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Effects of a water extract of *Lepidium meyenii* root in different models of persistent pain in rats

https://doi.org/10.1515/znc-2016-0251
Received December 21, 2016; revised June 28, 2017; accepted July 24, 2017

Abstract: *Lepidium meyenii* (Walp.), commonly called maca, is an Andean crop belonging to the Brassicaceae family. Maca hypocotils are habitually consumed as customary food as well as traditional remedies for pathological conditions such as infertility. Moreover, the characterization of maca extracts revealed the presence of compounds that are able to modulate the nervous system. Aimed to evaluate the efficacy of *L. meyenii* in persistent pain, the present study analyzed the effects of a commercial root extract from maca in different animal models reproducing the most common causes of chronic painful pathologies. A qualitative characterization of this commercial extract by high performance liquid chromatography-mass spectrometry and tandem mass spectrometry analyses allowed us to confirm the presence of some macamides known as bioactive constituents of this root and the absence of the main aromatic glucosinolates. The acute oral administration of maca extract is able to reduce mechanical hypersensitivity and postural unbalance induced by the intra-articular injection of monoiodoacetate and the chronic-constriction injury of the sciatic nerve. Furthermore, *L. meyenii* extract reverts pain threshold alterations evoked by oxaliplatin and paclitaxel. A good safety profile in mice and rats was shown. In conclusion, the present maca extract could be considered as a therapeutic opportunity to relieve articular and neuropathic pain.

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Keywords: arthritis; *Lepidium meyenii*; macamide; neuropathy; persistent pain.

1 Introduction

Persistent pain is defined as pain lasting for a period of 6 months or more. Primary care settings in Asia, Africa, Europe, and in the Americas had patients reporting persistent pain in approximately a quarter of the population [1]. Arthritis-related diseases, nerve trauma or compression, and neuropathies induced by metabolic syndromes or neurotoxic drugs are among the most common causes of persistent pain. Clinical evidence of neuropathic or inflammatory pain are hyperalgesia, thermal allodynia and spontaneous pain [2]. The choice of therapy depends on the type of pain, its intensity, and how the patient responds to treatment. Drug therapy, however, continues to be inadequate to completely control persistent pain [3]. In this context, plant-derived products characterized by a safety profile (especially those used as food) emerge as interesting resources for individuating novel therapeutic possibilities to manage chronic pain.

Maca (*Lepidium meyenii* Walp.) is a traditional Andean crop belonging to the Brassicaceae family and it is traditionally used for its nutritional and medicinal properties [4]. Maca's hypocotils are usually consumed fresh or dehydrated after boiling in water or milk, after being made into juices, maca coffee, or alcoholic beverages [5, 6]. The tubers of *L. meyenii* have been widely investigated during these last years, pointing out the copresence of two main groups of phytochemicals, the glucosinolates and the macamides and macaenes. The first group is typical of Brassicaceae; in maca, the main forms are aromatic glucosinolates [7], suggested by some authors as chemical markers to explain some biological effects of this plant [8] and denied by others. Over the last few years, different studies that have focused on the analysis and purification of macamides from this plant have been available in the literature. To this aim, lipophilic extracts of roots, such as those by petroleum ether [9] or by *n*-hexane [10], were selected. Nevertheless, Ganzera et al. [11] highlighted, using high

performance liquid chromatography (HPLC), the presence of some macamides also in root powder methanol extract. Because the macamides have not been found in other *Lepidium* species, they have been proposed as phytochemical markers of maca [10]. Recently, it has been highlighted that some aromatic glucosinolates, abundant in fresh roots, consistently decrease in the dried material because they are involved in the ex novo biosynthesis of macamides, together with free fatty acids [12].

