Universidade Federal do Pampa

CURSO DE GRADUAÇÃO EM FARMÁCIA

TRABALHO DE CONCLUSÃO DE CURSO

Maca peruana (*Lepidium meyenii*) causa reprotoxicidade em *Caenorhabditis elegans*.

LUIZ EDUARDO BEN PILISSÃO

URUGUAIANA, 2018

LUIZ EDUARDO BEN PILISSÃO

Maca peruana (*Lepidium meyenii*) causa reprotoxicidade em *Caenorhabditis elegans*.

Trabalho de Conclusão de Curso apresentado ao Curso de Farmácia, da Universidade Federal do Pampa, como requisito parcial para obtenção do título de Bacharel em Farmácia.

Orientador: Daiana Silva de Ávila.

URUGUAIANA 2018

Luiz Eduardo Ben Pilissão

MACA PERUANA (*Lepidium meyenii*) CAUSA REPROTOXICIDADE EM *Caenorhabditis elegans*

Trabalho de conclusão de curso apresentado ao curso de Farmácia da Universidade Federal do Pampa (UNIPAMPA), como requisito parcial para aprovação na disciplina de Trabalho de Conclusão de Curso.

Orientador (a): Profª. Drª. Daiana Silva de Àvila

Área de concentração: Farmácia

Trabalho de conclusão de curso defendido e aprovado em: 03 / 07 / 2018

Banca examinadora:

Profª. Drª. Daiana Silva de Àvila
Orientadora
Curso de Farmácia - UNIPAMPA

Profª. Drª. Fabiane Moreira Farias
Curso de Farmácia - UNIPAMPA

Profª. Drª. Francielli Weber Santos Cibin
Curso de Farmácia - UNIPAMPA

Resumo

A maca (*Lepidium meyenii*) é uma planta que cresce acima dos 4000 metros nos Andes Central do Peru e possui diferentes variedades de acordo com a cor do hipocótilo.De acordo com a população, suas propriedades medicinais estão conectadas à fertilidade e à vitalidade. Além dos efeitos benéficos relacionados à saúde sexual, a maca também vem sendo largamente utilizada por aqueles que desejam emagrecer de forma natural e saudável. Entretanto, há poucos estudos científicos validando estes usos. O presente estudo teve como objetivo avaliar os efeitos do extrato aquoso da maca sobre a reprodução, utilizando o *Caenorhabditis elegans* como modelo experimental. Adquiriu-se a maca peruana do comércio local, do qual se obteve um extrato aquoso. Osanimaisforam tratados e mantidos a 20 °C em NGM (meio de crescimento para nematoides), semeados com *Escherichia coli* OP50. Os Vermes (2000) foram expostos ao extratoaquoso de maca em diferentes concentrações (41,25; 123,75; 247,5; 330μg/μl) no estágio larval L1, durante 30 minutos em meio líquido contendo M9 e livre de bactéria. As análises de sobrevivência e de toxicidade reprodutiva (postura de ovos, ovos por verme) foram realizadas, em placas NGM/*E. coli*, 48h após o tratamento. Os ensaios bioquímicos foram realizados utilizando um homogeneizado dos tratamentos e kits comerciais. A analise estatística foi obtida pela ANOVA de uma via seguida de múltiplas comparações com post teste de Tukey. Os resultados mostraram que a maca causou uma redução na sobrevivência em todas as concentrações. Além disso, o extrato causou uma diminuição significativa no tamanho da ninhada. Nossos resultados também mostram que o extrato da maca causou alterações no perfil lipídico e na expressão de vitelogeninas e interferiu de forma acentuada no equilíbrio fisiológico da linhagem germinativado sistema reprodutor do verme. Com isso, conclui-se que a utilização do extrato da maca peruana, nestas concentrações em *C. elegans*tem efeitos contrários aos efeitos atribuídos e esperados desse vegetal, já que a maca é utilizada em virtude dos seus supostos beneficios sobre a vitalidade e fertilidade.

Palavras-Chave: *Lepidium meyenii*, reprodução, vitelogeninas, apoptose, *Caenorhabditis elegans.*

Abstract

Maca (Lepidium meyenii) is a plant that grows above 4000 meters in the Central Andes of Peru and has different varieties according to the color of the hypocotyl. According to the population, its medicinal properties are connected to fertility and vitality. Besides the beneficial effects related to sexual health, maca has also been widely used by those who wish to lose weight naturally and healthy. However, there are few scientific studies validating these uses. The objective of the present study was to evaluate the effects of aqueous extract of maca on reproduction, using Caenorhabditis elegans as an experimental model. The Peruvian maca from local commerce was obtained, from which an aqueous extract was obtained. The animals were treated and maintained at 20 ° C in NGM (nematode growth medium), seeded with Escherichia coli OP50. The worms (2000) were exposed to maca extracts at different concentrations (41.25, 123.75, 247.5, 330 μg / μl) in the L1 larval stage for 30 minutes in liquid medium containing M9 and free of bacteria. Survival and reproductive toxicity analyzes (egg laying, eggs per worm) were performed on NGM / E plates. coli, 48h after treatment. Biochemical assays were performed using a homogenate of the commercial treatments and kits. Statistical analysis was obtained by one-way ANOVA followed by multiple comparisons with Tukey's post-test. The results showed that maca caused a reduction in survival at all concentrations. In addition, the extract caused a significant decrease in litter size. Our results also show that the maca extract caused alterations in the lipid profile and expression of vitellogenins and interfered in a marked way in the physiological balance of the germinated lineage of the worm reproductive system. Therefore, it is concluded that the use of the extract of Peruvian maca in these concentrations in C. elegans has effects contrary to the attributed and expected effects of this vegetable, since maca is used because of its supposed benefits on vitality and fertility.

Key words: Lepidium meyenii, reproduction, vitellogenins, apoptosis, *Caenorhabditis elegans.*

Lista de figuras

Figura 1: Representação esquemática do ciclo de vida do *C. elegans*

Figure 2: *Maca* aqueous extract effects on N2 worms survival.

Figure 3: Worms development following exposure to different concentrations of the *maca* extract

Figure 4: Effect of maca exposure in the reproduction of wild-type N2 worms

Figure 5: Quantification of the number of apoptotic cells in the of the worms

Figure 6: A) Determination of triglyceride levels in wild type worms (N2) treated with different concentrations of the root.B) Determination of lipid peroxidation in wild type worms (N2).

Figure 7: Fluorescence quantification of *C. elegans* strain DH1033 treated with different concentrations of *maca* extract.

SUMÁRIO

1. Introdução 8
2. Objetivo 9
2.1 Objetivo geral 9
2.2 Objetivos específicos 9
3. Revisão Bibliográfica 9
3.1 Maca peruana e Função sexual 9
3.2 Caenorhabditis elegans 11
3.3 Vitelogênese 14
2. Materials and methods 19
2.1 Plant Material and extract preparation 19
2.2 Culture, strains and worms treatment 19
2.3 Survival test 20
2.4 Development test 20
2.5 Brood size 20
2.6 Number of eggs per worm 20
2.7 Triglyceride level 21
2.8 Reactive Substances test Thiobarbituric Acid (TBARS) 21
2.9 Number of apoptotic cells 21
2.10 Vitellogenin levels 21
2.11. Statistical analysis 22
3. Results 22
3.1 *Maca* extract reduced worms survival 22
3.2 Maca extract slightly affects larval development 22
3.3 Maca aqueous extract causes alterations on reproductive profile 23
3.4 Maca exposure induces apoptosis of germ cells 24
3.5 Maca aqueous extract affectsworms lipid profile 25
3.6 Maca extract reduces theexpression of vitellogenin-2 in worms embryo 26
4. Discussion 28
8. Conclusion 31
9. References ..... **Erro! Indicador não definido.**

1. Introdução

As terapias à base de plantas para disfunção sexual em machos e fêmeas incluem *Ginkgo(Ginkgo biloba*), ginseng vermelho (*Panax ginseng*) [1 , 2] e ioimbina (*Pausinvstalia vohimbe*), que possui efeitos adversos graves [1, 3 , 4] . Várias outras terapias botânicas para disfunção sexual também foram introduzidas [1 , 3 , 5]. Estes também são frequentemente utilizados para melhorar a função sexual em indivíduos saudáveis.

Dentre as espécies utilizadas, está a *Lepidium meyenii* (Maca peruana), uma planta da família Brassicaceae que cresce entre 4.000 e 4.500 metros acima do nível do mar nos Andes Centrais peruanos, particularmente em Junin e Pasco. A maca cresce nesses locais na faixa de temperaturas de um máximo de 12 ° C a um mínimo de 1,5 ° C. Esta gama de temperaturas ocorre nos Andes Centrais devido à sua proximidade com o equador. Por essa razão, a maca não cresce bem nos Andes do Sul peruanos, onde a temperatura é mais baixa com geadas frequentes [6]. Algumas evidências científicas mostraram que a maca tem propriedades nutricionais, energéticas e favoráveis à fertilidade, e atua sobre disfunções sexuais, osteoporose, hiperplasia prostática benigna, memória e aprendizado, metabolismo de lipídios e glicose e protege a pele contra a radiação ultravioleta [7 - 8].

Existem inúmeros estudos visando avaliar os possíveis efeitos benéficos dos extratos nutracêuticos e para este estudo escolhemos o modelo *Caenorhabditis elegans*. O *Caenorhabditis elegans* é um nematoide de vida livre, encontrado no solo rico em matéria orgânica. Experimentalmente, tem a vantagem de ser um animal simples, com sistemas nervoso, reprodutivo e digestivo presentes em um pequeno verme (os vermes adultos têm um comprimento de ~ 1,2 mm). Outras vantagens incluem ciclo de vida rápido e vida útil curta, que são relevantes para investigações de vida útil [9 , 10];a translação dos resultados observados em vermes para mamíferos, que se deve à alta homologia genética entre humanos e *C. elegans* (cerca de 70%) é muito relevante em toxicologia e farmacologia. Depois do período embrionário eles se desenvolvem através de quatro estágios larvais, a partir de L1 a L4. Vermes adultos hermafroditas podem produzir até 300 descendentes durante o período de reprodução e o ciclo de vida do *C. elegans* é relativamente curto. Possui 1mm de comprimento, corpo transparente o que

possibilita a visualização microscópica do animal vivo. Outra vantagem também identificada é o grande potencial para análises gênicas por meio de cepas transgênicas. Vários estudos fornecem evidências de que este verme responde após a exposição a vários compostos naturais com aumento da resistência ao estresse e/ou até mesmo a estendendo a vida útil [11].

Notavelmente, pouco se sabe sobre os mecanismos pelos quais a maca afeta a reprodução. Para abordar esta questão, avaliamos o número de progênie, bem como os efeitos do extrato sobre parâmetros relacionados a reprodução , avaliando os níveis de proteínas da vitelogenina, através da fluorescência GFP, as células germinativas e o perfil lipídico.

