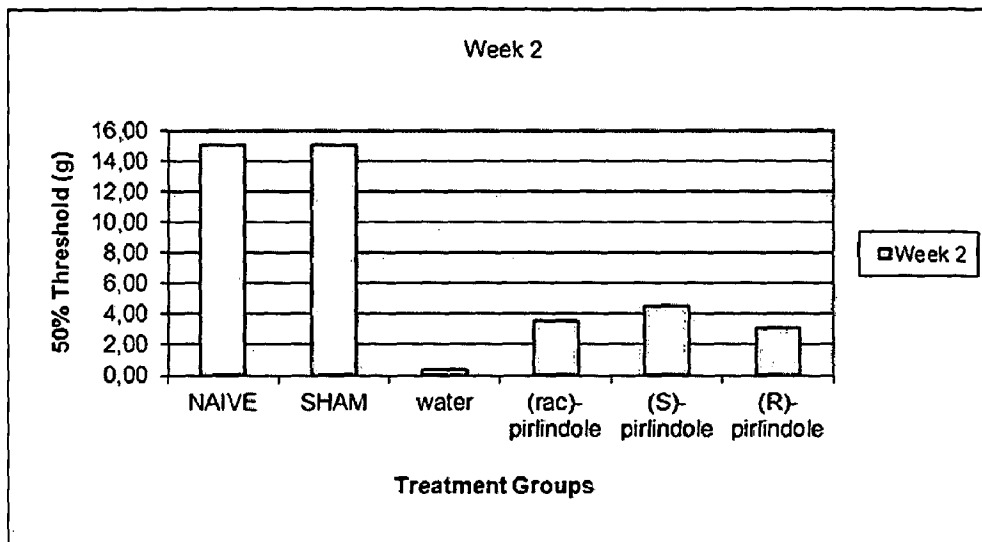




(86) Date de dépôt PCT/PCT Filing Date: 2014/05/09
 (87) Date publication PCT/PCT Publication Date: 2015/11/12
 (45) Date de délivrance/Issue Date: 2021/06/08
 (85) Entrée phase nationale/National Entry: 2016/11/09
 (86) N° demande PCT/PCT Application No.: PT 2014/000029
 (87) N° publication PCT/PCT Publication No.: 2015/171005

(51) Cl.Int./Int.Cl. *A61K 31/4985* (2006.01),
A61P 25/04 (2006.01)
 (72) Inventeurs/Inventors:
 PARDAL FILIPE, AUGUSTO EUGENIO, PT;
 EUFRASIO PEDROSO, PEDRO FILIPE, PT;
 ALMEIDA PECORELLI, SUSANA MARQUES, PT;
 CASIMIRO CAIXADO, CARLOS ALBERTO EUFRASIO,
 PT;
 LOPES, ANA SOFIA DA CONCEICAO, PT;
 DAMIL, JOAO CARLOS, PT
 (73) Propriétaire/Owner:
 TECNIMEDE SOCIEDADE TECNICO-MEDICINAL S.A.,
 PT
 (74) Agent: LAVERY, DE BILLY, LLP

(54) Titre : (S)-PIRLINDOLE OU SES SELS PHARMACEUTIQUEMENT ACCEPTABLES POUR UNE UTILISATION EN MEDECINE
 (54) Title: (S)-PIRLINDOLE AND ITS PHARMACEUTICALLY ACCEPTABLE SALTS FOR USE IN MEDICINE



(57) Abrégé/Abstract:

(S)-pirindole or its pharmaceutically acceptable salts, as well as pharmaceutical compositions comprising the same for use in the therapeutic treatment and prevention of pain.

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

(19) World Intellectual Property
Organization
International Bureau(10) International Publication Number
WO 2015/171005 A1(43) International Publication Date
12 November 2015 (12.11.2015)

- (51) **International Patent Classification:**
A61K 31/4985 (2006.01) *A61P 25/04* (2006.01)
- (21) **International Application Number:**
PCT/PT2014/000029
- (22) **International Filing Date:**
9 May 2014 (09.05.2014)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (71) **Applicant:** **TECNIMEDE SOCIEDADE TECNICO-MEDICINAL S.A.** [PT/PT]; Rua Tapada Grande, N.º 2, Abrunheira, P-2710-089 Sintra (PT).
- (72) **Inventors:** **PARDAL FILIPE, Augusto Eugénio;** Rua Sargento José Paulo dos Santos, N.º 8, P-1800-331 Lisboa (PT). **EUFRÁSIO PEDROSO, Pedro Filipe;** Rua Joaquim Rocha Cabral, N.º 14 - 9º B, P-1600-075 Lisboa (PT). **ALMEIDA PECORELLI, Susana Marques;** Rua Camilo Pessanha, N.º 138, Murches, P-2755-254 Alcabi-deche (PT). **CASIMIRO CAIXADO, Carlos Alberto Eufrásio;** Rua Sra Do Socorro, N.º 5, Longo da Vila, P-2640-433 Mafra (PT). **LOPES, Ana Sofia da Conceição;** Travessa do Valinho, N.º 3 - 1º trás-meio, Póvoa da Galega, P-2665-364 Milharado (PT). **DAMIL, João Carlos;** Rua Cidade Torres Vedras, N.º 15, Ermegeira, Maxial, P-2565-433 Torres Vedras (PT).
- (74) **Agent:** **FERREIRA PINTO, Francisca;** Av. da República, 25 - 1º, P-1050-186 Lisboa (PT).

(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, BN, 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, IR, IS, JP, KE, KG, 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, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, 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.

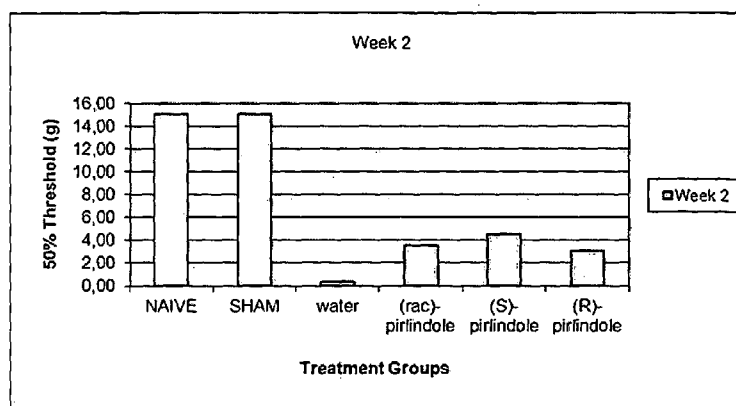
(84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, 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, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

Published:

— with international search report (Art. 21(3))

(54) **Title:** (S)-PIRLINDOLE OR ITS PHARMACEUTICALLY ACCEPTABLE SALTS FOR USE IN MEDICINE**Fig. 1A**(57) **Abstract:** (S)-pirindole or its pharmaceutically acceptable salts, as well as pharmaceutical compositions comprising the same for use in the therapeutic treatment and prevention of pain.