Maca extracts are used for the treatment of a wide range of pathological conditions such as sexual infertility, osteoporosis, and benign prostatic hyperplasia [13, 14]. On the contrary, limited evidence of efficacy against persistent pain has been described up to now [15]. However, in vitro studies confirmed macaenes family components' involvement in enhancing cannabinoid transmission [16]. The cholinergic stimulation induced by *L. meyenii* extract (mainly by polyphenols) has been related to memory improvement [17] as well as antioxidant properties that may sustain the antidepressant-like effects of the plant extract [18]. Moreover, maca improves the antioxidant cell machinery [19]. Cannabinoid and cholinergic systems [20, 21], as well as redox unbalance [22–25], play a relevant role in persistent pain processes. Maca consumption has been associated with lower serum levels of the proinflammatory and proalgic cytokine IL-6 in populations living in the Peruvian Central Andes [8]. Starting from the theoretical approach that maca is able to modulate endogenous systems normally involved in pain control, the aim of this study was to evaluate the efficacy, after acute intake (oral administration), of a commercial water extract of *L. meyenii*, controlled in its composition by high performance liquid chromatography with diode array detector (HPLC-DAD) and high performance liquid chromatography-mass spectrometry (HPLC-MS). The study was carried out in rat or mouse models of persistent pain induced by the intra-articular injection of monoiodoacetate (MIA), chronic constriction injury (CCI) of the sciatic nerve and chemotherapy-induced neuropathy.

2 Materials and methods

2.1 Sample preparation

The dried extract was purchased from KOS Spa, Italy, and was obtained from the roots of maca (*L. meyenii* Walp.) harvested in the Peruvian highlands in South America. According to the supplier's documentation, the extract was obtained by water extraction, and to achieve a final powdered homogeneous sample, maltodextrins were added, obtaining a final 4:1 ratio of dry extract/excipient. Further technical specifications were not available from the supplier. This sample was fractionated as described subsequently: 50 mg of powder were dissolved in 1 mL of water (W) or acetonitrile/methanol (AM) 1:1 v/v; after 1 h of stirring, the solid residues were removed by centrifugation and the solutions recovered to obtain W and AM extracts, respectively. These samples were then analyzed by HPLC-MS and tandem mass spectrometry (MS-MS).

2.2 HPLC-MS and MS-MS analysis

The HPLC-MS analyses were done using a Platin Blue UHPLC (Knauer, Germany) directly coupled to a 6410B triple quadrupole mass spectrometer (Agilent Technologies, USA) through an ESI interface. Two different columns were used: a Gemini C18 150×2 mm, 3 µm, 110 Å, and a Kinetex HILIC 150×2.1 mm, 2.6 µm, 100 Å (Phenomenex, Italy). For each column, a multistep linear gradient elution method was applied, in both cases using a 0.1% formic acid solution (eluent A) and acetonitrile, containing 0.1% formic acid (eluent B). The elution programs for the two columns are reported in the Supplementary information Table S1.

At the end of the run, the columns were re-equilibrated at the starting condition for 10 min. The injection volume was 10 µL; the column oven temperature was 30 °C. The diode-array UV detector recorded the full UV spectra in the range 210–500 nm, and the 240, 280 and 330 nm wavelengths. MS acquisitions were performed in scan mode (100–800 *m/z* scan range at 650 ms scan^{-1}), switching between positive and negative polarities. The ESI interface parameters were: nebulizer gas 30 psi, gas flow 10 L min^{-1}, gas temperature 310 °C, capillary voltages 4.2 kV and 4.0 kV in positive and negative modes, respectively.

2.3 Animals

Male Sprague-Dawley rats (Envigo, Varese, Italy) weighing approximately 200–250 g and male Swiss albino mice (Envigo, Varese, Italy) weighing approximately 23–25 g at the beginning of the experimental procedure were used. Animals were housed in CeSAL (Centro Stabulazione Animali da Laboratorio, University of Florence) and used at least 1 week after their arrival. Four rats and five mice were housed per cage (size 26–41 cm); animals were fed with standard laboratory diet and tap water ad libitum, and kept at 23 °C ± 1 °C with a 12-h light/dark cycle, light at 7 a.m. All animal manipulations were carried out according to Directive 2010/63/EU of the European parliament

and of the European Union council (22 September 2010) on the protection of animals used for scientific purposes. The ethical policy of the University of Florence complies with the Guide for the Care and Use of Laboratory Animals of the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996; University of Florence assurance number: A5278-01). Formal approval to conduct the experiments described was obtained from the Italian Ministry of Health (No. 54/2014-B) and from the Animal Subjects Review Board of the University of Florence. Experiments involving animals have been reported according to ARRIVE guidelines [26].