2. Objetivo

2.1 Objetivo geral

O presente trabalho teve como objetivo geral avaliar a toxicidade e os efeitos do extrato aquoso de maca peruana (*Lepidium meyenii*) sobre parâmetros relacionados a viabilidade e reprodução em *Caenorhabditis elegans*.

2.2 Objetivos específicos

-Determinar os efeitos per se do extrato do tubérculo sobre a sobrevivência e reprodução em *C. elegans;*

-Avaliar os efeitos do extrato sobre o perfil lipídico em *C. elegans*, contemplando os níveis de triglicérides, peroxidação lipídica e vitelogeninas;

- avaliar os efeitos do extrato de maca sobre a linhagem germinativa de *C. elegans*.

3. Revisão Bibliográfica

3.1 Maca peruana e Função sexual

Problemas sexuais (ou disfunções sexuais) são generalizados e afetam negativamente o humor, o bem-estar e as relações interpessoais [12]. Eles ocorrem em 20% -30% dos homens e 40-45% das mulheres de acordo com 18 estudos epidemiológicos descritivos de todo o mundo [13]. A maioria dos problemas sexuais relaciona-se com o desejo sexual (interesse sexual) tanto em mulheres quanto em homens e disfunção erétil masculina (ED) [13]. As intervenções farmacológicas atuais para o manejo de problemas sexuais incluem farmacos orais, terapias intra-penetrais (supositórios intra-uretrais e injeções intracavernosas), implante de prótese peniana para homens e terapia hormonal para mulheres. Embora tenham sido feitos avanços consideráveis, o tratamento ideal para ED não foi identificado. O tratamento para problemas sexuais nas mulheres também é problemático, já que envolve vários fatores emocionais, financeiros entre outros [14]. Além disso, demonstrou-se que os tratamentos farmacológicos resultam em vários efeitos adversos, incluindo risco de câncer, dor de cabeça, rinite e dispepsia [15,16,17]. Por estes motivos, terapias com uso de produtos naturais tem sido buscadas.

A maca peruana (*Lepidium meyenii*) é uma planta andina que pertence à família Brassicaceae (mostarda). A maca tem sido usada há séculos nos Andes para aumentar a fertilidade em seres humanos e animais [6 , 7]. As preparações desse tubérculo foram relatados para melhorar a função sexual em populações saudáveis [18]. Embora a maca seja uma planta e não uma substância ativa isolada, ela é uma das mais citadas na rede mundial para a melhoria do desejo sexual. A hipótese de que a maca pode ser eficaz na melhoria da função sexual é suportada por várias linhas de evidência. Experimentos com animais sugerem que a maca tem atividades espermatogênicas e favoráveis à fertilidade, provavelmente devido aos fitoesteróis ou fitoestrógenos presentes na maca [19]. Vários estudos in vivo mostraram que a maca pode melhorar o comportamento sexual e aumentar os efeitos semelhantes aos andrógenos em ratos [20 , 21]. Ensaios clínicos recentes também sugeriram efeitos significativos da maca para aumentar a contagem e motilidade de espermatozoides e melhorar a função sexual em seres humanos [22 , 23]. Os ingredientes bioativos potenciais na maca incluem macaridina, macamidas, macaene, gluosinolatos, alcalóides de maca e nutrientes de maca [19]. Contudo, estes dados são insuficientes para determinar se a maca é clinicamente eficaz. Atualmente, nenhuma revisão sistemática deste assunto está disponível.

Tradicionalmente, os hipocótilos desta planta são usados principalmente como nutrientes e para aumentar a fertilidade [24]. O hipocótilo é a parte comestível da planta e é caracterizada por suas propriedades nutricionais e medicinais [25]. Embora muitas tentativas tenham sido desenvolvidas para cultivar esta planta em outros lugares, incluindo o uso das mesmas sementes dos Andes Centrais peruanos, o tamanho do hipocótilo não se assemelha ao nativo peruano. Essas abordagens foram descritas para os Estados Unidos, Alemanha, República Tcheca [26] e, mais recentemente, na China [27]. Diferentes variedades de maca foram descritas de acordo com a cor dos seus hipocótilos.

3.2 Caenorhabditis elegans

O *Caenorhabditis elegans* é um nematoide de vida livre, habitante dos locais onde há matéria orgânica e comum em todo o mundo, o qual tem sido amplamente usado como organismo modelo para os mais diversos tipos de pesquisas [28,29]. O *C. elegans* pode proliferar-se em vários tipos de material vegetal em decomposição como, por exemplo, em frutas [30]. O *C. elegans* foi o primeiro animal multicelular a ter seu genoma completamente sequenciado. É um excelente organismo modelo para estudos de biologia do desenvolvimento (embriologia), pois é pequeno, de fácil criação, com ciclo de vida curto (de ovo a adulto em aproximadamente quatro dias), apresenta poucas células e sua manipulação genética é relativamente simples [31].

Muitos são os atributos experimentais que tornam o *C. elegans* um modelo animal tão bem sucedido e com um número cada vez maior de publicações em biologia do desenvolvimento, genética, envelhecimento e de ecotoxicologia [32]: apresenta pequeno comprimento (aproximadamente 1mm), o que possibilita a fácil manutenção de grandes quantidades de espécimes (10000 vermes/placa) em placas de petri contendo NGM (Nematode Growth Media) semeadas com *E. coli*; grande capacidade reprodutiva, pois um hermafrodita gera entre 200 e 300 descendentes; já quando um macho (0,02% da população) cruza com um hermafrodita este número pode passar de 1000 [33]; apresenta curto ciclo de vida, de aproximadamente 21 dias, possibilitando a execução de estudos relacionados à longevidade em um espaço temporal praticamente impraticável em modelos mamíferos clássicos; possibilita inúmeras possibilidades relacionadas à genética, já que cerca de 60% dos genes do *C. elegans* possuem homólogos em mamíferos [34], além do grande suporte e trabalho em conjunto de vários laboratórios

espalhados pelo mundo para a criação, manutenção e ampliação de bases de dados, como o WormBook, e armazenamento e distribuição gratuita de cepas mutantes e/ou transgênicas, como o CGC (Caenorhabditis Genetics Center).

Após o desenvolvimento embrionário dos *C. elegans*, o ovo eclode e liberta um jovem verme denominado larva L1, o qual se desenvolve por mais três sucessivas fases larvais (L2, L3 e L4) até chegar à fase adulta [32].

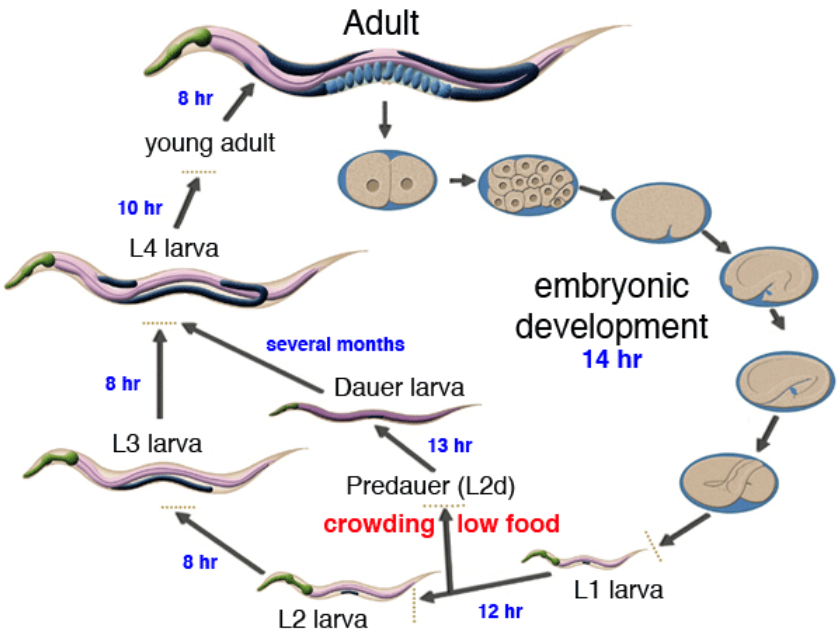


Figura 1: Representação esquemática do ciclo de vida do *C. elegans*: o ciclo de vida compreende as seguintes fases: ovo, larva (quatro estágios: L1, L2, L3, L4) e adulto. Dentro do ovo, o embriãose desenvolve rapidamente, dando origem à primeira forma larvária (L1) que eclode do ovo depois de aproximadamente 14 horas. Após a eclosão, o animal vai crescendo e se desenvolvendo, passando pelos 4 estágios larvários. Em 4 dias o animal chega à fase adulta. Eventualmente, sob condições adversas, pode ocorrer uma forma larval resistente chamada de Dauer ("permanente") que pode permanecer quiescente, sem se alimentar, por cerca de 3 meses. Porém, após encontrar condições adequadas, a larva Dauer se desenvolve direto para a fase L4 e continua o ciclo de vida.

Os adultos podem ser machos (muito raros) ou hermafroditas, sendo esses últimos os que liberam ovos fertilizados. Os ovos podem ser fertilizados por autofecundação ou por fecundação cruzada. No caso da autofecundação, os espermatozoides produzidos pelo hermafrodita na fase L4 são armazenados na espermateca e depois são liberados para fecundar os oócitos que são produzidos ao

longo da vida do animal [33]. No caso da fecundaçãocruzada, os espermatozoides liberados pelo verme macho têm preferência sobre os espermatozoides produzidos pelo hermafrodita para fecundar os oócitos [34]. Após a fecundação, os embriões são liberados no ambiente por meio de ovos, recomeçando o ciclo de vida.

Particularmente para estudos de toxicologia reprodutiva, quando se estuda a linhagem germinativa, esse modelo oferece a possibilidade de diversos ensaios. Em *C. elegans,* as células germinativas são um tecido único devido a três fatores importantes. Como em todos os metazoários, as células germinativas de *C. elegans* são pluripotentes e imortais, na medida em que dão origem a todos os tipos de células na próxima geração e também produzem todas as gerações subseqüentes. Em segundo lugar, a linha germinal é o único tecido adulto que é mantido por células-tronco que reabastecem constantemente a sua população, uma vez que os gametas em maturação são usados ou destruídos. A linha germinal de *C. elegans* é, portanto, mais semelhante aos tecidos auto-renováveis de mamíferos do que aos tecidos somáticos pós-mitóticos de adultos de *C. elegans* . Finalmente, em *C. elegans* a linha germinal é o único tecido em que a apoptose não é controlada por uma linhagem celular invariante. Em vez disso, a apoptose da linha germinal parece ser uma parte intrínseca do programa de oogênese [35].

