Antidepressant-Like Behavioral and Spatial Memory Effects in Peruvian Red Maca (Lepidium meyenii)-Treated Rats

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ABSTRACT

Introduction: Lepidium meyenii, known with the vernacular name of “Maca”, is a Brassicaceae family that has been widely used by Peruvian traditional medicine in cases of sexual dysfunction, memory loss, and several other diseases and pathologies. Objective: The aim of the study was to test the potential effects of aqueous extract of Red Maca (AQ-RM) on antidepressant-like behavior in male rats and spatial memory in ovariecetomized (OVX) rats. Methods: Forced swimming test was used to examine the potential antidepressant AQ-RM activity in male rats. Fluoxetine and amitryptyline were included as reference drugs. Morris Water Maze test was employed to examine the effect of AQ-RM on spatial memory in ovariecetomized (OVX) rats. Such effects were compared to exogenous estradiol administration. The potential role of oxidative stress on spatial memory loss was assessed by measuring malondialdehyde (MDA) levels in rats brain homogenates. Results: AQ-RM enhances swimming and climbing activities while reducing the time of immobility in male rats. Meanwhile, it prevents the decrease in the time spent in the target quadrant and displays higher values in the number of crossings in OVX-rats as compared to OVX-control rats. MDA levels in brain homogenates were decreased in OVX-rats receiving AQ-RM. Conclusion: Oral administration of AQ-RM has anti-depressive application in male rats; and increases the ability of learning and memory in OVX rats.

Key words: Lepidium meyenii, Red Maca, Antidepressant activity, Forced swimming test, Spatial memory, Morris water maze test.

INTRODUCTION

Over the last two decades, the interest and demand for Lepidium meyenii, best known with the vernacular name of “Maca”, have grown worldwide. This interest has established Maca as one of the flagship products of Peru, being sold as powder, pills, capsules, flour, liquor, and extracts at a variety of retail outlets such as health food stores and smoothie shops.1

Maca root, native to the Peruvian Andean region is botanically a member of the Brassicaceae family, growing in high altitude region, characterized by extreme weather conditions as rocky formations, intense sunlight and strong winds.2,6 There are diverse varieties according to the color of the hypocotyl, and many Maca root-based food supplements of different colors are available on the world market due to the possible effect on the treatment of a wide range of diseases and medical disorders.3 They include sexual dysfunction regulation,4 neuroprotective effects,5 memory enhancement, antidepressant,6,11 antioxidant, anticancer, and anti-inflammatory effects.12,14 and skin protection.15

In the Department of Junin (Carhuamayo), 13 varieties of Maca ranging from White to Black have been described. The most frequent was the Yellow color (47.8%), the most preferred commercially; due to its properties to increase energy, improve attentiveness, and stabilize hormones. Red Maca accounts for about 25% of the annual harvest being the sweetest and highest in phytochemical levels among Maca powders with all litter colors. It is known as the most effective type for women because of its hormonal balance effects and its action on bone health. The Black Maca is the rarest of all colors, accounting for about 15% of the annual harvest. Studies have shown that it is the most effective form for men, especially for muscle gain, endurance, brain focus, and libido.7

It has been reported that Red Maca reduces rat benign prostatic hyperplasia induced by testosterone likely by stimulating androgen signal pathways16 and/or inhibiting TNF-α17 By looking other potential pharmacological properties, the aim of the present work was to study the effects of aqueous extract from Red Maca (AQ-RM) as antidepressant in a depression model in male rats and as enhancer of learning and memory in ovariecetomized (OVX) rats.

MATERIALS AND METHODS

Plant material

The hypocotyls of Lepidium meyenii (Red Maca) were collected in September 2018 in the district of Carhuamayo, Province of Junin at 4000 m above sea level, in the Valle Mantaro, Peru. Once collected, the specimen was identified from the "Herbarium Truillense de la Facultad de Ciencias Biológicas de
la Universidad Nacional de Trujillo’. A voucher sample was deposited in this herbarium.

**Lyophilized aqueous extract preparation**

The aqueous extracts of hypocotyls of *Lepidium meyenii* (Red Maca) were prepared as follows: 500 g of dried hypocotyls were weighted and pulverized, then added to 1.5 L of aqueous, and the mixture was subjected to reflux for 2 hours. The extraction process was repeated two times with another 1.5 L of aqueous. The extracts were mixed and filtered to remove insoluble particles and then evaporated under reduced pressure. The extract was frozen at -80°C (Arctiko) and then lyophilized with a freeze-dryer (Labconco). The lyophilized was stored at +4°C until analysis and further testing.

