Anti-glycative and anti-inflammatory effects of macamides isolated from *Tropaeolum tuberosum* in skin cells

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**ABSTRACT**

*Tropaeolum tuberosum*, commonly known as Mashua, is an herbal remedy used on the skin in order to treat local pain and to heal wounds. This study aimed to evaluate the extracts and isolated compounds from *T. tuberosum* with anti-glycative and anti-inflammatory activities. Guided isolation by bioassay led to the isolation and characterisation by NMR and MS of \((S)-(\alpha\text{-methylbenzyl})\)-oleamide (1) and \((S)-(\alpha\text{-methylbenzyl})\)-linoleamide (2). Both compounds inhibited the production of TNF-\(\alpha\) with IC\(_{50}\) values of 9.38 \(\mu\)M (NIH/3T3 cells) and 10.06 \(\mu\)M (PA317 cells) for compound 1, and 5.30 \(\mu\)M (NIH/3T3 cells) and 6.48 \(\mu\)M (PA317 cells) for compound 2. Compounds 1 and 2 showed the inhibitory effect on the BSA-MGO formation at concentrations of 9.38 \(\mu\)M (3.39\%) and 5.30 \(\mu\)M (8.53\%), respectively. Moreover, both compounds showed significant breaking properties on the MGO-AGE-protein crosslink with percent modification of 6.58\% (9.38 \(\mu\)M) and 18.08\% (5.30 \(\mu\)M), respectively.

**ARTICLE HISTORY**

Received 19 September 2021
Accepted 29 November 2021

**KEYWORDS**

Tropaeolaceae; Tropaeolum tuberosum; macamides; glycoxidation; anti-inflammatory
1. Introduction

During recent years, the role of advanced glycation end products (AGEs) has been increasingly discussed in skin aging, and the potential of anti-AGE strategies has received high interest from pharmaceutical companies for the development of novel anti-aging compounds (Xin et al. 2021). AGEs interact with the cell receptors for advanced glycation end-products (RAGEs) (Ramasamy et al. 2008), and stimulate the macrophages to secrete an excessive quantity of pro-inflammatory biomarkers (COX-2, TNF-α and IL-6) (Davis et al. 2016). On the other hand, AGEs are accumulated in the skin as a result of physiological ageing, where they remain bounded to collagen for years, contributing to the stiffness of interstitial tissues and mitochondrial dysfunction (Li et al. 2017). Various AGE inhibitors (aminoguanidine, carnosine, homocarnosine, metformin) have been developed, and some of them have reached advanced clinical trials, although they have shown different degrees of toxicity (Yamagishi et al. 2008).

In this context, natural products are an alternative source of compounds with AGE-inhibitory effects. Examples include piceatannol-3-O-glucoside (Liu et al. 2020) and geigerianoloide (Fadul et al. 2020) that inhibit glycation. Therefore, the search for more effective and safer natural AGE inhibitors can be considered a key area of natural products research. *Tropaeolum tuberosum* Ruíz & Pavón (Tropaeolaceae), commonly known as Mashua, is an indigenous species from Peru and Bolivia. The coated compresses with the tuber decoction from Mashua have traditionally been used on the skin in order to treat local pain and to heal wounds (Apaza Ticona et al. 2020b). Recent studies have shown that specific macamides from *T. tuberosum* tubers can be related to the anti-inflammatory potential of this species by inhibiting TNF-α, NF-κB and STAT3 in THP-1, CCD-1109Sk, MRC-5 and RWPE-1 cells (Apaza Ticona et al. 2019; 2020a).

The current manuscript aims to provide a bio-guided phytochemical study to identify those compounds from *T. tuberosum* tubers samples with anti-glycative and anti-inflammatory effects on skin cells.
2. Results and discussion

The isolated compounds were identified as (S)-(-)-(α-methylbenzyl)-oleamide (1) and (S)-(−)-N-(α-methylbenzyl)-linoleamide (2) (Figure 1). Compounds 1 and 2 were previously described as synthesis products by D’Oca et al. (2010).