Um tipo de apoptose da linha germinal envolve a morte de muitas células germinativas precoces durante a oogênese normal, um fenômeno notável, mas pouco compreendido, que parece ser amplamente conservado. Uma segunda categoria é desencadeada por danos ao DNA ou vários estresses ambientais, uma propriedade típica de essencialmente todos os tipos de células em vertebrados. A apoptose da linhagem germinativa também pode ser desencadeada por infecção com certas bactérias patogênicas. Portanto, o *C. elegans* fornece um sistema valioso para abordar a questão indescritível de como e por que tantas células germinais oogênicas precoce morrem em diversas espécies e para analisar mecanismos que desencadeiam apoptose durante oogênese normal ou em resposta ao estresse [35].

Outra característica em particular para estudos de toxicidade reprodutiva é a fácil visualização da apoptose em células germinativas. Através da utilização de uma cepa que possui fusão de GFP com a bainha de células somáticas que envolvem as células germinativas, é possível visualizar em microscópio de fluorescência a célula morta [36].

O estudo da oôgenese também e facilitado, pois o ovócito desenvolve-se da parte distal para a proximal no braço da gônada do *C. elegans*, passando por fases características da prófase I meiótica [37].

3.3 Vitelogênese

Para os nematoides, assim como para outros grupos animais, o sucesso na ocupação de um ambiente depende estreitamente da eficiência reprodutiva da espécie. Assim, o conhecimento dos mecanismos envolvidos nas diferentes etapas do processo reprodutivo é importante para o estabelecimento de novas técnicas para o estudo e o controle de espécies desse filo [38].

Todo animal precisa ser alimentado durante o desenvolvimento embrionário. Nos vertebrados placentários, a alimentação é direta de mãe para fílho, além de ser constante. Porém, para os animais ovíparos, é necessário que se faça uma reserva alimentar dentro do ovo que garanta seu desenvolvimento embrionário e pós-embrionário. O vitelo tem a função de suprir as necessidades nutritivas destes embriões. Ele é composto em sua maioria por lipídeos, proteínas, carboidratos e íons, além de possuir fatores de controle de desenvolvimento maternos. Portanto, pode-se considerar que as proteínas do vitelo estejam entre as mais importantes proteínas para a reprodução, desenvolvimento e manutenção da maioria dos animais. A formação do vitelo é chamada de vitelogênese, e é controlada por diversos fatores em diferentes grupos de animais [39].

A vitelogênese começa com a mobilização de toda a energia necessária, incluindo lipídios, desde o armazenamento até a formação da gema, garantindo o crescimento de embriões em animais ovíparos. A gema é uma lipoproteína de partículas formadas por lípidos e proteínas de ligação de lípidios referidos como vitelogeninas(VTG) [40]. O conjunto de recursos energéticos contidos no ovo é denominado vitelo e seu principal componente é uma proteína denominada vitelina (VT), que deriva da VTG [41].

Em nematoides, a biossíntese acontece no intestino e o processamento no pseudoceloma. Em nematoides o controle não foi completamente elucidado, mas é aceito que ele seja dependente de hormônios derivados de colesterol [42]. Em *C.*

elegans, seis genes (*vit-1, vit-2, vit-3, vit-4, vit-5 e vit-6*) codificam os quatro polipeptídeos da vitelogenina (YP170A, YP170B, YP115 e YP88) [43].

Os genes *vit-1 e vit-2* codificam o polipeptídeo YP170B, os genes *vit-3, vit-4 e vit-5* codificam o polipeptídeo YP170A. Por último, o gene *vit-6* codifica o precursor dos polipeptídeos YP115 e YP88. Este precursor sofre clivagem no pseudoceloma para formar estes dois peptídeos, assim que é secretado do intestino e logo antes de ser tomado pelo ovócito. Estudos sugerem que a clivagem do precursor ocorra através de uma enzima do tipo pró-hormônio convertase, pois está presente, na região de clivagem, o par de aminoácidos básicos lisina-arginina [44,45]. Os quatro peptídeos de VTG em *C. elegans* formam dois complexos: um dímero de YP170B e um heterotrímero formado por YP170A, YP115 e YP88[46]. Estes complexos são tomados via receptorese armazenados em grânulos de vitelo [47].

As VTGs já foram amplamente estudadas em diversas espécies animais, incluindo vertebrados e invertebrados. Sua síntese, nos vertebrados, é controlada por estrógeno [48,49] e, nos insetos, pelo hormônio juvenil e pela ecdisona [50,51]. Nos nematoides rabditídeos, ainda não foi elucidado como ocorre o controle da vitelogênese, porém alguns estudos demostram que a vitelogênese é impactada em resposta a condições de estresse, incluindo metais pesados, hormônios esteroides e fatores de transcrição [42].

Os materiais e métodos, Resultados e Discussão estão na forma de artigo científico que será submetido para a revista Food and Chemical Toxicology após as considerações da banca.

Reprotoxicity induced by Peruvian Maca (*Lepidium meyenii*) in Caenorhabditis elegans

Luiz Eduardo Ben Pilissão[1], Cristiane Freitas Rodrigues[1], Cristiane Casagrande Denardin[1], Daiana Silva Ávila[1].

[1] Universidade Federal do Pampa, Uruguaiana, Rio Grande do Sul, Brazil.

*Corresponding author:

Daiana Silva Ávila

Universidade Federal do Pampa - UNIPAMPA

Programa de Pós-Graduação em Bioquímica

BR 472 – Km 592 – Caixa Postal 118

CEP 97500-970 Uruguaiana /RS

Email: avilads1@gmail.com

Abstract

Maca (Lepidium meyenii) is a plant that grows above 4000 meters in the Central Andes of Peru and has different varieties according to the color of the hypocotyl. According to the population, its medicinal properties are connected to fertility and vitality. Besides the beneficial effects related to sexual health, maca has also been widely used by those who wish to lose weight naturally and healthy. However, there are few scientific studies validating these uses. The objective of the present study was to evaluate the effects of aqueous extract of maca on reproduction, using *Caenorhabditis elegans* as an experimental model. The Peruvian maca from local commerce was obtained, from which an aqueous extract was obtained. The animals were treated and maintained at 20 ° C in NGM (nematode growth medium), seeded with Escherichia coli OP50. The worms (2000) were exposed to maca extracts at different concentrations (41.25, 123.75, 247.5, 330 μg / μl) in the L1 larval stage for 30 minutes in liquid medium containing M9 and free of bacteria. Survival and reproductive toxicity analyzes (egg laying, eggs per worm) were performed on NGM / E plates. coli, 48h after treatment. Biochemical assays were performed using a homogenate of the commercial treatments and kits. Statistical analysis was obtained by one-way ANOVA followed by multiple comparisons with Tukey's post-test. The results showed that maca caused a reduction in survival at all concentrations. In addition, the extract caused a significant decrease in litter size. Our results also show that the maca extract caused alterations in the lipid profile and expression of vitellogenins and interfered in a marked way in the physiological balance of the germinated lineage of the worm reproductive system. Therefore, it is concluded that the use of the extract of Peruvian maca in these concentrations in *C. elegans* has effects contrary to the attributed and expected effects of this vegetable, since maca is used because of its supposed benefits on vitality and fertility.

1. Introduction

Herbal therapies for sexual dysfunction in males and females include Ginkgo (Ginkgo biloba), red ginseng (Panax ginseng) (Aung et al., 2004; Jang et al., 2008) and yohimbine (Pausinvstalia vohimbe), the last with serious adverse effects (Aung et al., 2004; Shamloul, 2010). Several other botanical therapies for sexual dysfunction have also been introduced (Aung et al., 2004; Shamloul, 2010; Tharakan and Manyam, 2005). These are also often used to improve sexual function in healthy individuals.

Among the species used is Lepidium meyenii (*maca peruana*), a plant from the Brassicaceae family that grows between 4,000 and 4,500 meters above sea level in the central Andes of Peru, particularly in Junin and Pasco. Maca grows in these locations in the temperature average of 1.5 ° C to 12 °. This range of temperatures occurs in the Central Andes because of its proximity to the equator. For this reason, *maca* does not grow well in the Peruvian South Andes, where the temperature is lower with frequent frosts (Valerio and Gonzales, 2005). Some scientific evidence has shown that *maca* has nutritional, energetic and fertility-enhancing properties and acts on sexual dysfunctions, osteoporosis, benign prostatic hyperplasia, memory and learning, lipid and glucose metabolism and protects the skin against ultraviolet radiation (Gonzales et al., 2005; Vecera et al., 2007).

There are numerous studies to evaluate the possible beneficial effects of nutraceutical extracts and for this study we chose the *Caenorhabditis elegans* model. *C. elegans* is a free-living nematode found in organic matter. Experimentally, it has the advantage of being a simple animal with nervous, reproductive and digestive systems present in a small organism (adult worms have a length of ~ 1.2 mm). Other advantages include fast life-cycle, which are relevant for lifespan investigations (Brenner, 1974); the prediction of observed results in mammalian worms, due to the high genetic homology between humans and *C. elegans* is very relevant in toxicology and pharmacology. After the embryonic period they develop through four larval stages, from L1 to L4. Hermaphrodite adult worms can produce up to 300 offspring during the short breeding period (4 days). Its, transparent body allows the microscopic visualization of the living animal. Another advantage also identified is the great potential for gene analysis through transgenic strains. Several studies provide evidence

that this worm responds following exposure to various natural compounds with increased resistance to stress and / or even extending lifespan (Possik and Pause, 2015).

Notably, little is known about the mechanisms by which *maca* affects the reproductive system *in vivo*. To address this issue, we evaluated the number of progeny as well as the effects of the extract on reproduction-related parameters, evaluating the levels of vitellogenin proteins, through GFP fluorescence, germ cells and lipid profile in *C. elegans*.

2. Materials and methods

2.1 Plant Material and extract preparation

The maca powder was obtained from local market from which was prepared a water extract. 1g of the powder was diluted in 10 ml of water which was homogenized with the aid of a turrax apparatus. The extract was centrifuged for 5 min at 7000 rpm. At the end of this process only the supernatant was separated filtered and stored at -20°C in microtubes to be used in the tests.

2.2 Culture, strains and worms treatment

The strains of Caenorhabditis elegans used in the study were: N2 (wild type), DH1033 (bls1[*vit-2::GFP+ rol-6 (su1006)*]) and MD701 (bcIs39 [lim-7p::ced-1::GFP + lin-15(+)]). *C. elegans* were kept in incubators at 20 ° C in Petri dishes containing solid growth medium (NGM) and Escherichia coli OP50 as food source (Brenner et al., 1974). The nematodes were exposed to treatments from the larval stage (L1), which are obtained by a synchronization process, in which eggs of pregnant hermaphrodites are obtained using a solution of sodium hypochlorite (1% NaOCl, 2,4 % NaOH) to break the cuticle of the worms. After 12-14 hours, the isolated eggs hatch and release L1 larvae. The worms in the L1 larval stage were exposed to different concentrations of *maca extract* (41.25, 123.75, 247.5, 330 μg of extract/ μl of water) for 30 minutes in liquid medium. At the end of the exposure, the treatment was centrifuged and washed 3 times with M9 buffer to stop the action of the extract and worms were transferred to NGM plates with *E. coli* OP50. For the biochemical assays, the worms were sonicated

and homogenized followed by centrifugation at 10,000 rpm for 10 min at 4 ° C and the supernatant was used for the assays.

