing mainly weakness in the lower

limbs, although some people may experience clumsiness in the hands or speech problems. It occurs when specific nerve cells in the motor regions of the cerebral cortex gradually degenerate, causing the movements to be slow and effortful. Difficulty with balance may lead to falls. PLS is more common in men than in women, with a very gradual onset that generally occurs between ages 40 and 60. The cause is unknown. The symptoms progress gradually over years, leading to progressive stiffness and clumsiness of the affected muscles. The disorder is not fatal but may affect quality of life, if it develops into ALS.

Spinal muscular atrophy (SMA) is an autosomal, hereditary recessive disorder caused by defects in the gene SMN1. In SMA, insufficient levels of the SMN protein lead to degeneration of the lower motor neurons, producing weakness and wasting of the skeletal muscles. This weakness is often more severe in the trunk and upper leg and arm muscles than in muscles of the hands and feet.

Kennedy's Disease, also known as progressive spinobulbar muscular atrophy, is an X-linked recessive progressive disorder of the motor neurons caused by mutations in the gene for the androgen receptor. Symptoms include weakness and atrophy of the facial, jaw, and tongue muscles, leading to problems with chewing, swallowing, and changes in speech. Early symptoms may include muscle pain and fatigue. Individuals with Kennedy's disease also develop sensory loss in the feet and hands. It only affects men, but women may carry the mutation. The course of the disorder is generally slowly progressive. Individuals tend to remain ambulatory until late in the disease. The life expectancy for individuals with Kennedy disease is usually normal.

Post-polio syndrome (PPS) is a condition that can strike polio survivors decades after their recovery from poliomyelitis. Polio is an acute viral disease that destroys motor neurons. PPS and Post-Polio Muscular Atrophy (PPMA) are thought to occur when the surviving motor neurons are lost in the aging process or through injury or illness. Symptoms include fatigue, slowly progressive muscle weakness, muscle atrophy, fasciculations, cold intolerance, and muscle and joint pain.

These symptoms appear most often among muscle groups affected by the initial disease, and may consist of difficulty breathing, swallowing, or sleeping. PPS is not usually life threatening. Doctors estimate that 25 to 50 percent of survivors of paralytic poliomyelitis usually develop PPS.

Multifocal motor neuropathy (MMN) is a progressively worsening condition where muscles in the extremities gradually weaken. MMN is thought to be autoimmune and involves only lower motor nerves. MMN usually involves very little pain however muscle cramps, spasms and twitches can cause pain for some sufferers. MMN is not fatal, and does not diminish life expectation.

Monomelic amyotrophy (MMA) is an untreatable, focal motor neuron disease that primarily affects young males in India and Japan. MMA is marked by insidious onset of muscular atrophy, which stabilizes at a plateau after two to five years from which it neither improves nor worsens.

Paraneoplastic motor neuron disease is a disease affecting the motor neurons.

Lambert-Eaton Myasthenic Syndrome (LEMS) is a rare autoimmune disorder characterized by muscle weakness of the limbs. Around 60% of those with LEMS have an underlying malignancy, most commonly small cell lung cancer; it is therefore regarded as a paraneoplastic syndrome.

Myasthenia gravis (MG) leads to fluctuating muscle weakness and fatigue. In the most common cases, muscle weakness is caused by circulating antibodies that block acetylcholine receptors at the postsynaptic neuromuscular junction, inhibiting the excitatory effects of the neurotransmitter acetylcholine on nicotinic receptors at neuromuscular junctions. Alternatively, in a much rarer form, muscle weakness is caused by a genetic inherited defect in some portion of the neuromuscular junction.

Botulism, a rare and potentially fatal illness caused by a toxin produced by the bacterium *Clostridium botulinum*, prevents muscle

contraction by blocking the release of acetyl choline, thereby halting postsynaptic activity of the neuromuscular junction.

In certain embodiments of the invention, the methods and compositions treat, limit development or reduce the progression of ALS in particular.

As used herein, "treating" ALS means providing any clinical benefit to a subject with ALS. The clinical benefit may be temporary or long-lasting. In various non-limiting embodiments, the treatment results in one or more clinical outcome selected from the group consisting of:

(a) decrease in ALS disease progression;

(b) decrease in ALS disease severity;

(c) decrease in ALS clinical symptoms;

As used herein, "reducing the progression" or "limiting development" of ALS means providing a limitation in development of symptoms or disease in a subject that is at risk of developing ALS.

In some embodiments, there is a method of treating or limiting development of a neuromuscular disease in a subject, comprising the step of administering to the subject a therapeutic amount of a plant extract composition, such that said neuromuscular disease in a subject is treated or its development limited, wherein said composition contains a non toxic plant extract of *Withania somnifera.*

The neuromuscular diseases comprise MN diseases, ALS, PBP, PMA, PLS, SMA, Kennedy's disease, PPS, PPMA, MMN, MMA, paraneoplastic motor neuron disease, LEMS, MG and botulism.

Preferably, the subject is a human.

The composition according to the invention is formulated for oral or parenteral administration.

A person skilled in the art of pharmaceutical formulations will implement the various useful forms for administration of the compositions and/or supplements of the invention. The compositions may be in liquid, gel, emulsion, solid or injectable form.

The composition used may additionally include suspensions, emulsions, syrups containing conventionally used inert diluents, and possibly other substances such as wetting agents, sweeteners,

preservatives, thickeners, colourings or any other substance known to a person skilled in the art suitable for oral administration, in particular ((sodium sorbate (E20 1) (Sigma-Aldrich), anthocyanin (E 163) (FBC Industries, USA), sodium metabisulphite (E223) (Sigma-Aldrich), alpha-tocopherol (E307) (FBC Industries, USA).

The composition used may also comprise solvents or other excipients such as water, propylene glycol, vegetable oils or other suitable organic solvents.

The term "excipient" is used to mean any compound which does not interfere with the effectiveness of the biological activity of the composition according to the invention, and which is not toxic to the host to which it is administered.

The composition used may also contain adjuvants, such as wetting agents, isotoning agents, emulsifiers, salts or any other substances known to a person skilled in the art that can be used as adjuvants (Polydimethylsiloxane, polyvinyl alcohol (PVA), hydrogels (Carbopol), polyvinylpyrrolidone, hydroxypropyl cellulose (HPC), poloxamer 188, EDTA, chlorobutanol) (Lubrizol, France, Dow Corning, USA).

Advantageously, the composition may comprise other substances such as vitamins, mineral salts, pharmaceutically acceptable vectors, stabilizers, antioxidants, or any other substance known to a person skilled in the art and intended to be integrated into a drug.

Preferably, the composition is liquid, orally administrable and contains at least a non-toxic extract of *Withania somnifera,* some preservatives, vitamins, water and salt.

More preferably, the preservatives are potassium sorbate or benzoate. Preferably, the vitamin is riboflavin (vitamin B2).

The therapeutic composition used in the method of the invention is administered in a pharmaceutically acceptable vehicle.

The terms "pharmaceutically acceptable vehicle" is used to mean any vehicle which does not interfere with the effectiveness of the biological activity of the composition according to the invention and which is not toxic to the host to which it is administered.

The composition obtained is usable as a medicinal product for a mammal, and more particularly for humans, to assist in the treatment or limitation of development of MNDs and in particular ALS.

The term "medicinal product" is used to mean a product containing an accurate dose of said preparation according to European directive 65/65/EC, namely any substance or composition described as possessing curative or preventive properties with respect of human or animal disease. For example, the medicinal product containing said preparation at therapeutic doses can be administered orally as a capsule or a tablet, or injected via any other route to confer the beneficial effects.

An appropriate dosage of the therapeutic composition can be determined by one of skill in the art, taking into consideration the findings described herein together with typical factors such as the body mass of the patient, the physical condition of the patient, and so on. The dosage should contain the therapeutic composition in an amount that is effective for treating or limiting development of MNDs and in particular ALS.

The drug can be administered daily, weekly, or on an intermittent basis. For example, the drug can be administered for three weeks on, followed by one week off, or for two weeks on, followed by one week off, or under other dosing schedules as can be determined by one skilled in the field.

The particular dose selected will depend upon the mode of administration and dosing regimen selected. One preferred schedule is a once daily oral dosing schedule. When longer periods of time are prescribed between each application (typically the case for i.v. administration), each unit dose may be larger than when daily dosages are provided.

The daily dose of the compositions used may vary according to the needs and severity of symptoms of the patient and according to the route. Typically, the daily dose is between 10 mg/mL and 300 mg/mL of the composition after fermentation.

Preferably, the daily dose for an adult human is between 30 and 100 mg/mL of the composition after fermentation.

The present invention will be explained in further detail by way of non-limiting examples below, which make reference to the appended drawings. The following methods were used in the experiments described in the examples that follow the description of the methods.

Example 1 : Composition WEB-1 before fermentation

The composition WEB-1 contains a commercial extract of *Withania Somnifera* at a concentration of 20 g/L, of *Emblica officinalis* at a concentration of 15 g/L, of *Bacopa monnieri* at a concentration of 15 g/L.

A solution of 100 mL is made in water. After lyophilization, 3.8 g of a beige powder is obtained.

Example 2 : Strain of filamentous fungus *Beauveria bassiana*

The strain *Beauvaria Bassiana* (reference ATCC 7159) has been cultivated in a medium containing 0,5 g/L KH_2P0_4 ; 1 g/L KH_2P0_4 ; 1 g/L $MgS0_4$; 2 g/L $NaN0_3$; 0,5 g/L KC1 ; 0,02 g/L $FeS0_4$; 30 g/L glucose (all reagents from Sigma-Aldrich, France) and 10 g/L of corn steep liquor (Roquette, France).

The culture was then agitated at 200 rotations per minute, for 72 hours at 27 °C. It was then filtered by non-sterile methods on a filter paper to separate the fungal biomass from the culture medium. The fungal biomass was then washed thoroughly with water.

Example 3 : Composition WEB-2 used in the invention

The composition WEB-1 as in example 1 is added to the fresh fungal biomass of example 2 using 60 g of biomass per liter of composition WEB-1 containing 50g of glucose.

After incubation, this seeded composition was agitated at 200 rpm for 5 days at a temperature of 27 °C.

