Cell Biol Toxicol 2006; **22:** 91–99.
DOI: 10.1007/s10565-006-0033-0

The *in vitro* biological activity of *Lepidium meyenii* extracts

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Received 18 August 2005; accepted 11 November 2005

Keywords: Lepidium meyenii, maca, DPPH, rat hepatocyte, MCF-7, estrogenicity

Abstract

The biological activity of methanolic and aqueous extracts from dehydrated hypocotyls of *Lepidium meyenii* (Brassicaceae, vernacular name "maca"), was studied on rat hepatocytes and human breast cancer MCF-7 cells. The extracts did not exhibit cytotoxicity in hepatocyte primary cultures up to 10 mg/ml as measured by the MTT viability test, and lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) leakage. Moreover, after 72 h, extracts inhibited LDH and AST leakage from the hepatocytes. When hepatocytes were intoxicated by *t*-butyl hydroperoxide, neither extract prevented oxidative damage. Both extracts showed weak antioxidant activity in the DPPH radical scavenging test with IC_{50} values of 3.46 ± 0.16 and 0.71 ± 0.10 mg/ml, for aqueous and methanolic extracts, respectively. Thus, the observed effect on spontaneous enzyme leakage is probably mediated through mechanisms other than antioxidant activity. Both methanolic and aqueous extracts have shown estrogenic activity comparable with that of silymarin in MCF-7 cell line. Maca estrogenicity was exhibited in the range from 100 to 200 μg of extract per ml. The findings in the present study show that maca does not display *in vitro* hepatotoxicity. In contrast, a slight cytoprotective effect, probably not mediated by antioxidant capacity, was noted. Maca extracts exhibited estrogenic activity comparably to the effect of silymarin in MCF-7 cells.

Abbreviations: AST, aspartate aminotransferase; DMEM, Dulbecco's modified Eagle medium; DMSO, dimethyl sulfoxide; DPPH, 1,1-diphenyl-2-picrylhydrazyl; GC-MS, gas chromatography – mass spectrometry; IC_{50}, 50% inhibitory concentration; LDH, lactate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TBA, 2-thiobarbituric acid; tBH, *t*-butyl peroxide

Introduction

Lepidium meyenii Walp. (Brassicaceae, vernacular name "maca", sometimes considered synonymous with *L. peruvianum* Chacón) is a traditional crop from the Andean highlands that became renowned for the ability of its underground organs (hypocotyls) to improve potency and stamina. Maca-derived products are recommended as fertility enhancers and aphrodisiacs for men and livestock (Popovici, 1997; Quirós and Aliaga, 1997; Balick and Lee, 2002; Flores et al., 2003; Valentová and Ulrichová, 2003). Many food supplements containing dried

powdered maca hypocotyls are available on the world market.

Maca is known to contain phytosterols (Dini et al., 1994), alkaloids (Popovici, 1997; Muhammad et al., 2002; Cui et al., 2003), isothiocyanates (Li et al., 2001; Piacente et al., 2002), glucosinolates (Dini et al., 2002), and macaenes and macamides (Ganzera et al., 2002; Muhammad et al., 2002). The biological activity of maca hypocotyls has been studied extensively in recent years. In various animal and human models, researchers have found in particular enhanced sexual performance and stimulation of spermatogenesis (Gonzales et al., 2001a, 2002, 2003a, 2003b), and also nutritional, energizing, and stress-reducing properties, and improvement of growth rates and survival (Canales et al., 2000; Lee et al., 2004; López-Fando et al., 2004; Lee K.J. et al., 2005). However, although at least three of the studies were performed on healthy volunteers (Gonzales et al., 2001a, 2002, 2003a), no "safety" parameters were evaluated. The present study was undertaken to evaluate the effects of high doses of maca extracts on hepatocytes and their potential estrogenic effects in human breast cancer MCF-7 cells with the aim of elucidating a possible mechanism of action.

Methods

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH, 90%), 2-thiobarbituric acid (TBA, 98%), *t*-butyl hydroperoxide (tBH, 70% in water), trypan blue, charcoal-coated dextran, Hepes, 17β-estradiol, dimethyl sulfoxide (DMSO) for cell cultures, Williams' medium E, RPMI medium, bovine serum and fetal calf serum (FCS), and additives were purchased from Sigma-Aldrich Ltd., Czech Republic. DMEM medium was from Gibco-Life Technologies, Grand Island, NY, USA; silymarin (standardized extract of the seeds of *Silybum marianum* containing 44.5% silybin, 5.0% isosilybinin, 1.4% silydianin, 10.9% silychristin, 2.5% taxifolin) was from Favea Ltd., Kopřivnice, Czech Republic; collagenase was from Sevapharma, Czech Republic; and WST-1 proliferation test was from Roche-Diagnostic, Czech Republic. Other chemicals and solvents, all of analytical grade, were from Pliva-Lachema, Czech Republic.