2.3 Survival test

Worms at larval stage L1 (2,000 worms) were treated as previously described and then placed on NGM plates of NGM with bacteria. 48h after the end of treatments, the number of surviving worms on each plate was counted. The experiments were performed in duplicates and repeated at least three times.

2.4 Development test

The effects on nematodes development were evaluated by counting the number of animals at each stage of their life cycle: egg, L1, L2, L3, L4 and adult, at a time interval after treatment. Development through the larval stages can be determined using the following differentiation criteria. In this way after 48h of the treatment the scoring of animals in each larval stage was performed.

2.5 Brood size

After 48 hours of acute exposure to different concentrations of maca extract, only one worm from each treatment (in triplicates) was individually transferred to a new N50 plate seeded with OP50. To evaluate egg laying, the nematodes were monitored and transferred to a new plate every day, until no more eggs were layed, and the total number of eggs released on the plates was counted. Data were expressed as percentage of control. The experiments were repeated three times.

2.6 Number of eggs per worm

To complement the study on egg laying, another method was used. After 72 hours of treatment, 5 worms of each concentration of the extract and also the control oneswere transferred to a glass slide containing 20 ul of the lysis solution (4 ml of bleach, 1 ml of NaOH and 5 ml of water). After a few minutes, the body of this worm was broken and its eggs, until then inside its body, were released and counted.

2.7 Triglyceride level

Triglyceride levels were determined using the colorimetric method through a triglyceride-specific kit (LabTest). Briefly, 50 µl of sample were mixed with 150 µl of color reagent in 96-well plates and then incubated for 10 min at 37 ° C. Then the absorbance was read at 500 nm in the plate reader. Triglyceride levels were calculated from a standard curve and normalized by the amount of protein per sample. Proteins were determined by the Bradford method.

2.8 Reactive Substances test Thiobarbituric Acid (TBARS)

Lipid peroxidation was determined by the measurement of species reactive to thiobarbituric acid (TBARS). Briefly, a mixture of 100 µl of standard sample and 100 µl of SDS was prepared for the first time. To this mixture, 60 µl of thiobarbituric acid and 60 µl of phosphoric acid were added and boiled for one hour. The reaction was stopped on ice for 10 minutes and centrifuged for 1 min. The supernatant (150 µl) was transferred to a 96-well plate and the absorbance was read at 540 nm in the plate reader. The TBARS concentration was calculated from a standard malondialdehyde (MDA) curve and normalized by the amount of total protein per sample (Ohkawa et al., 1979).

2.9 Number of apoptotic cells

The determination of the number of apoptotic cells was evaluated using the strain MD701 which possesses apoptotic cells in the region of the vulva labelled with GPF. The number of apoptotic cells was determined using a fluorescence microscope (FLoid Cell Image Station -Thermo Scientific).

2.10 Vitellogenin levels

To understand the mechanism involved in the reprotoxic effects we evaluated the levels of vitellogenins, which are proteins transported to the embryo to maintain energy input during its development, labeled with GFP. Fluorescence levels of

transgenic worms expressing the GFP reporter for proteins of our interest were obtained by transferring worms to glass slides containing levamisole (0.02%), imaging them in a FLoid Cell Image Station (Thermo Scientific) and then fluorescence was quantified using ImageJ.

2.11. Statistical analysis

Statistical analysis was obtained by One-way ANOVA, followed by multiple comparisons Tukey test when $p<0.05$.

3. Results

3.1 *Maca* extract reduced worms survival

Initially, we evaluated the effects of acute *maca* exposure on survival (Figure 2). Theresults have shown that *maca* extract exposure caused a reduction in survival at all concentrations tested, especially at the three highest concentrations.

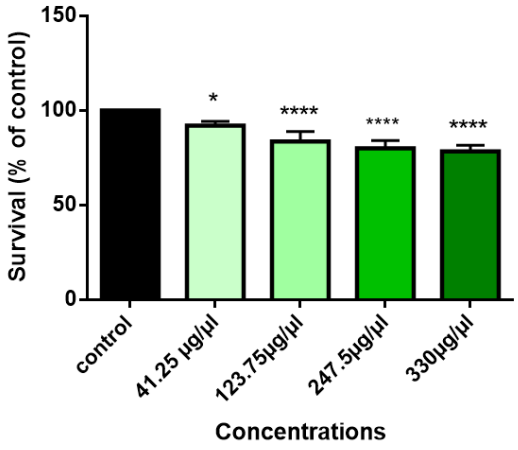


Figure 2: *Maca* aqueous extract effects on N2 worms survival. Data are expressed as mean ± SEM. * Indicates $p<0.05$ compared to the control group and **** indicates $p<0.0001$ in comparison to control group.

3.2 Maca extract slightly affects larval development

The effects of acute exposure of *maca* on worms development were evaluated 48 hours after exposure The results showed that the extract did not cause a significant delay on this parameter (Figure 3).

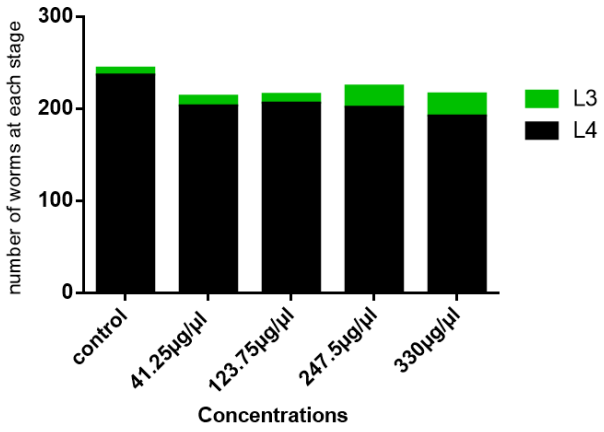
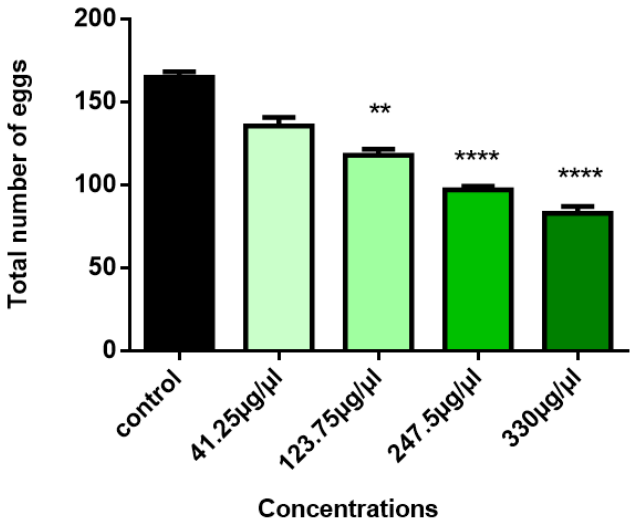
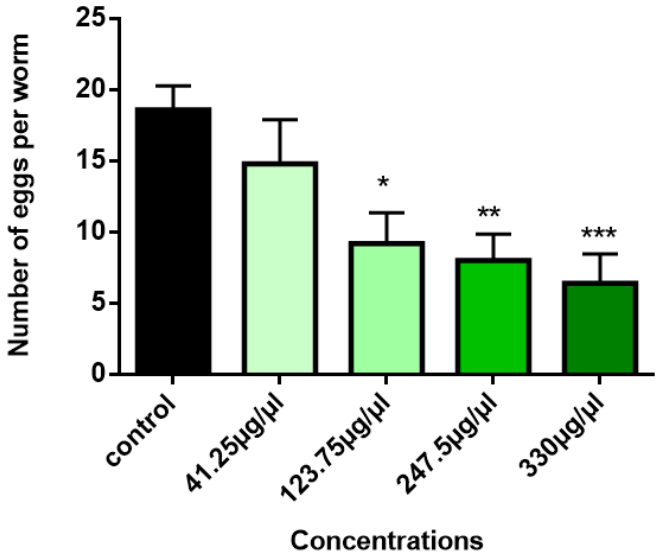


Figure 3: Worms development following exposure to different concentrations of the *maca* extract. Data are expressed as number of worms at each larval stage.

3.3 Maca aqueous extract causes alterations on reproductive profile

We then discuss whether acute exposure to the extract interferes with egg production. In Fig. 4A we can observe that after the treatment, the number of the eggs was significantly reduced in the concentrations from 123.47µg / µl. Just as the number of eggs produced per worm was decreased when compared to the control group (Fig. 4B) . In Figure 4C the number of eggs layed are distributed per day and is possible to observea decrease in egg laying on days 1 and 2.





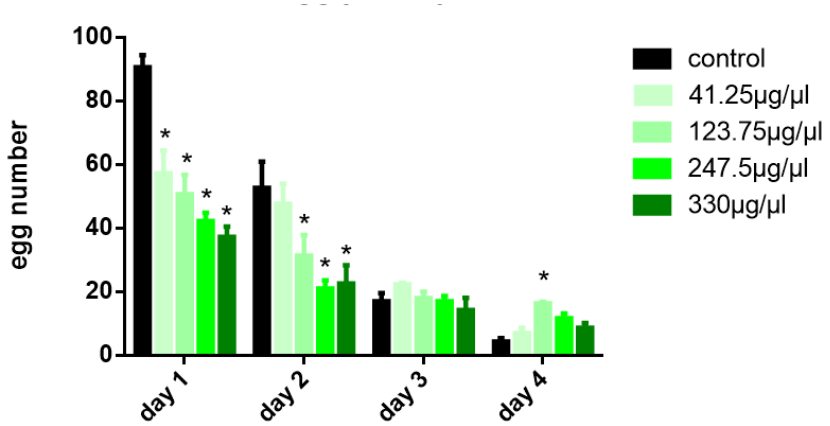


Figure 4: Effect of maca exposure in the reproduction of wild-type N2 worms. A) total number of eggs;B) number of eggs per worm;C) number of eggs layed per day. * Indicates p <0.05 compared to the control. ** Indicates p <0.005 compared to the control group and.*** indicates p <0.001 in comparison to control group .**** indicates p <0.0001 in comparison to control group.

3.4 Maca exposure induces apoptosis of germ cells

Next, we approached the different mechanisms associated with the decrease in the egg production induced by the extract. Thus, using the MD701 strain (which

possesses apoptotic cells labeled with GFP in the gonad region) we counted the number of apoptotic cells of the control group and the animals treated with different concentrations of the maca extract. Our results demonstrated an expressive increase in the number of apoptotic cells in this region, suggesting a toxic effect of the extract by interfering in the normal physiology of the reproductive system (Figure 5).