WO 2015/171005 A1

DESCRIPTION**(S)-PIRLINDOLE AND ITS PHARMACEUTICALLY ACCEPTABLE
SALTS FOR USE IN MEDICINE****Field of the invention**

The present invention relates to (S)-pirlindole enantiomer or its pharmaceutically acceptable salts, as well as compositions comprising the same for use in the therapeutic treatment and prevention of pain.

Prior Art background

The treatment of pain conditions is of utmost importance in medicine, leading to a worldwide need for additional therapies for its treatment and prevention.

Pain is the most common symptom for which patients seek medical attention. Although there is no exact definition it can be defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (International Association for the Study of Pain, IASP).

Pain is a very relevant symptom that presents a complex pathogenesis. Its presence is described in a large number of syndromes and diseases in medicine. In severe forms, pain translates into disabilities of diverse nature, difficulty in performing daily activities, interference with mood, professional performance and social relations.

Furthermore, the interpretation of pain varies from individual to individual, and within the individual himself, at different times of his life, depending on varying physical, biological, socio-cultural and emotional conditions.

Regarding the topographic distribution it is possible to distinguish three types of pain: peripheral pain, central pain and psychogenic pain.

Peripheral pain is the result of an organic disorder in peripheral nerve fibers, which, while transmitting nerve impulses, originate the sensation of pain. Peripheral pain further divides into superficial peripheral pain and deep peripheral pain. The first is acute and penetrating and it is located at the point of origin. Deep peripheral pain may be classified as somatic or visceral. Somatic deep peripheral pain can be localised (pain is located at the point of origin of the painful stimulus) or radiating (pain is diffuse and distant from the point of origin of the painful stimulus). Finally, deep visceral peripheral pain has a difficult topography to limit.

Central pain is a spontaneous pain resulting from neurological injury of central nerve centres such as the spinal cord, *medulla oblongata*, the thalamus or the cerebral cortex.

Psychogenic pain is defined as a painful sensation that does not have an organic substrate. It is any pain that is exclusively mental, which acquires a specific anatomical location.

From the pathophysiological point of view, pain can be nociceptive or neuropathic.

Nociceptive pain can be defined as pain that arises from actual or threatened damage to non-neural tissue and is due to the activation of nociceptors. Nociceptive pain can be classified as somatic or visceral. Somatic pain is due to activation of the nociceptive receptors in somatic tissues, such as bone, joint, muscle or skin. In visceral pain the visceral nociceptors are activated by different pathological mechanisms (e.g. mechanical injury, inflammation, radiation, toxic agents). Both visceral and somatic nociceptive pain can be acute or chronic. Visceral pain is more difficult to characterise and less sensitive to usual pain treatment. Some pain syndromes

(e.g., cancer pain) include elements of both visceral and somatic nociceptive pain.

Neuropathic pain can be defined as pain arising as a direct consequence of a lesion or disease affecting the somatosensory system (i.e. peripheral nerve, the dorsal root ganglion or dorsal root or the central nervous system).

Almost any of the pathological processes known to create damage or dysfunction to neural tissue can be considered as potential causes for neuropathic pain: viral, bacterial, aseptic inflammation, pressure due to neoplasm or other structural lesions, degenerative, ischemia, autoimmune, toxic, traumatic or endocrine/metabolic mechanisms all have been implicated in the production of neuropathic pain.

However, the most commonly studied neuropathic pain conditions include diabetic neuropathic pain (diabetic NP), post-herpetic neuralgia (PHN), trigeminal neuralgia and central neuropathic pain (spinal cord injury (SCI), central post-stroke pain (CPSP) and multiple sclerosis associated pain).

Other neuropathic pain conditions include neuropathy associated with HIV infection (HIV neuropathy), post-traumatic or post-surgical neuropathic pain, chronic radiculopathy, cancer associated neuropathic pain, phantom pain and multi-aetiology neuropathic pain.

Neuropathy is a functional disorder or pathological change that affects the nerves. It is called mononeuropathy if it involves a single nerve trunk, multiple mononeuropathy if it successively involves several nerve trunks and polyneuropathy if it involves, diffusely and bilaterally, several nerve trunks.

Peripheral neuropathies may be sensory, motor or autonomic. The most frequent motor manifestations are muscle spasms, clonus, fasciculations, amyotrophy and the loss of muscle strength or dexterity.

Negative sensory manifestations include hypoalgesia and hypoaesthesia. In turn, positive sensory manifestations include paresthesia, dysesthesia, hyperpathia, hyperalgesia and allodynia, aside from the sensation of pricking, tingling or tinnitus. Examples of neuropathy include, peripheral polyneuropathy, characterised by a burning or needle pricking sensation in the feet and fingertips, which is worsened by walking, allodynia, feet hypoaesthesia, and absence of Achilles reflex; trigeminal neuralgia, characterised by severe acute pain, shock-like, with duration of seconds in the upper lip and nose, which worsens with chewing or brushing and which is not accompanied by changes in the neurological examination; carpal tunnel syndrome, characterised by acute pain, tingling sensations and reduced sensitivity in the 1st, 2nd and 3rd fingers and palmar surface of the hand, that worsens at night, with decreased muscle strength in thumb abduction and positive Phalen test; mononeuropathy of the lateral cutaneous nerve of the thigh, characterised by a burning and pricking sensation to the side of the thigh and presence of a circumscribed area of cutaneous hypersensitivity; postherpetic neuralgia, characterised by a burning pain with banner-like distribution on the thoracic region after the appearance of vesicles, which does not improve after skin healing, hyperchromic skin spots and allodynia.

The impact of pain on individual and social welfare has increased in recent decades.

To this regard, neuropathic pain is widely recognised as one of the most difficult pain syndromes to manage, and outcomes often are unsatisfactory. Neuropathic pain is estimated to afflict as high as 7-8% of the general population in Europe (N. Attal, G. Cruccu, R. Baron, M. Haanpaa, P. Hansson, T.S. Jensen and T. Nurmikko, EFNS guidelines on the pharmacological treatment of neuropathic pain: 2010 revision, *European Journal of Neurology* 2010, 17 : 1113-1123).

Management of neuropathic pain is a complicated endeavour and often is frustrating to patient and physician alike. Drugs with mechanisms of action that are not aimed at changing the underlying physiopathological processes, or that are administered at sub-therapeutic doses, are frequently used in the treatment of pain, and *a fortiori* of neuropathic pain.

This stems from the relatively incomplete understanding of mechanisms of actions of drugs and the limited efficacy of currently available analgesics. Therapeutic approaches vary greatly among physicians and recalcitrant chronic pain syndromes warrant an interdisciplinary approach and more potent pharmacotherapies.