Plant material

Maca Andina Naturalfa, a dehydrated powder from the hypocotyls, was obtained from Quimica Suiza (Lima, Peru). The product is declared to contain 8.0% protein, 2.0% fat, 5.0% ash, 5.0% fiber, 60.0% carbohydrates and 1256 kJ/100 g. Our analysis revealed that the powder contained 11.6% protein, 1.09% fat (of which 86.7% steroids), 9.08% fiber, 60.0% carbohydrates (23.4% sucrose, 1.55% glucose, 4.56% oligosaccharides, and 30.4% polysaccharides), 5.0% ash and 663 kJ/100 g. Ash consisted mainly of potassium (16.2 mg/g), sodium (260 mg/kg), zinc (58.4 mg/kg), iron (72.3 mg/kg), copper (5.14 mg/kg) and nickel (0.49 mg/kg). The contents of arsenic, lead, cadmium and nitrates were below the limit set for foodstuffs.

Preparation of the extracts

Dried powder of the maca hypocotyls (100 g) was macerated with distilled water or methanol (three times during 24 h); both extracts were dried until constant weight was achieved. Extraction yields of 45.0% and 26.3% respectively were obtained.

Analysis of steroids and fatty acids

Before analysis of lipid compounds, the maca extracts (1 g) were extracted three times with $CHCl_3$/MeOH (1:1, 300 ml) for 24 h with stirring. The resulting extract was filtered and evaporated to dryness (40°C). Then 50 ml NaOH (1 mol/L) was added and the mixture was boiled (10 min). After cooling, the mixture was acidified

(20% HCI) to pH$\sim$ 2 and extracted three times with $CHCl_3$ (10 ml). The extract was evaporated to dryness and the methyl esters were prepared by reaction with BF_3-MeOH. The reaction product was diluted with water and extracted three times with hexane (1 ml). The resulting solution was applied into GC-MS. Both fatty acids and lipids were identified under the same conditions. The samples were separated on a gas chromatograph GC-HP 6890 (Hewlett Packard, USA), capillary JW-DB5 25 m $\times$ 0.250 mm i.d, film 0.25 μm, temperature gradient of 15°C/min, starting at 50°C, final temperature 300°C; split 1:50. The flow rate of helium was maintained constant at 1.2 ml/min. Detection was performed on an Autospec Ultima mass spectrometer (Micromas, UK) magnetic scan 20–600 amu.

Animals

Male Wistar rats weighing 200–250 g were conditioned in standard boxes for 15 days before the experiments. They were fed a standard laboratory diet, provided with water *ad libitum*, and kept on a 12/12 h light–dark cycle.

Rat hepatocyte primary cultures

Rat hepatocytes were isolated by two-step collagenase perfusion of rat liver (Moldeus et al., 1978). The cell viability was determined by measuring trypan blue exclusion. Yields of 2–4 $\times$ 10^8 cells/liver with viability greater than 80% were routinely obtained. The hepatocytes were then dispersed in sterile conditions under Williams' medium E supplemented with penicillin, streptomycin, dexamethasone, insulin, and glutamine and then cultivated in collagen-coated 12-well dishes in a humidified atmosphere of 5% CO_2 at 37°C. For culture stabilization, bovine serum (final concentration 10%) was added to the cultivation medium for the first 4 h (Valentová et al., 2004). The tested maca extracts (final concentrations 1–10 000 μg/ml) were added to the incubation medium in DMSO (maximum concentration 0.5%).

For cytotoxicity studies, the hepatocyte monolayers were incubated with the test samples for 4, 24 or 48 h and cell viability was assessed by the MTT test (Sieuwerts et al., 1995); potential damage to the hepatocytes was determined by extracellular activity of lactate dehydrogenase (LDH) (Bergmeyer and Bernt, 1974) and aspartate amino-transferase (AST) (Bergmeyer and Horder, 1980).

For study of potential cytoprotective effects against tBH-induced damage, the primary cultures were intoxicated with tBH for 1.5 h (final concentration 0.5 mmol/L after preincubation with the maca extracts (0.5 h). The quality of the culture was controlled using cell viability (MTT test; Sieuwerts et al., 1995) and levels of released LDH (Bergmeyer and Bernt, 1974) and lipoperoxidation products (TBARS, thiobarbituric acid-reactive substances; Buege and Aust, 1978) in the medium.

DPPH scavenging

Methanolic solutions (375 μl) of the tested maca extract (12.5, 25, 50, 100, 1000, 2000, and 10000 μg/ml) were mixed with 750 μl of a methanolic solution of (DPPH 20 mg/l). After 30 min, absorbance at 517 nm was measured and $1C_{50}$ values were obtained from the inhibition curves (Mensor et al., 2001).

MCF-7 estrogenicity testing (E-screen)

The MCF-7, an estrogen-positive cell line derived from human breast cancer (Soto et al., 1992), used in this study was a gift from *Z*. Kovařík (Masaryk Memorial Cancer Institute Brno, Czech Republic). The cells were cultivated in RPMI medium with 5% FCS, penicillin, streptomycin, Hepes and L-glutamine in an atmosphere of 5% CO_2/95% air at 37°C. For the experiments, RPMI medium was replaced by DMEM without phenol red. Steroids were removed from FCS with 5% heat-inactivated

FCS. Steroids were removed from the medium by incubation with charcoal-coated dextran for 30 min at 55°C; the chacoal particles were removed by centrifugation at 4500 *g* at 4°C for 20 min. The stripped serum was sterile–filtered and stored at aliquots at–20°C.