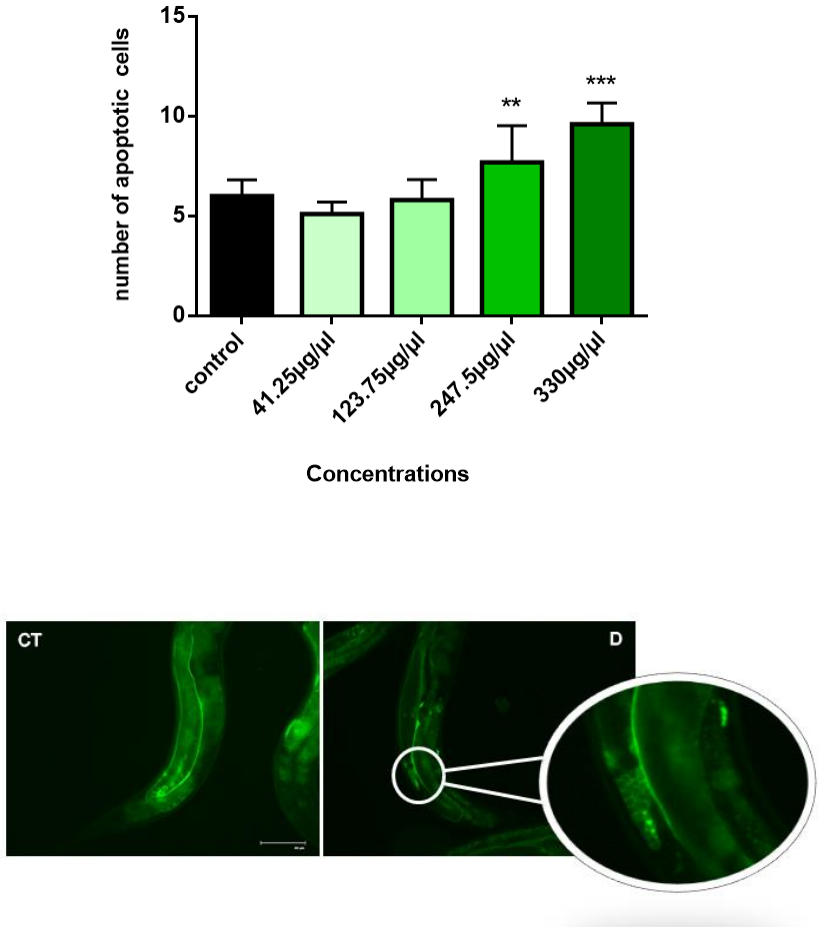


Figure 5: Quantification of the number of apoptotic cells in the of the worms. Below are presented representative images from control group (CT) versus worms exposed to *maca* extract at the highest concentration (D 330µg/µl). ** Indicates p <0.005 compared to the control group and *** indicates p <0.001 in comparison to control group.

3.5 Maca aqueous extract affects worms lipid profile

Considering that eggs contains high levels of lipids, we evaluated the lipid profile of wildtype worms treated with different concentrations of the *maca* extract. Our

results demonstrated a significant decrease in triglyceride levels from the concentration of 123.75µg / µl, showing a change in the lipid profile of the worm (Figure 6A). After this observation, we evaluated whether this decrease would be associated to lipid peroxidation. In Figure 6B, we demonstrate an increase in TBARS levels, which suggests an increase in the production of reactive species, thus implying damage to the reproductive system of the worm.

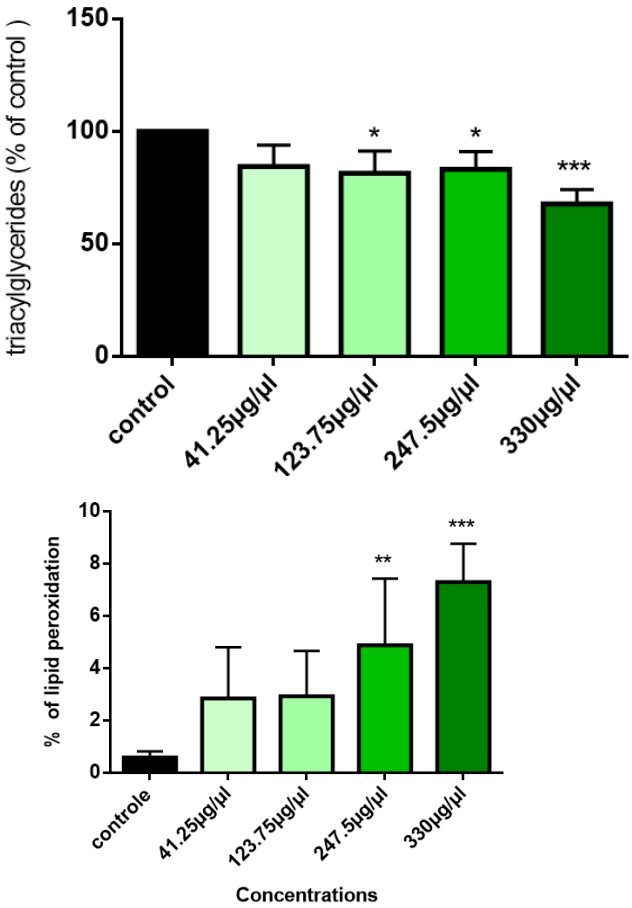


Figure 6: A) Determination of triglyceride levels in wild type worms (N2) treated with different concentrations of the root * Indicates p <0.05 (statistically significant) compared to the control group. *** indicates p <0.001 (statistically significant).B) determination of lipid peroxidation in wild type worms (N2) treated with different concentrations of the tuber.

3.6 Maca extract reduces the expression of vitellogenin-2 in worms embryo

In order to seek a mechanism by which the extract would be affecting eggs production, we observed the expression of the GFP-labeled vitellogenin-2 (DH1033). In Figure 7 is depicted that the expression of VIT-2 is significantly decreased when compared to the control. The representative images show how this decrease is dependent on the concentrations.

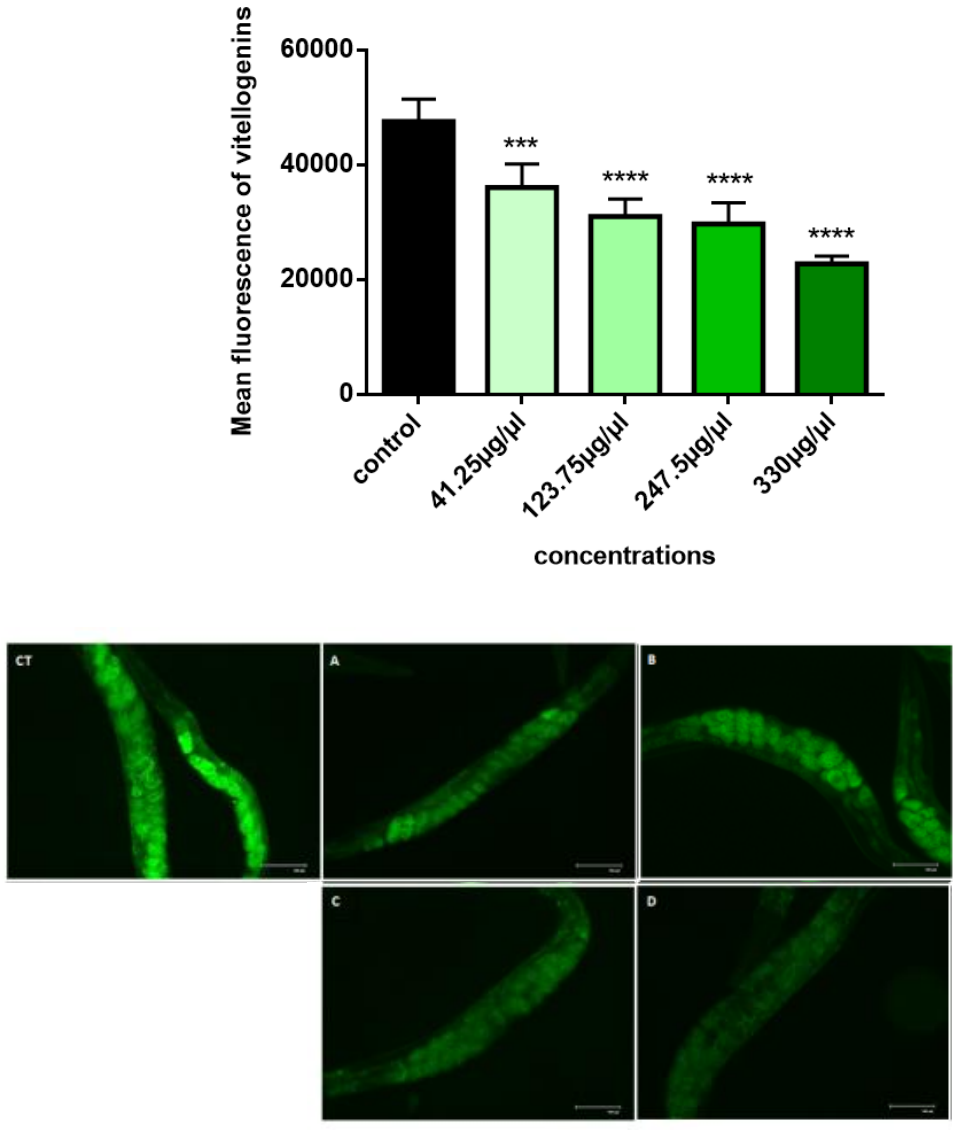


Figure 7: Fluorescence quantification of *C. elegans* strain DH1033 treated with different concentrations of *maca* extract. Data are expressed as mean ± SEM. . * Indicates p <0.05 (statistically significant); . ** Indicates p <0.005 (statistically significant); . *** Indicates p <0.001 (statistically significant). .**** indicates p <0.0001 in comparison to control group. In the panel bellow are shown images of these worms after treatment

with maca extract at different concentrations. CT (control), A (41.25µg / µl), B (123.75µg / µl), C (247.5µg / µl), D (330µg / µl).

4. Discussion

In the present study, we evaluated the toxicity of Peruvian *maca* using an in vivo model assay system. Considering the properties of the tuber that is traditionally used as a fertility enhancer for humans and cattle (Gonzales et al., 2005; Meissner et al., 2006b; Oshima et al., 2003), our findings are very contradictory, especially considering the acute exposure. Our data demonstrate that acute exposure to aqueous *maca* extract caused reduced survival and a very pronounced toxicity reproduction profile. Notably, exposure to the extract caused an increase in germline apoptosis and a decrease in the expression level of vitellogenins, where this deficiency was also observed in nematodes exposed to heavy metals (Wang et al., 2009; Wu et al., 2011; Wu et al., 2012), which suggests that the change in progeny production may be a common phenotype for effects adverse effects of toxic substances on nematodes. In addition, after acute exposure to maca, an increase in lipid peroxidation was associated to a decrease in triacylglycerols, which suggests an increase in the production of reactive species, thus implying damage to the reproductive system of the worm.