**Antidepressant activity**

**Animals**

Male Sprague-Dawley rats weighing between 150 and 200 g kept under controlled conditions (12-h dark/12-h light cycle, 23-25°C, and 50-60% humidity) obtained from the animal house of the Instituto Nacional de Salud of Peru were used for the study. All experiments were conducted in accordance with international standards of animal welfare, and the experiments were performed in accordance with protocols approved by Ethics Committee for animal research of the Universidad Nacional de Trujillo (Res. Cons. Univ. No. 0361-2018/UNT). Groups of five animals were selected and, to reduce the influence of diurnal variation, all assays were conducted from 09:00 to 13:00 h, in a special noise-free room with controlled illumination. The animals received a standard food pellet, and before experiments, they were fasted overnight with water *ad libitum*.

The rats were randomly distributed into five experimental groups (n=6 per group). They were orally received: only vehicle (a); fluoxetine (FLX) at 10 mg/kg (b); amitriptyline (AMT) at 30 mg/kg (c); aqueous extract of Red Maca (AQ-RM) at 0.5 g/kg (d); and 1.0 g/kg of AQ-RM (e). The administration of vehicle or aqueous extract of Red Maca was during 28 days from 9:00 to 11:00 am.

**Forced swimming test (FST)**

The FST is the most widely used *in vivo* model for assessing pharmacological antidepressant activity.13,19 The development of immobility when the rats are placed in a cylinder filled with water, from which there is no escape, reflects the cessation of persistent escape-directed behavior. The apparatus consisted of a clear Plexiglas cylinder (40 cm high x 20 cm diameter) filled to 30 cm with water (24 ± 1°C). In the pre-test session, each animal was placed individually into the cylinder for 15 min, 24 h prior to the 5 min swimming test. AQ-RM, fluoxetine and amitriptyline were administered once day during 28 days starting at different release positions to find the location of the plexiglas platform (located one cm below the water level). Rats not finding the platform within 60 sec of the acquisition phase are either placed on the platform or guided to it. During the training days (acquisition test), the time spent to find the platform (escape latency) was assessed and compared as behaviors between the groups. Probe test was done on the 5th day without the platform. The animal was placed in a new starting position in the maze, facing the pool wall, 180° from the original position of the platform. The rat was allowed to swim for 60 seconds in a single trial, and the time in the target quadrant and the number of times it crossed that quadrant was recorded.20

**Ovariectomy**

Rats at the age of three months were anesthetized by intraperitoneal injection of 110 mg/kg of ketamine using standard procedures.21 Briefly, anesthesia was confirmed by reduced respiratory rate and absence of response to gentle pinching the footpad. A ventral incision was made through the skin on the right flank. In the OVX groups, the ovary, oviduct, and top of the fallopian tubes were clamped and removed. Skins and abdominal walls of animals of OVX groups were sutured, and animals were returned to their cages. Animals were included in the experiments two months after they were ovariectomized.

**Morris Water Maze (MWM) Test**

The MWM test was carried out using the black circle pool (60 cm depth, and 150 cm diameter) that is filled half-way with water (26 ± 1°C).21,22 Briefly, the procedure included four consecutive days of training with another day for a probe test. In this experiment, three trials per day were performed for each rat starting at different release positions to find the location of a plexiglas platform (located one cm below the water level). Rats not finding the platform within 60 sec of the acquisition phase are either placed on the platform or guided to it. During the training days (acquisition test), the time spent to find the platform (escape latency) was assessed and compared as behaviors between the groups. Probe test was done on the 5th day without the platform. The animal was placed in a new starting position in the maze, facing the pool wall, 180° from the original position of the platform. The rat was allowed to swim for 60 seconds in a single trial, and the time in the target quadrant and the number of times it crossed that quadrant was recorded.20

