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Macamides: A review of structures, isolation, therapeutics and prospects

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Abstract

Macamides, the major bioactive compounds of *Lepidium meyenii* (Walp.) or Maca, are a unique class of non-polar, long chain fatty acid N-benzylamides with fertility-enhancing, neuroprotective, neuro-modulatory, anti-fatigue and anti-osteoporosis effects. However, the relationship between the structures and pharmacological effects of macamides have not been established so far. In addition, little is known regarding their biosynthetic pathways and the mechanisms underlying the biological activities. In this review, we have summarized the methods currently used for the extraction, purification and synthesis of macamides. Their pharmacological effects, clinical prospects and biomedical applications have also been discussed. Current data strongly suggest that macamides are a promising bio-active agent, and further studies are warranted to elucidate their mechanisms of action.

Keywords: Aryl alkamides; phytochemistry; *Lepidium meyenii*; secondary metabolites; pharmacological effects; endocannabinoid system

1. Introduction

Lepidium meyenii Walp. or Maca is a biennial herbaceous plant belonging to the *Brassicaceae* family, and is currently cultivated in the Peruvian highlands at central Andes Sierra (Y. Wang, Wang, McNeil, & Harvey, 2007), as well as in the Qinghai-Tibet Plateau in China (L. F. Chen, Li, & Fan, 2017). It is adapted to the extreme high-altitude (3500-4500 m) conditions such as cold, strong ultraviolet radiation and low air pressure (Gustavo F. Gonzales, 2012). It is consumed worldwide - mainly in the Americas, Canada, UK, China and Japan - as a nutritional and functional food, fertility enhancer (Sunny O. Abarikwu, Chigozie Linda Onuah, & Shio Kumar Singh, 2020; G. F. Gonzales et al., 2003; Melnikovova, Fait, Kolarova, Fernandez, & Milella, 2015), aphrodisiac (G. F. Gonzales et al., 2003), immunostimulant (S. Wang & F. Zhu, 2019) and anabolic or hormonal balancer (Cruz et al., 2017; Valentova & Ulrichova, 2003). In addition, regular intake of Maca has been shown to slow the progression of chronic neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD) (Banjari, Marcek, Tomic, & Waisundara, 2018; Patel, Raghuwanshi, Masood, Acharya, & Jain, 2018).

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Several bioactive components have been isolated from Maca, such as non-starch polysaccharides, polyphenols (flavonolignans), glucosinolates, alkaloids, essential amino acids and minerals (S. A. Wang & F. Zhu, 2019). The polysaccharide fraction of Maca stimulates CD^{4+} T cell expansion and secretion of interferon-γ (IFN-γ) (Chang et al., 2020), and exhibits a stronger prebiotic and anti-inflammatory effect compared to inulin in a dose-dependent manner (Y.-K. Lee, Jung, & Chang, 2020). In addition, the cysteine and proline-rich Maca protein isolate (MPI) is also immune-active (L. Wu, Zhang, Xin, Lai, & Wu, 2019), whereas the high levels of antioxidants like polyphenols and glucosinolates (GLs) protect the skin of rats against UV irradiation (Campos, Chirinos, Barreto, Noratto, & Pedreschi, 2013; Gonzales-Castaneda & Gonzales, 2008). The tuberous roots of Maca are also rich in secondary metabolites like macamides, macaenes, macahidantoins, glucosinolates and alkaloids, of which the first two are unique to this species of *Lepidium* (S.-Z. Zhang et al., 2020). Hormone signaling pathways are activated in response to biotic or abiotic stresses via whole-genome duplication (WGD; similar to 6.7 Ma), resulting in the biosynthesis of secondary metabolites. They mainly accumulate during the pre-harvest, harvest and post-harvest drying stages (J. Zhang et al., 2016). Reyes et al. (Huarancca Reyes et al., 2020) showed that the Maca plants can adapt to the strong UV radiations at high altitudes through the coordinated remobilization and relocation of these secondary metabolites. Macamides are consumed as part of a balanced diet or supplements during pregnancy (Zeng et al., 2012), as well as for its neuro-modulatory (Z. J. Yu et al., 2019), energy boosting (Choi et al., 2012) and osteogenic effects (H. Liu et al., 2015). In addition, network pharmacological analysis has predicted 57 disease targets of different macamides (Yi, Tan, Yan, & Liu, 2016).