Firstly, we investigated the survival of worms in relation to maca extract, where we used different concentrations (41.25, 123.75, 247.5, 330 µg / µl) of the extract in an acute administration (30 minutes). The survival rate varied according to concentrations, and for that reason we decided to use all concentrations for the later experiments. Previous studies demonstrated that aqueous and methanolic extracts of *maca* did not present hepatotoxicity *in vitro* (Valentova et al., 2006). Likewise, aqueous extract of maca at a dose of 1 g / kg in mice did not alter the normal development of pre-implanted embryos (D´Arrigo et al., 2004). In rats and mice, 22 days oral treatment to a purified lipid maca extract increased the sexual function of these animals. (Gasco et al., 2007). In mice, the LD50 is greater than 15 g maca / kg (Meissner et al., 2006a) without histopathological changes in the liver, pancreas, spleen, testes or ovaries (Meissner et al., 2006a; Meissner et al., 2006b). It is important to emphasize that the higher toxicity observed in the present study may be due to the early exposure, considering that at the L1 stage many systems are not fully developed. In the cited studies, exposures were

done in adults. In addition, the demonstration of the presence of alkaloids such as carboline-3-carboxylic acid and 1-methyltetrahydro-β in *maca* preparations evidences the concern of the consumption of maca, which is why more studies are needed to evaluate the fraction containing this alkaloid and thus to verify its efficacy and safety (Piacente et al., 2002).

In nematodes, the reproductive organ is one of the most important organs for toxic actions (Chang et al., 2012; Nouara et al., 2013; Zhao et al., 2013). In this sense, we investigated the effects of acute extract exposure on reproduction by evaluating the number of layed eggs. Acute exposure at all concentrations, especially at the highest doses, notably influenced this endpoint. More interestingly, this alteration appeared mainly on day 1 during the reproductive period of adult nematodes. In addition, we observed a small delay in egg laying, where we could see that maca treated worms layed more eggs than control ones. It is important to emphasize that no significative changes in worms development were observed (Figure 2), then reduction in reproduction were not a consequence of development delay.

In order to further clarify the studies on the molecular effects of the extract on reproduction, we analyzed whether exposure to maca induces apoptosis in germline cells through the use of ced-1::GFP strain that possesses apoptotic cells labeled with GFP. Our results demonstrated a very significant increase in the number of apoptotic cells in the germline, expressing a toxic effect on these cells and interfering in the physiology and normal health of this reproductive system. By exposing the nematoid to increasing concentrations of maca, we have shown here that germinal apoptosis is exhibited in a dose-dependent manner.

The germ line provides the crucial link between generations, and its proper function depends on the precise execution of the process of cellular meiosis and chromosome division. Deregulation of the meiotic process can result in reduced fertility and the production of gametes and embryos with an abnormal number of chromosomes, a condition called aneuploidy. Errors of chromosomal segregation in meiosis are highly relevant to human health. Chromosomal abnormalities are common, often 1 in 150 live births, Trisomy 21, 18 and 13, as well as X and Y chromosomal errors are the most prevalent (Fragouli et al., 2011). The idea that environmental influences can affect segregation and chromosomal behavior is not new (Hunt, 2006), but it is still poorly

understood. Therefore, it is crucial to investigate which of the chemicals introduced into our environment are interfering with human fertility, early development and overall reproductive health.

Seeking a mechanism that explains the toxicity of the reproductive system, we raised the possibility that the extract is interfering with the lipid profile of the worm. Lipids and gem-derived proteins provide crucial nutrients needed to support the rapid development of the embryo. The vitellogenesis of *C. elegans* is the most expensive energy process for worms, requiring nutrients from the gut to form buds. C. elegans express a class of VIT lipoproteins encoded by six different genes (Baker, 1988). *C. elegans* VITs YP170, YP115 and YP88 are homologous to VIT from vertebrates and ApoB-100, the main component of mammalian LDL particles (Grant and Hirsh, 1999; Spieth et al., 1991). Endocytosis of gem particles in oocyte membrane vesicles is mediated by receptors of the LDL receptor superfamily in C. elegans (Goldstein et al., 1985), insects and vertebrates. It is believed that the endocytic traffic of gem and yolk receptors proceeds along pathways very similar to those used by LDL in somatic cells (Goldstein et al., 1985; Schneider, 1996). To highlight possible mechanisms on the effects of maca on lipoproteins, we used *C. elegans* as a powerful tool to observe its reproduction as the culminating profile of vitellogenin alterations. Taking advantage of the transgenic strain VIT-2p :: VIT-2 :: GFP, we visualized VIT-2 at various stages of development and the effect of maca on its expression. Given the expression of VIT proteins begins in the late larval L4 stage, no GFP fluorescence was observed 24 h after exposure of maca to young adult worms. Thus, we chose 72 h after exposure to the extract to evaluate the expression of VIT-2. Assays showed that vitellogenin levels were significantly reduced after exposure to the aqueous extract of *maca*. This finding corroborates the results of the lipid profile, in which there was an increase of the lipid peroxidation added to the decrease of the triacylglycerols generating an imbalance in the lipid levels and with that interfering in the amount of lipids needed to be sent to the yolk to supply the development of the embryo. In this way we suggest that the aqueous extract of maca is interfering with these processes, preventing the internalization of vitellogenins by the decrease of lipids through the increase of the lipid peroxidation and with that, generating the lack of nutrients for the normal development of the embryos, and thus also affecting throughout the germiline process and the normal physiological organization of the eggs, causing a decrease of the progeny. For instance, Gubert et al

established the ability of Mn to reduce the size of young in worms (Gubert et al., 2016), showing that this system is very sensitive to the action of toxic substances.

Even though there are some studies using mice ans rats that provide some positive effects of maca extract on sexual function, a clinical trial conducted by Gonzales et al demonstrated that a 12-week treatment with maca did not improved luteinizing hormone, follicle-stimulating hormone, prolactin, 17-alpha hydroxyprogesterone, testosterone and 17-beta estradiol levels (Gonzales et al., 2003). Our study points out that early exposure to maca in a reproductive immature organism can cause serious reproduction defects caused by reduced vitelogenesis and increased germline apoptosis.

---

5. Conclusion

In summary, we have shown, for the first time, that the aqueous extract of litter affects both egg production and germiline behaviors. In addition, interference in the vitellogenesis process is associated with *maca*-induced toxicity. These results suggest that the acute and early exposure of *C. elegans* to maca at these concentrations impairs the reproductive profile of hermaphrodites.

6. References

Aung, H.H., Dey, L., Rand, V., Yuan, C.S., 2004. Alternative therapies for male and female sexual dysfunction. Am J Chin Med 32, 161-173.

Baker, M.E., 1988. Is vitellogenin an ancestor of apolipoprotein B-100 of human low-density lipoprotein and human lipoprotein lipase? Biochem J 255, 1057-1060.

Brenner, S., 1974. The genetics of Caenorhabditis elegans. Genetics 77, 71-94.

Chang, C.W., Leu, Y.L., Horng, J.T., 2012. Daphne Genkwa sieb. Et zucc. Water-soluble extracts act on enterovirus 71 by inhibiting viral entry. Viruses 4, 539-556.

D´Arrigo, G., Benavides, V., Pino, J., 2004. Preliminary Evaluation Effect of Lepidium meyenii Walp on the embryonic development of mouse. Revista Peruana de Biología 11, 103-106.

Fragouli, E., Wells, D., Delhanty, J.D., 2011. Chromosome abnormalities in the human oocyte. Cytogenetic and genome research 133, 107-118.

Gasco, M., Aguilar, J., Gonzales, G.F., 2007. Effect of chronic treatment with three varieties of Lepidium meyenii (Maca) on reproductive parameters and DNA quantification in adult male rats. Andrologia 39, 151-158.

Goldstein, J.L., Brown, M.S., Anderson, R.G., Russell, D.W., Schneider, W.J., 1985. Receptor-mediated endocytosis: concepts emerging from the LDL receptor system. Annu Rev Cell Biol 1, 1-39.

Gonzales, G.F., Cordova, A., Vega, K., Chung, A., Villena, A., Gonez, C., 2003. Effect of Lepidium meyenii (Maca), a root with aphrodisiac and fertility-enhancing properties, on serum reproductive hormone levels in adult healthy men. The Journal of endocrinology 176, 163-168.

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

Grant, B., Hirsh, D., 1999. Receptor-mediated endocytosis in the Caenorhabditis elegans oocyte. Mol Biol Cell 10, 4311-4326.

Gubert, P., Puntel, B., Lehmen, T., Bornhorst, J., Avila, D.S., Aschner, M., Soares, F.A.A., 2016. Reversible reprotoxic effects of manganese through DAF-16 transcription factor activation and vitellogenin downregulation in Caenorhabditis elegans. Life Sci 151, 218-223.

Hunt, P.A., 2006. Meiosis in mammals: recombination, non-disjunction and the environment. Biochemical Society transactions 34, 574-577.

Jang, D.J., Lee, M.S., Shin, B.C., Lee, Y.C., Ernst, E., 2008. Red ginseng for treating erectile dysfunction: a systematic review. British journal of clinical pharmacology 66, 444-450.

Meissner, H.O., Mrozikiewicz, P., Bobkiewicz-Kozlowska, T., Mscisz, A., Kedzia, B., Lowicka, A., Reich-Bilinska, H., Kapczynski, W., Barchia, I., 2006a. Hormone-Balancing Effect of Pre-Gelatinized Organic Maca (Lepidium peruvianum Chacon): (I) Biochemical and Pharmacodynamic Study on Maca using Clinical Laboratory Model on Ovariectomized Rats. Int J Biomed Sci 2, 260-272.

Meissner, H.O., Mscisz, A., Reich-Bilinska, H., Mrozikiewicz, P., Bobkiewicz-Kozlowska, T., Kedzia, B., Lowicka, A., Barchia, I., 2006b. Hormone-Balancing Effect of Pre-Gelatinized Organic Maca (Lepidium peruvianum Chacon): (III) Clinical responses of early-postmenopausal women to Maca in double blind, randomized, Placebo-controlled, crossover configuration, outpatient study. Int J Biomed Sci 2, 375-394.

Nouara, A., Wu, Q., Li, Y., Tang, M., Wang, H., Zhao, Y., Wang, D., 2013. Carboxylic acid functionalization prevents the translocation of multi-walled carbon nanotubes at predicted environmentally relevant concentrations into targeted organs of nematode Caenorhabditis elegans. Nanoscale 5, 6088-6096.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry 95, 351-358.

Oshima, M., Gu, Y., Tsukada, S., 2003. Effects of Lepidium meyenii Walp and Jatropha macrantha on blood levels of estradiol-17 beta, progesterone, testosterone and the rate of embryo implantation in mice. J Vet Med Sci 65, 1145-1146.