2.4 MIA-induced osteoarthritis

Unilateral osteoarthritis was also induced in rats by injection of MIA (Sigma-Aldrich, Italy) into the knee joint according to a described method [27, 28]. Exactly 2 mg MIA in 25 µL saline was delivered into the left articular cavity. Control rats received 25 µL of saline solution (day 1) in the knee joint. Behavioral and biochemical measures were performed on day 14.

2.5 CCI-induced neuropathy

Neuropathy was induced in rats according to a procedure described by Bennett and Xie [29]. Another group of rats were subjected to sham surgery in which the sciatic nerve was only exposed but not ligated. The animals were allowed to recover from surgery and then housed one per cage with free access to water and standard laboratory chow. Behavioral tests were performed on day 14.

2.6 Oxaliplatin-induced neuropathy

Mice were treated with 2.4 mg kg^{-1} oxaliplatin, administered i.p. for five consecutive days every week for 2 weeks (10 i.p. injections) [30, 31]. Oxaliplatin was dissolved in 5% glucose solution. Control animals received an equivalent volume of vehicle. Behavioral tests were performed on day 14.

2.7 Paclitaxel-induced neuropathy

Mice were treated with 2.0 mg kg^{-1} paclitaxel, administered i.p. four times on alternating days 1, 3, 5, and 7. Paclitaxel was dissolved in 10% Cremophor E-L of saline solution [32]. Control animals received an equivalent volume of vehicle. Behavioral and biochemical tests were performed on day 14.

2.8 Maca extract administration

Maca extract (KOS Spa, Italy) was suspended in 1% carboxymethylcellulose sodium salt and administered orally. Acute administrations (0.5, 1.5, 3 and 10 g kg^{-1}; conventionally, 1 kg was considered as corresponding to 10 mL) were performed on day 14 after damage induction (CCI and MIA) or anticancer drug treatment start (oxaliplatin and paclitaxel). Behavioral tests were performed before and over time (60 min) after maca extract administration.

2.9 Paw pressure test

The nociceptive threshold in the rat was determined with an analgesimeter (Ugo Basile, Varese, Italy), according to a method described by Leighton et al. [33]. Rats scoring less than 40 g or more than 75 g during the test before drug administration were rejected (25%). For analgesia measures, mechanical pressure application was stopped at 120 g.

2.10 Incapacitance test

Weight-bearing changes were measured using an incapacitance apparatus (Linton Instrumentation, UK) detecting changes in postural equilibrium after a hind limb injury [34]. Rats were trained to stand on their hind paws in a box with an inclined plane (65° from horizontal). The value considered for each animal was the mean of five consecutive measurements. Data are expressed as the difference between the weight applied on the limb contralateral to the injury and the weight applied on the ipsilateral one (ΔWeight).

2.11 Cold plate test

The animals (mice) were placed in a stainless steel box (12 cm × 20 cm × 10 cm) with a cold plate as floor. The temperature of the cold plate was kept constant at 4 °C ± 1 °C. Pain-related behaviors (i.e. lifting and licking of the hind paw) were observed and the time(s) of the first sign was recorded. The cutoff time of the latency of paw lifting or licking was set at 60 s [35].

2.12 Rotarod test

The rotarod apparatus for rats (Ugo Basile, Varese, Italy) consisted of a base platform and a rotating rod with a diameter of 6 cm and a nonslippery surface. The rod was placed at a height of 25 cm from the base. The rod, 36 cm in length, was divided into four equal sections using five disks. Thus, up to four rats were tested simultaneously on the apparatus, with a rod-rotating speed of 10 rpm. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 30 s. Those animals scoring less than three and more than six falls in the pretest were rejected. The performance time was measured every 15 min for four times. Animals with normal coordination progressively reduced the number of falls during the experimental sessions.