Most reviews on Maca have focused on its chemical composition and the medicinal effects of the powdered form, crude extracts and polysaccharide fraction (da Silva Leitao Peres et al., 2020; S. Wang & F. Zhu, 2019; Yujuan et al., 2018). In contrast, the secondary metabolites of Maca (Apaza Ticona, Tena Pérez, & Bermejo Benito, 2020) like polyphenols (Ying Li et al., 2018), glucosinolates (Huang, Peng, & Qiu, 2018) and others (Carvalho & Ribeiro, 2019) are not well characterized. In this review, we have summarized the diverse structures, isolation techniques, pharmacological effects, therapeutic potential, and the underlying mechanisms of macamides. In addition, the clinical and other applications of macamides have also been discussed.

2. Macamides structures

2.1. Chemical structures and characterization

Macamides are secondary amides composed of benzylamine and fatty acids that vary in terms of hydrocarbon chain length and degree of unsaturation. The structural core of all macamides is benzylamine (Ph-CH_2-NH-CO-) or m-methoxybenzylamine (CH_3-O-Ph-CH_2-NH-CO-) (Fig. 1a, b) that is attached via an amide bond to the alkyl group of fatty acids like oleic, linoleic and linolenic acids with the common formula R=/-$(CH_2)_n$-CH_3, where n ranges from 8 to 24. Twenty-six macamides have been identified in Maca

roots (Table 1). N-benzylacetamides are highly stable due to presence of inter-molecular N-H…O hydrogen bonds (Yan et al., 2013), and the bond energy, dipole moment and stability of the molecules increase linearly with the number of C atoms in the side chain (F. E. Chain, Ladetto, Grau, Catalan, & Brandan, 2016). The biological effects of macamides largely depend on their chemical, structural and topological properties (F. Chain, Iramain, Grau, Catalan, & Brandan, 2017). The antioxidant activity of N-benzylamides depends on their ability to donate electrons (Petruska, Capcarova, & Sutovsky, 2014; Visioli & Hagen, 2011). In addition, the hydrophobic and hydrophilic regions in the macamides can also scavenge free radicals due to presence of donor hydrogen-bonds (F. Chain et al., 2017). Macamides inhibit fatty acid amide hydrolase (FAAH) by forming an unsaturated double bond with arachidonic acid tail via the alkyl side chain. As the structural analog of anandamide (Fig. 1c), macamides can bind to the CB_1 receptor and prevent neurotoxicity (Alasmari, Bhlke, Kelley, Maher, & Pino-Figueroa, 2019; Almukadi et al., 2013). Apaza T. et al. (Luis et al., 2019) identified macamides analogues N-oleoyldopamine and N-(2-hydroxyethyl)-7Z, 10Z, 13Z, 16Z-docosatetraenamide in the heptanic extract of purple and black *T. tuberosum* tubers (Fig. 2), which also grows in high-altitude conditions like Maca, and therefore produces metabolites similar to that of macamides (Apaza Ticona et al., 2020).

2.2. Identification of macamides

2.2.1. Mass spectroscopy

The content of total macamides in the dry hypocotyls of Maca is 0.1-2% (J. L. Zhong, Yan, Xu, Muhammad, & Yan, 2019). Mass spectroscopy (MS) and high resolution MS (HRMS) using electrospray ionization (ESI) can detect such low amounts with high sensitivity and specificity. Zhao et al. (J. Zhao, Muhammad, Dunbar, Mustafa, & Khan, 2005) identified five new macamides - N-Benzyl-9-oxo-12Z-octadecenamide (4 mg/kg), N-Benzyl-9-oxo-12Z,15Z-octadecadienamide (3 mg/kg), N-Benzyl-13-oxooctadeca-9E,11E-dienamide (2 mg/kg), N-Benzyl-15Z-tetracosenamide (3.5 mg/kg) and N-(m-Methoxybenzyl)hexadecanamide (8 mg/kg) - from dried Maca tubers using ESI-MS and ESI-HRMS, and elucidated their structures using ^{1}H, ^{13}C and 2D NMR. Likewise, Xia et al identified and characterized N-benzyl-9-oxo-10E,12E-octadecadienamide (93 mg/kg) and N-benzyl-9-oxo-10E,12Z-octadecadienamide (18 mg/kg) through a similar approach (Xia et al., 2018). Time-of-flight (TOF) detectors used in HRMS decrease the error limit to 0.001 amu. Thus, HRMS can measure the exact mass of macamides and distinguish between minor structural variations through fragmentation when coupled to a quadrupole (S. H. Yang et al., 2019). Gas chromatography MS (GC-MS) and ultra-high performance liquid chromatography MS (UPLC-QqQ-MS/MS) combine the high resolution of chromatography with the quantitative ability of MS, and have identified several novel macamides (Alasmari et al., 2019) such as N-Benzyloctadeca-9Z,12Z-dienamide (Alves et al., 2020; Mamat, Azizan, Baharum, Noor, & Aizat, 2020).