MCF-7 cells were used in the E-screen test according to the technique originally described by Soto (Soto et al., 1992). Briefly, cells were trypsinized and plated onto 96-well plates. The cells were allowed to attach for 24 h, then test compounds in a range of concentrations were added in fresh medium. 17β-Estradiol dissolved in ethanol was used as a positive control. Proliferative activity was assessed by WST-1 test after 144 h of incubation. Results were expressed as means from four culture wells; mean cell numbers were normalized to the steroid-free control, (taken as 1) to correct differences in the initial plating density.

Statistics

The data were analyzed by one-way ANOVA using the StatView Statistical Package. Differences were considered statistically significant $p < 0.05$ and $p < 0.01$.

Results

Steroid and fatty acid analyses

Palmitic, stearic, oleic, linoleic, and linolenic acids were identified as predominant in maca extracts; we also found 9-oxo-nonadecanoic acid. β-Sitosterol was found to be the main steroid; we also determined 3,5-stigmastadiene and 3-hydroxy-5-ergostene. 3,5-Stigmastadien-7-one and homo-β-sitosterol were found in methanol extract, and brassicasterol, its isomer (precise structure not determined), and avenastrol were present in water extract. 3-Hydroxy-5-ergostene was not found in water extract (Table 1).

Table 1. Fatty acids and steroids in maca extracts (GC-MS as methyl esters, expressed as percentage of total fatty acid or steroid content)

	Extract	
Compound	Methanol[a]	Water[b]
Phenylhydroxyacetic	4.1	–
2-Oxononadecanoic	2.6	–
Octadecanoic	2.2	–
Nonadecanoic	21.8	–
Palmitic	36.8	44.0
Stearic	13.0	23.9
Oleic	7.2	11.6
Linoleic	3.6	8.7
Linolenic	5.4	5.8
Eicosanoic	1.3	6.0
Tetracosanoic acid	2.0	–
3,5-Stigmastadiene	1.6	3.8
3-Hydroxy-5-ergostene	19.2	–
β-Sitosterol	69.8	48.5
3,5-Stigmastadien-7-one	6.0	2.6
Homo-β-sitosterol	3.4	0.8
Brassicasterol	–	16.8
Isomer of brassicasterol	–	23.1
Avenasterol	–	4.4

[a] 2.5 mg/g fatty acids and 14.9 mg/g steroids.
[b] 6.2 mg/g fatty acids and 9.1 mg/g steroids.

Rat hepatocyte primary cultures

Both aqueous and methanolic maca extracts were tested for *in vitro* hepatotoxicity in rat hepatocyte primary cultures. The cell cultures were incubated in the presence of maca extracts (1, 10, 1000 μg/ml and 1, 5, 10 mg/ml) for 24, 48, and 72 h. The extracts did not influence cell morphology and viability (detrmined in the MTT test) at any of the concentrations tested; at the highest concentration, used after 72 h, cell viability was still 93.3 ± 11.1% and 100.2 ± 2.7% of the control culture for aqueous and methanolic extracts, respectively.

During 24 and 48 h incubation, no significant differences in the enzyme levels in the incubation media were noted (data not shown). After 72 h of cultivation, AST and LDH levels in the medium of the control culture were 7.36 ± 0.57 and 7.99 ± 0.87 μkat/L, respectively. When the hepatocytes were incubated with 10 mg/ml of maca aqueous extract, these levels were 5.93 ± 0.65 ($p < 0.05$)

Table 2. Effect of *Lepidium meyenii* extracts on primary cultures of rat hepatocytes incubated for 72 h

Extract	Concentration (mg/ml)	MTT ($A_{540\,nm}$)	AST (μkat/L)	LDH (μkat/L)
Control		0.925 ± 0.018	7.36 ± 0.57	7.99 ± 0.87
Water	0.1	1.075 ± 0.131	7.19 ± 0.12	6.76 ± 2.10
	0.5	0.997 ± 0.112	7.61 ± 0.76	7.39 ± 1.40
	1	0.979 ± 0.046	7.11 ± 1.21	7.13 ± 1.14
	5	0.918 ± 0.019	$5.37 \pm 0.78^{*}$	8.92 ± 0.58
	10	0.865 ± 0.117	$5.93 \pm 0.65^{*}$	8.19 ± 0.70
Methanol	0.1	0.857 ± 0.081	6.85 ± 1.45	8.08 ± 1.35
	0.5	0.893 ± 0.100	6.95 ± 1.57	7.17 ± 1.31
	1	0.847 ± 0.045	$5.59 \pm 1.23^{*}$	$4.92 \pm 1.94^{*}$
	5	0.864 ± 0.019	$4.95 \pm 0.49^{**}$	$4.89 \pm 0.91^{**}$
	10	0.926 ± 0.025	$4.51 \pm 0.62^{**}$	$4.45 \pm 1.52^{**}$

The data are expressed as means ± SD, $n = 4$.
* Values differ from control at $p < 0.05$.
** Values differ from control at $p < 0.01$.

and 8.19 ± 0.70 μkat/L. For methanol extract, enzyme activities were even lower; 4.45 ± 1.52 and 4.51 ± 0.62 μkat/L for LDH and AST, respectively ($p < 0.01$, Table 2).