Piacente, S., Carbone, V., Plaza, A., Zampelli, A., Pizza, C., 2002. Investigation of the tuber constituents of maca (Lepidium meyenii Walp.). J Agric Food Chem 50, 5621-5625.

Possik, E., Pause, A., 2015. Measuring oxidative stress resistance of Caenorhabditis elegans in 96-well microtiter plates. Journal of visualized experiments : JoVE, e52746.

Schneider, W.J., 1996. Vitellogenin receptors: oocyte-specific members of the low-density lipoprotein receptor supergene family. Int Rev Cytol 166, 103-137.

Shamloul, R., 2010. Natural aphrodisiacs. J Sex Med 7, 39-49.

Spieth, J., Shim, Y.H., Lea, K., Conrad, R., Blumenthal, T., 1991. elt-1, an embryonically expressed Caenorhabditis elegans gene homologous to the GATA transcription factor family. Mol Cell Biol 11, 4651-4659.

Tharakan, B., Manyam, B.V., 2005. Botanical therapies in sexual dysfunction. Phytother Res 19, 457-463.

Valentova, K., Buckiova, D., Kren, V., Peknicova, J., Ulrichova, J., Simanek, V., 2006. The in vitro biological activity of Lepidium meyenii extracts. Cell Biol Toxicol 22, 91-99.

Valerio, L.G., Jr., Gonzales, G.F., 2005. Toxicological aspects of the South American herbs cat's claw (Uncaria tomentosa) and Maca (Lepidium meyenii) : a critical synopsis. Toxicological reviews 24, 11-35.

Vecera, R., Orolin, J., Skottova, N., Kazdova, L., Oliyarnik, O., Ulrichova, J., Simanek, V., 2007. The influence of maca (Lepidium meyenii) on antioxidant status, lipid and glucose metabolism in rat. Plant Foods Hum Nutr 62, 59-63.

Wang, S., Wu, L., Wang, Y., Luo, X., Lu, Y., 2009. Copper-induced germline apoptosis in Caenorhabditis elegans: the independent roles of DNA damage response signaling and the dependent roles of MAPK cascades. Chem Biol Interact 180, 151-157.

Wu, Q., He, K., Liu, P., Li, Y., Wang, D., 2011. Association of oxidative stress with the formation of reproductive toxicity from mercury exposure on hermaphrodite nematode Caenorhabditis elegans. Environ Toxicol Pharmacol 32, 175-184.

Wu, Q., Qu, Y., Li, X., Wang, D., 2012. Chromium exhibits adverse effects at environmental relevant concentrations in chronic toxicity assay system of nematode Caenorhabditis elegans. Chemosphere 87, 1281-1287.

Zhao, Y., Wu, Q., Lia, Y., Wang, D., 2013. Translocation, transfer, and in vivo safety evaluation of engineered nanomaterials in the non-mammalian alternative toxicity assay model of nematode Caenorhabditis elegans. RSC Advances 3, 5741-5757.

5. Conclusão

Em resumo, mostramos, pela primeira vez, que o extrato aquoso de maca afeta a produção de ovos e o comportamento germinativo. Além disso, a interferência no processo de vitelogênese está associada à toxicidade induzida pela maca. Estes resultados sugerem que a exposição precoce de C. elegans a maca nessas concentrações prejudica o perfil reprodutivo dos hermafroditas.

6. Referências

1. Shamloul R. Natural aphrodisiacs. J Sex Med. 2010;7(1 Pt 1):39–49. doi: 10.1111/j.1743-6109.2009.01521.
2. Ernst E, Pittler MH. Yohimbine for erectile dysfunction: a systematic review and meta-analysis of randomized clinical trials. J Urol. 1998;159(2):433–436. doi: 10.1016/S0022-5347(01)63942-9.
3. Aung HH, Dey L, Rand V, Yuan CS. Alternative therapies for male and female sexual dysfunction. Am J Chin Med. 2004;32(2):161–173. doi: 10.1142/S0192415X04001837.
4. Ang DJ, Lee MS, Shin BC, Lee YC, Ernst E. Red ginseng for treating erectile dysfunction: a systematic review. Br J Clin Pharmacol. 2008;66(4):444–450. doi: 10.1111/j.1365-2125.2008.03236.x.
5. Tharakan B, Manyam BV. Botanical therapies in sexual dysfunction. Phytotherapy Research. 2005;19(6):457–463. doi: 10.1002/ptr.1634.
6. Toxicological aspects of the South American herbs cat's claw (Uncaria tomentosa) and Maca (Lepidium meyenii): a critical synopsis.Valerio LG Jr, Gonzales GF
7. Gonzales GF, Miranda S, Nieto J, et al. Red Maca (Lepidium meyenii) reduced prostate size in rats. Reprod Biol Endocrinol. 2005;3(1):5.
8. Vecera R, Orolin J, Skottová N, et al. The influence of maca (*Lepidium meyenii*) on antioxidant status, lipid and glucose metabolism in rat. Plant Foods Hum Nutr. 2007;62:59–63.

9. Halliwell B., Guterride J.M.C. Free Radicals in Biology and Medicine. Oxford University Press; New York, NY, USA: 2007. Chapters 3–5.
10. Brenner S. The genetics of *Caenorhabditis elegans*. Genetics. 1994;77:71–94.
11. Possik, E., Pause, A. Measuring Oxidative Stress Resistance of Caenorhabditis elegans in 96-well Microtiter Plates. J. Vis. Exp. (99), e52746, doi:10.3791/52746 (2015).
12. Laumann EO, Paik A, Rosen RC. Sexual dysfunction in the United States: prevalence and predictors. JAMA. 1999;281(6):537–544. doi: 10.1001/jama.281.6.537
13. Lewis RW, Fugl-Meyer KS, Corona G, Hayes RD, Laumann EO, Moreira ED Jr, Rellini AH, Segraves T. Definições / epidemiologia / fatores de risco para disfunção sexual. J Sex Med. 2010; 7(4 Pt 2): 1598-1607. doi: 10.1111 / j.1743-6109.2010.01778.x.
14. Phillips NA. Disfunção sexual feminina: avaliação e tratamento. Am Fam Médico. 2000; 62 (1): 127-136. 141-122.
15. Morales AM, Mirone V, Dean J, Costa P. Vardenafil for the treatment of erectile dysfunction: an overview of clinical evidence. Clin Interv Aging. 2009; 4 : 463-472.
16. Tsertsvadze A, Fink HA, Yazdi F, MacDonald R, Bella AJ, Ansari MT, Garritty C, Soares-Weiser K, Daniel R, Sampson M. et al. Oral inhibitors of Phosphodiesterase-5 and hormonal treatments for erectile dysfunction: a systematic review and meta-analysis. Ann Intern Med. 2009; 151 (9): 650-661.
17. Zakhari R. Female sexual dysfunction: a primary care perspective. J Am Acad Nurse Pract. 2009; 21 (9): 498-505. doi: 10.1111 / j.1745-7599.2009.00440.x.
18. Hudson T. Maca: novas idéias sobre uma antiga fábrica. Medicina integrativa: um periódico do clínico. 2008; 7 (6): 54-57.
19. Wang Y, Wang Y, McNeil B, Harvey LM. Maca: uma safra andina com funções multi-farmacológicas. Food Res Intern. 2007; 40 : 783-792. doi: 10.1016 / j.foodres.2007.02.005.
20. Gonzales GF, Ruiz A, Gonzales C, Villegas L, Cordova A. Effect of roots of Lepidium meyenii (maca) on spermatogenesis of male rats. Asian J Androl. 2001; 3 (3): 231-233

21. Zheng BL, He K, Kim CH, Rogers L, Shao Y, Huang ZY, Lu Y, Yan SJ, Qien LC, Zheng QY. Efeito de um extrato lipídico de lepidium meyenii em comportamento sexual em ratos e ratos. Urologia. 2000; 55 (4): 598-602. doi: 10.1016 / S0090-4295 (99) 00549-X.
22. Gonzales GF, Cordova A, Gonzales C, Chung A, Vega K, Villena A. Lepidium meyenii (Maca) melhoraram os parâmetros de sêmen em homens adultos. Asian J Androl. 2001; 3 (4): 301-303.
23. Gonzales GF, Cordova A, Vega K, Chung A, Villena A, Gonez C. Effect of Lepidium meyenii (Maca), a root with aphrodisiac properties and fertility-friendly, in serum levels of reproductive hormones in healthy adult men. J Endocrinol. 2003; 176 (1): 163-168. doi: 10.1677 / joe.0.1760163.
24. Gasco, M., Aguilar, J. and Gonzales, G. F. (2007), Effect of chronic treatment with three varieties of *Lepidium meyenii* (Maca) on reproductive parameters and DNA quantification in adult male rats. Andrologia, 39: 151–158. doi:10.1111/j.1439-0272.2007.00783.x
25. Inoue, N., Farfan, C. and Gonzales, G. F. (2016), Effect of butanolic fraction of yellow and black maca (*Lepidium meyenii*) on the sperm count of adult mice. Andrologia, 48: 915–921. doi:10.1111/and.12679
26. Melnikovova, Ingrid et al. "Effect of *Lepidium Meyenii* Walp. on Semen Parameters and Serum Hormone Levels in Healthy Adult Men: A Double-Blind, Randomized, Placebo-Controlled Pilot Study." *Evidence-based Complementary and Alternative Medicine : eCAM* 2015 (2015): 324369. *PMC*. Web. 2 Aug. 2017
27. Zhang, J., Wang, H. M., Zhao, Y. L., Zuo, Z. T., Wang, Y. Z., & Jin, H. (2015). Comparison of mineral element content in a functional food Maca (*Lepidium meyenii* Walp.) from Asia and South America. *Journal of Analytical Methods in Chemistry*.
28. Donald, D. L. (Ed.). C. elegans II. New York, NY: Cold Spring Harbor Laboratory Press, 1997
29. Schierenberg, E.; Wood, W. B. Control of cell-cycle timing in early embryos of Caenorhabditis elegans. Dev Biol 107(2): 337-354, 1985
30. FÉLIX, Marie-Anne; BRAENDLE, Christian. The natural history of Caenorhabditis elegans. Current Biology, v. 20, n. 22, p. R965-R969, 2010.