2.2.2. Gas chromatography-mass spectrometry

Macamides are volatile compounds that can be detected by dynamic head-space adsorption (H. Zheng, Zhang, Xu, Zhang, & Gan, 2013). Li et al. (A. Li et al., 2019) used SPME-GC/MS with an electronic nose and BP neutral network algorithm to analyze the Maca volatiles and establish an odor database. GC-MS can simultaneously purify and identify compounds based on the MS spectra (Pan, Zhang, Li, Wang, & Li, 2016b), and allows high-throughput identification of compounds in a complex plant extracts (Cosio, Esparza, Kofer, Bendezu, & Gonzales, 2009). For instance, GC-MS and LC-MS were used to isolate and identify several primary and secondary metabolites of mangosteen fruit during harvest (Mamat et al., 2020).

2.2.3. Liquid chromatography-mass spectrometry

Ultra-high-performance liquid chromatography (UHPLC) coupled with triple quadrupole mass spectrometry (UPLC-QqQ-MS/MS) is routinely used to analyze macamides with high sensitivity and efficiency (X. Zhao, Jianhe, & Meihua, 2018). Alasmari et al. (Alasmari et al., 2019) identified N-Benzyloctadeca-9Z,12Z-dienamide using HPLC/ESI-MS. Sharma et al. (Sharma et al., 2011) purified 7 macamides from the ethanol extract of Maca using a gradient reversed phase on an embedded polar column. Zhou et al. (Y. Y. Zhou et al., 2018) profiled 95% ethanol extracts of Maca tubers using UHPLC-ESI-Orbitrap MS coupled with UHPLC-ESI-QqQ MS, and identified ≥ 95% pure N-benzylhexadecamide using carbon nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR). Macaenes can be also be characterized using the above approaches (S. X. Chen et al., 2017). LC with tandem ultraviolet (LC-UV) or MS (LC-MS) detection is a routine technique for analyzing fruit extracts (Pardo-Mates et al., 2017). Macamides were first screened using chromatographic fingerprinting in 2005 (Jin et al., 2005), and Pan et al. (Pan, Zhang, Li, Wang, & Li, 2016a) recently identified 12 macamides in the Maca hypocotyls of different geographical origins by LC-UV/MS/MS and partial least square (PLS) regression. With more advancements in chemometric approaches, the combination of ^{1}H NMR and LC–MS datasets can discriminate and identify samples with greater accuracy (Alves et al., 2020). Recently, an approach of flow injection mass spectrometry (FIMS) was applied to obtain spectral fingerprints of compounds (macamides, glucosinolates, etc.) in Maca in a rapid manner (Geng et al., 2020). Compared with LC-MS, FIMS provides rapid non-targeted spectra with no prior separation. The different methods of analyzing macamides are summarized in Table 2.

3. Extraction, purification and synthesis of macamides

3.1. Extraction and purification methods

3.1.1. Solvent reflux extraction

Since macamides and macaenes are non-polar compounds on account of the benzyl group and unsaturated fatty acid chains, they are commonly reflux extracted using organic solvents like 100% n-hexane (1:10 w/v, 30 ℃, ultrasonic bath, 2×15 min)