In a typical nontreated cell culture, the MTT test gave $A_{540\,nm} = 1.009 \pm 0.030$; the activity of LDH was 4.70 ± 0.50 μkat/L, and TBARS level in culture medium was 0.35 ± 0.05 μmol/L (100%). After intoxication with tBH, these values changed to 0.193 ± 0.010 (MTT test), 21.65 ± 1.74 μkat/L (LDH) and 4.44 ± 0.19 μmol/L (TBARS). After 30 min of preincubation with *Lepidium meyenii* extracts, no significant protective effet of the primary cultures was noted (data not shown). The only exception was the level of released LDH after treatment with 1 mg/ml of the methanolic extract, where we observed protection of $14.51\% \pm 4.37\%$ (to 19.28 ± 2.58 μkat1^{-1}), which was significant ($p < 0.05$).

DPPH scavenging

Both *Lepidium meyenii* extracts tested in this study had a limited solubility in methanol and at the concentrations of 1 mg/ml and higher we observed precipitation of the extracts in the reaction system. This was probably due to the high content of saccharides in the extract; pure sucrose represented about 50% of dry weight precipitated from the methanolic extract after one month at room temperature. However, both extracts were able to reduce the DPPH radical with IC_{50} values of 3.46 ± 0.16 and 0.71 ± 0.10 mg/ml for the aqueous and methanolic extracts, respectively.

MCF-7 (E-screen)

The addition of maca extracts had no deleterious effect on the growth of MCF-7 cells, as in the case of silymarin. Cell proliferation was stimulated by both maca extracts and silymarin in concentrations ranging from 0.1 to 100 μg/ml and from 0.1 to 200 μg/ml, respectively. The effect of maca methanolic extract (Figure 1) was expressed as multiples of control values. The effect of the aqueous extract was similar (data not shown). For comparison, graphic expression of stimulation of MCF-7 cells by 17β-estradiol is shown in (Figure 2), where the horizontal axis represents in log molar units. Maximal stimulation of MCF-7 by 17β-estradiol occured at concentrations ranging from 10^{-10} to 10^{-12} mol/L. Maca extract estrogenicity was exhibited in the range from 100 to 200 μg/ml.

Discussion

Lepidium meyenii (maca) has become very popular in recent years for use in various dietary supplements. Maca is often called Peruvian or Andean ginseng; it is also recommended as an adaptogen, an imunostimulant, an anabolic, in menopause and for influence on hormonal balance (Quirós and Aliaga, 1997). Chacón de Popovici recommends maca for treating malabsorption syndrome and protein deficiency disease, during chemotherapy for leukemia, in AIDS treatment, and for alcoholism and menopausal anemia (Popovici, 1997). Others report its use to treat chronic polyarthritis, during allergy attacks, and as a laxative (Aliaga and Aliaga, 1998). Reliable pharmacological confirmation of all cited effects has been lacking until very recently, but a number of studies

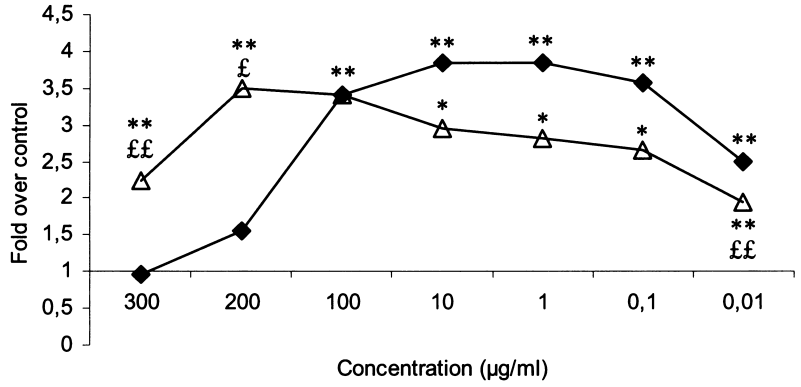

Figure 1. Effect of maca methanolic extract (△) and silymarin (♦) on MCF-7 cell proliferation (E-screen). The cells in 96-well plates were allowed to attach for 24 h, and then a range of concentrations of tested compounds were added in fresh medium. Proliferative activity was assessed by WST-1 test after 144 h of incubation. Results are expressed as means from four culture wells; mean cell numbers were normalized to the steroid-free control, taken as 1. $^{*}p. < 0.05$ vs. control; $^{**}p < 0.01$ vs. control; $^{£}p < 0.05$; $^{ff}p < 0.01$ vs. silymarin.