2.13 Statistical analysis

Results were expressed as means ± SEM and the analysis of variance was performed by ANOVA test. A Bonferroni's significant difference procedure was used as a post hoc comparison. *P* values less than 0.05 were considered significant. Data were analyzed using the "Origin 8.1" software.

3 Results

Several analytical methods were proposed to investigate macamides [11] and glucosinolates [7] recognized as the main bioactive phytochemicals of maca. According to these findings, the aim of this work was to check the presence of these two groups of molecules in the commercial extract selected for this in vivo study. The screening was done by HPLC-DAD-MS working on two extracts, at different polarities, obtained from the same commercial sample. The chromatographic profiles of the W extract on the Gemini® column did not reveal the presence of analytes detectable in the 220–330 nm range. The same test on the HILIC column, specifically selected to highlight the glucosinolates, confirmed the absence of these compounds and the presence of several diglycosides and oligoglycosides, as expected. On the contrary, the AM extract after the HPLC-DAD-MS screening in positive and negative ionization mode, showed the best result in positive mode. By LC-MS several macamides were detected by their intense $[M+H]^+$ and/or $[M+Na]^+$ ions. Supplementary Figure S1A shows the base peak chromatogram in the 300–450 *m/z* range, selected in agreement with a recent article focused on the comparison of macamides in different batches of maca hypocotyls [9]. To summarize, some representative extract ion profiles at the selected masses are reported in Supplementary Figure S1B, whereas all the detected macamides are listed in Table 1.

The DAD profile also allowed us to point out a group of lipophilic metabolites with retention time values close to that of macamides and characterized by their UV-Vis spectra and ion molecular weight as reported in Supplementary Figure S2. To date, further information is not available on these compounds.

The pain reliever effect of *L. meyenii* extract was evaluated in several models of persistent pain by behavioral tests. In Figure 1, the effect of MIA administration in rats is shown. Fourteen days after MIA injection, the paw pressure test lead us to appreciate that the weight tolerated on the ipsilateral paw was significantly reduced compared with the contralateral (data not shown). A total of 10 g kg^{-1} of *L. meyenii* extract time dependently reduced MIA-evoked mechanical hypersensitivity increasing the weight tolerated up to 70.8 ± 2.2 g (Figure 1A). Figure 1B shows the difference between the weight burdened on the contralateral and the ipsilateral limb. ΔWeight was significantly increased in MIA + vehicle-treated rats

Table 1: List of the detected macamides in the AM extract of *L. meyenii* in positive ion mode and potential identification.

Rt (min)	$[M+H]^+$ (% intensity)	$[M+Na]^+$ (% intensity)	Identity
42.20	384 (5)	406 (95)	*N*-Benzyl-9-oxo-(12*Z*, 15*Z*)-octadecadienamide
42.36	384 (5)	406 (95)	*N*-Benzyl-13-oxo-(9*E*, 11*E*)-octadecadienamide
42.54	386 (5)	408 (95)	*N*-Benzyl-9-oxo-12*Z*-octadecanamide
43.09	368 (80)	390 (20)	*N*-Benzyl-13-oxo-(9*Z*, 12*Z*, 15*Z*)-octadecatrienamide
43.61	370 (100)	–	*N*-Benzyl-(9*Z*, 12*Z*)-octadecadienamide
44.20	346 (50)	368 (50)	*N*-Benzyl-hexadecanamide
44.29	372 (80)	394 (20)	*N*-Benzyl-9*Z*-octadecanamide
42.88	374 (100)	–	*N*-Benzyl-octadecanamide