(Hajdu et al., 2014), methanol (1:10 w/v, 40 ℃, ultrasonication, 1h) (Xia et al., 2019) and ethanol [95% (1/10, w/v) at 70 ℃ for 2h twice, or 95% (1/16, w/v) at 70 ℃ for 2.8h] (Z. J. Yu et al., 2019; Y. Zheng et al., 2019). Petroleum ether can extract macamides with greater efficiency between 60-90 ℃ (S. X. Chen et al., 2017), and can selectively extract amide alkaloids and other bio-active compounds from whole fruits (Han, Li, Hao, Tang, & Wan, 2012). Given the low thermal stability of macamides however, low temperature extraction under reduced pressure is a more viable option, except the rapid autoxidation process (J. L. Zhong et al., 2019). Yang et al. (Q. Yang et al., 2016) reflux-extracted the lipid-soluble components of air-dried and powdered roots of Maca with 12 volumes of petroleum ether for 30 min at 50℃, followed by ultrasonication for 15 min. The yield of the Maca petroleum ether extract was 1.96%. Ye et al. (Ye et al., 2019) extracted dried Maca powder thrice with 80% aqueous acetone at 25℃. The pooled extracts were evaporated in vacuo and then extracted thrice with dichloromethane and ethyl acetate respectively. The different extracts were pooled and eluted through a silica gel column using CH_2C_{l2}-acetone gradient, and the macamides were further purified by HPLC. Some macamides, such as N-Benzyl-9-oxo-10E,12Z-octadecadienamide and N-Benzyl-9-oxo-10E,12E-octadecadienamide, are formed during the drying and extraction of macanes (9-Oxo-10E,12Z-octadecadienoic acid and 9-Oxo-10E,12E-octadecadienoic acid), and are thus not detected in fresh Maca tubers (S. X. Chen et al., 2017).

3.1.2. Ultrasound-assisted extraction

Ultrasound-assisted extraction (UAE) lowers the duration and temperature of conventional solvent refluxing and maceration, and is therefore widely used in biomedical and food industries. Clement et al obtained up to 1.98μM macamides and 0.17-2.67 μM macaenes per gram DM of Maca hypocotyls following sonication in methanol for 20 min (Clement et al., 2010). Xia et al. (Xia et al., 2019) extracted macamides by ultrasonicating Maca powder in 10 volumes of methanol (w/v) for 1h at 40 ℃. Likewise, Chen et al. (S. X. Chen et al., 2017) obtained a high yield of 1175.18μg/g macamides by sonicating at 200 W using 10 volumes of the solvent at 40 ℃ for 30 min. UAE is always coupled with solvent reflux extraction to increase the macamide yield as well as bioactive amide synthesis in Maca (J. J. Chen et al., 2018). Effective moisture diffusivity and migration of biosynthetic precursors are beneficial for the biosynthesis of macamides in Maca during ultrasonication.

3.1.3. Other methods

Supercritical fluid extraction (SCFE) uses pressure and temperature above the critical values of the specific compound, and is a cost-effective and sustainable method on an industrial scale (Torres-Valenzuela, Ballesteros-Gomez, & Rubio, 2020). Significantly higher amounts of macamides have been extracted from Maca roots using supercritical carbon dioxide (CO_2) as the solvent (Cho, Choi, & Kang, 2013; Choi et al., 2012). The extraction efficiency can be further elevated using an intermediate co-solvent. For instance, Zhang et al. (Y. Zhang, Zhou, & Ge, 2019) found that the yield of macamides

and macaenes from the mixture of Chinese chive seed and Maca root extract (202.44 mg/g) was 45-fold higher compared to Maca root extract alone (4.51 mg/g) using SCFE (CO_2, 35 MPa, 50 ℃, 1.5h), since the extracted Chinese chive oil acted as a co-solvent for supercritical CO_2. This markedly increased the extraction efficacy, especially for the lipophilic components like (9Z,12Z)-octadecadienamide. Some of the macamides are unstable above 35℃, as well as in the molten state, which corresponds to the stability profile of unsaturated fatty acids. However, dissolved macamides are remarkably stable. High-speed counter-current chromatography (HSCCC) in combination with semi-preparative HPLC can achieve a higher yield and purity (≥ 98%) macamides from Maca roots (J. L. Zhong et al., 2019) on a larger scale and at lower costs (Kan et al., 2020). As shown in Fig.3, the upper and lower phases of the selected two-phase solvent system are simultaneously pumped into the coiled column at a certain flow rate, which is subsequently detected by HPLC using a UV-VIS detector. To prevent the oxidation of macamides in acetonitrile-water solution during evaporation under high temperatures, the compounds were extracted by ethyl acetate and methanol and evaporated under vacuum at 50℃. Nonetheless, since the extraction and purification processes are complicated, it has not been adapted for industrial production (Table 2).