The use of analgesic medications follows a step-wise approach. The 1st step corresponds to mild pain, and should be treated with non-opioid analgesic drugs (anti-inflammatory drugs and paracetamol); the 2nd is moderate pain, for which weak opioids are indicated and, in the third step, for severe pain, strong opioids are indicated. Depending on the intensity of pain, therapy should be initiated at the corresponding level, with appropriate doses. If the pain persists or worsens, drug doses should be optimised.

Analgesic drugs can be distributed in two large groups: non-opioid and opioid analgesic drugs.

Non-opioid analgesic drugs include paracetamol and nonsteroidal anti-inflammatory drugs (NSAIDs). Paracetamol does not have a well known mechanism of action although a central action is assumed. The administration of large doses either acutely or chronically, involves a risk of hepatotoxicity associated with depletion of sulfhydryl radicals.

Nonsteroidal anti-inflammatory drugs act by inhibiting cyclooxygenase (COX), inhibiting the synthesis of prostaglandins (PG) involved in the inflammatory process and in pain. They may be non-selective, inhibiting COX₁ and COX₂, as acetylsalicylic acid (AAS), ibuprofen or diclofenac, or selective, inhibiting only COX₂, as celecoxib, rofecoxib or etoricoxib. Nonsteroidal anti-inflammatory drugs present several side effects, including gastrointestinal (nausea, epigastralgia, ulceration, bleeding), renal (acute renal failure, interstitial nephritis), haematological (platelet anti-aggregation, haemorrhage) or hypersensitivity reactions. These effects are most notorious with non-selective NSAIDs. The main side effect with COX₂ inhibitors is an increased cardiovascular risk, by inhibiting

prostacyclin. Nonsteroidal anti-inflammatory drugs treatment is associated with an increased risk of gastrointestinal adverse reactions.

The so-called weak opioid drugs include drugs like tramadol, hydrocodone and dextropropoxyphene. These drugs have a maximum posology due to the increased incidence of adverse reactions at high doses. Tramadol is a synthetic derivative of codeine that acts by binding to opioid μ receptors and inhibiting neuronal reuptake of serotonin. Tramadol is metabolised in the liver and undergoes renal elimination, therefore it may require dose decrease or prolonged interval between doses in patients with kidney or renal failure.

Hydrocodone is a synthetic derivative of codeine and dextropropoxyphene and presents a structure similar to methadone. Patients with mild to moderate pain should be treated with a weak opioid associated with a NSAID or paracetamol. If pain cannot be controlled at therapeutic doses, a switch to another drug in this group should not be considered and more potent opioids should be used.

Opioids act by binding to receptors μ , κ , and δ and may be classified, according to their intrinsic activity, in agonists (morphine, fentanyl, methadone, oxycodone, hydromorphone and pethidine), partial agonist/antagonist (buprenorphine) and agonist/antagonist (pentazocine). A pure agonist is a drug which exerts effect on μ receptors and on the other receptors. A partial agonist/antagonist exerts a partial agonist effect on a receptor and antagonist effect in at least one receptor, and an agonist/antagonist exerts a pure agonist effect on a receptor and an antagonist effect on at least one receptor.

Opioids induce predictable undesirable side effects, which if not minimised can hamper dose titration and the patient's treatment compliance. Side effects include respiratory depression, nausea and vomiting, constipation, urinary retention, euphoria, sedation, miosis, antitussive action, hypotension, bradycardia, cognitive changes (hallucinations, delusional ideas), hyperalgesia, myoclonus, dyspepsia, pruritus, tolerance and dependence.

Morphine can be administered by oral or parenteral route (subcutaneous and intravenous). Intravenous administration requires specific care and its use is limited to the inpatient setting. For subcutaneous administration infusion pumps are used.

Tricyclic antidepressants (amitriptyline, nortriptyline and desipramine) are used in combination with analgesic drugs, particularly in combination with opioids and mostly in the treatment of neuropathic pain. Due to their anti-cholinergic action, adverse reactions include constipation, xerostomia, urinary retention and tachycardia.

Anticonvulsants (carbamazepine, oxcarbazepine, phenytoin, sodium valproate, clonazepam, lamotrigine, gabapentin, pregabalin) are also used in the treatment of pain, especially neuropathic pain. Carbamazepine and phenytoin can cause liver toxicity, leukopaenia and thrombocytopaenia, thus requiring monitoring of serum concentrations.

Corticosteroids, of which dexamethasone is most often used for the treatment of pain, are associated with the onset of gastrointestinal disorders, *diabetes mellitus*, neuro-psychiatric disorders and proximal myopathy.

Therefore it is still a need in the state of the art to find new therapeutic approaches for the treatment of pain, not only as a symptom of disease, but as a disease itself.

The problem that the present invention solves is the provision of an alternative, new and effective agent for the treatment of pain, which alone or in combination with other analgesic agents provides an effective treatment of pain with reduced side effects while maintaining the desirable analgesic activity.

Brief description of the invention

The present invention is based on the findings that the optically active compound (S)-pirlindole enantiomer shows an advantageous analgesic activity, useful for the therapeutic treatment and prevention of pain.

The inventors of the present invention have surprisingly found that the optically active (S)-pirlindole enantiomer shows analgesic activity in an animal model of pain, which in turn explains the unexpected activity of (S)-pirlindole enantiomer, in the therapeutic treatment and prevention of pain, in particular, neuropathic pain.

Furthermore the analgesic effect of (S)-pirlindole enantiomer is shown to be unexpectedly higher when compared to (R)-pirlindole enantiomer and (rac)-pirlindole.

(S)-pirlindole is shown to be an effective agent for the treatment and prevention of pain, in particular, neuropathic pain, when used alone as the sole active ingredient or in a combination therapy with additional analgesic agents, where (S)-pirlindole has been proven to also potentiate the effect of these additional analgesic agents.

The object of the present invention is therefore directed to the provision of (S)-pirlindole or a pharmaceutically acceptable salt thereof as an analgesic agent, for the treatment of pain.

A further object of the present invention is also directed to the provision of (S)-pirlindole or a pharmaceutically acceptable salt thereof alone or as part of a combination therapy with at least one additional analgesic agent, for the treatment of pain.

An additional object of the present invention is also directed to the provision of a pharmaceutical composition comprising (S)-pirlindole or a pharmaceutically acceptable salt

thereof together with acceptable carriers, vehicles and/or suitable excipients, for the treatment of pain.

A further object of the present invention is also directed to the provision of a pharmaceutical composition comprising (S)-pirlindole or a pharmaceutically acceptable salt thereof, in combination with at least one additional analgesic agent and together with acceptable carriers, vehicles and/or suitable excipients, for the treatment of pain, where both of (S)-pirlindole or a pharmaceutically acceptable salt thereof and the other analgesic agents are formulated so as to be administered in a fixed-dose or separate dose, such as, for simultaneous or sequential administration.

A still further object of the present invention is also directed to (S)-pirlindole or a pharmaceutically acceptable salt thereof, for use in a combination therapy with additional analgesic agents, for potentiating the effects of these additional analgesic agents in the treatment of pain.

