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Short Communication

Maca (*Lepidimium meyenii*) and the Prostate Cancer

[Maca (*Lepidimium meyenii*) y el cáncer de próstata]

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Abstract: The extract of *Lepidium meyenii* (red Maca) has attracted considerable interest in ethnomedicine due to their medicinal properties such as fertility improvement, antioxidant activities, antinflammatory or vasoactive properties. Maca is a Peruvian high Andean plant that belongs to the Brassicaceaes (copper) family and is grown between 4,000 and 5,000 m at a temperature of 1.5-12ºC in preferably acidic soils. Maca is naturally present in different varieties that are characterized by the external color of their hypocolites which have different biological properties. In phytomedicine, it is clearly demonstrated that maca is capable of increasing fertility and sexual desire. Our laboratory is using the LNCaP androgen-sensitive human prostate cancer cell line to evaluate the anticancer activity of red maca aqueous extracts. Our endpoints involved effects on cell viability and changes in the expression of target genes for estradiol. We show that red Maca did not affect viability of the LNCaP, but treatment with red maca changed mRNA expression for the estrogen-target genes, estrogen receptor-alpha (ESR1) in LNCaP. The phytochemical analysis using GC/MS showed the presence of alkaloids, lipids, carbocyclic acids or saponins in the Maca extract. We conclude that red Maca aqueous extract does not have toxic effects but stimulate estrogen signaling in prostate cancer cells. These results highlight the presence of active compounds with biological properties on reproductive cancers.

Keywords: Maca; Prostate; Cancer; LNCaP cells; Estrogen receptor

Resumen: El extracto de *Lepidimium meyenii* (Maca roja) ha atraído considerablemente la atención en la etnomedicina debido a sus propiedades medicinales especialmente en mejorar la infertilidad y como antioxidante. La Maca es una planta altoandina que pertenece a la familia de las Brassicaceaes (cooper) que crece entre los 4000 y 5000 metros sobre el nivel del mar a una temperatura de 1.5-12 ºC en suelos preferiblemente ácidos. Esta planta está naturalmente presente in diferentes variedades que se caracterizan por el color externo de sus hipocolitos y sus diferentes propiedades biológicas. En la fitomedicina, se ha demostrado que Maca is capaz de incrementar la fertilidad y el deseo sexual. Nuestro laboratorio, es usando como modelo experimental anticáncer a la línea celular de cáncer de próstata humana sensible a andrógenos y en este contexto, es que hemos utilizado esta línea celular para evaluar si el extracto acuoso de maca roja tiene actividad anticancerígena. Para ello, evaluamos si este extracto afecta la viabilidad de las células LNCaP y si cambia la expresión del receptore de estrógeno ESR1. Encontramos que el extracto acuosos de Maca roja no afecto la viabilidad de las células LNCaP pero aumento la expresión de ESR1, además, un análisis por cromatografía de gases acoplado a espectrometria de masas encontró la presencia de alcaloides, lípidos, ácidos carbociclicos y saponinas. Se concluye que Maca roja no tiene efectos tóxicos pero estimulan la señalización estrogénica en células cancerosas de próstata.

Palabras clave: Maca; Próstata; Cáncer; Células LNCaP; Receptor de estrógenos

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INTRODUCCIÓN

Traditional native plants are a rich source to isolate and characterize new drugs exhibiting therapeutic activity for various pathologies of the human reproductive tract (Talalay & Fahey, 2001). Cancer of the reproductive tract explains for 12% of all female neoplasia and 15% of mortalities are associated with this pathology; furthermore, prostate and endometrial cancer are the most common and with high indices of mortality in men and women. Risk factors include obesity, diabetes mellitus, exposure to high levels of estrogen, and old age. The use of classical antineoplastic drugs is associated with side effects that affect the quality of life of patients. Survival prognostic for prostate and endometrium cancer patients is critical because most patients will suffer from recurrence and subsequent metastasis (Fleshner, 2005). Thus, it is imperative to find new effective therapies for these malignancy with minimal toxicity risks to normal cells.

Lepidium meyenii also called "red Maca", is a cruciferous plant that grows exclusively over 4000 m in the Peruvian central Andes. Administration of red Maca aqueous extract attenuated prostate hyperplasia in rodent models (Gonzales *et al.*, 2008). In accordance with this assumption, some reports show that Maca could have anticancer properties or improve fertility in various animal models (Li *et al.*, 2001; Nachshon-Kedmi *et al.*, 2001). Therefore, it is probable that both red Maca could be used as a source for active principles against a wide range of human pathologies involving the reproductive tract.