After 5 days, the incubation medium was filtered on a filter paper, the samples for HPLC assay were also filtered using a 0.45 micron filter (Ait-France, ref: SFNY 013045N).

The brownish solution obtained which was then lyophilized during 5 days to produce dried beige powder.

Example 4 : Composition WE-2 used in the invention

The composition WE-1 contains commercial extracts of *Withania Somnifera* at a concentration of 20 g/L, and of *Emblica officinalis* at a concentration of 15 g/L.

To 100 mL of such a solution, are added 5 g of glucose and 6 g of biomass of example 2.

After having treated and lyophilized the solution like in example 3, 4.13 g of a beige powder is obtained.

The markers identified in the composition WE-2 were Withanoside IV, Withanoside VI and gallic acid.

Example 5 : Composition WB-2 used in the invention

The composition WB-1 contains an extract of *Withania Somnifera* at a concentration of 20 g/L, and of *Bacopa Monnieri* at a concentration of 15 g/L.

To 100 mL of such a solution, are added 5 g of glucose and 6 g of biomass of example 2.

After having treated and lyophilized the solution like in example 3, 2,62 g of a beige powder is obtained.

The markers identified in the composition WB-2 were Withanoside IV, Withanoside VI, Bacoside A3, Bacopaside X and Bacopa, saponin C, Bacosaponin C.

Example 6 : Composition BE-2 used in the invention

The composition BE-2 contains an extract of *Bacopa Monnieri* at a concentration of 15 g/L, and of *Emblica officinalis* at a concentration of 15 g/L.

To 100 mL of such a solution, are added 5 g of glucose and 6 g of biomass of example 2.

After having treated and lyophilized the solution like in example 3, 2,62 g of a beige powder is obtained.

The markers identified in the composition BE-2 were Bacopaside X, Bacopa, saponin C, Bacosaponin C and gallic acid.

Example 7 : Composition WEB-4 according to the invention

The composition WBE-4 contains an extract of *Withania Somnifera* at a concentration of 40 g/L, of *Bacopa Monnieri* at a concentration of 15 g/L, and of *Emblica officinalis* at a concentration of 15 g/L.

To 100 mL of such a solution, are added 5 g of glucose and 6 g of biomass of example 2.

After having treated and lyophilized the solution like in example 3, 4.23 g of a beige powder is obtained.

Example 8 :Composition WEB-6 used in the invention

The composition WEB-6 contains an extract of *Withania Somnifera* at a concentration of 20 g/L, of *Bacopa Monnieri* at a concentration of 15 g/L, and of *Emblica officinalis* at a concentration of 30 g/L.

To 100 mL of such a solution, are added 5 g of glucose and 6 g of biomass of example 2.

After having treated and lyophilized the solution like in example 3, 4.22 g of a beige powder is obtained.

Example 9: Composition WEB-8 used in the invention

The composition WEB-8 contains an extract of *Withania Somnifera* at a concentration of 20 g/L, of *Bacopa Monnieri* at a concentration of 30 g/L, and of *Emblica officinalis* at a concentration of 15 g/L.

To 100 mL of such a solution, are added 5 g of glucose and 6 g of biomass of example 2.

After having treated and lyophilized the solution like in example 3, 3.76 g of a beige powder is obtained.

Example 10 : primary motor neuron culture survival

The aim of this study was to study the effect of WEB-2 on primary motor neuron culture from rat spinal cord (SC) injured by glutamate exposure (a well validated *in vitro* ALS model and model of motor neuron diseases).

a) Culture of Spinal cord (SC) motor neurons

Rat SC motor neurons were cultured as described by Martinou *et al.*, Neuron. 1992 Apr;8(4):737-44 and Wang *et al.*, Hum Mol Genet. 2013 Dec 1;22(23):4706-19. Briefly, pregnant female rats (Wistar, Janvier labs) of 14 days gestation were killed by cervical dislocation. Foetuses were collected and immediately placed in ice-cold L15 Leibovitz medium (Batch: 400 1014, Pan Biotech) with a 2% penicillin (10,000 U/mL) and streptomycin (10 mg/mL) solution (PS; Pan Biotech, batch: 30909 14) and 1% bovine serum albumin (BSA; Pan Biotech, batch: hl40603). SC were treated for 20 min at 37°C with a trypsin- EDTA (Pan Biotech, batch: 58903 14) solution at a final concentration of 0.05% trypsin and 0.02% EDTA. The dissociation was stopped by addition of Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/liter of glucose (Pan Biotech, batch: 603 12 14), containing DNAse I grade II (final concentration 0.5 mg/mL; Pan Biotech, batch: H140508) and 10% fetal calf serum (FCS; Invitrogen, batch: 41Q72 18K). Cells were mechanically dissociated by three forced passages through the tip of a 10-mL pipette. Cells were then centrifuged at 180g for 10 min at +4°C on a layer of BSA (3.5%) in L15 medium. The supernatant was discarded, and the pellet was resuspended in Neurobasal medium (Invitrogen, batch: 1704746) with a 2%> solution of B27 supplement (Invitrogen, batch: 1668967), 2 mmol/L of L-glutamine (Pan Biotech, batch: 66203 14), 2% of PS solution, and 10 ng/mL of brain-derived neurotrophic factor (BDNF; Pan Biotech, batch: H140 108). Viable cells were counted in a

Neubauer cytometer, using the trypan blue exclusion test. The cells were seeded at a density of 20,000 per well in 96-well plates precoated with poly-L-lysine (Biocoat, batch: 21614030) and were cultured at 37°C in an air (95%)-C02 (5%) incubator. The medium was changed every day.

b) WEB-2 and glutamate exposure

On day 13 of culture, WEB-2 was pre-incubated with cells for 1 hour before glutamate exposure. Then, glutamate (Sigma, Batch: SLBL7326V) was added into cell culture to a final concentration of 10 µM diluted in control medium in presence of WEB-2 for 20 min. After 20 min, glutamate was washed and fresh culture medium with WEB-2 was added for additional 48 hours.

The following conditions were assessed:

- Control
- + glutamate (10 µM, 20min)
- + glutamate (10 µM, 20min) + WEB-2(500 ng/mL)
- + glutamate (10 µM, 20min)+ - WEB-2(1 µg/mL)
- + glutamate (10 µM, 20min)+ - WEB-2(5 µg/mL)
- + glutamate (10 µM, 20min) + WEB-2(10 µg/mL)
- + glutamate (10 µM, 20min) + WEB-2(50 µg/mL)
- + glutamate (10 µM, 20min) + WEB-2(100 µg/mL)
- + glutamate (10 µM, 20min) + WEB-2(500 µg/mL)
- + glutamate (10 µM, 20min) + WEB-2(1 mg/mL)

c) Immunostaining of cells

48 hours after intoxication, the cell culture supernatants were taken off and the SC motor neurons were fixed by a cold solution of ethanol (95%, Sigma, batch: SZBD3080V) and acetic acid (5%, Sigma, Batch: SZBD1760V) for 5 min. After permeabilization with 0.1% of saponin, cells were incubated for 2 hours with an anti microtubule-associated-protein 2 monoclonal antibody (MAP-2; Sigma, batch: (063M4802) at dilution of 1/400 in PBS containing 1% foetal calf

serum and 0.1% of saponin. This antibody specifically stains cell bodies of neurons, allowing study of neuron survival in the culture.

This antibody was revealed with Alexa Fluor 488 goat anti-mouse IgG (Molecular probe, batch: 1613346) at the dilution of 1/400 in PBS containing 1% foetal calf serum and 0.1% of saponin for 1 hour at room temperature.

d) Results

For each condition, 6 wells were assessed, 30 pictures per well were taken using ImageXpress (Molecular Devices) with 20x magnification, to assess motor neuron survival (MAP-2). Analysis of picture was done using Custom Module Editor (Molecular Devices). Results were expressed in terms of mean survival neuron, labeled for MAP-2. Data were expressed in percentage of control conditions (no intoxication, no glutamate = 100 %) in order to express the glutamate injury. All values were expressed as mean +/- SEM (s.e.mean) of the culture (n = 6 wells per condition per culture). Graphs and statistical analyses are made on the different conditions (ANOVA followed by PLSD Fisher's test when allowed, using Statview software version 5.0).

Glutamate (10 µM for 20 min) applied on motor neuron culture induced a large and significant cell death (> 40 %). WEB-2displayed a protective effect of motor neuron when it was added 1h before glutamate, especially for the range dosing of 1-10 µg/mL (Figure 1).

Example 11 glutamate exposure

The aim of this study was to test the effect of WEB-2 on nerve /muscle co-culture injured by glutamate exposure. Riluzole (5 µM) was used as reference compound. Evaluation of neuromuscular junction (NMJ) integrity (number and mean size) and neurite network innervating the muscular cells were assessed in presence of the treatment.

a. Primary cultures of nerve muscle co-culture

Human muscle (Promocell, Batch: 306 1107) was prepared according to a previously described method from portions of a biopsy from a healthy subject (Braun *et al.,* 1996, J Neurol Sci. 136: 17-23, Combes *et al,* 20 15, J Neurosci Res. 93(4):633-43). Briefly, the human muscle cell line was established from dissociated cells (2 1 000 cells per wells), plated in gelatin-coated 0.1% (Sigma, Batch: slbkl4 10v) in water on 48 wells plate (Greiner, Batch: E 13111ME) and grown in a proliferation medium consisting of mix of 62 % of MEM medium (PAN, Batch: 74 102 15) and 25 % of M 199 medium (PAN, Batch: 458 1114) supplemented with glutamine 2mM (PAN, Batch: 66203 14), human insulin l(^g/mL (PAN, Batch: 44804 13), Human recombinant Epidermal growth factor l Ong/mL (EGF, GIBCO, Batch: 129 1552A), human recombinant Fibroblast growth factor basic 2ng/mL (bFGF, PAN, Batch: H080 113), foetal calf serum 10% (FCS, GIBCO, Batch: 41Q72 18K), 2% of Penicillin 10.000 U/mL and Streptomycin 10.000 µg/mL (PS, PAN, Batch: 145 1013). The medium was changed every 2 days. Five days after the start of culture, immediately after satellite cell fusion, whole transverse slices of 13-day-old rat Wistar embryos (Janvier, France) spinal cords with 4 dorsal root ganglia (DRG) attached are placed on the muscle monolayer (one explant per well in the central area). DRG are necessary to achieve a good ratio of innervation. Innervated cultures were maintained in a mixed (67%/25%) medium composed of MEM and medium 199, supplemented with 5% FCS, insulin 5 µg/mL, glutamine 2mM and 2% PS. After 24h of co-culture, neurites were observed growing out of the spinal cord explants. These neurites made contacts with myotubes and induced the first contractions after ~ 8 days. Quickly thereafter, innervated muscle fibres located in the proximity to the spinal cord explants, were virtually continuously contracting. Innervated fibers were morphologically and spatially distinct from the non-innervated ones and could easily be distinguished from them.