**Malondialdehyde (MDA) measurements in brain**

MDA levels were quantified using a minor modification of the method published by Esterbauer & Cheeseman.23 Briefly, the rat brain was excised and kept to 4°C. It was further homogenized with 20 mM of phosphate buffer (pH 7.4) containing 140 mM of KCl. The mixture was centrifuged at 3000 rpm for 10 min at 4°C and the supernatant was kept to 37°C in a water bath in dark for 60 min. Proteins were precipitated by adding 20 µL of trichloroacetic acid (10%, v/v) and further centrifugation at 1000 rpm for 10 min at 4°C. To one mL of the supernatant obtained it was added 2 mL of thiobarbituric acid (0.7%; w/v) and the mixture was incubated for 60 min at 90°C. Next, 4.0 mL of n-butanol/pyridine (15:1; v/v) and 0.5 mL of distilled water were added. Then, the mixture was shaken and centrifuged for 10 min at 4°C at 1000 rpm. Finally, MDA levels were spectrometrically recorded at 532 nm. The standard calibration curve was calculated by using 1,1,3,3-Tetraethoxyxpropane as reference and the results were expressed in µmol/g tissue.

**Statistical analysis**

The results are present as means ± SEM (standard error of the mean). All data were analyzed using one-way analysis of variance (ANOVA) with the Tukey *post hoc* test. A *p*-value ≤ 0.05 was considered statistically significant.
RESULTS AND DISCUSSION

Antidepressant activity

Depression is a common mental disorder, which has dramatically increased affecting more than 264 million people worldwide while the lack of an effective treatment is a permanent challenge for psychopharmacological research today.24,25 Nowadays, the search for novel pharmacotherapies against depressant activity from medicinal plants has significantly progressed as show by the large number of herbal medicines under current evaluation for their psychotherapeutic potential in a variety of animal models.14,15 These studies provide useful information for the development of novel therapeutic agents from medicinal plants to be used in antidepressant activity.

The forced swimming test (FST) was employed to assess the potential antidepressant effect by aqueous extract Red Maca (AQ-RM). The results were compared to those obtained by a selective serotonin reuptake inhibitor (fluoxetine at 10 mg/kg), and a non-selective serotonin and norepinephrine reuptake inhibitor (amitriptyline at 30 mg/kg). Figure 1 shows active responses, namely climbing, swimming and reduction of immobility, a behavior profile indicating an antidepressant effect.

The results show in the Figure 1A that AQ-RM (0.5 and 1.0 g/kg) and fluoxetine significantly increased the swimming when compared with the control-treated group. The amitriptyline was not different when compared with the control-treated group. The results also indicated a dose-dependent effect by AQ-RM. Zheng et al.26 discussed the increased of swimming would be directly related to the increase in the content of polysaccharides in the aqueous extract of Maca.

Figure 1B shows a slight but no statistically significant effect on climbing time, by AQ-RM as compared to control-treated group (5.5 ± 1.1 sec), while fluoxetine and amitriptyline increased it by 18.17 ± 2.8 and 31.33 ± 1.1 sec, respectively. Indeed, serotoninergic compounds like fluoxetine have been reported to influence swimming whereas amitriptyline and drugs with selective effects on noradrenergic transmission are affecting climbing.27 In this context, it was suggested that climbing implies the adrenergic neurotransmission, while swimming involves the serotonergic (5-HT) neurotransmission.28

As expected, the immobility time of rats was strongly reduced when they were administered with either fluoxetine or amitriptyline (Figure 1C). As compared to control-treated group (32.0 ± 0.6 sec), AQ-RM reduced in a dose-dependent way the immobility time at 11.67 ± 4.0 s and 3.17 ± 1.2 s, at doses of 0.5 and 1.0 g/kg respectively. Meanwhile other studies have shown that petroleum ether extract from Maca (collected in Xizang, China) at doses of 250 and 500 mg/kg, significantly decreased the duration of immobility in mice, indicating that Maca extract had antidepressant activity.29 Moreover, Rubio et al.11 reported that Yellow, Red and Black Maca reduced in similar extents the immobility time in OVX mice. Moreover, since quercetin displays antidepressant activity,30 it is tempting to suggest that the effect by Maca is likely due to the presence of phytoestrogens such as quercetin and anthocyanins.