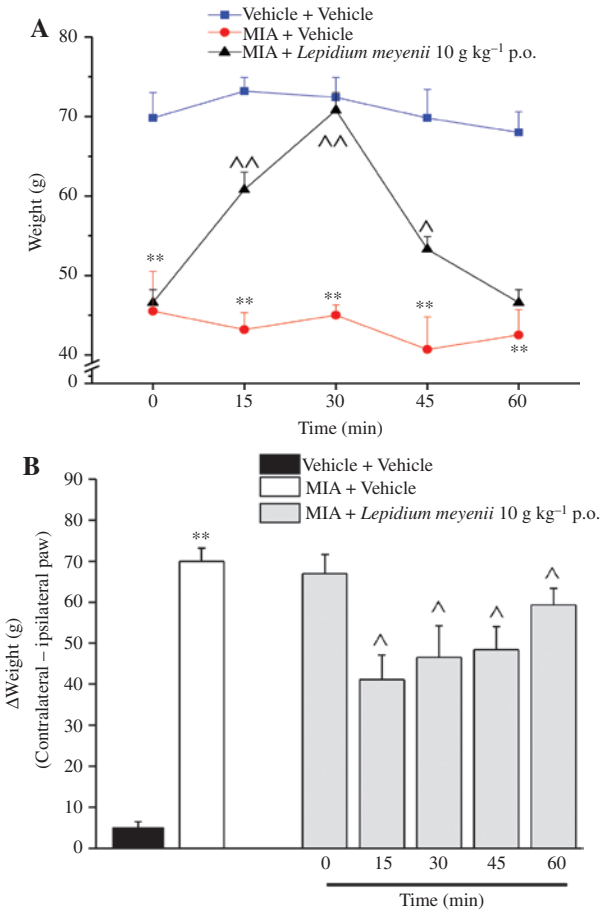

Figure 1: Articular pain.
On day 14 after MIA injection, *L. meyenii* extract (10 g kg^{-1}) was p.o. administered and pain threshold was evaluated over time. (A) Response to a noxious mechanical stimulus evaluated by paw pressure test on the ipsilateral paw. (B) Hind limb weight-bearing alterations were measured by incapacitance test. $n = 10$; $^{*}P < 0.01$ versus vehicle + vehicle-treated rats; $^{\wedge}P < 0.05$ and $^{\wedge\wedge}P < 0.01$ versus MIA + vehicle-treated rats.

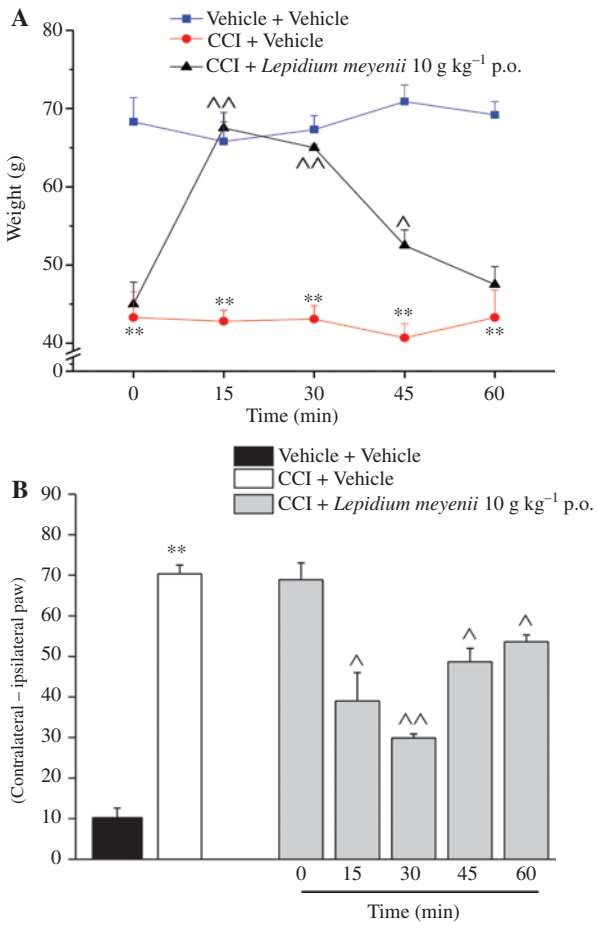

Figure 2: CCI-induced neuropathic pain.
On day 14 after nerve surgery, *L. meyenii* extract (10 g kg^{-1}) was p.o. administered and pain threshold was evaluated over time. (A) Response to a noxious mechanical stimulus evaluated by paw pressure test on the ipsilateral paw. (B) Hind limb weight-bearing alterations were measured by incapacitance test 14 days after surgery. $n = 10$; $^{**}P < 0.01$ versus vehicle + vehicle-treated rats; $^{\wedge}P < 0.05$ and $^{\wedge\wedge}P < 0.01$ versus CCI + vehicle-treated rats.