b. WEB-2 and glutamate exposure

Briefly, on day 27 of co-culture, WEB-2 or riluzole (Sigma, Batch: 44804 13) were pre-incubated for 1 hour with co-culture before glutamate exposure. Then, glutamate (Sigma, Batch: SLBL7326V) was added into co-culture to a final concentration of 60 μM diluted in control medium in presence of WEB-2 or riluzole for 20 min. After 20 min, glutamate was washed and fresh culture medium with WEB-2 or riluzole was added for additional 48 hours.

c) Immunostaining of NMJs and area of innervation and neurite network

After 48H of intoxication, cells were incubated with 500 nM a-bungarotoxin coupled with Alexa 488 (Molecular probes, Batch: 1579040) during 15 min in culture innervations medium at 37 °C to detect NMJ. After 2 washing in PBS (Pan Biotech, Batch: 18704 15), cells were fixed by a solution of 4 % of paraformaldehyde (Sigma Aldrich, Batch: SLBF7274V) in PBS, pH =7.3 for 20 min at room temperature.

The cells were washed 2 times in PBS and then permeabilized and non-specific sites were blocked with a solution of PBS containing 0.1% of saponin (Sigma-Aldrich, Batch: BCBJ84 17V) and 1% FBS (Gibco, Batch: 41Q16 13K) for 15 min at room temperature, co-cultures were incubated with a mouse monoclonal anti-neurofilament 200 KD antibody (NF, Sigma Aldrich, Batch: 053M4756) at the dilution of 1/400 in PBS containing 1% FCS, 0.1 % saponin, for 2 h at room temperature. Antibody against NF stained the axon of motor neuron. These antibodies were revealed with Alexa Fluor 568 goat anti-mouse IgG (Invitrogen, Batch: 14 197 15) at the dilution 1/400 in PBS containing 1% FCS, 0.1 % saponin, for 1 h at room temperature. Nuclei of neurons were labeled by Hoechst solution, a nuclear fluorescent marker at 1 μιη/mL in the same solution (Hoechst solution, Sigma, batch: 011M4004V).

d) Results

The NMJ number and mean size (area) of NMJs and the total length of neurite were assessed under the experimental conditions described above. For each condition, 63 pictures per well were taken in the center area using ImageXpress (Molecular device) with 10x magnification. All images were taken under the same conditions.

The following endpoints were automatically evaluated

-the total number of NMJs was counted to measure the level of innervation of the co-culture

-the mean size of the NMJs was measured to assess the quality of innervations

-the length of neurites was measured to assess the extent of the neurite network in the co-culture.

Data were expressed in percentage of control conditions (no induced injury, no glutamate = 100 %) in order to express the glutamate injury. All values were expressed as mean +/- SEM (s.e. mean) (n=6 wells per condition per culture). Graphs and statistical analyses are made on the different conditions (ANOVA followed by PLSD Fisher's test when allowed, using Statview software version 5.0).

The results are shown in Figures 2a and 2b. Glutamate (60 µM - 20 min), applied on nerve/muscle cocultures, induced a significant NMJ mean size decrease (fig 2a) as well as a significant decrease of their number (fig 2b).

In presence of WEB-2used at 1 and 5µg/mL, a significant protective effect on NMJ number and integrity was observed.

A large protective effect was seen with riluzole (5 µM) used as reference compound. No significant difference was observed between riluzole and WEB-2 (1 and 5 µg/mL) in the protective effect.

The 2 lowest doses of WEB-2 (100 and 500 ng/mL) displayed protective effect on the NMJ integrity but this effect was not significantly different with glutamate condition.

The effect of WEB-2 has been assessed on neurite network. The results are in Figure 3.

On this figure, Glutamate (60 µM - 20 min) induced a large and significant decrease of the total neuronal network innervating muscle cells.

WEB-2 (all concentrations tested except the lowest dose: 100 ng/mL) showed a protective effect. A similar effect was observed with riluzole (5 µM) used as reference compound.

WEB-2 (from 500 ng/mL to 5 µg/mL) showed a significant protective effect of the neuritic network.

Example 12

The aim of this study was to study the effect of WEB-2 on primary motor neuron culture survival and neurite outgrowth (as a model of neurodegeneration of spinal cord MN).

a. Culture of Spinal cord (SC) motor neurons

Rat SC motor neurons were cultured as previously described in example 10. Viable cells were counted in a Neubauer cytometer, using the trypan blue exclusion test. The cells were seeded at a density of 20,000 per well in 96-well plates precoated with poly-L-lysine and were cultured at 37°C in an air (95%)-C02 (5%) incubator.

b. Immunostaining of Motor neurons

Immediately after seeding, WEB-2 was added on the culture.

The following conditions were assessed:

- Control
- + WEB-2 (100 ng/mL)
- + WEB-2 (500 ng/mL)
- + WEB-2 (1 µg/mL)
- + WEB-2 (5 µg/mL)
- + WEB-2 (10 µg/mL)
- + WEB-2 (50 µg/mL)
- + WEB-2 (100 µg/mL)
- + WEB-2 (500 µg/mL)
- + WEB-2 (1 mg/mL)

Then, on day 3 and on day 5, the cell culture supernatants were taken off and the SC motor neurons were fixed by a cold solution of ethanol (95%, sigma, batch: SZBD3080V) and acetic acid (5%, Sigma, Batch: SZBD 1760V) for 5 min. After permeabilization with 0.1% of saponin, cells were incubated for 2 hours with a monoclonal antibody anti MAP-2 (MAP-2; Sigma, batch: 063M4802) at dilution of 1/400 in PBS containing 1% foetal calf serum and 0.1% of saponin. This antibody stains specifically cell bodies of neurons (MAP-2) allowing study of neuron survival in the culture.

This antibody was revealed with Alexa Fluor 488 goat anti-mouse IgG (Molecular probe, batch: 1613346) at the dilution of 1/400 in PBS containing 1% foetal calf serum and 0.1% of saponin for 1 hour at room temperature.

For each condition, 6 wells were assessed, 30 pictures per well were taken using ImageXpress (Molecular Devices) with 20x magnification, to assess motor neuron survival (MAP-2). Analysis of picture was done using Custom Module Editor (Molecular Devices). Results were expressed in terms of mean survival neuron, labeled for a given marker (here MAP-2).

Data were expressed in percentage of control conditions (no WEB-2= 100 %). All values were expressed as mean +/- SEM (s.e. mean) of the culture (n = 6 wells per condition per culture). Graphs and statistical analyses are made on the different conditions (ANOVA followed by PLSD Fisher's test when allowed, using GraphPadPrism version 5.0).

c) Results

The results on motor neuron survival and neurite network at Day 3 are shown on Figure 4

Primary neurons in culture displayed spontaneous death due to apoptosis mechanisms. This phenomenon immediately occurs after the seeding and stops in the first days of culture (Horisberger MA. In Vitro Cell Dev Biol Anim. 2006 May-Jun;42(5-6): 143-8.

WEB-2 added for 3 days significantly prevented this spontaneous death (especially at the doses of 100, 500 ng/mL and 1 µg/mL). The neuroprotective effect increased the neuron survival by 20 %. At the highest doses (from 50 µg/mL and above), WEB-2 was toxic.

Significant effect was observed on the neurite outgrowth (fig 4b). The neurite network was increased by 30 % in presence of WEB-2 (100 ng/mL).

The results on motor neuron survival and neurite network at Day 5 are shown on Figure 5.

Similarly, WEB-2 added for 5 days significantly prevented the spontaneous death (especially at the doses of 100, 500 ng/mL and 1 µg/mL), the survival was increase by 30 %. The WEB-2 neuroprotective effect was increased compared to 3 days treatment.

Significant effect was observed on the neurite outgrowth (fig 5b). The neurite network was increased by -30 % in presence of WEB-2 (100 ng/mL).

Indeed, WEB-2 induced a large increase of neurite network (WEB-2 promotes the neurite outgrowth) of spinal cord motor neurons. In addition, protective effect against the spontaneous cell death in culture was significantly reduced in presence of WEB-2.

Example 13 : primary motor neuron culture survival

The aim of this study was to study the effect of WEB-2 on primary motor neuron culture from rat spinal cord (SC) injured by glutamate exposure.

The culture of Spinal cord (SC) motor neurons has been performed in the same conditions as in example 10.

a) WEB-2 and glutamate exposure

On day 13 of culture, glutamate (Sigma, Batch: SLBL7326V) and WEB-2 were added into cell culture or glutamate and riluzole were added into cell culture. After 20 min, glutamate was washed and fresh

culture medium with either WEB-2 of riluzole was added for additional 48 hours.

The following conditions were assessed:

- Control
- + glutamate (5 µM, 20min)
- + glutamate (5 µM, 20min) + WEB-2(500 ng/mL)
- + glutamate (5 µM, 20min)+ WEB-2(1 µg/mL)
- + glutamate (5 µM, 20min)+ WEB-2(2 µg/mL)
- + glutamate (5 µM, 20min) + riluzole (500 nM/L)
- + glutamate (5 µM, 20min) + riluzole (1 µM/L)
- + glutamate (5 µM, 20min) + riluzole (2 µM/L)

The immunostaining of cells has been performed as in example 10.

e) Results

For each condition, 6 wells were assessed, 30 pictures per well were taken using ImageXpress (Molecular Devices) with 20x magnification, to assess motor neuron survival (MAP-2). Analysis of picture was done using Custom Module Editor (Molecular Devices). Results were expressed in terms of mean survival neuron, labeled for MAP-2. Data were expressed in percentage of control conditions (no intoxication, no glutamate = 100 %) in order to express the glutamate injury. All values were expressed as mean +/- SEM (s.e.mean) of the culture (n = 6 wells per condition per culture). Graphs and statistical analyses are made on the different conditions (ANOVA followed by PLSD Fisher's test when allowed, using Statview software version 5.0).