The present invention further provides a method for the treatment of pain in a subject in need thereof, characterized by administering to the subject an effective amount of (S)-pirlindole or a pharmaceutically acceptable salt thereof, alone or in combination with at least one additional analgesic agent.

The present invention further provides a method for the treatment of pain in a subject in need thereof, by potentiating the effect of the additional analgesic agents, characterized by administering to the subject an effective amount of (S)-pirlindole or a pharmaceutically acceptable salt thereof in combination with additional analgesic agents

Description of the drawings

Figure 1A illustrates the mean lowest force (g) that evoke a brisk withdrawal response during treatment week 2 (TW2) in groups: naive control (negative control), sham control, SNI plus water (positive control), SNI plus (rac)-pirlindole 20 mg/kg, SNI plus (S)-pirlindole 20 mg/kg and SNI plus (R)-pirlindole 20 mg/kg.

Figure 1B illustrates the mean lowest force (g) that evoke a brisk withdrawal response during treatment week 3 (TW3) in groups: naive control (negative control), sham control, SNI plus water (positive control), SNI plus (rac)-pirlindole 20 mg/kg, SNI plus (S)-pirlindole 20 mg/kg and SNI plus (R)-pirlindole 20 mg/kg.

Detailed description of the invention

In the context of the present invention, the following terms have the meaning described below:

"Pain" as used in the present invention encompasses any unpleasant sensory and emotional experience associated with actual or potential damage, or described in terms of such damage. It includes: peripheral pain, central pain and psychogenic pain, and comprises superficial peripheral pain, deep peripheral pain, somatic deep peripheral pain, visceral deep peripheral pain, pain resulting from neurological injury of central nerve centres such as the spinal cord, *medulla oblongata*, the thalamus or the cerebral cortex, and psychogenic pain that does not have an organic substrate.

Further, "pain" comprises nociceptive or neuropathic pain. Nociceptive pain being pain that arises from actual or threatened damage to non-neural tissue and which is due to the activation of nociceptors, and being classified as somatic or visceral. Somatic pain is due to activation of the nociceptive receptors in somatic tissues (e.g. bone, joint, muscle or skin). In visceral pain the visceral nociceptors are activated by different pathological mechanisms (e.g. mechanical injury, inflammation, radiation, toxic agents). Both visceral and somatic nociceptive pain can be acute or chronic, and include, cancer pain.

"Neuropathic pain" according to the present invention can be defined as pain arising as a direct consequence of a lesion or disease affecting the somatosensory system (i.e. peripheral nerve, the dorsal root ganglion or dorsal root, or the central nervous system) and includes pain associated to:

- Neuropathies, which are a functional disorder or pathological change that affects the nerves, such as mononeuropathy if it involves a single nerve trunk, multiple mononeuropathy if it successively involves several nerve trunks and polyneuropathy if it involves, diffusely and bilaterally, several nerve trunks.

- Peripheral neuropathies which may be sensory, motor or autonomic. The most frequent motor manifestations include muscle spasms, clonus, fasciculations, amyotrophy and the loss of muscle strength or dexterity.

- Negative sensory manifestations such as, hypoalgesia and hypoesthesia.

- Positive sensory manifestations, such as, paresthesia, dysesthesia, hyperpathia, hyperalgesia and allodynia, aside from the sensation of pricking, tingling or tinnitus.

Among others, examples of neuropathic pain include: diabetic neuropathic pain (diabetic NP), post-herpetic neuralgia (PHN), trigeminal neuralgia and central neuropathic pain (spinal cord injury (SCI), central post-stroke pain (CPSP) and multiple sclerosis associated pain, neuropathy associated with HIV infection (HIV neuropathy), post-traumatic or post-surgical neuropathic pain, chronic radiculopathy, cancer associated neuropathic pain, phantom pain and multi-aetiology neuropathic pain, peripheral polyneuropathy, characterised by a burning or needle pricking sensation in the feet and fingertips, which is worsened by walking, allodynia, feet hypoesthesia, and absence of Achilles reflex; trigeminal neuralgia, characterised by severe acute pain, toxic neuropathies, compression neuropathies and trauma, which are characterized by spontaneous lancinating, burning pain and shock-like pain as well as pain hypersensitivity including tactile allodynia, pin prick hyperalgesia and hyperpathia, shock-like pain with duration of seconds in the upper lip and nose, which worsens with chewing or brushing and which is not accompanied by changes in the neurological examination; carpal tunnel syndrome, characterised by acute pain, tingling sensations and reduced sensitivity in

the 1st, 2nd and 3rd fingers and palmar surface of the hand, that worsens at night, with decreased muscle strength in thumb abduction and positive Phalen test; mononeuropathy of the lateral cutaneous nerve of the thigh, characterised by a burning and pricking sensation to the side of the thigh and presence of a circumscribed area of cutaneous hypersensitivity; postherpetic neuralgia, characterised by a burning pain with banner-like distribution on the thoracic region after the appearance of vesicles, which does not improve after skin healing and hyperchromic skin spots.

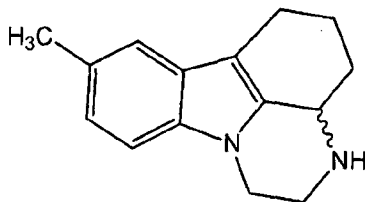
The term "potentiation of the effect of additional analgesic agents", means that the coadministration, in a fixed-dose or separate dose, such as, in simultaneous or sequential form of (S)-pirlindole and at least one additional analgesic agent, including opioids and opiates, enhances the analgesic effect of the latter, allowing for a lower dose of active agents to be administered and reducing the risk of occurrence of adverse reactions, dependence and tolerance.

For purposes of this invention, it is considered as enantiomerically pure when enantiomeric purity as calculated by chiral chromatography or specific optical rotation is equal to or greater than 97%.

The term "pharmaceutically acceptable salt" refers to those salts that are within the scope of proper medicinal assessment, suitable for use in contact with human tissues and organs and those of lower animals, without undue toxicity, irritation, allergic response or similar and are consistent with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are technically well known. A list of preferred salts, in the context of the present invention, is mentioned in this description.

The term "pharmaceutically acceptable carrier, vehicle or excipient" as used here, means a solid, semi-solid or inert fluid excipient, filler, encapsulating or formulation aiding material of any kind already known by one skilled in the art.

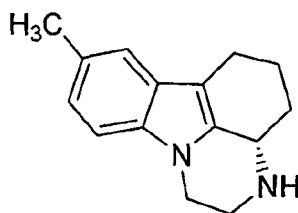
Pirlindole, 2,3,3a,4,5,6-hexahydro-8 methyl-1H-pyrazino [3, 2, 1-j, k] carbazole, is a tetracyclic compound having the following formula:



Pirlindole is a reversible inhibitor of monoamine oxidase A (MAO-A) so far useful as a medicine in the treatment of depression.