MATERIALES Y METODOS

Preparation of the aqueous extract of red Maca

For this work, 1 g of the atomized red variety of *Lepidium meyenii* (provided in a local market in Santiago de Chile) was diluted with saline solution to a stock solution of 1 mg/mL. For all experiments, Maca extract was diluted in culture medium.

Cell culture

LNCaP cells were maintained in DMEM/High Modified medium with 4.0 mM L-Glutamine and 4.500 mg/L Glucose free of Phenol Red (Thermo Fisher Scientific, Waltham, USA) supplemented with 10% (vol/vol) of Foetal Bovine Serum (Thermo Fisher Scientific), sodium pyruvate 1mM, penicillin 100 UI/mL and streptomycin 100 μg/mL (Sigma Chemical, St. Louis, USA). For all experiments 1.000.000 LNCaP or Ishikawa cells/well were seeded.

Treatments

LNCaP cells were treated with 0 (control), 10, 30 or 100 μg/mL of the aqueous extract of red Maca or the hydroethanolic extract of maqui. Culture medium was used as vehicle of the extracts.

Measurement of cell viability

Cell viability was assessed by the MTS dye reduction assay using the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay kit (Promega, Madison, USA) according to manufacturer's instructions.

Real-Time Polymerase Chain Reaction

Total RNA from LNCaP cells was isolated using Trizol Reagent (Invitrogen, Carlsbad, USA). One μg of total RNA of each sample was treated with Dnase I Amplification grade (Invitrogen). The single-strand cDNA was synthesized by reverse transcription using the Superscript III Reverse Transcriptase First Strand System for RT-PCR (Invitrogen), according to the manufacturer's protocol. The Light Cycler instrument (Roche Diagnostics, GmbH Mannheim, Germany) was used to quantify the relative mRNA level for ESR1 in the LNCaP cells; Gapdh was chosen as the housekeeping gene to be used as load control. SYBR® Green I double-strand DNA binding dye (Roche Diagnostics) was used for these assays. Primers for ESR1 were 5´ GCTTACTGACCAACCTGGCAGA 3´ (sense) and 5´ GGATCTCTAGCCAGGCACATTC 3´ (antisense); and for Gapdh were 5' TGCCAAATATGATGACATCAAGAA 3' (sense) and 5' GGAGTGGGTGTCGCTGTTG 3' (anti sense). All real time PCR assays were performed in duplicate (Livak & Schmittgen, 2001).

GC-MS analysis of the Red Maca aqueous extract

GM-MS analysis was performed using an electrospray ionization triple quad tandem mass spectrometer (Agilent 6410, Palo Alto, CA, USA) in negative mode, with 300 ºC temperature, 3500 V ionization voltage, and 8 L/min of nitrogen flow. Quantitation was performed using the multiple reaction monitoring mode, with determination of mass-charge

ratios (m/z). Reference compounds were identified by comparing their UV-visible spectra with previously published data.

Statistical Analysis

All data are presented as mean ± SE. These data followed a non-normal distribution (Kolmogorov-Smirnov test) and significant differences between groups were determined through the use of variance analysis by Friedman's test with subsequent post-hoc Wilcoxon signed-rank test. Significance was accepted at $p<0.05$. Values for the experiments of cell viability are expressed as percentage from the control.

RESULTADOS

Aqueous extract of red Maca did not affect viability of LNCaP cells

Figure No. 1a shows that viability of LNCaP cells treated 48 h with different concentrations of red Maca extracts was not affected significantly compared with control group.