3.2. Synthesis methods

3.2.1. Biosynthesis during natural air drying

Since macamides are formed during postharvest drying, a feasible strategy for increasing their yield is to improve bio-synthesis in fresh Maca roots and optimize the drying rate. For instance, postharvest ultrasound-assisted freeze-thaw (UFP) increased the efficiency of macamide extraction through six freeze-thaw cycles at 40 ℃, with a 2h ultrasonication at the end of each cycle (J. J. Chen et al., 2018). Prolonging (>30 min) the freeze-thaw procedure can further increase cell-wall disruption (Fig. 4) (Syrpas et al., 2020). Benzylamine or m-methoxybenzylamine is formed in Maca roots during postharvest drying from benzyl glucosinolate and m-methoxybenzyl glucosinolate respectively (Huang et al., 2018) (E. Esparza, Hadzich, Kofer, Mithofer, & Cosio, 2015). Alkyl groups of macamides are hydrolysis products of free fatty acid (FFA) that naturally exist as storage lipids and in the cell membranes of Maca roots (H. Wu, Kelley, Pino-Figueroa, Vu, & Maher, 2013). Almost all macamides are generated by dehydration and condensation of N-benzylamides or m-methoxybenzylamine and FFAs with different degrees of unsaturation. It involves removal of a hydrogen ion (H) and an oxhydryl (OH) from N-benzyl or m-methoxybenzyl amides and acids (S.-Z. Zhang et al., 2020). In addition, macamides can also be transformed into other macamides via double bond isomerization (Xia et al., 2019). As shown in Fig. 5, macamide biosynthesis during natural air drying of Maca roots involves tissue maceration, release of FFAs, molecular dehydration, hydrolysis and recombination of glucosinolates. The main biosynthetic route of macamides includes the following 3 steps: 1) hydrolysis of glucosinolates by myrosinase into benzyl isothiocyanates, 2)

conversion of the intermediates to benzylamines and hydrolysis of membrane and storage lipids into FFAs, and 3) condensation of benzylamine and fatty acid residues (J. J. Chen et al., 2017; E. Esparza et al., 2015). During natural air drying, the ratio of benzylamine deamination to amide formation that determines the eventual yields of macamides in relation to benzenoids and their esters in Maca flour (Eliana Esparza, Yi, Limonchi, & Cosio, 2020).

3.2.2. Chemical synthesis

The isolation and purification of macamides from plant tissues is challenging given their ultra-low levels. Therefore, chemical synthesis of macamides using benzylamines and palmitoyl chloride has gained precedence (McCollom, Gafner, & Craker, 2004). It is a fairly simple procedure wherein palmitoyl chloride (0.008 mol, soluble in diethyl ether) and oleyl chloride (0.008 mol in 10 mL diethyl ether, 85% pure, with small amounts of linoleoyl chloride and stearoyl chloride) are mixed with chilled benzylamine for 15 min. Benzyl-hexadecamide and benzyl-oleamide are formed in a vial through the septum of the Claisen adapter and precipitated using 10% sodium hydroxide . The capsules and tablets produced by this reaction contain N-benzyl-5-oxo-6E,8E-octadecadienamide, N-benzyl-hexadecanamide and three kinds of macaenes at high purity (Ganzera, Zhao, Muhammad, & Khan, 2002). However, given the limited fatty acid chlorides and their low purity, this method cannot be used to synthesize all types of macamides.

Macamides can also be synthesized by direct condensation of carboxyl groups and the amino components. Zhao et al. (J. Zhao et al., 2005) synthesized N-Benzyl-15Z-tetracosenamide from benzylamine and cis-15-tetracosenoic acid dissolved in dry methylene chloride using dimethylamino pyridine (DMAP) and dicyclohexyl carbodiimide (DCC) as the catalysts, and the duration of the reaction was 90 min. Cosio et al. (Cosio et al., 2009) synthesized macamides by combining benzylamine or 4-methoxy-benzylamine with caprilic, palmitic, stearic, oleic, linoleic and linolenic acids, with pyrrolidine-pyridine (PPy) as the catalyst. Finally, carbonyl diimidazole (N, N′-carbonyldiimidazole, CDI) (H. Wu et al., 2013) and 1-ethyl-3(3-dimethylpropylamine) carbodiimide (EDC) (Ye et al., 2019) have also been used for macamide synthesis via condensation reactions. Nevertheless, macamide synthesis, whether through biological or chemical method, is a highly complex and erratic process given the variations in structure, solubility and other chemical properties.