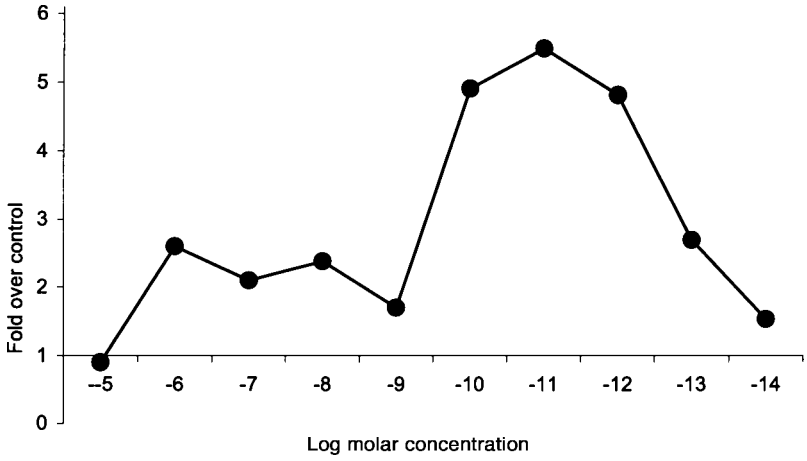

Figure 2. Effect of 17β-estradiol on MCF-7 cell proliferation (E-screen). The cells in 96-well plates were allowed to attach for 24 h, and then a range of concentrations of 17β-estradiol were added in fresh medium. Proliferative activity was assessed by WST-1 test after 144 h of incubation. Results are expressed as means from four culture wells; mean cell numbers were normalized to the steroid-free control, taken as 1.

confirming some of the repeated activities have appeared in the last few years. Maca extracts increased the number of complete intromissions and sperm-positive females in normal animals and decreased the latent period of erection in rats with erectile dysfunction (Zheng et al., 2000). It also induct an increase in testis size and stimulation of spermatogenesis in rats and mice (Cicero et al., 2001; Gonzales et al., 200lb; Cicero et al., 2002). Maca reduced spermatogenic damage in mice and prostate size in rats (Bustos-Obregon et al., 2005; Gonzales et al., 2005), restored homeostasis impaired by restraint stress in mice (López-Fanso et al., 2004), and improved growth rate and survival in rainbow trout (Lee K. J. et al., 2004, 2005). In adult men, increases in sexual desire, sperm volume, total sperm count, and sperm motility were observed, but serum biochemical parameters—except for reproductive hormones—were not evaluated (Gonzales et al.,

2001a, 2002, 2003a). The only study to evaluate the safety of maca in high doses was performed by measuring the weight of different organs in adult rats, where no toxicity was noted (Chung et al, 2005).

Because a slight but significant increase in plasma AST level was registred in our pilot clinical study with 0.6 *g* of maca powder per day (unpublished results), the effect of maca extracts on rat hepatocyte primary cultures was evaluated. Not only were no cytotoxic effects observed up to 10 mg/ml, the extract inhibited leakage of LDH and AST from the cells at the highest doses. This observation led us to test the hypothesis that maca extracts have cytoprotective effects against damage to hepatocytes; *t*-butyl hydroperoxide was used as a model toxin. In our hands, no protective effect was noted and quite low antioixidant activity was measured in the DPPH scavenging test (IC_{50} values 3.46 ± 0.16 and 0.71 ± 0.10 mg/ml, for the aqueous and methanol extracts, respectively). This finding is in contrast with the IC_{50} value of 0.61 mg/ml found for maca aqueous by Sandoval et al.(2002). The difference is probably due to different methods of preparation; while we chose maceration at room temperature, Sandoval used warm water. DPPH scavenging activity was also observed using a Soxhlet methanolic maca extract with IC_{50} between 100 and 500 μg/ml (Lee K. J. et al., 2005). In comparison with other plant extracts tested in our laboratory, the values found for silymarin (Psotová et al., 2002), *Prunella vulgaris* (Psotová et al., 2003) and *Smallanthus sonchifolius* (Valentová et al., 2003) aerial part ethyl acetate extracts were 93.3 ± 2.5 μmol/L (as silybin), 0.90 ± 0.07 and 16.14 ± 3.38 μg/ml, respectively. Although no protective effect was noted, our experiments provide evidence that *Lepidium meyenii* extracts, even at very high concentration, do not display *in vitro* hepatotoxicity. The observed protection against spontaneous enzyme leakage is probably mediated through other than antioxidant mechanisms.

The estrogen-positive cell line MCF-7 is a suitable cell model for study of estrogenic effects. For example, *Panax quinquefolius* (Duda et al., 1999), *Cimicifuga racemosa* (Liu et al., 2001), *Pueraria mirifica* (Lee Y. S. et al., 2002), food additives (Okubo and Kano, 2003), genistein (Almstrup et al., 2002; Chen et al., 2003), and phytosterols (Baker et al., 1999), have been studied in this model. Maca extracts exhibited estrogenicity in the range 100–200 μg/ml. The addition of silymarin, known to act as a partial estrogen receptor β-agonist (Seidlova-Wuttke et al., 2003; Plíšková et al., 2005), also led to stimulation of MCF-7 cells proliferation at a similar level to maca extracts ($p < 0.05$; Figure 1). The maximum stimulation was one order of magnitude higher. However, the overall stimulation of MCF-7 cells by maca extracts was rather low in comparison with the effects of genistein and resveratrol (our unpublished data) or 17β-estradiol (Figure 2). Neither the maca extracts nor silymarin had activity comparable with that of 17β-estradiol ($p < 0.1$).