(67.6 ± 1.6 g) in comparison to vehicle + vehicle-treated rats (5.0 ± 1.5 g). *Lepidium meyenii* (10 g kg^{-1}) was able to statistically reduce hind limb weight-bearing alterations peaking 15 min after treatment (41.1 ± 6.0 g). *Lepidium meyenii* extract was also effective on neuropathic pain induced in rats by lesion to the peripheral nervous system. Rat paw pressure test revealed that 14 days after CCI, the weight tolerated on the ipsilateral paw significantly decreased (43.3 ± 3.3 g) with respect to the contralateral (68.3 ± 3.1 g) (data not shown). As depicted in Figure 2A, 10 g kg^{-1} of *L. meyenii* completely reverted the CCI-induced hyperalgesia for 30 min after administration. Moreover, the effect of the extract was also evaluated by measuring spontaneous pain of CCI rats with the incapacitance test. As shown in Figure 2B, 10 g kg^{-1} of *L. meyenii* extract reduced the difference between the weight burdened on the contralateral and the ipsilateral limb by approximately 45%. A different kind of neuropathic pain was induced in mice by the administration of the neurotoxic drugs oxaliplatin and paclitaxel. The platin-derivative anticancer compound was repeatedly administered and, on day 14, it induced a lowered threshold to cold stimuli that was measured by cold plate test (Figure 3A the licking latency of oxaliplatin + vehicle-treated mice is 11.6 ± 0.7 s vs. 21.2 ± 1.2 s of vehicle + vehicle-treated mice). *Lepidium meyenii* extract, acutely administered in a dose range of 0.5–10 g kg^{-1}, reverted oxaliplatin-induced neuropathic pain in a dose-dependent manner. A total of 10 g kg^{-1} evoked the longest significant effect peaking 30 min after administration (20 ± 0.9 s). Moreover, 3 and 1.5 g kg^{-1} induced a statistically significant effect. In Figure 3B, the effect of repeated