Glutamate (5 µM for 20 min) applied on motor neuron culture induced a large and significant cell death (> 40 %). WEB-2 displayed a protective effect of motor neuron when it was added with glutamate, especially for the range dosing of 1-2 µg/mL (see Figure 6).

WEB-2 is even better than riluzole to protect the motor neurons from glutamate-induced cell death.

Example 14

The aim of this study was to study the effect of WEB-2 or riluzole on primary motor neuron culture survival.

The protocol is the same as in example 11. Briefly, after culture of Spinal cord (SC) motor neurons and immunostaining of Motor neurons, WEB-2 was added on the culture, immediately after seeding

The following conditions were assessed:

- Control
- + WEB-2 (100 ng/mL)
- + WEB-2 (500 ng/mL)
- + WEB-2 (1 µg/mL)
- + riluzole (100 nM)
- + riluzole (500 nM)
- + riluzole (1 µM)

Then, on day 1 and on day 5, the cell culture supernatants were taken off and the SC motor neurons were fixed by a cold solution of ethanol (95%, sigma, batch: SZBD3080V) and acetic acid (5%, Sigma, Batch: SZBD 1760V) for 5 min. After permeabilization with 0.1% of saponin, cells were incubated for 2 hours with a monoclonal antibody anti MAP-2 (MAP-2; Sigma, batch: 063M4802) at dilution of 1/400 in PBS containing 1% foetal calf serum and 0.1% of saponin. This antibody stains specifically cell bodies of neurons (MAP-2) allowing study of neuron survival in the culture.

This antibody was revealed with Alexa Fluor 488 goat anti-mouse IgG (Molecular probe, batch: 1613346) at the dilution of 1/400 in PBS containing 1% foetal calf serum and 0.1% of saponin for 1 hour at room temperature.

For each condition, 6 wells were assessed, 30 pictures per well were taken using ImageXpress (Molecular Devices) with 20x magnification, to assess motor neuron survival (MAP-2). Analysis of picture was done using Custom Module Editor (Molecular Devices).

Results were expressed in terms of mean survival neuron, labeled for a given marker (here MAP-2).

Data were expressed in percentage of control conditions (no WEB-2= 100 %). All values were expressed as mean +/- SEM (s.e. mean) of the culture (n = 6 wells per condition per culture). Graphs and statistical analyses are made on the different conditions (ANOVA followed by PLSD Fisher's test when allowed, using GraphPadPrism version 5.0).

c) Results

The results on motor neuron survival at Day 1 are shown on Figure 7

Primary neurons in culture displayed spontaneous death due to apoptosis mechanisms. This phenomenon immediately occurs after the seeding and stops in the first days of culture (Horisberger MA. In Vitro Cell Dev Biol Anim. 2006 May-Jun;42(5-6): 143-8.

WEB-2 added for 5 days significantly prevented this spontaneous death at the doses of 100, 500 ng/mL and 1 µg/mL. The neuroprotective effect increased the neuron survival by 20 %.

The results on motor neuron survival at Day 5 are shown on Figure 7.

Similarly, WEB-2 added for 5 days significantly prevented the spontaneous death and the survival was increase by 30 %. The WEB-2 neuroprotective effect was similar to the effect observed with the riluzole.

CLAIMS

1. Composition containing a *Withania somnifera* extract for its use to treat or limit development of neuromuscular diseases in a mammal, preferably a human.

2. Composition according to claim 1, in which the *Withania somnifera* extract has been fermented by its incubation with a filamentous fungus in a suitable environment.

3. Composition according to claim 1 or 2, in which the fermentation is carried out with a filamentous fungus of the family *Cordycipitaceae,* preferably from the genus Beauveria and, more particularly, *Beauveria bassiana.*

4. Composition according to any of claims 1 to 3, further containing at least one extract from the following plants: *Emblica officinalis, Bacopa monnieri, Punica granatum, Curcuma longa, Piper longum,* or *Calendula officinalis* .

5. Composition according to any of claims 1 to 3, further containing an extract of *Emblica officinalis* and an extract of *Bacopa monnieri.*

6. Composition according to any of claims 1 to 5, comprising an amount of *Withania somnifera* of between 5 and 100 g/L of *Withania somnifera,* and preferably 20 g/L.

7. Composition according to any of claims 1 to 6, comprising an amount of *Withania somnifera* at a concentration of 20 g/L, of *Emblica officinalis* at a concentration of 15 g/L and of *Bacopa monnieri* at a concentration of 15 g/L.

8. Composition according to any of claims 1 to 7, for its use to treat or limit development of MN diseases, ALS, PBP, PMA, PLS, SMA, Kennedy's disease, PPS, PPMA, MMN, MMA, paraneoplastic motor neuron disease, LEMS, MG and botulism.

9. Composition according to any of claims 1 to 8, for its use to treat or limit development of ALS, wherein the treatment results in one or more clinical outcomes, compared to ALS subjects no treated with the composition, selected from the group consisting of:

(a) decrease in ALS disease progression;

(b) decrease in ALS disease severity;

(c) decrease in ALS clinical symptoms;

10. A method of treating or limiting development of a neuromuscular disease in a subject, comprising administering to a subject a therapeutic amount of a plant extract composition, such that said neuromuscular disease in a subject is treated or its development limited, wherein said composition contains a plant extract of *Withania somnifera* according to claims 1 to 8.

11. The method of claim 10, wherein said disease is MN disease, ALS, PBP, PMA, PLS, SMA, Kennedy's disease, PPS, PPMA, MMN, MMA, paraneoplastic motor neuron disease, LEMS, MG and botulism.

12. The method according to any of claims 10 to 11, wherein said subject is a human.

13. The method of any of claims 10 to 12, wherein the treatment results in one or more clinical outcomes, compared to ALS subjects no treated with the composition, selected from the group consisting of:

(a) decrease in ALS disease progression;

(b) decrease in ALS disease severity;

(c) decrease in ALS clinical symptoms;

14. The method according to any one of claims 10 to 13, wherein the therapeutic compound is administered orally or parenterally.

15. The method according to any one of claims 10 to 14, wherein said therapeutic compound is administered in a pharmaceutically acceptable vehicle.

AMENDED CLAIMS
received by the International Bureau on 23 January 2017 (23.01.17)

1. Composition containing a *Withania somnifera* extract fermented by its incubation with a filamentous fungus in a suitable environment for its use to treat or limit development of neuromuscular diseases in a mammal, preferably a human.

2. Composition according to claim 1, in which the fermentation is carried out with a filamentous fungus of the family *Cordycipitaceae,* preferably from the genus Beauveria and, more particularly, *Beauveria bassiana.*

3. Composition according to claims 1 or 2, further containing at least one extract from the following plants: *Emblica officinalis, Bacopa monnieri, Punica granatum, Curcuma longa, Piper longum,* or *Calendula officinalis.*

4. Composition according to claims 1 or 2, further containing an extract of *Emblica officinalis* and an extract of *Bacopa monnieri.*

5. Composition according to any of claims 1 to 4, comprising an amount of *Withania somnifera* of between 5 and 100 g/L of *Withania somnifera,* and preferably 20 g/L.

6. Composition according to any of claims 1 to 5, comprising an amount of *Withania somnifera* at a concentration of 20 g/L, of *Emblica officinalis* at a concentration of 15 g/L and of *Bacopa monnieri* at a concentration of 15 g/L.

7. Composition according to any of claims 1 to 6, for its use to treat or limit development of MN diseases, ALS, PBP, PMA, PLS, SMA, Kennedy's disease, PPS, PPMA, MMN, MMA, paraneoplastic motor neuron disease, LEMS, MG and botulism.

8. Composition according to any of claims 1 to 7, for its use to treat or limit development of ALS, wherein the treatment results in one or more clinical outcomes, compared to ALS subjects no treated with the composition, selected from the group consisting of:

(a) decrease in ALS disease progression;

(b) decrease in ALS disease severity;

(c) decrease in ALS clinical symptoms;

9. A method of treating or limiting development of a neuromuscular disease in a subject, comprising administering to a subject a therapeutic amount of a plant extract composition, such that said neuromuscular disease in a subject is treated or its development limited, wherein said composition contains a plant extract of *Withania somnifera* according to claims 1 to 7.

10. The method of claim 9, wherein said disease is MN disease, ALS, PBP, PMA, PLS, SMA, Kennedy's disease, PPS, PPMA, MMN, MMA, paraneoplastic motor neuron disease, LEMS, MG and botulism.

11. The method according to any of claims 9 to 10, wherein said subject is a human.

12. The method of any of claims 9 to 11, wherein the treatment results in one or more clinical outcomes, compared to ALS subjects no treated with the composition, selected from the group consisting of:

(a) decrease in ALS disease progression;

(b) decrease in ALS disease severity;

(c) decrease in ALS clinical symptoms;

13. The method according to any one of claims 9 to 12, wherein the therapeutic compound is administered orally or parenterally.

14. The method according to any one of claims 9 to 13, wherein said therapeutic compound is administered in a pharmaceutically acceptable vehicle.