This activity supports the hypothesis (Gonzales et al., 2001a, 2002, 2003a) that maca contains bioactive compounds, probably steroids, the concentration of which in hypocotyls is important. The estrogenicity of β-sitosterol has been described previously in T-47D cells but it was inactive in MCF-7 (Rosenblum et al., 1993); only weak activity was noted in ovariectomized rats (Mellanen et al., 1996) and no activity was found after postnatal hypophysectomy (Cummings and Laws, 2000). An estrogenic effect of β-sitosterol has been reported in several fish species and it has been suggested that phytosterols could be responsible for maca's effect on growth and survival of rainbow trout (Lee K.J. et al., 2004, 2005). In view of recent studies concerning the role of estrogen and phytoestrogen in both male and female reproduction (Simpson et al., 2000; O'Donnell et al., 2001; Robertson et al., 2002; Aquila et al., 2004; Tsai et al., 2005), the estrogenic activity of maca must be taken into consideration for evaluation of the *in vivo* activity of maca on fertility, potency, and growth rate as reported in the literature and in traditional medicine.

In conclusion, the present findings show that maca does not display *in vitro* hepatotoxicity. In contrast, a slight cytoprotective effect, probably not mediated by antioxidant capacity, was noted. The extracts exhibited estrogenic activity comparable with that of silymarin MCF-7 cells.

Acknowledgments

This work was supported by the Ministry of Education, Youth and Sport (MSM 6198959216) and Ministry of Health of the Czech Republic (IGA NJ7463-3/2003).

References

Aliaga E, Aliaga R. Guia para el cultivo, aprovechamiento y conservacion de la maca, *Lepidium meyenii* Walpers. Santafé de Bogotá: Convenio Andres Bello; 1998.

Almstrup K, Fernandez MF, Petersen JH, Olea N, Skakkebaek NE, Leffers H. Dual effects of phytoestrogens result in u-shaped dose-response curves. Environ Health Perspect. 2002; 110:743–8.

Aquila S, Sisci D, Gentile M, Middea E, Catalano S, Carpino A, Rago V, Ando S. Estrogen receptor (ER) alpha and ER beta are both expressed in human ejaculated spermatozoa: evidence of their direct interaction with phosphatidylinositol-3-OH kinase/Akt pathway. J Clin Endocrinol Metab. 2004;89:1443–51.

Baker VA, Hepburn PA, Kennedy SJ, Jones PA, Lea LJ, Sumpter JP, Ashby J. Safety evaluation of phytosterol esters. Part 1. Assessment of oestrogenicity using a combination of *in vivo* and in *vitro* assays. Food Chem Toxicol. 1999;37:13–22.

Balick MJ, Lee R. Maca: from traditional food crop to energy and libido stimulant. Altern Ther Health Med. 2002;8:96–8.

Bergmeyer HU, Bernt E. Lactate dehydrogenase: UV-Assay with pyruvate and NADH. In: Bergmeyer HU, ed. New York: Academic Press; 1974:574–6.

Bergmeyer HU, Horder M. International federation of clinical chemistry. Scientific committee. Expert panel on enzymes. IFCC document stage 2, draft 1; 1979-11-19 with a view to an IFCC recommendation. IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase. J Clin Chem Clin Biochem. 1980; 18:521–34.

Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978;52:302–10.

Bustos-Obregon E, Yucra S, Gonzales GF. *Lepidium meyenii* (Maca) reduces spermatogenic damage induced by a single dose of malathion in mice. Asian J Androl. 2005;7:71–6.

Canales M, Aguilar J, Prada A, Marcelo A, Huaman C, Carbajal L. Nutritional evaluation of *Lepidium meyenii* (MACA) in albino mice and their descendant. Archivos Latinoamericanos De Nutricion. 2000;50:126–33.

Chen WF, Huang MH, Tzang CH, Yang M, Wong MS. Inhibitory actions of genistein human breast cancer (MCF-7) cells. Biochim Biophys Acta. 2003; 1638:187–96.

Chung F, Rubio J, Gonzales C, Gasco M, Gonzales GF. Dose-response effects of *Lepidium meyenii* (Maca) aqueous extract on testicular function and weight of different organs in adult rats. J Ethnopharmacol. 2005;98:143–7.

Cicero AF, Bandieri E, Arletti R. *Lepidium meyenii* Walp. improves sexual behaviour in male rats independently from its action on spontaneous locomotor activity. J Ethnopharmacol. 2001;75:225–9.