31. SULSTON, John E.; HORVITZ, H. Robert. Post-embryonic cell lineages of the nematode, Caenorhabditis elegans. Developmental biology, v. 56, n. 1, p. 110-156, 1977.
32. Mota, M.M.; Diogo, A. Caenorhabditis elegans: Biological model for the 21st century.Laboratório de nematologia/ICAM. Deartamento de Biologia-Universidade de Évora, 2001
33. Nayak, S., J. Goree & T. Schedl, S; Goree, J; Schedl, T. "Fog-2 and the Evolution of Self-Fertile Hermaphroditism in Caenorhabditis". PLoS Biology 3 (1): e6, Jan. 2004
34. Kaletta, T.; Hengartner, M. O. Finding function in novel targets: C-elegans as a model organism. Nature Reviews Drug Discovery 5(5): 387-398, 2006
35. Xuan Ma et al. Transformation: how do nematode sperm become activated and crawl? Protein Cell 2012, 3(10): 755–761 DOI 10.1007/s13238-012-2936-2.
36. Anton Gartner , Peter R Boag e T. Keith Blackwell: WormBook: The Online Review of *C. elegans* Biology.
37. Gasco, M., Aguilar, J. and Gonzales, G. F. (2007), Effect of chronic treatment with three varieties of *Lepidium meyenii* (Maca) on reproductive parameters and DNA quantification in adult male rats. Andrologia, 39: 151–158. doi:10.1111/j.1439-0272.2007.00783
38. Hubbard, E.J. and Greenstein D. 2000. The *Caenorhabditis elegans* gonad: a test tube for cell and developmental biology. Dev. Dyn. 218: 2-22
39. BOWES, M.; PATHIRANA, S. The yolk proteins of higher In: RAIKHEL, A.S.; SAPPINGTON, T.W. (Ed.). Reproductive Biology of Invertebrates: Progress in vitellogenesis. Enfield: Science Publishers, 2002. p. 103-130.
40. CAROLINA ROSSI.AS PROTEÍNAS DO VITELO DO NEMATOIDE ENTOMOPATOGÊNICO Heterorhabditis baujardi LPP7. Dissertação apresentada ao Programa de PósGraduação em Biologia da Relação Patógeno Hospedeiro do Instituto de Ciências Biomédicas da Universidade de São Paulo.
41. P. Gubert et al. Reversible reprotoxic effects of manganese through DAF-16 transcription factor activation and vitellogenin downregulation in Caenorhabditis elegans. Life Sciences 151 (2016) 218–223
42. LIM, E.H.; TEO, E.Y.; LAM, T. J.; DING, J.L. Sequence analysis of a fish vitellogenin cDNA with a large phosvitin domain. Gene, v. 277, p. 175-186, 2001.

43. MATYASH, V.; ENTCHEV, E. V.; MENDE, F.; WILSCH-BRÄUNINGER, M.; THIELE, C.; SCHMIDT, A. W.; KNöLKER, H.-J.; WARD, S.; KURZCHALIA, T. V. Sterol-derived hormone(s) controls entry into diapause in Caenorhabditis elegans by consecutive activation of daf-12 and daf-16.PLoSBiol, Public Library of Science, v. 2, n. 10, p. e280, 2004.
44. BLUMENTHAL, T.; SQUIRE, M.; KIRTLAND, S.; CANE, J.; DONEGAN, M.; SPIETH, J.; SHARROCK,W.Cloningofayolkproteingenefamilyfrom Caenorhabditiselegans.Journal ofMolecularBiology, v. 174, n. 1, p. 1 – 18, 1984.
45. ANDREONI-NICO, J. Evidências de redundância funcional entre as pró-hormônio convertases no processamento pós-traducional do precursor da vitelogenina VIT-6 do nematóide Caenorhabditis elegans. Tese (Doutorado) — Tese de doutorado apresentada aoInstituto de Ciências Biomédicas da Universidade de São Paulo, 2008
46. WINTER,C.E.; PENHA,C.; BLUMENTHAL,T.Comparisonofavitellogeningenebetween two distantly related rhabditid nematode species. Molecular Biology and Evolution, v. 13, n. 5, p. 674–684, 1996
47. SHARROCK, W. J. Yolk proteins of Caenorhabditis elegans. Developmental Biology, v. 96, n. 1, p. 182 – 188, 1983
48. SCHNEIDER, W. J. Vitellogenin receptors: Oocyte-specific members of the low-density lipoprotein receptor supergene family. In: JEON, K. W. (Ed.). [S.l.]: Academic Press, 1996, (International Review of Cytology, v. 166). p. 103 – 137.
49. WALLACE, R.A. Vitellogenesis and oocyte growth in non mamallian vertebrates. In: BROWDER, L.W. (Ed). Developmental Biology: a Comprehensive Synthesis (Oogenesis). New York: Plenum Publishing Corporation, 1985. p. 127-177
50. WAHLI, W. Evolution and expression of vitellogenin genes. Trends in Genetics, v. 4, p. 227232, 1988.
51. DHADIALLA, T.S.; RAIKHEL, A.S. Biosynthesis of mosquito vitellogenin. Journal of Biological Chemistry, v. 265, p. 9924-9933, 1990.
52. Oshima M, Gu Y, Tsukada S. Effects of *Lepidium meyenii* Walp and *Jatropha macrantha* on blood levels of estradiol-17β, progesterone, testosterone and the rate of embryo implantation in mice. J Vet Med Sci. 2003;65(10):1145-6.

53. Gonzales GF, Miranda S, Nieto J, Fernandez G, Yucra S, Rubio J, et al. Red Maca (*Lepidium meyenii*) reduced prostate size in rats. Reprod Biol Endocrinol. 2005;3(1):5
54. Meissner HO, Mrozikiewicz P, Bobkiewicz-Kozlowska T, Mscisz A, Kedzia B, Lowicka A, et al. Hormone-Balancing Effect of Pre-Gelatinized Organic Maca (*Lepidium peruvianum* Chacon): (I) Biochemical and Pharmacodynamic Study on Maca using Clinical Laboratory Model on Ovariectomized Rats. Int J Biomed Sci. 2006;2(3):260-72.
55. Wu QL, Ele KW, Liu PD, YX Li, Wang DY (2011) Association of oxidative stress with the formation of reproductive toxicity of mercury exposure in the hermaphrodite nematode Caenorhabditis elegans. . Environ Toxicol Pharmacol 32 : 175-184
56. Wang DY, Wang Y (2008) Defeitos fenotípicos e comportamentais causados pela exposição ao bário no nematoide *Caenorhabditis elegans* . Arch Environ Contam Toxicol 54 : 447-453.
57. Valentová K, Buckiova D, Kren V., Peknicova J, Ulrichova J, Simanek V. The biological activity in vitro of extracts of Lepidium meyenii. Cell Biol Toxicol. 2006; 22 (2): 91-9.
58. D'Arrigo G, Benavides V, Pine J. Preliminary assessment of the effect of Lepidium meyenii Walp in the embryonic development of mice. Rev Per Biol. 2004; 11 (1): 103-6.
59. Gasco M, Aguilar J, Gonzales GF. Effect of chronic treatment with three varieties of Lepidium meyenii (Maca) on reproductive parameters and DNA quantification in adult male rats. Andrology. 2007; 39 (4): 151-8.
60. Meissner HO, Mrozikiewicz P, Bobkiewicz-Kozlowska T, Mscisz A, Kedzia B, Lowicka A, et al. Effect of Hormone balancer Pre-Gelatinized Organic Maca (Lepidium peruvianum Christenson Chacon): (I) Biochemical and pharmacodynamic Study on Gurney using Model of clinical laboratory in Ovariectomized Rats. Int J Biomed Sci. 2006; 2 (3): 260-72.
61. Meissner HO, Mscisz A, Reich-Bilinska H, Kapczynski W, Mrozikiewicz P, Bobkiewicz-Kozlowska T, et al. Hormone Balancing effect of Pre-Gelatinized Organic Maca (Lepidium peruvianum Christenson Chacon): (II) physiological responses and Symptomatic women in post menopause with Standardized Doses

of Maca in Multi-center clinical trial, placebo-controlled, Randomized, Placebo. Int J Biomed Sci. 2006; 2 (4): 360-74.

62. Piacente S, Carbone V, Praça A, Zampelli A, Pizza C. Research of constituents of tuber of maca (Lepidium meyeniiwalp). J Agric Food Chem. 2002; 50 (20): 5621-5.
63. ZhaoY – L, Wu Q – L, Li Y – P, Wang D – Y (2013) Translocation, transfer and safety evaluation in vivo of artificial nanomaterials in alternative toxicity test model of non-mammals of the gonad-Caenorhabditis elegans. RSC Adv 3 : 5741-5757.
64. Chang C, Y Leu, Horng J (2012) *Daphne genkwa* Sieb. et Zucc. extratos solúveis em água atuam sobre o enterovírus 71 inibindo a entrada viral . Vírus 4 : 539-556.
65. Nouara A, Wu QL, YX Li, Tang M, Wang HF, et al. (2013The funcionalization carboxylic acid) prevents the translocation of multiple walled carbon nanotubes in predicted concentrations relevant to the environment in target organs of Caenorhabditis elegans gonad. Nanoscale 5 : 6088-6096.
66. Fragouli E, Wells D, Delhanty JD. Anormalidades cromossômicas no oócito humano. Citogenet Genome Res. 2011; 133 (2-4): 107–118.
67. Hassold T, Hunt PA, Sherman S. Trissomia em humanos: incidência, origem e etiologia. Curr Opin Genet Dev. 1993; 3 (3): 398-403.
68. Hunt PA. Meiosis in mammals: recombination, non-disjunction and the environment. Biochem Soc Trans. 2006;34(Pt 4):574–577.
69. Parodi, D.A., Damoiseaux, R., Allard, P. Comprehensive Assessment of Germline Chemical Toxicity Using the Nematode Caenorhabditis elegans. J. Vis. Exp. (96), e52445, doi:10.3791/52445 (2015).
70. M.E. BakerIs vitellogenin an ancestor of apolipoprotein-b-100 of human low-density lipoprotein and human lipoprotein-lipase.Biochem. J., 255 (1988), pp. 1057-1060
71. J. Spieth, Y.H. Shim, K. Lea, R. Conrad, T. Blumenthalelt-1, an embryonically expressed *Caenorhabditis elegans* gene homologous to the GATA transcription factor Family.Mol. Cell. Biol., 11 (1991), pp. 4651-4659
72. B. Grant, D. HirshReceptor-mediated endocytosis in the *Caenorhabditis elegans* oocyte.Mol. Biol. Cell, 10 (1999), pp. 4311-4326

73. J.L. Goldstein, M.S. Brown, R.G. Anderson, D.W. Russell, W.J. SchneiderRece ptor-mediated endocytosis: concepts emerging from the LDL receptor system.Annu. Rev. Cell Biol., 1 (1985), pp. 1-39
74. W.J. SchneiderVitellogenin receptors: oocyte-specific members of the low-density lipoprotein receptor supergene Family.Int. Rev. Cytol., 166 (1996), pp. 103-137
75. T.R. GuilarteManganese and Parkinson's disease: a critical review and new findings.Environ. Health Perspect., 118 (2010), pp. 1071-1080
76. Spieth, K. Denison, E. Zucker, T. Blumenthal.The nucleotide sequence of a nematode vitellogenin gene.Nucleic Acids Res., 13 (1985), pp. 7129-7138