FIG.1

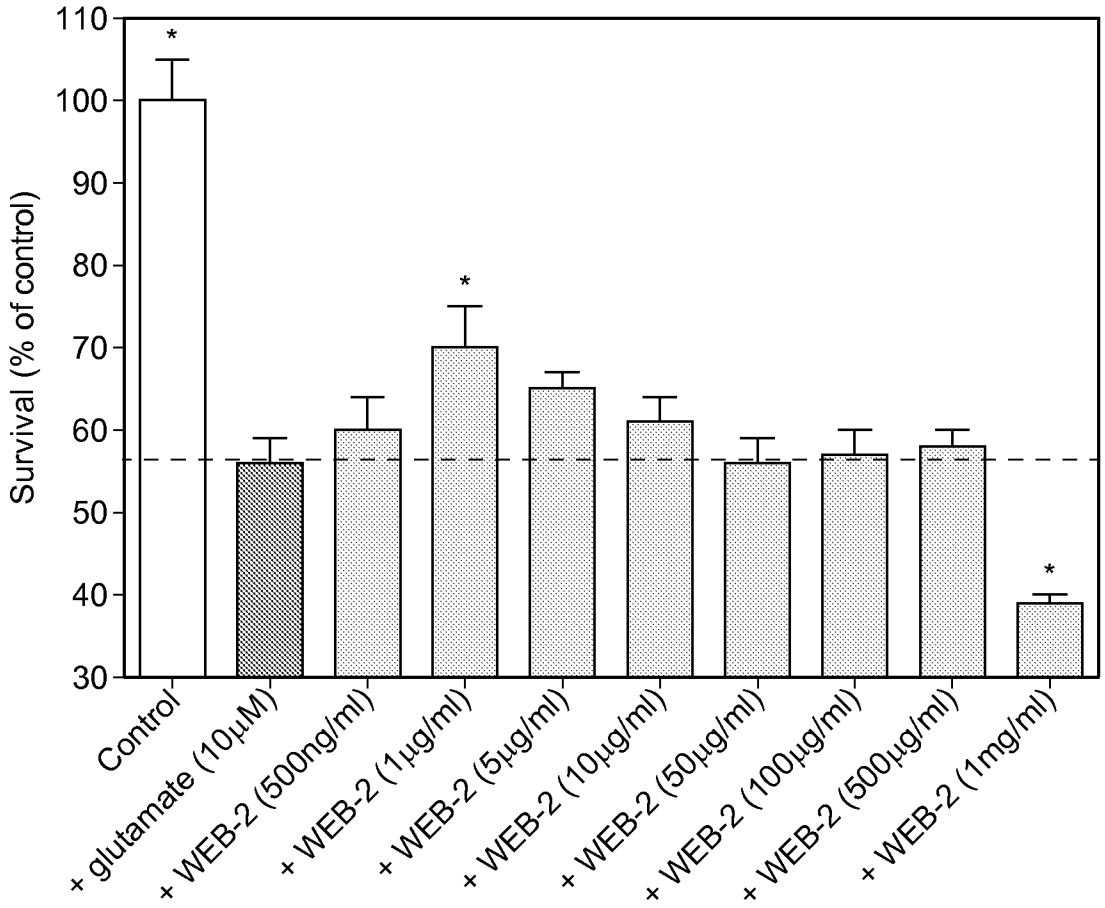

Survival (% of control)
110
100
90
80
70
60
50
40
30
*
*
*
Control
+ glutamate (10µM)
+ WEB-2 (500ng/ml)
+ WEB-2 (1µg/ml)
+ WEB-2 (5µg/ml)
+ WEB-2 (10µg/ml)
+ WEB-2 (50µg/ml)
+ WEB-2 (100µg/ml)
+ WEB-2 (500µg/ml)
+ WEB-2 (1mg/ml)

FIG.2a

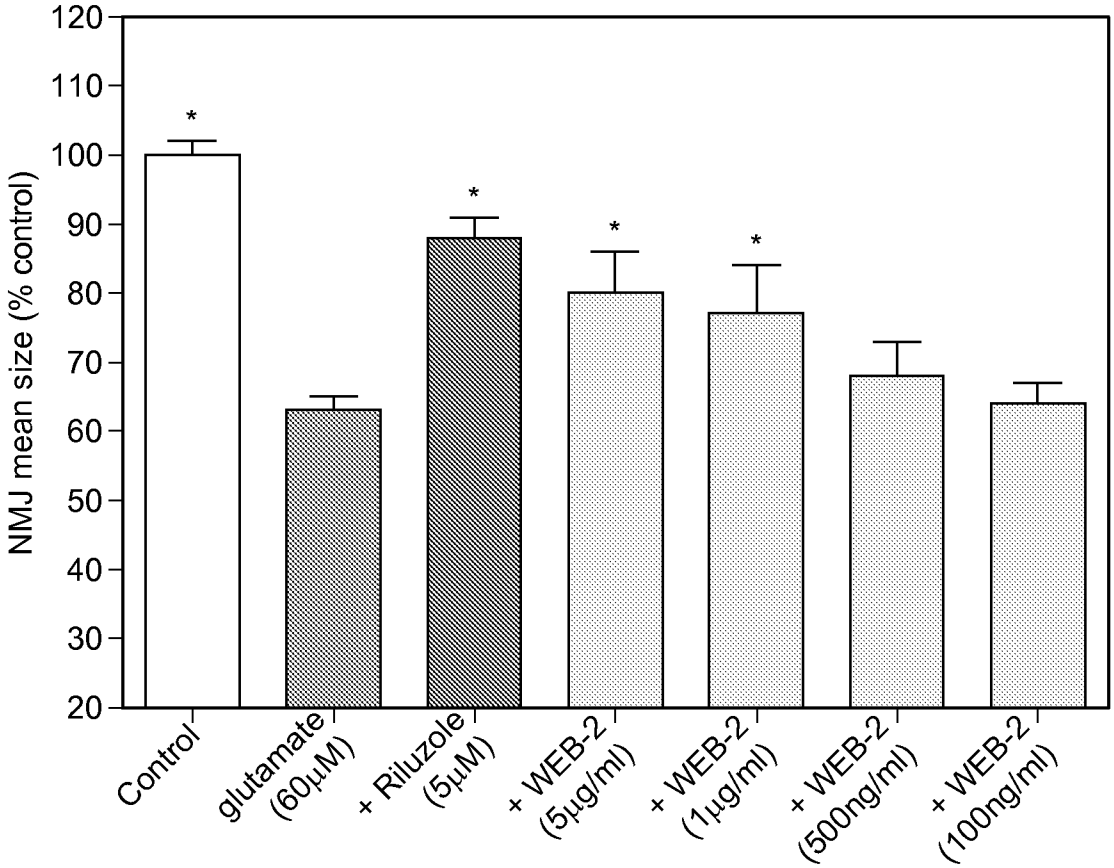

NMJ mean size (% control)
120
110
100
90
80
70
60
50
40
30
20
*
*
*
*
Control
glutamate (60µM)
+ Riluzole (5µM)
+ WEB-2 (5µg/ml)
+ WEB-2 (1µg/ml)
+ WEB-2 (500ng/ml)
+ WEB-2 (100ng/ml)

FIG.2b

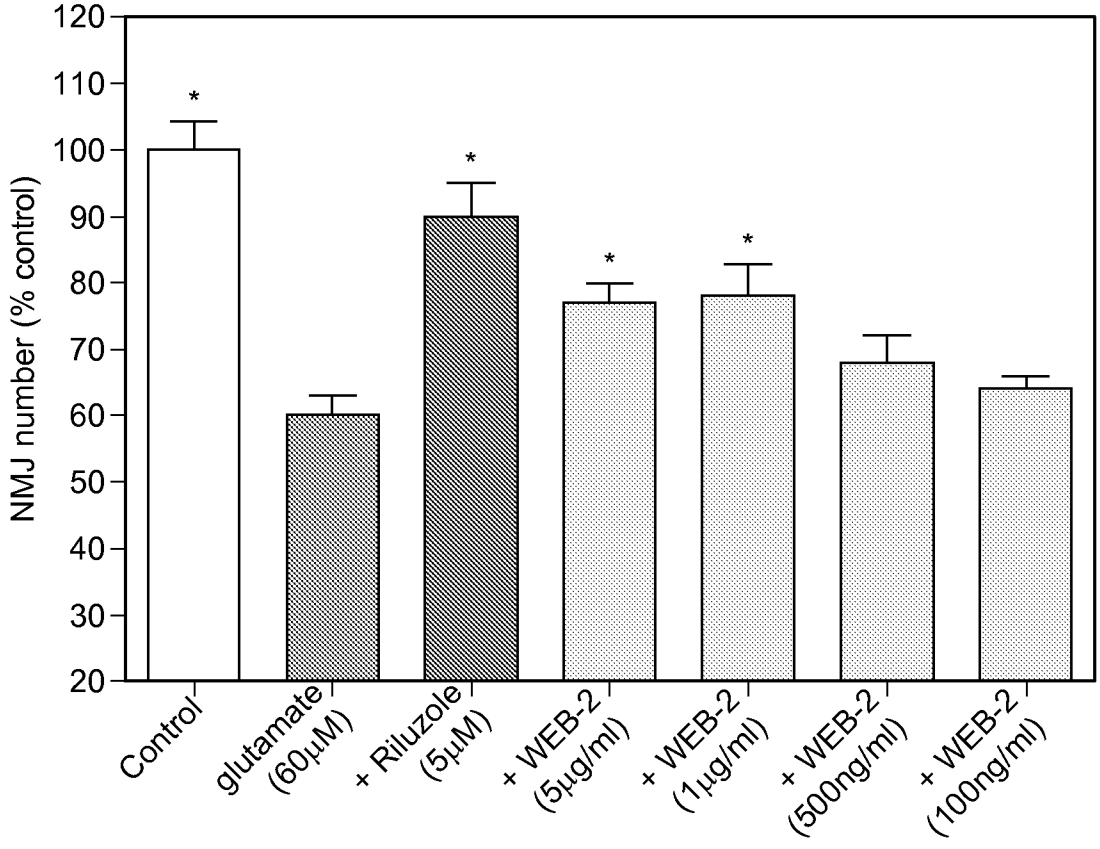

NMJ number (% control)
120
110
100
90
80
70
60
50
40
30
20
*
*
*
*
Control
glutamate (60µM)
+ Riluzole (5µM)
+ WEB-2 (5µg/ml)
+ WEB-2 (1µg/ml)
+ WEB-2 (500ng/ml)
+ WEB-2 (100ng/ml)