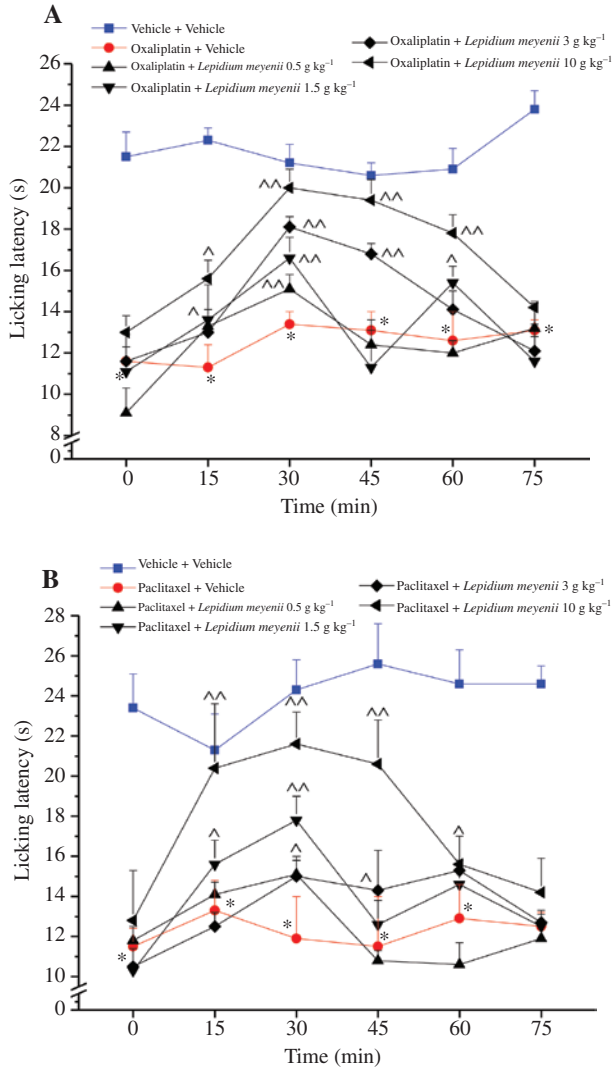

Figure 3: Chemotherapy-induced neuropathic pain.
(A) Oxaliplatin-induced neuropathic pain. Mice were treated i.p. daily with 2.4 mg kg^{-1} oxaliplatin. (B) Paclitaxel-induced neuropathic pain. Mice were treated i.p. (on days 1, 3, 5, and 7) with 2 mg kg^{-1} paclitaxel. The response to a thermal non-noxious stimulus was evaluated by cold plate test. Behavioral test was performed on day 14. Before and after *L. meyenii* extract (0.5–10 g kg^{-1}) p.o. administration. $n = 10$; $^{*}P < 0.05$ versus vehicle + vehicle; $^{\wedge}P < 0.05$ and $^{\wedge\wedge}P < 0.01$ versus oxaliplatin + vehicle; $^{\wedge}P < 0.05$ and $^{\wedge\wedge}P < 0.01$ versus paclitaxel + vehicle.

paclitaxel administrations in mice is shown. On day 14, the taxane derivative decreased the time that animals tolerate on a cold surface (11.5 ± 0.9 s, paclitaxel + vehicle vs. 23.4 ± 1.7 s, vehicle + vehicle). *Lepidium meyenii* extract was administered at a dose range of 0.5–10 g kg^{-1}. As depicted in Figure 3B, 0.5, 1.5, and 3 g kg^{-1} doses induced a significant effect, whereas the highest dose significantly increased licking latency peaking (21.6 ± 1.6 s) 30 min after the administration. Table 2 displays the effect of the maximum tested dose of *L. meyenii* (10 g kg^{-1}) on animals' motor coordination as evaluated by rotarod test. Fifteen minutes after extract administration, the animals were placed on the rotating rod (10 rpm) for 10 min and the number of falls was counted. Administration of *L. meyenii* extract did not induce significant alterations of motor coordination in comparison with the control group.

4 Discussion

The present results describe the pain-relieving effects of an aqueous extract of *L. meyenii*, in three different models of persistent pain. In particular, the intra-articular injection of MIA provides a monolateral osteoarthritis characterized by a persistent inflammatory pain which, starting on day 14 after injection, possesses a neuropathic component [36]. Ten gram per kilogram of maca extract reduces pain induced by a mechanical noxious stimulus on the ipsilateral paw. Moreover, maca-dependent pain relief allows also to reduce postural unbalance, an outward of osteoarthritis progression, as measured by hind limb weight bearing alterations [34]. The anti-neuropathic properties of the *L. meyenii* extract are confirmed after the ligation of the sciatic nerve, a neuropathy model where the alterations of the nerve morphology are accompanied by remarkable inflammatory state emerged in terms of cellular infiltrate and edema [37, 38]. Maca extract, at maximum dosage, is able to revert mechanical hypersensitivity for 30 min, after injection, as shown by paw pressure test. Ten gram per kilogram of *L. meyenii*

Table 2: Effect of *L. meyenii* extract on mouse motor coordination (rotarod test).

Treatment	Dosage (g kg^{-1})	Falls number (min)				
		0	15	30	45	60
Vehicle		7.0 ± 0.8	5.0 ± 1.0	1.0 ± 0.2	1.0 ± 0.4	0.0 ± 0.0
Lepidium meyenii	10	5.3 ± 1.3	2.0 ± 1.0	0.6 ± 0.3	1.6 ± 0.3	0.0 ± 0.0

Animals received *L. meyenii* extract (10 g kg^{-1}) per os; 15 min (time 0) after the administration they were placed on the rotating rod (10 rpm) for 30 s and the number of falls was evaluated. The performance time was measured every 15 min to evaluate the ability of the animals to acquire the movement. Each value represents the mean ± SEM of eight rats per group, performed in two different experimental sets. $P > 0.05$ between groups.