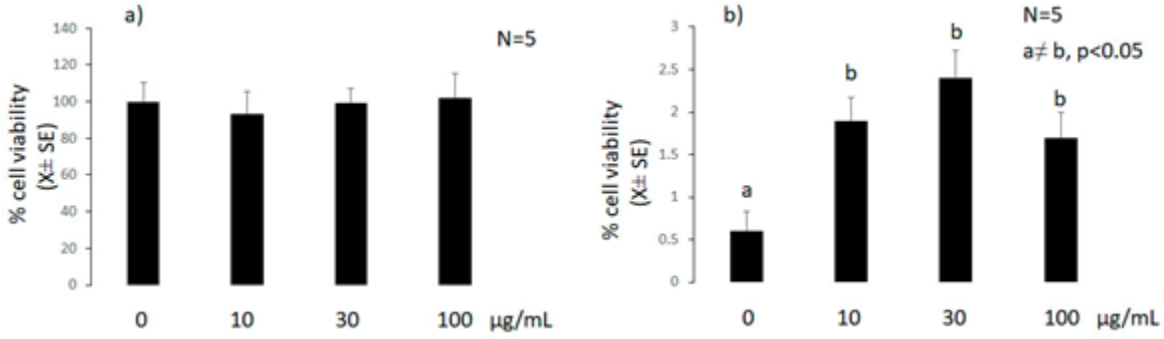

Figure No. 1

a) Cultures of LNCaP cells were treated with Maca extract 0 (control group), 10, 30 or 100 µg/mL. At 48 h after treatment, cultured cells were processed to measure their viability as described in the Material and Methods section. b) Cultures of LNCaP cells were treated with Maca extract 0, 30 or 100 µg/mL. At 12 h after treatment, cultured cells were processed to measure the relative expression of the mRNA for ESR1 as described in the Material and Methods section

Aqueous extract of red Maca increased ESR1 gene expression in LNCaP cells

Figure No. 1b shows that red Maca extract increased dramatically mRNA level for ESR1 at all concentrations compared with the control group.

GC-MS analysis of the aqueous extract of red Maca

The results obtained showed a huge variety of organic compounds including lipids, carboxylic acids, alkaloids or saponins (Table No. 1).

Table No. 1

GC-MS Analysis of the red Maca aqueous extract

RT	Area	Formula	MW	Compound
12.63	123005	C22H2605N2S	430	4-Aminothiocolchicine
16.17	3477511	C22H46	310	Heneicosane, 5-methyl
17.32	611415	C21H44	296	Heneicosane
17.44	518662	C36H74	506	Hexatriacontane
17.53	100190	C6HCl5	248	Benzene, pentachloro
17.52	2482607	C12H36O4Si5	384	Trisiloxane 1,1,1,5,5,5 hexamethyl-3,3-bis[(trimethylsilyl)oxy]
19.47	462477	C3H7ON	73	Propanamide

19.73	298170	C32H40O8N2	580	Tetraethyl 4,4-(1,3 phenylene) bis(1,4-dihydro-2,6-dimethyl)-3,5-pyri
20.91	1418190	C16H30O4	286	Pentanoic acid,2,2,4-trimethyl-3-carboxyisopropyl,isobutylest
21.01	618646	C20H42O2S	346	Di-N-decylsulfone
21.49	210844	C7H5O3N5	207	Pterin-6-carboxylic acid
21.69	119872	C18H20O3N2	312	2-benzoyl-3-isobutylidene-hexahydro-pyrrolo[1,2-A] pyrazin-1,4-D1
22.68	892570	C16H50O7Si8	578	Octaxiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 hexadecamethyl
22.92	1297641	C14H42O5Si6	458	Hexasiloxane, tetradecamethyl

DISCUSSION

Contrary to previous findings showing presence of glucosinolates and anthocyanines (Fahey *et al*., 2001; Li *et al*., 2001 in the aqueous Maca extract, which could exert antiproliferative and proapoptotic activity on various models of cancer (Wong *et al*., 2014), we found that treatment with red Maca aqueous extract did not affect viability in LNCaP cells suggesting that this type of extract is not toxic for human prostate cancer cell lines. As shown in the GC-MS analysis, red Maca aqueous extract contains a variety of organic compounds such as lipids, carboxylic acids, alkaloids or saponins which could attenuate the proapototic activity of the glucosinolates and anthocyanines previously described for another plants (Le *et al*., 2003). On the other hand, analysis of mRNA level for ESR1 in LNCaP cells showed that this transcript increased their expression after Maca treatment. This indicate that compounds that activate estrogenic signaling could be present in the red Maca aqueous extract. The presence of polyphenols and flavonoids with estrogenic activity has been reported in red Maca and other species of Brassicaceae family (Valentová *et al*., 2006). These estrogenic properties could explain some of the biological effects on the human reproductive tract previously described (Fano *et al*., 2017). In conclusion, red Maca aqueous extract does not have toxic effects but stimulate estrogen signaling in prostate cancer cells and contains a variety of chemical compounds that can influence the biology of reproductive cancers.

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