FIG.3

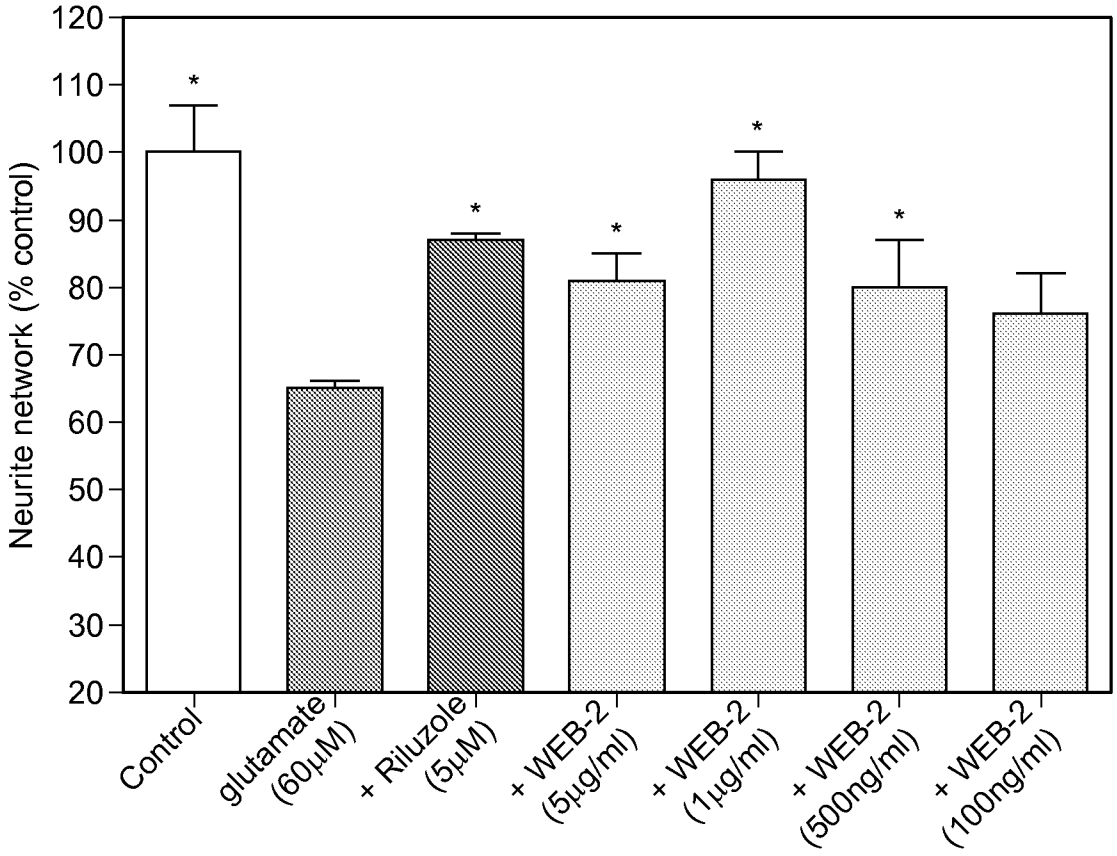

Neurite network (% control)
120
110
100
90
80
70
60
50
40
30
20
*
*
*
*
*
Control
glutamate (60µM)
+ Riluzole (5µM)
+ WEB-2 (5µg/ml)
+ WEB-2 (1µg/ml)
+ WEB-2 (500ng/ml)
+ WEB-2 (100ng/ml)

FIG.4a

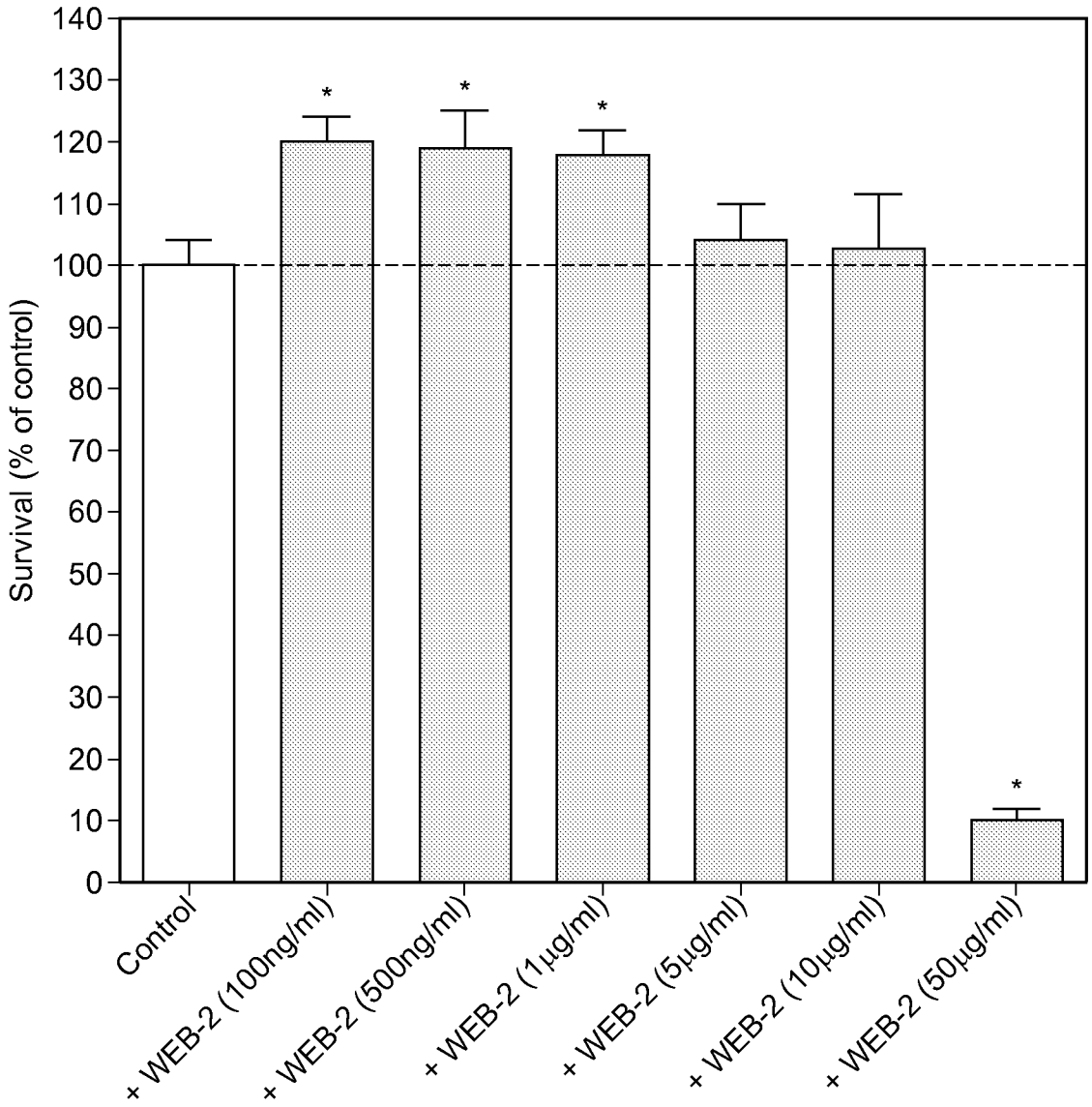

Survival (% of control)
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
*
*
*
*
Control
+ WEB-2 (100ng/ml)
+ WEB-2 (500ng/ml)
+ WEB-2 (1µg/ml)
+ WEB-2 (5µg/ml)
+ WEB-2 (10µg/ml)
+ WEB-2 (50µg/ml)

FIG.4b

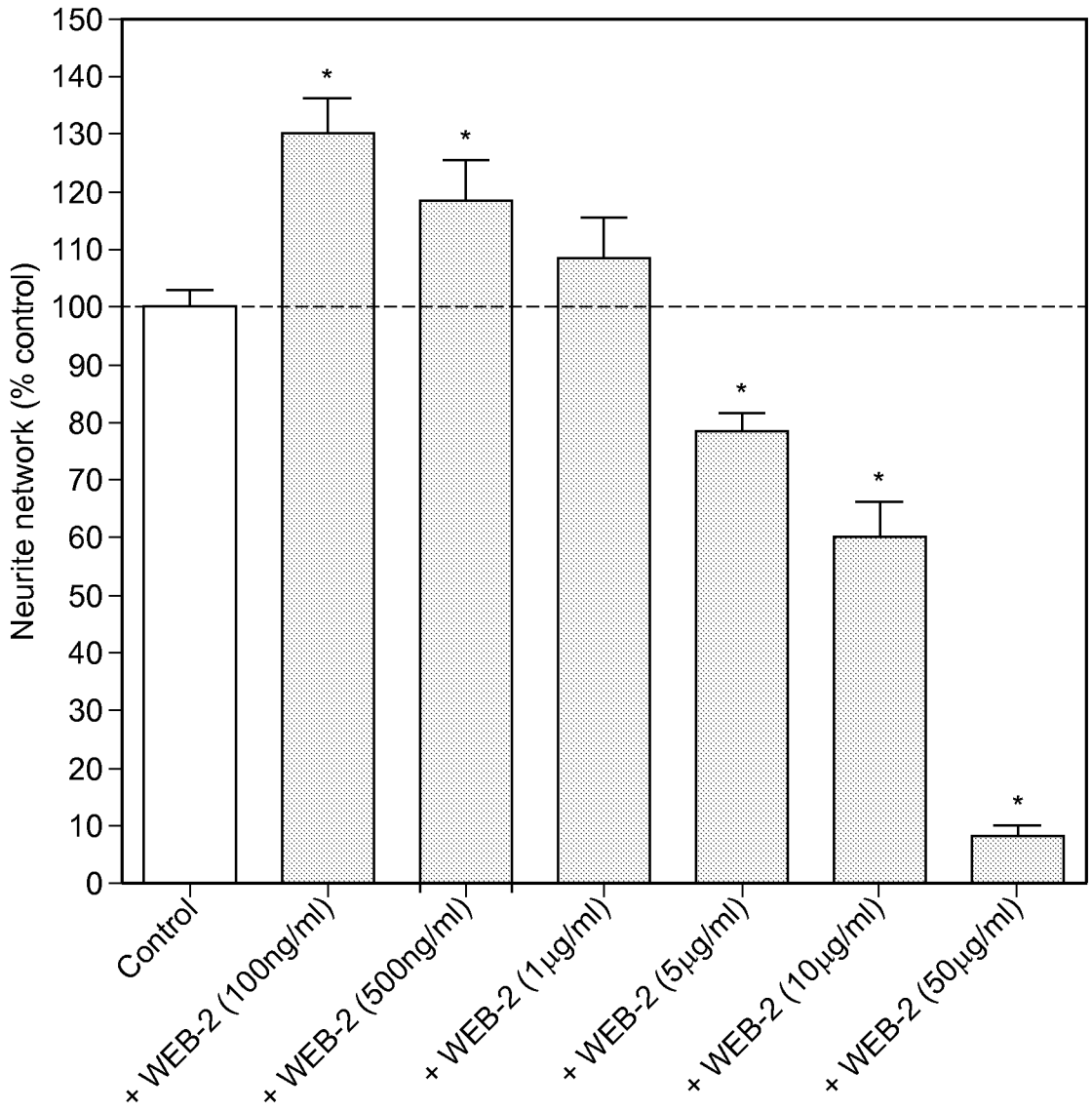

Neurite network (% control)
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
*
*
*
*
*
Control
+ WEB-2 (100ng/ml)
+ WEB-2 (500ng/ml)
+ WEB-2 (1µg/ml)
+ WEB-2 (5µg/ml)
+ WEB-2 (10µg/ml)
+ WEB-2 (50µg/ml)