Pirlindole has an asymmetric carbon atom, but to date only the racemate, (rac)-pirlindole, corresponding to a mixture of both enantiomers (R) and (S) in equal amounts, is used in medicine.

(S)-Pirlindole enantiomer has the following formula:



For the purpose of the present invention, (S)-pirlindole enantiomer may, for example, be obtained according to the method described by P.Chiap et al. in "Automated determination of pirlindole enantiomers in plasma by on-line coupling of a pre-column packed with restricted access material to a chiral liquid chromatographic column" *Journal of Pharmaceutical and Biomedical Analysis*, 27(2002) 447-455.

According to this method, (S)-pirlindole may be synthesised by derivatisation of racemic pirlindole with R-phenethylisocyanate, separation of the corresponding diastereoisomers by preparative LC, hydrolysis and recrystallization.

The (S)-pirlindole provided in the present invention is enantiomerically pure.

The present invention is based on the findings that the optically active (S)-pirlindole enantiomer shows analgesic activity in an animal model of pain, which in turn explains the unexpected activity of (S)-pirlindole enantiomer, in the therapeutic treatment and prevention of pain, in particular, neuropathic pain.

Therefore the subject invention provides (S)-pirlindole enantiomer and its pharmaceutically acceptable salts as an alternative and new agent for the preventive and therapeutic treatment of pain, more specifically, neuropathic pain, including pain associated with any type of peripheral neuropathy.

The inventors of the present application have found that (S)-pirlindole enantiomer or its pharmaceutically acceptable salts may be used alone as the sole active ingredient or as part of a combination therapy with at least one additional analgesic agent in the therapeutic treatment and prevention of pain.

(S)-pirlindole enantiomer provides improved efficacy in the preventive and therapeutic treatment of pain, more specifically, neuropathic pain. Additionally, (S)-pirlindole exhibits a safer profile in comparison with other analgesic drugs. (S)-pirlindole doesn't present the side effects of opioids, among which are particularly relevant urinary retention, nausea and vomiting, respiratory effects (respiratory depression) and tolerance. Additionally, the use of (S)-pirlindole will overcome the side effects related with nonsteroid anti-inflammatory drugs (NSAIDs) including renal injury, gastrointestinal bleeding, platelet inhibition, hypertension and congestive heart failure. Advanced

age is a primary risk factor for adverse gastrointestinal events associated with NSAID administration. The mortality rate among patients hospitalized for NSAID-induced upper gastrointestinal bleeding is approximately 5-10%.

In fact when (S)-pirlindole enantiomer or its pharmaceutically acceptable salts are used in a combination therapy with additional analgesic agents, the analgesic effect of these additional analgesic agents is potentiated, so that a lower dose of active ingredients is needed to achieve the desirable therapeutic effect thereby reducing the risk of occurrence of adverse reactions, dependence and tolerance to these analgesic drugs.

The coadministration of (S)-pirlindole enantiomer or its pharmaceutically acceptable salts together with at least one additional analgesic agent allows reducing the dose of the analgesic agents from 25 - 50% from the dose taken in a non combination therapy.

The coadministration or use in a combination therapy of (S)-pirlindole and additional analgesic agents includes the coadministration of (S)-pirlindole or a pharmaceutically acceptable salt thereof with at least one, (two, three, four, etc.) additional analgesic agents, where the formulation of the active ingredients is either in a fixed-dose (single product) or in separate dose products, where each active ingredient is formulated in a suitable form to be administered in simultaneous or sequential form.

The present invention contemplates the presentation of the said product for combination therapy in a combination pack together with instructions of use.

The additional analgesic agents or drugs contemplated to be used according to the present invention in a combination therapy with (S)-pirlindole or its pharmaceutically acceptable salts, comprise at least one compound or drug from the following list: compounds that decrease neuronal hyperexcitability, such as, inhibitors of the sodium channels (carbamazepine, oxcarbazepine,

eslicarbazepine, phenytoin, valproic acid), calcium channel antagonists (pregabalin), ionotropic and metabotropic glutamate receptor antagonists, γ -aminobutyric acid activity enhancers (gabapentin) or μ , κ , and δ opioid receptor agonists, partial agonists/antagonists and antagonists.

Additionally, a patient who suffers from pain or that might suffer from pain in the future, more specifically of neuropathic pain associated with any type of peripheral neuropathy may benefit from using, according to the present invention, in a fixed or a separate combination therapy, (S)-pirlindole or its pharmaceutically acceptable salts and at least one compound or drug from the following list: compounds with any mechanism of action other than decreasing neuronal hyperexcitability, such as, paracetamol, nonsteroidal anti-inflammatory drugs (acetylsalicylic acid, diclofenac, nabumetone, nimesulide, nabumetone, etodolac, piroxicam, lysine clonixinate, diflunisal, acemetacin, glucametacin, indometacin, proglumetacin, oxametacin, sulindac, aceclofenac, fentiazac, ketorolac, zomepirac, meloxicam, tenoxicam, lornoxican, fenoprofen, fenbufeno, flurbiprofen, benoxaprofen, ibuprofen, ketoprofen, dexketoprofen, pirprofen, indoprofen, naproxen, oxaprozin, tiaprofen, dexibuprofen, meclofenamic acid, mefenamic acid, flufenamic acid, tolfenamic acid, niflumic acid, etofenamate, azapropazone, orgotein, feprazone, morniflumate, tenidap, glycosaminoglycan, polysulfate, celecoxib, rofecoxib, parecoxib, valdecoxib, and etoricoxib), glucosamine or diacerein.

The present invention contemplates the provision of compositions comprising an effective amount of (S)-pirlindole compound or a pharmaceutically acceptable salt thereof which are formulated into pharmaceutical compositions in combination with one or more pharmaceutically acceptable carriers according to known methods for preparing pharmaceutically useful compositions.

Such formulations are described in detail in a number of sources which are well known and readily available to those skilled in the art. For example, Remington's The Science and Practice of Pharmacy, edited by Allen, Loyd V., Jr, 22nd edition, describe the making of formulations which can be used in connection with the subject invention.

Pharmaceutical compositions of the present invention can be formulated to be administered to humans and other mammals orally, (in liquid or solid forms) rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (in the form of powders, ointments or drops), buccally or in the form of an oral or nasal spray. The term "parenterally", as used here, refers to modes of administration, including intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous, intra-articular injection and infusion.

Pharmaceutical compositions for parenteral injection include aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, pharmaceutically acceptable sterile or non-sterile, and powders for reconstitution in sterile injectable solutions or dispersions.

If it is required, and for a more effective distribution, the compounds of the invention can be embedded in extended controlled release or directed administration systems, such as polymeric matrices, liposomes and microspheres.

The dosage of (S)-pirlindole compound or the pharmaceutically acceptable salt thereof administered to an individual will be dependent upon the response desired and may be dependent upon the subject to be treated, its age, health, weight, frequency of treatment etc. For example, dosage levels contemplated according to the present invention comprise, from 0.1 to 10 mg/kg for oral administration, from 0.01 to 10 mg/kg for intravenous administration.