Spatial Memory

Ovariectomy is a chirurgical procedure widely used to generate an in vivo condition mimicking post-menopausal pathophysiological changes in women associated with learning and memory.31 It is characterized by progressive memory deficits, central cholinergic nerve system degeneration, excessive oxidative stress and differentiation/apoptosis imbalance,32 brain oxidative stress33 and alteration of cholinergic and monoaminergic function.32,34

The Morris water maze is a well-established paradigm for evaluating deficits in hippocampal-dependent memory. In particular, learning and memory deficit is demonstrated by the extended time in acquisition and retention. The Morris water maze has been used to test spatial memory in many studies, for instance exogenous estrogen replacement improve spatial memory in ovariectomized rats.35

In the present study, ovariectomy resulted in impairment on memory function as observed in the water Morris maze test. These results are in accordance with those reported in previous studies.11,36 Although hormone deprivation due to ovariectomy causes spatial memory
deficits, it is important to notice that estrous cycle may influence the performance in memory tests. 

Figure 2 shows that OVX rats showed lower time in the target quadrant and lower number of crossings than rats in naïve group. Meanwhile, estrogen administration restores spatial memory to normal values (naïve rats). The effects by AQ-RM were similar to that observed by estrogen in OVX-rats. Indeed, AQ-RM also restores, in a dose-dependent manner, the time spent in the target quadrant (19.67 ± 0.6 sec and 28.17 ± 0.9 sec, respectively) as compared to OVX-rats Figure 2A. Meanwhile, AQ-RM, only at 2.0 g/kg, increased the number of crossings of the previous location of the platform when compared to OVX-rats (Figure 2B). These results clearly show that AQ-RM was able to alleviate the effect of ovariectomy. Since the water Morris maze is dealing with spatial learning and memory abilities, it may be concluded that AQ-RM improve spatial learning and memory deficits provoked by ovariectomy.

In addition to disturbances in both spatial learning and memory, ovariectomy of rats and surgical post-menopause increases ROS production via the mitochondrial respiratory chain and impaired antioxidant defense system. The human body has several mechanisms to counteract oxidative stress through the production of antioxidants. This production plays an important role in preventing or alleviating chronic diseases by reducing the oxidative damage to cellular components caused by reactive oxygen species. It should be stressed that brain is susceptible to be oxidized by free radicals. Lipid peroxidation (LPO) readily decomposes to liberate carbonyl fragments, the most prominent being MDA, which are highly reactive and responsible for cytotoxic effects and neuronal death. From this, MDA levels can be taken as an indicator for the state of LPO and a recognized indicator of oxidative stress. The reported abnormal alteration in MDA levels and its relation to memory impairment have been showed in previous studies.

In this context, Figure 3 shows that MDA brain levels was enhanced in OVX rats when compared with naïve group. Exogenous estrogen replacement hidden such oxidative process as seen by the same MDA levels in both naïve and OVX-estradiol groups \(p > 0.05\). AQ-RM decreased, in a dose dependent way, the formation of MDA in OVX-rats.

Our results confirmed the capacity of AQ-RM to reduce oxidative stress, suggesting that improvement in learning and memory abilities in OVX-rats is likely due to its capacity to reduce oxidative stress.

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**Figure 2:** Effect of aqueous extract of Red Maca on (A) the time in the target quadrant (s) and (B) number of crossing in OVX rats in the Morris water maze during the spatial memory test. Data are expressed as the means ± SEM. Comparisons test: *** \(p < 0.001\), ** \(p < 0.01\), * \(p < 0.05\) versus naïve group and ### \(p < 0.001\), ## \(p < 0.01\), # \(p < 0.05\) versus OVX-control.

**Figure 3:** The effect of aqueous extract of Red Maca on MDA levels on rats brain. Data are expressed as the means ± SEM. Comparisons test: *** \(p < 0.001\), ** \(p < 0.01\), * \(p < 0.05\) versus naïve group and ### \(p < 0.001\), ## \(p < 0.01\), # \(p < 0.05\) versus OVX-control.
CONCLUSION

In summary, the results obtained after oral administration of aqueous extract of Red Maca in male rats demonstrated possible applications in CNS conditions, such as depression. It was further show that AQ-RM may enhance learning and memory in O VX rats. Additional studies are in progress in order to disclose its physiological mechanism, as well as its applicability as antidepressant-like and positive memory effects in behavioral disorders.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTIONS

ROYJ, IMQD, PBC and J.B. supervised the experimental work, and interpretation of the data, literature search, and wrote the manuscript. EAVC and RJLA collected the plant species and carried out the preparation of extracts. DAA carried out the statistical analysis and the preparation of images. KSM and AEVG, performed the antidepressant assays. JIMG and RDYM performed ovarioctomy and Spatial Memory experiments. All authors read and approved the final manuscript.

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GRAPHICAL ABSTRACT

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