FIG.5a

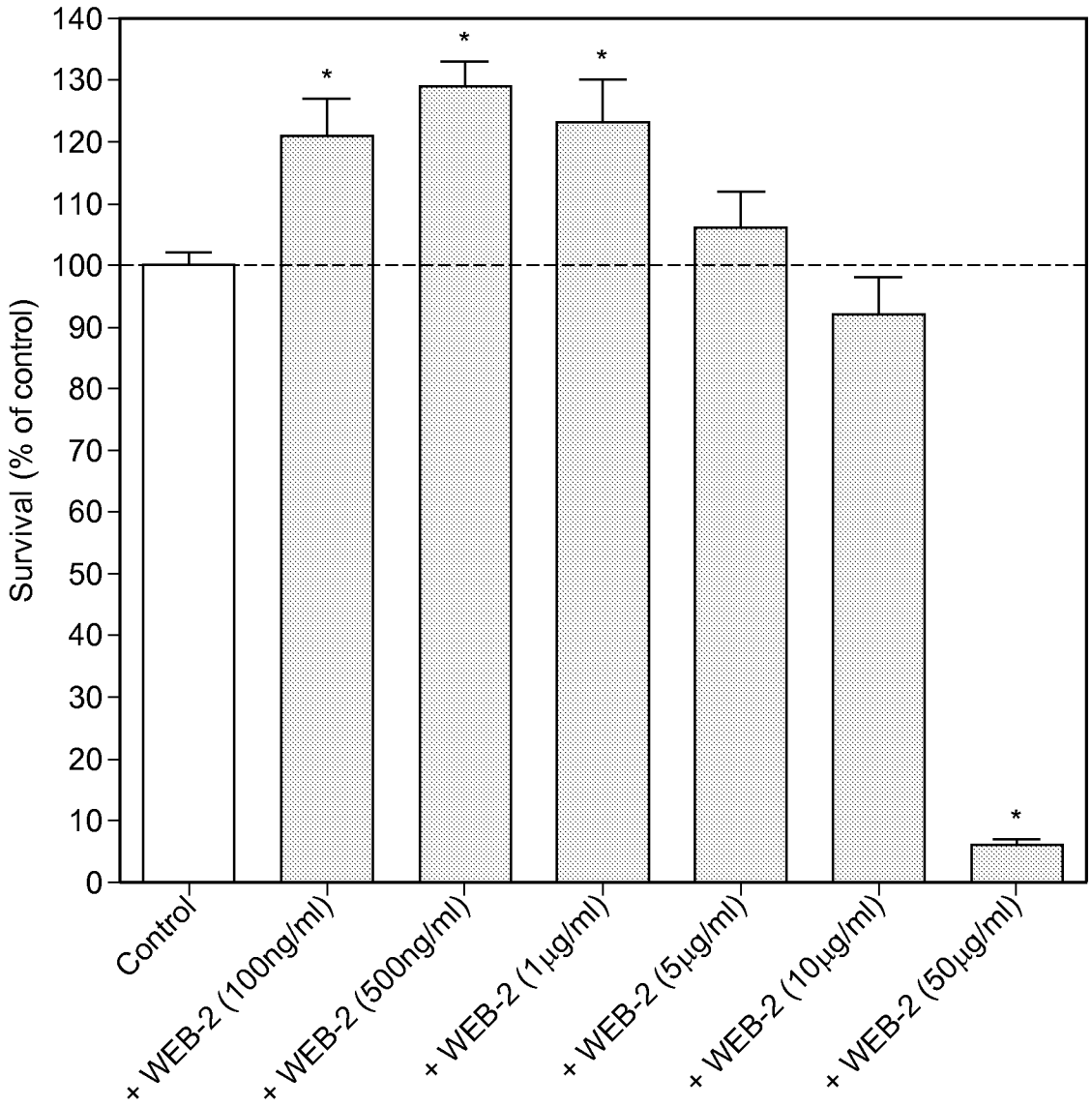

Survival (% of control)
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
*
*
*
*
Control
+ WEB-2 (100ng/ml)
+ WEB-2 (500ng/ml)
+ WEB-2 (1µg/ml)
+ WEB-2 (5µg/ml)
+ WEB-2 (10µg/ml)
+ WEB-2 (50µg/ml)

FIG.5b

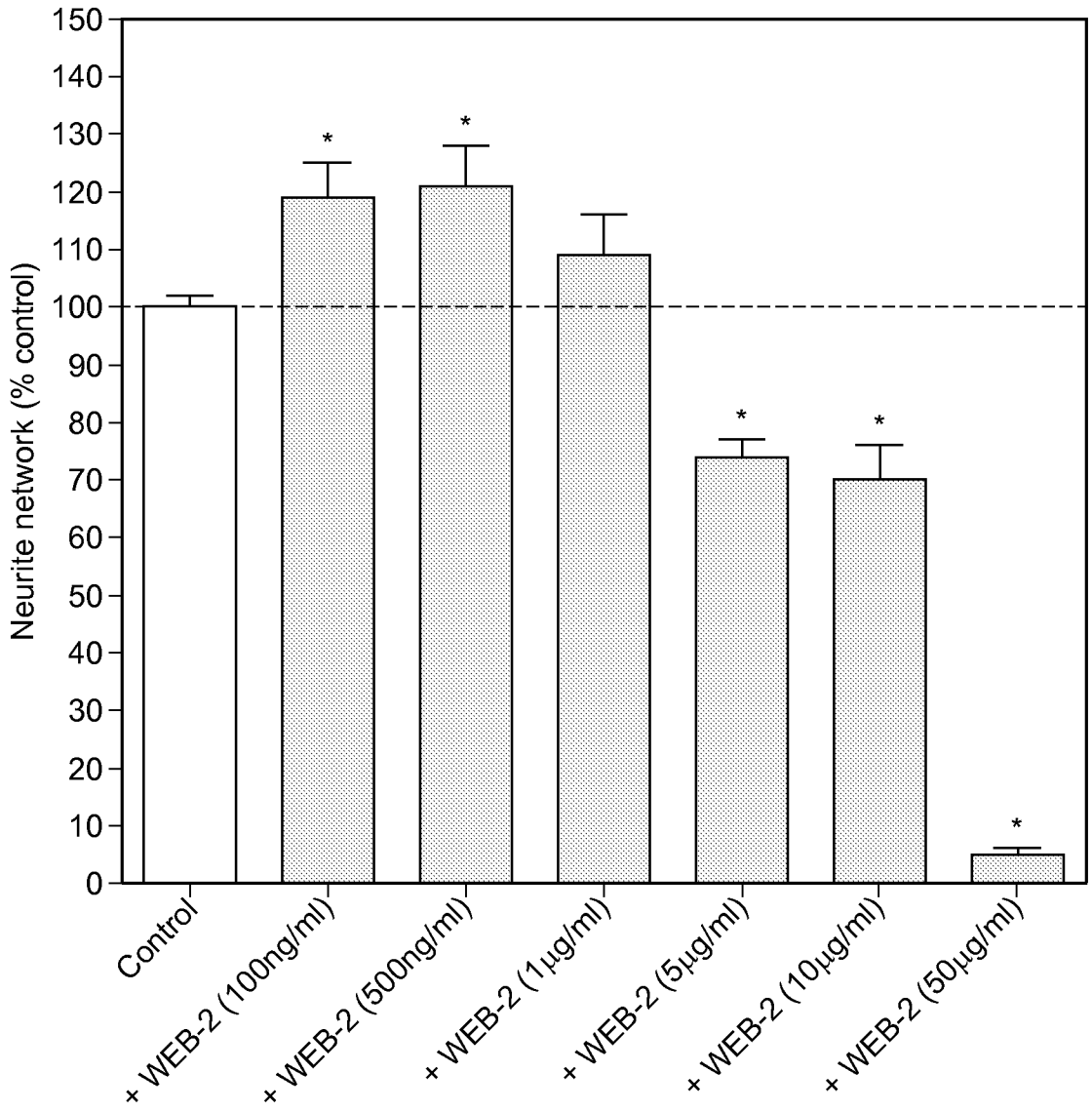

Neurite network (% control)
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
*
*
*
*
*
Control
+ WEB-2 (100ng/ml)
+ WEB-2 (500ng/ml)
+ WEB-2 (1µg/ml)
+ WEB-2 (5µg/ml)
+ WEB-2 (10µg/ml)
+ WEB-2 (50µg/ml)