4. Pharmacological effects of macamides

Although previous studies have commented on the bioactive extracts of Maca, the pharmacological effects of its secondary metabolites and the underlying mechanisms are still lacking. In this section, the various pharmaco-biological effects of macamides are discussed (Table 1).

4.1. Fertility-enhancing activity

Maca was first identified as an aphrodisiac and fertility enhancer in both humans (M. S. Lee, Lee, You, & Ha, 2016; Santos, Howell, & Teixeira, 2019) and domestic

livestock (Del Prete et al., 2018). The major fertility-related bioactives in Maca are macamides and macaenes (B. L. Zheng et al., 2000). Recent studies show that Maca can alleviate antidepressant-induced sexual dysfunction (AISD) (Dording et al., 2015; Tafuri et al., 2019). Zhang et al. (Y. Zhang et al., 2019) showed that the lipophilic extracts of Maca and *Allium tuberosum Rottler* containing 10 macamides synergistically improved erectile function in male mice. Since erectile dysfunction (ED) might involve the upregulation of nitric oxide (NO) and cyclic guanosine monophosphate (cGMP), it is possible that the macamides target these compounds. Furthermore, the petroleum ether extract of Maca significantly reduced corticosterone (CORT) levels in mouse serum, and increased the levels of noradrenaline (NE) and dopamine (DA) in the brain tissue, along with inhibiting reactive oxygen species (ROS) generation (AiZhong, ChengAiFang, YuYuanTao, YuLongJiang, & JinWenwen, 2014). Uchiyama et al. (Uchiyama, Jikyo, Takeda, & Ogata, 2014) found that the ether fraction of Maca consisting of 10 macamides enhanced the serum levels of luteinizing hormone (LH) in female rats in a dose-dependent manner. Nevertheless, it is unclear whether the macamides act as both noradrenergic and dopaminergic agonists to improve sperm motility and fertility in mice. In a recent study, Zhang et al. (N. X. Zhang et al., 2019) found that N-benzylhexadecanamide increased Leydig cell proliferation and testosterone secretion in mice by modulating amino acid, carbohydrate and lipid metabolism. However, no study so far has shown a direct effect of macamides on sexual function and fertility, or the exact mechanisms.

4.2. Neuroprotective activity

Maca spray-dried extracts and macamides are known to have mood alleviating effects, which can be attributed to increased 5-hydroxytryptamine (5-HT) and norepinephrine transmission through the endocannabinoid system (ECS), which promotes hippocampal neurogenesis (Gonzales-Arimborgo et al., 2016). Gugnani et al. (Gugnani et al., 2018) found that macamides protected U-87 MG glioblastoma cells against $MnCl_2$-induced toxicity and mitochondrial depolarization by binding to the CB_1 receptor, which restored mitochondrial function by downregulating the p38 MAPK/$PPAR_\gamma$ axis. Zhou et al. (Y. Zhou et al., 2018) showed that N-benzylhexadecamide promoted recovery of zebrafish dopaminergic neurons following 1-methyl-4-phenylpyridine (MPP^+) exposure by regulating sphingolipid metabolism and mitochondrial function. In addition, N-(3-methoxybenzyl)-hexadecanamide, N-benzy-(9Z,12Z)-octadecadienamide and N-benzy-(9Z,12Z,15Z)-octadeca-trienamide also showed neuroprotective effects in H_2O_2-induced (50μM) PC12 cells (Z. Yu et al., 2020). In addition, neurotransmitters including taurine, norepinephrine and choline are also closely related to the neuroprotective effects of macamides. As chemically neutral lipids, macamides can easily cross the intestinal wall and the blood brain barrier (Russo, 2016). In addition, macamides can ameliorate the neuronal damage produced by amyloid-beta (Aβ) peptide deposits, suggesting a therapeutic potential against neurodegenerative diseases as well (Apaza Ticona et al., 2020; Lewis & Pino-Figueroa, 2013). Daily supplementation of Maca extracts (1-5 g/day) can alleviate symptoms of

neurodegenerative diseases like, AD, PD and HD (Banjari et al., 2018; Patel et al., 2018), and hence there is considerable interest at present in the identification and screening of neuroprotective components from Maca (Zhou et al., 2017).