The (S)-pirlindole molecule has a secondary amine group, which has basic characteristics, thereby easily forming acid salts, which can be, in the context of the present invention, pharmaceutically acceptable acid salts.

Representative pharmaceutically acceptable acid salts include, but are not limited to acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate, fumarate, glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydrogen iodide, 2-hydroxyethanesulfonate (isethionate), lactate, maleate,

methanesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, phosphate, glutamate, bicarbonate, p-toluenesulfonate, undecanoate and mandelate.

~~Examples of acids that can be used to form preferred~~
pharmaceutically acceptable acid salts with the (S)-pirlindole compound of the invention, include inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid and phosphoric acid and organic acids such as methanesulfonic acid, citric acid, mandelic acid, succinic acid and citric acid.

Preferred pharmaceutically acceptable salts are: (S)-pirlindole-(R)mandelate salt, (S)-pirlindole mesylate salt and (S)-pirlindole citrate salt.

The following examples are intended to illustrate the invention and should not be understood as limiting the scope of the present invention.

EXAMPLES

Example 1:

Tablets of S-Pirlindole hydrochloride

Manufacture of the tablets proceeded by direct compression and included mixing all components, passing them through a sieve and pressing with a suitable compressing force at the required relative atmospheric humidity.

Qualitative and Quantitative composition (amounts are given as weight % of the total composition)

(S) - Pirlindole hydrochloride	25,00
Colloidal Silicon Dioxide	1,50
Lactose monohydrate	45,50
HPMC	2,00
Cellulose microcrystalline	21,00
Croscarmellose sodium	4,00
Calcium stearate	1,00

Tablets of S-Pirlindole hydrochloride

Manufacture of the tablets proceeded by wet granulation followed by compression, including granulating the active ingredients with a suitable solution, drying and passing through a sieve, adding the necessary excipients and pressing with a suitable compression force at the required relative atmospheric humidity.

Qualitative and Quantitative composition (amounts are given as weight % of the total composition)

(S) - Pirlindole hydrochloride	25,00
Cellulose microcrystalline	15,00
Lactose monohydrate	50,00
HPMC	4,00
Croscarmellose sodium	5,00
Calcium stearate	1,00

Example 2:

This *in vivo* assay shows the beneficial effects of (S)-pirlindole enantiomer in the treatment of pain.

For this purpose, the inventors have chosen the animal model designed by Decosterd and Woolf (Pain, 2000), which developed a partial denervation model, technically easy to perform and subject to minimal variability in the degree of damage produced. Most important was that this is a model that will enable a direct measurement of changes in both injured

primary sensory neurons and in neighbouring intact sensory neurons so that their relative contribution to the pathophysiology of pain could be investigated.

The essential idea of the model is to see what happens if two of the three terminal distal branches of the sciatic nerve are axotomized (tibial and common peroneal nerves) sparing one (sural nerve); hence, this model is called the spared nerve injury (SNI) model.

The spared nerve injury model has proved to be robust, with substantial and prolonged changes in mechanical sensitivity and thermal responsiveness that closely mimic many features of clinical neuropathic pain.

All procedures were performed on adult (200-250 g) male Sprague-Dawley rats. Under halothane (2%) anesthesia, the skin on the lateral surface of the thigh was incised and a section made directly through the biceps femoris muscle exposing the sciatic nerve and its three terminal branches: the sural, the common peroneal and the tibial nerves. The spared nerve injury procedure comprised an axotomy and ligation of the tibial and common peroneal nerves leaving the sural nerve intact. The common peroneal and the tibial nerves were tight-ligated with 5.0 silk and sectioned distal to the ligation, removing 2±4 mm of the distal nerve stump. Great care was taken to avoid any contact with or stretching of the intact sural nerve. Muscle and skin were closed in two layers. Sham controls involved exposure of the sciatic nerve and its branches without any lesion.

The Von Frey test is a mechanical sensitivity assay that consists of thin calibrated plastic filaments, called Von Frey hairs, which are applied to the plantar surface of the hindpaw of the tested animal. The filaments present different degrees of stiffness that are used to determine the one that evokes a hindpaw withdrawal. Each filament, starting with the less stiff, is applied to the plantar surface of both hindpaws of each rat. The process is repeated with Von Frey hairs of increasing stiffness until one evokes a reflexive and sustained hindpaw withdrawal. This is defined as the withdrawal threshold, is measured in grams and is an indicator of mechanical allodynia.

In the present example the animals were divided in 6 (six) groups (n= 7 per group): naive control (negative control), sham control, SNI plus water (positive control), SNI plus (rac)-pirlindole 20 mg/kg, SNI plus (S)-pirlindole 20 mg/kg, SNI plus (R)-pirlindole 20 mg/kg and SNI plus (rac)-pirlindole 20 mg/kg.

After the application of surgical procedure to the groups of sham control, SNI plus water (positive control), SNI plus (rac)-pirlindole, SNI plus (S)-pirlindole and SNI plus (R)-pirlindole, the animals were confined during a 7 weeks (7 wk) period aiming the full induction of mechanical allodynia. Following a 3 week (3 wk), treatment period was started and water or one of the test items (rac-pirlindole, S-pirlindole and R-pirlindole) were administered by intraperitoneal (ip) route, on a daily basis.

The formulations used in the present assay were the following:

Intraperitoneal solution of S-Pirlindole hydrochloride

Compounds name	Formula		Functional Category
	µg/ml	%	
Active compound:	µg/ml	%	
(S) - Pirlindole hydrochloride	5720	100,00	Active compound
Excipients:	µg/ml	%	
Purified water	---	---	Solvent agent

Intraperitoneal solution of R-Pirlindole hydrochloride

Compounds name	Formula		Functional Category
	µg/ml	%	
Active compound:	µg/ml	%	
(R) - Pirlindole hydrochloride	5720	100,00	Active compound
Excipients:	µg/ml	%	
Purified water	---	---	Solvent agent

Intraperitoneal solution of Pirlindole hydrochloride

Compounds name	Formula		Functional Category
	$\mu\text{g/ml}$	%	
Active compound:	$\mu\text{g/ml}$	%	
(rac)-Pirlindole hydrochloride	5720	100,00	Active compound
Excipients:	$\mu\text{g/ml}$	%	
Purified water	---	---	Solvent agent

The (S)-Pirlindole Hydrochloride, (R)-Pirlindole Hydrochloride and (rac)-Pirlindole Hydrochloride were prepared by dissolving the active ingredient in water until complete dissolution.

At the 2nd and 3rd week of treatment the mechanical allodynia was tested. The animals were placed on an elevated wire grid and the plantar surface of the paw stimulated with a series of ascending force Von Frey monofilaments. The threshold was taken as the lowest force that evoked a brisk withdrawal response to one of the repetitive stimuli (Tal and Bennett, 1994). The lateral and medial plantar surfaces of the paw as well as its dorsal surface were tested.