FIG.6

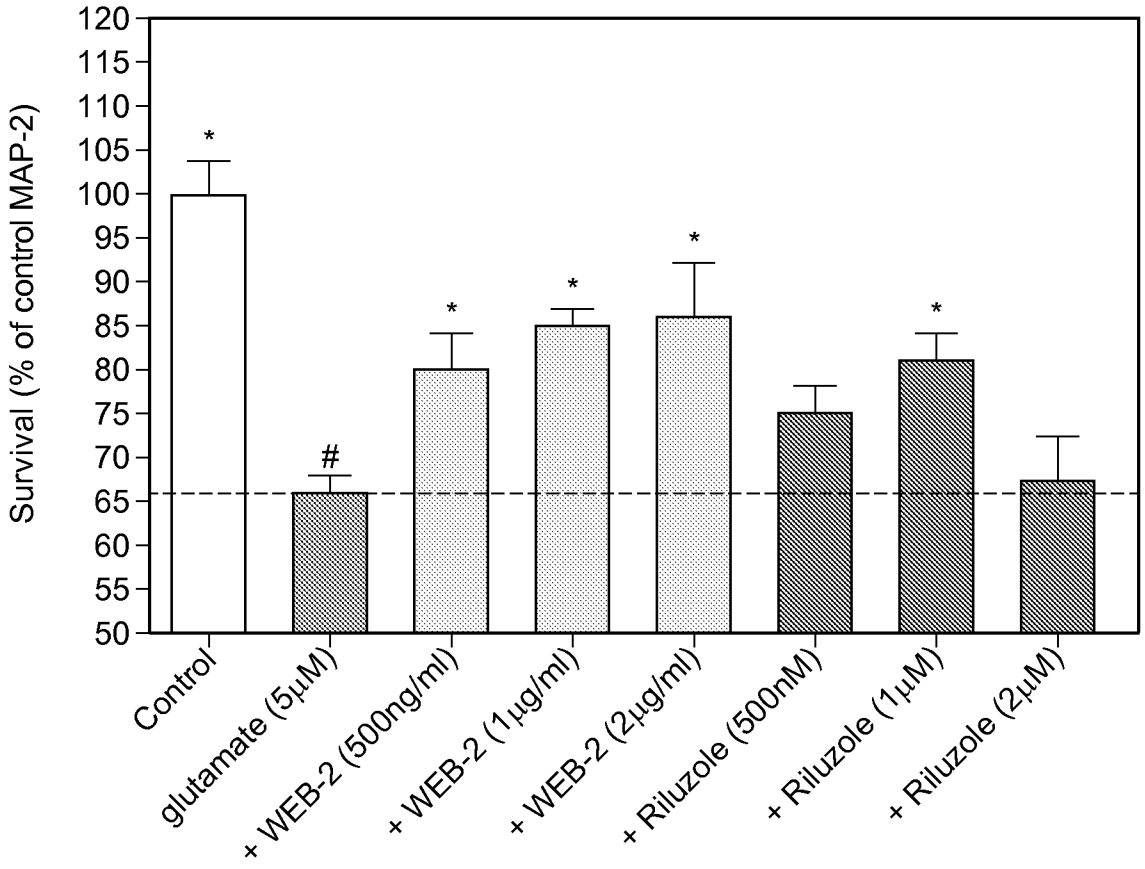
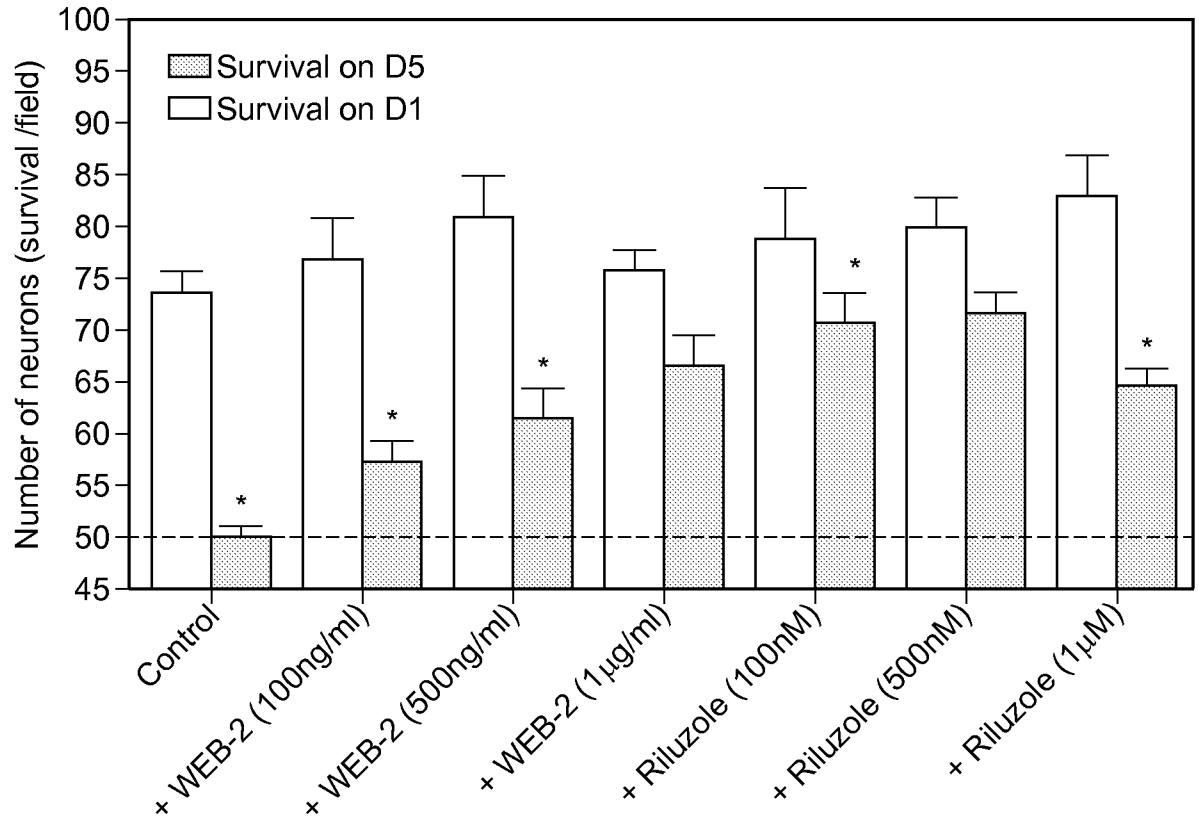

Survival (% of control MAP-2)
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
*
#
*
*
*
*
Control
glutamate (5µM)
+ WEB-2 (500ng/ml)
+ WEB-2 (1µg/ml)
+ WEB-2 (2µg/ml)
+ Riluzole (500nM)
+ Riluzole (1µM)
+ Riluzole (2µM)

FIG.7


Number of neurons (survival /field)
100
95
90
85
80
75
70
65
60
55
50
45
Survival on D5
Survival on D1
*
*
*
*
*
Control
+ WEB-2 (100ng/ml)
+ WEB-2 (500ng/ml)
+ WEB-2 (1µg/ml)
+ Riluzole (100nM)
+ Riluzole (500nM)
+ Riluzole (1µM)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2016/0OQ561

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K36/80 A61K36/81 A61K36/9066 A61K36/185 A61K36/28
A61K36/47 A61K36/67 A61P21/00

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal , BIOSIS, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2012/244235 Al (MYHI LL PAUL R [US] ET AL) 27 September 2012 (2012-09-27) paragraph [0152] - paragraph [0154]	1,4-6, 8-15
X	US 2011/097427 Al (RAMAKRISHNAN SHYAM [US] ET AL) 28 Apri l 2011 (2011-04-28) page 5, paragraph 0065 - paragraph 0066 page 2, paragraph 0026	1,6,8, 10-12 , 14, 15

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 November 2016	21/11/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Thalmai r , Mi chaela

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2016/0OQ561

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TOMOHARU KUBOYAMA ET AL: "Effects of Ashwagandha (Roots of Withania somnifera) on Neurodegenerative Diseases" , BIOLOGICAL & PHARMACEUTICAL BULLETIN (OF JAPAN) , vol . 37, no. 6 , 1 January 2014 (2014-01-01) , pages 892-897, XP55250382, JP ISSN : 0918-6158, DOI : 10. 1248/bpb.bl4-00022 page 895 ; table 1	I , 8 , 10, I I , 14, 15

X	MANJUNATH M J ET AL: "Standardized extract ofWithania somnifera (Ashwagandha) markedly offsets rotenone-induced locomotor deficits, oxidative impairments and neurotoxicity inDrosophila melanogaster" , JOURNAL OF FOOD SCIENCE AND TECHNOLOGY, SPRINGER (INDIA) PRIVATE LTD, INDIA, vol . 52, no. 4 , 1 December 2013 (2013-12-01) , pages 1971-1981 , XP035475884, ISSN : 0022-1155, DOI : 10. 1007/S13197-013-1219-0 [retrieved on 2013-12-01]	10, 11 , 14, 15
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Y	page 241 - page 244	1-15

Y	JAGDISH MANWAR ET AL: "Comparative antioxidant potential of Withania somnifera based herbal formulation prepared by traditional and non-traditional fermentation processes" , INTEGRATIVE MEDICINE RESEARCH , vol . 2 , no. 2 , 18 April 2013 (2013-04-18) , pages 56-61, XP055086855 , ISSN : 2213-4220, DOI : 10. 1016/j . imr.2013.04.002 the whole document	2 , 3

	-/ -	

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2016/0OQ561

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WANKHEDE SACHIN ET AL: "Exami ning the effect of Wi thani a somni fera suppl ementati on on muscle strength and recovery: a randomi zed control led tri al", JOURNAL OF THE INTERNATIONAL SOCI ETY OF SPORTS NUTRITION, BIOMED CENTRAL LTD, LO, vol . 12, no. 43, 1 January 2015 (2015-01-01) , pages 1-11, XP009192173, ISSN: 1550-2783, D0I: 10. 1186/S12970-015-0104-9 the whole document	1-15
Y	TRI PATHI S ET AL: "3-15 : PROTECTIVE POTENTIAL OF Bacopa monni era (Brahmi) EXTRACT ON ALUMINUM INDUCED CEREBELLAR TOXICITY AND ASSOCIATED NEUROMUSCULAR STATUS I N AGED RATS", CELLULAR AND MOLECULAR BIOLOGY, CMB ASSOCIATIONS, NOISY-LE-GRAND, FR, vol . 57, no. 1, 1 January 2011 (2011-01-01) , pages 3-15, XP009192177, ISSN: 0145-5680 the whole document	1-15
Y	HEIDI R FULLER ET AL. : "Natural l y occurring plant polyphenol s as potenti al therapies for inheri ted neuromuscular di sease" , FUTURE MEDICINAL CHEMISTRY, vol . 5, no. 17, 1 November 2013 (2013-11-01) , pages 2091-2101 , XP009192230, D0I: 10.4155/FMC. 13. 165 page 2096 - page 2097 ; tables 2,3	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2016/0OQ561

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		US 2011151030 Al	23-06-2011
		US 2012244235 Al	27-09-2012
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