significantly reduces the postural unbalance induced by the nerve ligation as revealed by the incapacitance test. Our results also demonstrate that a single oral administration of maca extract (10 g kg^{-1}) is able to completely revert the thermal allodynia induced in mice after oxaliplatin or paclitaxel chronic treatment. The platinum and the taxane derivatives are used for treatment of several types of cancer as colorectal cancer, lung cancer or ovarian cancer [39, 40]. Their chronic administration causes a peripheral neuropathy characterized by hypersensitivity to cold stimuli [41, 42]. These models of neuropathic pain are free from inflammatory components and are characterized by specific molecular alteration of the peripheral and central nervous system [35]. The maintenance of efficacy in these different kinds of pain suggests an interesting pharmacodynamic profile. The dosages used are quite high but compatible with the use as food supplement. Furthermore, no signs of evident toxicity are highlighted in all treatment groups; in particular, as shown by the rotarod test performances, no motor or neurological alteration was detectable. This maca sample was obtained using an aqueous extraction of the root, as declared in the label, and consequently was not rich in lyphophilic components. Nevertheless, the high sensitivity of a mass spectrometer, differently from the DAD, allowed to detect the unexpected presence of several macamides tentatively identified according to their molecular weight [9].

Furthermore, by use of the HILIC column, suitable to analyzing very polar compounds, the absence of glucosinolates in our sample was confirmed. This finding is in agreement with a recent work that correlates the low content of glucosinolates in dried maca roots to the increase of macamides after the drying process. The hydrolytic phenomena associated with open-air drying are responsible of the formation of the precursors of macamides, which continue to be synthetized until a water content close to 13% is achieved [12]. Even if in low amounts, the macamides can contribute to the observed effect in rats.

At the end, it is worth noting the presence of a group of unknown metabolites with UV-Vis spectra indicating a chromophoric system that can indicate a possible antioxidant capacity that can contribute to the biological effects of this commercial maca sample. At the same time, the shape of these spectra did not confirm the presence of typical flavonoids or flavones, some of them previously found in maca. In vitro studies have demonstrated that macaenes and macamides are able to inhibit the FAAH enzyme, normally responsible for inactivation of anandamide, the main endogenous endocannabinoid. Anandamide is involved in several physiological processes such as painful sensitivity and neuroprotection [16]. Selective FAAH inhibitors enhance anandamide endogenous levels that cause antinociceptive effect by agonism on the CB_2 receptor [21]. Furthermore, many preclinical studies demonstrated that FAAH inhibitors reduce chronic pain without the development of tolerance after repeated administrations [43]. The absence of flavonoids in the present extract was confirmed by the investigation on the UV-vis and mass spectra registered, respectively, by HPLC-DAD and by MS at different fragmentation energies. Only a recent work cited two new flavolignans [44]. By the mass experiments, it was possible to exclude the presence of these compounds in our extract. At the same time, other known phenolic compounds were also reported in the same work, as pinoresinol and tricine were not detected. These results strongly suggest the exclusion of both cholinergic system modulation and antioxidant effects of phenolic compounds from the pharmacodynamic mechanisms. The absence of the glucosinolates, confirmed by mass spectrometric analyses, allowed us to exclude the contribution of these phytochemicals for the biological effect observed for the maca extract.

In conclusion, *L. meyenii* extract is effective as a pain reliever in different models of persistent pain, both in those with a predominant inflammatory component and in those with a predominant neuropathic aspect. The absence of evident toxicity, confirmed by its common use as a customary refreshment as well as a traditional remedy by Peruvian people, shows that *L. meyenii* extract is a possible treatment against osteoarticular and neuropathic pain.

Acknowledgments: This research was funded by the Italian Ministry of Instruction, University and Research (MIUR) and by the University of Florence.

Disclosure statement: No competing financial interests exist.

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Supplemental Material: The online version of this article offers supplementary material (https://doi.org/10.1515/znc-2016-0251).