Cicero AF, Piacente S, Plaza A, Sala E, Arletti R, Pizza C. Hexanic Maca extract improves rat sexual performance more effectively than methanolic and chloroformic Maca extracts. Andrologia. 2002;34:177–9.

Cui B, Zheng BL, He K, Zheng QY. Imidazole alkaloids from *Lepidium meyenii*. J Nat Prod. 2003;66:1101–3.

Cummings AM, Laws SC. Assessment of estrogenicity by using the delayed implanting rat model and examples. Reprod Toxicol. 2000;14:111–7.

Dini A, Migliuolo G, Rastrelli L, Saturnino P, Shettino O. Chemical composition of *Lepidium meyenii*. Food Chem. 1994;49: 347–9.

Dini I, Tenore GC, Dini A. Glucosinolates from maca (*Lepidium meyenii*). Biochem Syst Ecol 2002;30:1087–90.

Duda RB, Zhong Y, Navas V, Li MZ, Toy BR, Alavarez JG. American ginseng and breast cancer therapeutic agents synergistically inhibit MCF-7 breast cancer cell growth. J Surg Oncol. 1999;72:230–9.

Flores H, Walker T, Guimaraes R, PalBais H, Vivanco J. Andean root and tuber crops: underground rainbows. HortScience. 2003;38:161–7.

Ganzera M, Zhao J, Muhammad I, Khan IA. Chemical profiling and standardization of *Lepidium meyenii* (maca) by reversed phase high performance liquid chromatography. Chem Pharm Bull. 2002;50:988–91.

Gonzales GF, Cordova A, Gonzales C, Chung A, Vega K, Villena A. *Lepidium meyenii* (Maca) improved semen parameters in adult men. Asian J Androl. 2001a;3:301–3.

Gonzales GF, Ruiz A, Gonzales C, Villegas L, Cordova A. Effect of *Lepidium meyenii* (maca) roots on spermatogenesis of male rats. Asian J Androl. 2001b;3:231–3.

Gonzales GF, Cordova A, Vega K, Chung A, Villena A, Gonez C, Castillo S. Effect of *Lepidium meyenii* (MACA) on sexual desire and its absent relationship with serum testosterone levels in adult healthy men. Andrologia. 2002;34:367–72.

Gonzales GF, Cordova A, Vega K, Chung A, Villena A, Gonez C. Effect of *Lepidium meyenii* (Maca), a root with aphrodisiac and fertility-enhancing properties, on serum reproductive hormone levels in adult healthy men. J Endocrinol. 2003a; 176: 163–8.

Gonzales GF, Rubio J, Chung A, Gasco M, Villegas L. Effect of alcoholic extract of *Lepidium meyenii* (Maca) on testicular function in male rats. Asian J Androl. 2003b;5:349–52.

Gonzales GF, Miranda S, Nieto J, Fernandez G, Yucra S, Rubio J, Yi P, Gasco M. Red maca (*Lepidium meyenii*) reduced prostate size in rats. Reprod Biol Endocrinol. 2005;3:5.

Lee KJ, Dabrowski K, Rinchard J, Gomez C, Guz L, Vilchez C. Supplementation of maca (*Lepidium meyenii*) tuber meal in diets improves growth rate and survival of rainbow trout *Oncorhynchus mykiss* (Walbaum) alevins and juveniles. Aquacult. Res. 2004;35:215–23.

Lee KJ, Dabrowski K, Sandoval M, Miller MJS. Activity-guided fractionation of phytochemicals of maca meal, their antioxidant activities and effects on growth, feed utilization, and survival in rainbow trout (*Oncorhynchus mykiss*) juveniles. Aquaculture. 2005;244:293–301.

Lee YS, Park JS, Cho SD, Son JK, Cherdshewasart W, Kang KS. Requirement of metabolic activation for estrogenic activity of *Pueraria mirifica*. J Vet Sci. 2002;3:273–7.

Li G, Ammermann U, Quirós CF. Glucosinolate contents in maca (*Lepidium peruvianum* Chacón) seeds, sprouts, mature plants and several derived commercial products. Econ Bot. 2001;55:255–62.

Liu Z, Yang Z, Zhu M, Huo J. Estrogenicity of black cohosh (*Cimicifuga racemosa*) and its effect on estrogen receptor level in human breast cancer MCF-7 cells. Wei Sheng Yan Jiu. 2001;30:77–80.

López-Fando A, Gómez-Serranillos MP, Iglesias I, Lock O, Upamayta UP, Carretero ME. *Lepidium peruvianum* Chacon restores homeostasis impaired by restraint stress. Phytother Res. 2004;18:471–4.

Mellanen P, Petanen T, Lehtimaki J, Makela S, Bylund G, Holmbom B, Mannila E, Oikari A, Santti R. Wood-derived estrogens: studies *in vitro* with breast cancer cell lines and *in vivo* in trout. Toxicol Appl Pharmacol. 1996;136:381–8.

Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS, Leitao SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res. 2001;15:127–30.

Moldeus P, Hogberg J, Orrenius S. Isolation and use of liver cells. Methods Enzymol. 1978;52:60–71.