4.3. Neuromodulatory activity

Maca water extracts (10g/kg, containing eight kinds of macamides) can exert analgesic, anti-inflammatory and anti-depressant effects by controlling the release of certain neurotransmitters through the ECS (Tenci et al., 2017). Administration of arachidonyl ethanolamine (AEA) or exogenous anandamide, an endocannabinoid that is structurally analogous to macamides (Fig. 1c), protected the brains of neonatal rodents from excitotoxic damage (Shouman et al., 2006). Likewise, macamides (5-10μM) showed a similar neuromodulatory activity both *in vitro* and *in vivo* (Almukadi et al., 2013). Pino-Figueroa et al. (Pino-Figueroa, 2011) found that the pentane extract of macamides (5-100μM) protected rat neuroblastoma from H_2O_2 oxidation by inhibiting fatty acid amide hydrolase (FAAH) in a concentration-dependent manner. Macamides can mimic plant-derived N-alkylamides like N-benzyloctadeca-9Z, 12Z-dienamide that is a known substrate of FAAH (Alasmari et al., 2019), and the inhibitory effect of macamides increases with the number of unsaturated double bonds (Hajdu et al., 2014). Thus, macamides act on the central nervous system by inhibiting the release of neurotransmitters by FAAH, which may have therapeutic effects on anxiety, depression and pain.

4.4. Anti-fatigue activity

Lipid-soluble extracts of Maca can attenuate oxidative stress during exercise-induced fatigue (Choi et al., 2012), and supplementation with yellow Maca extracts (0.1-0.5 mg/kg) (Q. Yang et al., 2016; Y. Zheng et al., 2019) improve endurance capacity, eliminate metabolites, reduce the skeletal and myocardial damage in mice during prolonged exercise. Adenosine monophosphate-activated protein kinase (AMPK) regulates glucose uptake, fatty acid oxidation, mitochondrial biogenesis and ROS scavenging, and is known to increase endurance during exercise (Y. Liu et al., 2020). AMPK activation in the skeletal muscles prevents ROS overproduction and accumulation following the increase in mitochondrial respiration. Macamides neutralized the ROS byproducts of mitochondrial respiratory chain in the skeletal muscle of rats, reversing fatigue and oxidative damage to cells (Luis et al., 2019). Furthermore, macamides improved exercise endurance in rats by increasing the expression of AMPK and its downstream antioxidant genes (Gugnani et al., 2018; R.-m. Wu et al., 2014). Macamide supplementation (12 and 40 mg/kg) can also protect the central nervous system from excitotoxicity and reduce mental fatigue caused by accumulation of ammonia during exercise (Q. Yang et al., 2016). King-Himmelreich et al. (King-Himmelreich et al., 2017) concluded that AMPK is an intermediate effector in endocannabinoid-mediated exercise-induced antinociception. Macamides alleviated exercise-induced inflammatory nociception by inhibiting FAAH, and the binding of AEA to its receptor (CB_1). In addition, Maca polysaccharides (20 and 100 mg/kg)

showed significant anti-fatigue effects in a mouse weight-loaded swimming model (J. Li et al., 2017; Yujuan Li et al., 2018; Tang et al., 2017). The mechanisms underlying the anti-fatigue activity of macamides need to be investigated further.

4.5. Anti-osteoporosis activity

Black, yellow and red Maca have been widely used to alleviate the symptoms of menopause (C. Gonzales et al., 2010). The ethanol extract of Maca with 0.6% macamides and macaenes reversed postmenopausal osteoporosis (PMOP) in ovariectomized (OVX) rats (Y. Z. Zhang, Yu, Ao, & Jin, 2006), and the alkaloid bis-benzylisoquinoline alkaloid was identified as the major bioactive compound (Z. Y. Zhong et al., 2020). Furthermore, macamides like N-benzyl-palmitamide and N-(3-methoxybenzyl)-(9Z,12Z,15Z)-octadecatrienamide also show anti-osteoporosis effects (H. Liu et al., 2015; T. Wang et al., 2019) by modulating osteoblast proliferation, differentiation and mineralization. At the molecular level, they can upregulate osteogenesis-related genes like bone morphogenetic protein-2 (BMP-2), core binding factor alpha 1 (Cbfa1), type 1 collagen (COL) and alkaline phosphatase (ALP) through an estrogen receptor-mediated signaling (Jayakar et al., 2012). Nevertheless, the specific macamides involved in osteogenic differentiation remain to be identified.