The mean lowest force (g) that evoked a brisk withdrawal response was for the positive group (control positive group) 0,41g at the end of wk2 and 0,14g at the end of wk3. These values are much lower when compared with those obtained from naïve group (negative control group) and the sham group that presented both, the mean lowest force of 15 g at the end of wk2 and wk3. See Fig. 1A and Fig.1B.

In the active treatment group of SNI plus (S)-pirlindole 20 mg/kg i.p. the mean lowest force was surprisingly improved (4,51 g and 7,43 g, respectively at wk2 and wk3) in comparison with the active treatment group of SNI plus (R)-pirlindole 20 mg/kg i.p. (where the mean lowest force were reported as 3.08 g and 1.39 g, at wk2 and wk3, respectively) and with the active treatment group of SNI plus (rac)-pirlindole 20 mg/kg (where the mean lowest force were reported as 3.50 g and 2.08 g, respectively, at wk2 and wk3). See Fig.1A and Fig.1B.

These unexpected results sustain the benefit of (S)-pirlindole for the treatment of pain, specifically the neuropathic pain syndromes, and moreover, show the unexpected superior effect of (S)-pirlindole versus (rac)- and (R)-pirlindole compounds in the said treatment.

Lisbon, May 8, 2014.

CLAIMS

1. An (S)-pirlindole enantiomer or a pharmaceutically acceptable salt thereof for use in the treatment or prevention of neuropathic pain.
2. The (S)-pirlindole enantiomer or pharmaceutically acceptable salt thereof for use according to claim 1, characterized in that the (S)-pirlindole is enantiomerically pure, wherein enantiomeric purity as calculated by chiral chromatography or specific optical rotation is equal to or greater than 97%.
3. The (S)-pirlindole enantiomer or pharmaceutically acceptable salt thereof for use according to claim 1 or 2, in a combination therapy with at least one additional analgesic agent.
4. The (S)-pirlindole enantiomer or pharmaceutically acceptable salt thereof for use according to claim 3, wherein the combination therapy is a fixed-dose combination therapy or a separate combination therapy that comprises sequential or simultaneous administration of active agents.
5. The (S)-pirlindole enantiomer or pharmaceutically acceptable salt thereof for use according to claim 3 or 4, for further use in potentiating the effects of the at least one additional analgesic agent in the treatment of neuropathic pain.
6. The (S)-pirlindole enantiomer or pharmaceutically acceptable salt thereof for use according to any one of claims 3 to 5, characterized in that the at least one additional analgesic agent is a compound that decreases neuronal hyperexcitability, and that is an inhibitor of sodium channel; a calcium channel antagonist; an ionotropic glutamate receptor antagonist; a metabotropic glutamate receptor antagonist; a γ -aminobutyric acid activity enhancer; a μ opioid receptor agonist; a κ opioid receptor agonist; a δ opioid receptor agonist; a μ opioid receptor partial agonist; a κ opioid receptor partial agonist; a δ opioid receptor partial agonist; a μ opioid receptor partial antagonist; a κ opioid receptor partial antagonist; a δ opioid receptor partial

antagonist; a μ opioid receptor antagonist; a κ opioid receptor antagonist; or a δ opioid receptor antagonist.

7. The (S)-pirlindole enantiomer or pharmaceutically acceptable salt thereof for use according to claim 6, characterized in that the inhibitor of sodium channel is carbamazepine, oxcarbazepine, eslicarbazepine, phenytoin or valproic acid.

8. The (S)-pirlindole enantiomer or pharmaceutically acceptable salt thereof for use according to claim 6, characterized in that the calcium channel antagonist is pregabalin.

9. The (S)-pirlindole enantiomer or pharmaceutically acceptable salt thereof for use according to claim 6, characterized in that the γ -aminobutyric acid activity enhancer is gabapentin.

10. The (S)-pirlindole enantiomer or pharmaceutically acceptable salt thereof for use according to any one of claims 3 to 5, characterized in that the at least one additional analgesic agent is paracetamol, glucosamine, diacerein or a nonsteroidal anti-inflammatory drug.

11. The (S)-pirlindole enantiomer or pharmaceutically acceptable salt thereof for use according to claim 10, wherein the nonsteroidal anti-inflammatory drug is acetylsalicylic acid, diclofenac, nabumetone, nimesulide, nabumetone, etodolac, piroxicam, lysine clonixinate, diflunisal, acetaminophen, glucametacin, indometacin, proglumetacin, oxametacin, sulindac, aceclofenac, fentiazac, ketorolac, zomepirac, meloxicam, tenoxicam, lornoxican, fenoprofen, fenbufen, flurbiprofen, benoxaprofen, ibuprofen, ketoprofen, dexketoprofen, piroprofen, indoprofen, naproxen, oxaprozin, tiaprofen, dexibuprofen, meclofenamic acid, mefenamic acid, flufenamic acid, tolfenamic acid, niflumic acid, etofenamate, azapropazone, orgotein, feprazone, morniflumate, tenidap, glycosaminoglycan, polysulfate, celecoxib, rofecoxib, parecoxib, valdecoxib, or etoricoxib.

12. The (S)-pirlindole enantiomer or pharmaceutically acceptable salt thereof for use according to any one of claims 1 to 11, in the form of (S)-pirlindole (R)-mandelate salt.

13. The (S)-pirlindole enantiomer or pharmaceutically acceptable salt thereof for use according to any one of claims 1 to 11, in the form of (S)-pirlindole mesylate salt.

14. The (S)-pirlindole enantiomer or pharmaceutically acceptable salt thereof for use according to any one of claims 1 to 11, in the form of (S)-pirlindole citrate salt.

15. Pharmaceutical composition for use in the treatment of neuropathic pain comprising a (S)-pirlindole enantiomer or a pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 14, together with a pharmaceutically acceptable carrier, vehicle or excipient.

16. The use of (i) a compound that is a (S)-pirlindole enantiomer or a pharmaceutically acceptable salt thereof; or (ii) a pharmaceutical composition comprising (i), together with a pharmaceutically acceptable carrier, vehicle or excipient, for the treatment or prevention of neuropathic pain.

17. The use of (i) a compound that is a (S)-pirlindole enantiomer or a pharmaceutically acceptable salt thereof; or (ii) a pharmaceutical composition comprising (i), together with a pharmaceutically acceptable carrier, vehicle or excipient, in the manufacture of a medicament for the treatment or prevention of neuropathic pain.

18. The use according to claim 16 or 17, characterized in that the (S)-pirlindole is enantiomerically pure, wherein enantiomeric purity as calculated by chiral chromatography or specific optical rotation is equal to or greater than 97%.

19. The use according to any one of claims 16 to 18, in a combination therapy with at least one additional analgesic agent.