Muhammad I, Zhao J, Dunbar DC, Khan IA. Constituents of *Lepidium meyenii* 'maca'. Phytochemistry. 2002;59:105–10.

O'Donnell L, Robertson KM, Jones ME, Simpson ER. Estrogen and spermatogenesis. Endocr Rev. 2001;22:289–318.

Okubo T, Kano I. Studies on estrogenic activities of food additives with human breast cancer MCF-7 cells and mechanism of estrogenicity by BHA and OPP. Yakugaku Zasshi. 2003;123:443–52.

Piacente S, Carbone V, Plaza A, Zampelli A, Pizza C. Investigation of the tuber constituents of maca (*Lepidium meyenii* Walp.). J Agric Food Chem. 2002;50:5621–5.

Plíšková M, Vondráček J, Křen V, Gažák R, Sedmera P, Walterová D, Psotová J, Šimánek V, Machala M. Effects of silymarin flavonolignans and synthetic silybin derivatives on estrogen and aryl hydrocarbon receptor activation. Toxicology. 2005;215:80–9.

Popovici GC. La importancia de *Lepidium peruvianum* ("Maca") en la alimentación y salud del ser humano y animal 2,00 anos antes y desputa del Cristo y en el siglo XXI. Lima: Servicios Grá Gficos "ROMERO"; 1997.

Psotová J, Chlopčíková S, Grambal F, Šimánek V, Ulrichová J. Influence of silymarin and its flavonolignans on doxorubicin-iron induced lipid peroxidation in rat heart microsomes and mitochondria in comparison with quercetin. Phytother Res. 2002;16 (Suppl 1) :S63–7.

Psotová J, Kolář M, Soušek J, Švagera Z, Vičar J, Ulrichová J. Biological activities of *Prunella vulgaris* extract. Phytother Res. 2003;17:1082–7.

Quirós CF, Aliaga RC Maca. *Lepidium meyenii* Walp. In: Hermann M, Heller J, eds. Rome: IPGRI;1997:173–97.

Robertson KM, O'Donnell L, Simpson ER, Jones ME. The phenotype of the aromatase knockout mouse reveals dietary phytoestrogens impact significantly on testis function. Endocrinology. 2002;143:2913–21.

Rosenblum ER, Stauber RE, Van Thiel DH, Campbell IM, Gavaler JS. Assessment of the estrogenic activity of phytoestrogens isolated from bourbon and beer. Alcohol Clin Exp Res. 1993;17:1207–9.

Sandoval M, Okuhama NN, Angeles FM, Melchor VV, Condezo LA, Lao J, Miller MJS. Antioxidant activity of the cruciferous vegetable Maca (*Lepidium meyenii*). Food Chem. 2002;79:207–13.

Seidlova-Wuttke D, Becker T, Christoffel V, Jarry H, Wuttke W. Silymarin is a selective estrogen receptor beta (ERbeta) agonist and has estrogenic effects in the metaphysis of the femur but no or antiestrogenic effects in the uterus of ovariectomized (ovx) rats. J Steroid Biochem Mol Biol. 2003;86:179–88.

Sieuwerts AM, Klijn JG, Peters HA, Foekens JA. The MTT tetrazolium salt assay scrutinized: how to use this assay reliably to measure metabolic activity of cell cultures *in vitro* for the assessment of growth characteristics, IC_{50}-values and cell survival. Eur J Clin Chem Clin Biochem. 1995;33:813–23.

Simpson E, Rubin G, Clyne C, Robertson K, O'Donnell L, Jones M, Davis S. The role of local estrogen biosynthesis in males and females. Trends Endocrinol Metab. 2000;11:184–8.

Soto AM, Lin TM, Justicia H, Silvia RM, Sonnenschein C. An "in culture" bioassay to assess the estrogenicity of xenobitics (E-screen). In: Colborn T, Ckenent C, eds. Princeton, NJ: Princeton Scientific Publishing; 1992:295–309.

Tsai PS, Kessler AE, Jones JT, Wahr KB. Alteration of the hypothalamic–pituitary–gonadal axis in estrogen- and androgen-treated adult male leopard frog, *Rana pipiens*. Reprod Biol Endocrinol. 2005;3:2.

Valentová K, Ulrichová J. *Smallanthus sonchifolius* and *Lepidium meyenii*—prospective Andean crops for the prevention of chronic diseases. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2003;147:119–30.

Valentová K, Cvak L, Muck A, Ulrichová J, Šimánek V. Antioxidant activity of extracts from the leaves of *Smallanthus sonchifolius*. Eur J Nutr. 2003;42:61–6.

Valentová K, Moncion A, de Waziers I, Ulrichová J. The effect of *Smallanthus sonchifolius* leaf extracts on rat hepatic metabolism. Cell Biol Toxicol. 2004;20:109–20.

Zheng BL, He K, Kim CH, Rogers L, Shao Y, Huang ZY, Lu Y, Yan SJ, Qien LC, Zheng QY. Effect of a lipidic extract from *Lepidium meyenii* on sexual behavior in mice and rats. Urology. 2000;55:598–602.

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