4.6. Other activities

Jin et al. (Jin et al., 2018) found that the aerial parts of Maca (45.1-70.33 mg macamide per 100 g DW) promoted gastric emptying (GE) and intestinal propulsion (IP) in human subjects. In addition, compared with the stomach, lipophilic macamides can remain in lung tissue for a longer time due to good lipophicity of lung tissue, which provide a basis for their application in the human respiratory system. Actually, after absorbing N-3-methoxybenzyl-palmitamide, the highest distribution was in the stomach, but the absorption and eliminate rate was slow and incomplete, which is followed by lung, displaying a lung targeting property (Q. H. Zhang et al., 2017). The methanol extract of Maca also cleared influenza virus A and B viruses from the upper respiratory tract (Mendoza, Pumarola, Gonzales, & del Valle, 2014). The secondary metabolites including phenols and macamides prevented virus attachment and fusion with the host cells, eventually decreasing the viral load. In addition, Maca extracts decreased plasma glucose levels and oxidative stress in a rat model of diabetes mellitus (DM), although the antioxidant effect was independent of the maceaenes and macamides (Qiu, Zhu, Lan, Zeng, & Du, 2016). In addition, Lin et al. (Lin et al., 2018) found that the polysaccharides and not macamides of black Maca protected human erythrocytes from oxidative damage.

5. Potential applications

Maca, also known as “South American ginseng” or “Plant Viagra”, has gained considerable attention over the last two decades as s superfood (Beharry & Heinrich, 2018). However, the health claims of Maca are not fully supported by functional studies and clinical trials. Recent chemical and biological profiling of Maca has identified macamides and other bioactive compounds of Maca that display low or no toxicity

(Hudson, Lopez, Almalki, Roe, & Calderon, 2018). The LD_{50} of the Maca ethanol extract (756.62 mg/100 g total macamides) is higher than 2000 mg/kg, indicating very low toxicity. The extracts did not show any particular hepatorenal toxicity (S. O. Abarikwu, C. L. Onuah, & S. K. Singh, 2020). Since macamides are digested to non-toxic amino acids in the body, Maca can also be incorporated in several food products with medicinal value. For instance, adult human subjects that took supplements of red or black Maca spray-dried extracts (3g/day) for 12 weeks showed improved mood, energy and general health, along with a reduced chronic mountain sickness (CMS) score (Gonzales-Arimborgo et al., 2016). Most vendors indicate the macamide content in the commercially available Maca products. The patented healthcare supplement CN109864328 (http://www.wipo.int/pctdb/en/) comprising of tea leaves and Maca shows anti-fatigue and the hormone regulatory effects. Similarly, a commercially marketed beverage of Maca leaves and citrus has an anti-fatigue effect (https://b2b.baidu.com). In addition, macamides can even be applied in health and medicine. CN106563117 is a formulation of glutinous rice and cereal husk with macamides for treating viral pneumonia. Taken together, macamides are promising analgesics, anti-depressants and anti-osteoporotic agents with potential biomedical applications.

Conclusion

Macamides are secondary metabolites and the major bioactive compounds of Maca roots with fertility-enhancing, neuroprotective, anti-fatigue and anti-osteoporotic effects. Macamides are routinely extracted using organic solvents and ultrasonication, and several biological and chemical strategies have been devised to enhance their yield. In addition, high resolution chromatographic separation and MS have helped identify several novel macamides in recent years. The biological and pharmacological activities of macamides have to validated through functional studies as well as clinical trials. This review summarizes the current knowledge regarding macamides and can greatly aid in translating the preliminary information into biomedical and clinical applications.

Credit Author Statement

Hongkang Zhu: Conceptualization, Writing-Original draft preparation, Writing-Reviewing and Editing. Bin Hu: Software, Validation. Hanyi Hua: Data curation. Chang Liu: Formal analysis. Yuliang Cheng: Supervision. Yahui Guo: Supervision. Weirong Yao: Supervision, Validation. He Qian: Conceptualization.

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Highlights

- Maca is known for its medicinal properties since ancient times.
- Chemical structures of macamides and their isolation, synthesis, identification methods are reviewed.
- The present review focuses on the unique bioactive components of macamides.
- Relationships between structures of macamides and pharmacological effects are initially established.
- Future prospects and industrial applications of macamides are forecast.