20. The use according to claim 19, wherein the combination therapy is a fixed-dose combination therapy or a separate combination

therapy that comprises a sequential or simultaneous administration of active agents.

21. The use according to claim 19 or 20, wherein the (S)-pirlindole enantiomer, pharmaceutically acceptable salt thereof or pharmaceutical composition potentiates the effects of the at least one additional analgesic agent in the treatment of neuropathic pain.

22. The use according to any one of claims 19 to 21, characterized in that the at least one additional analgesic agent is a compound that decreases neuronal hyperexcitability, and that is an inhibitor of sodium channel; a calcium channel antagonist; an ionotropic glutamate receptor antagonist; a metabotropic glutamate receptor antagonist; a γ -aminobutyric acid activity enhancer; a μ opioid receptor agonist; a κ opioid receptor agonist; a δ opioid receptor agonist; a μ opioid receptor partial agonist; a κ opioid receptor partial agonist; a δ opioid receptor partial agonist; a μ opioid receptor partial antagonist; a κ opioid receptor partial antagonist; a δ opioid receptor partial antagonist; a μ opioid receptor antagonist; a κ opioid receptor antagonist; or a δ opioid receptor antagonist.

23. The use according to claim 22, characterized in that the inhibitor of sodium channel is carbamazepine, oxcarbazepine, eslicarbazepine, phenytoin or valproic acid.

24. The use according to according to claim 22, characterized in that the calcium channel antagonist is pregabalin.

25. The use according to according to claim 22, characterized in that the γ -aminobutyric acid activity enhancer is gabapentin.

26. The use according to any one of claims 19 to 21, characterized in that the at least one additional analgesic agent is paracetamol, glucosamine, diacerein or a nonsteroidal anti-inflammatory drug.

27. The use according to claim 26, wherein the at least one additional analgesic agent is acetylsalicylic acid, diclofenac, nabumetone, nimesulide, nabumetone, etodolac, piroxicam, lysine clonixinate, diflunisal, acemetacin, glucametacin, indometacin,

proglumetacin, oxametacin, sulindac, aceclofenac, fentiazac, ketorolac, zomepirac, meloxicam, tenoxicam, lornoxican, fenoprofen, fenbufeno, flurbiprofen, benoxaprofen, ibuprofen, ketoprofen, dexketoprofen, piroprofen, indoprofen, naproxen, oxaprozin, tiaprofen, dexibuprofen, meclofenamic acid, mefenamic acid, flufenamic acid, tolfenamic acid, niflumic acid, etofenamate, azapropazone, orgotein, feprazone, morniflumate, tenidap, glycosaminoglycan, polysulfate, celecoxib, rofecoxib, parecoxib, valdecoxib, or etoricoxib.

28. The use according to any one of claims 16 to 27, wherein the compound is a (S)-pirlindole (R)-mandelate salt.

29. The use according to any one of claims 16 to 27, wherein the compound is a (S)-pirlindole mesylate salt.

30. The use according to any one of claims 16 to 27, wherein the compound is a (S)-pirlindole citrate salt.

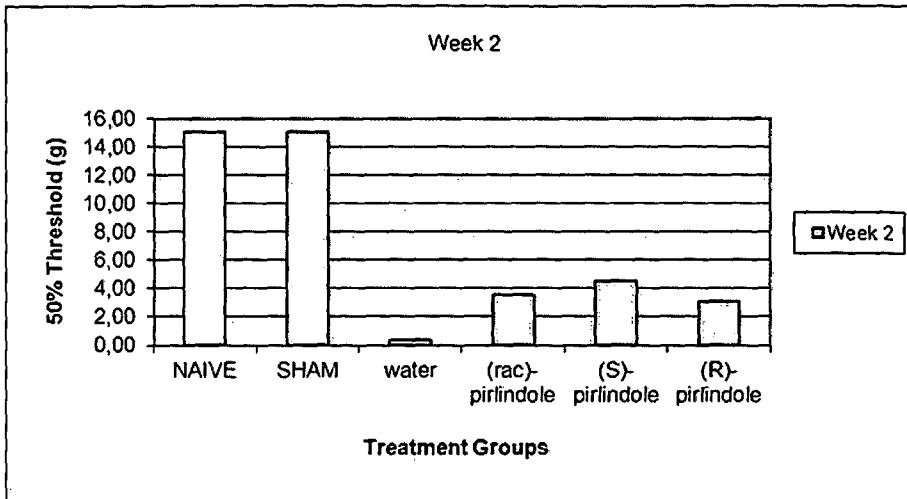


Fig. 1A

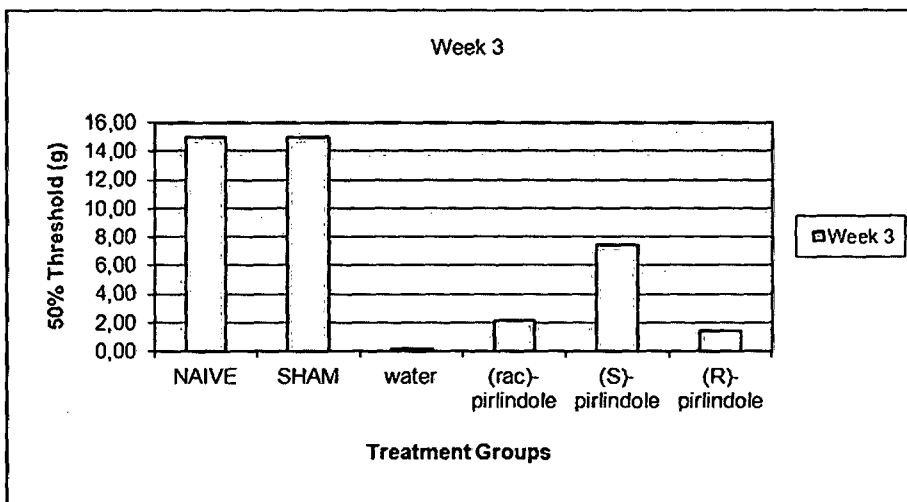
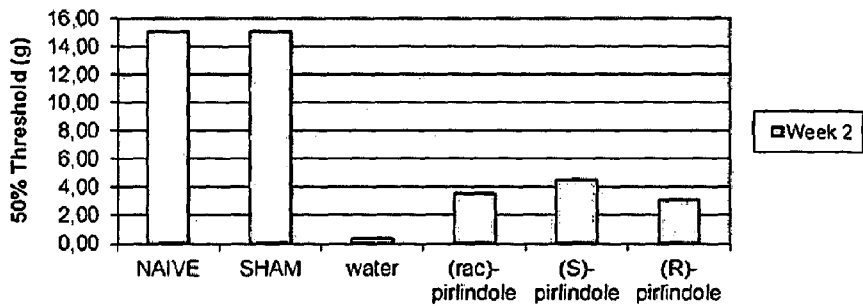


Fig. 1B

Week 2



Treatment Groups