The viability effect of compounds 1 and 2 were assessed by the XTT assay in NIH/3T3 and PA317 cells for 12 h (Table S7). Analysing the results, we observed that the compounds did not reduce the viability of the cells; in the case of compound 1, its CC50 was 90.15 μM (NIH/3T3 cells) and 91.15 μM (PA317 cells), and for compound 2, its CC50 was 88.71 (NIH/3T3 cells) μM and 90.49 μM (PA317 cells), both values being higher than the CC50 of actinomycin D, the positive control (ACT, CC50 = 0.008 μM) (**p < 0.01).

Analysing the viability (XTT) in the NIH/3T3 and PA317 cell lines, we observed that compounds 1 and 2 showed less cytotoxicity than ACT. This effect is due to the increase in double bonds in the side chain, which leads to an increase in lipophilicity, thus confirming the relationship between cytotoxicity and lipophilic capacity (Arnott and Planey 2012). Regarding the lipophilic capacity, this is a parameter that determines the lipophilicity of a molecule as LogP (an increase in LogP enhances cell permeability) (Apaza Ticona et al. 2020c). In our case, compound 2 showed a cLogP of 8.20 that allowed it to cross the membrane more easily than compound 1, which showed a cLogP of 7.22. Therefore, compounds with a side chain and with a higher number of double bonds can more easily cross the cell barrier by diffusion.

Concerning the anti-inflammatory activity over the NIH/3T3 and PA317 cells lines, both compounds showed TNF-α inhibition potential, with IC50 values of 9.38 μM (NIH/3T3 cells) and 10.06 μM (PA317 cells) for compound 1 and 5.3 μM (NIH/3T3 cells) and 6.48 μM (PA317 cells) for compound 2, respectively, both values being higher than that of the positive control (C87, IC50 = 0.11 μM) (**p < 0.01) (Table S8).

The difference in anti-inflammatory activity between compound 1 and 2 is the result of the different number of double bonds of the fatty acid chain (Apaza Ticona et al. 2019; 2020a). Thus, compound 2 was more active because of the additional double bond. As shown by mechanistic studies, the increase of double bonds in polyunsaturated fatty acids (PUFAs) leads to an increased activity in specific inflammatory
disorders, through the interaction with the JAK-STAT3 signalling pathway (Yan et al. 2013). By performing a computational prediction of compound 2 with the SwissTargetPrediction program, we observed that this macamide exhibited increased molecule-protein interaction with the tumour necrosis factor receptor 1 and 2; and NF-κB. In this sense, the fatty acid chain of compound 2 is very important for its potential of inhibiting TNF-α.

Regarding the ability of inhibiting the glycation, Figure S23 shows that the compounds inhibited AGE formation by 3.39% (compound 1) and 8.53% (compound 2) at the concentration of 9.38 μM and 5.30 μM, respectively, which was lower than that of the aminoguanidine (AG), the positive control, that showed an AGE inhibitory effect of 23.50% at a concentration of 1000 μM.

Regarding the effect of the compounds on increasing free amine levels, compounds 1 and 2 showed significant AGEs breaking activity of 6.58% and 18.08% at concentrations of 9.38 μM and 5.30 μM, respectively, which was lower than that of the positive control that showed an increase in the free amine level by 87.40% at a concentration of 1000 μM (Figure S24).

Although the isolated compounds belong to the same family, they showed different pharmacological potential. This difference comes from the acyl chains that are bound to the amine and that give different thermodynamic and kinetic properties to the compounds, thus affecting their pharmacological activity (D’Oca et al. 2010; Ye et al. 2019).

The finding that these macamides exert anti-inflammatory and anti-glycative activities can lead to the creation of a library of compounds for the treatment against skin aging. Likewise, to improve this pharmacological activity, different fatty acyl chains must be studied, performing a separation of their enantiomeric mixtures, since the enantiomers differ in their pharmacodynamics and pharmacokinetics (Apaza Ticona et al. 2020c).

3. Conclusions

The isolated compounds exhibit a higher anti-inflammatory and anti-glycative potential than actinomycin D and aminoguanidine. Although the effect observed is smaller than that for aminoguanidine, the concentration at which they have produced such effect is significantly lower. In order to determine whether they are more or less effective than aminoguanidine, an assay should have been carried out using the same concentration (1000 μM). Nevertheless, the assay was not carried out to determine solely the anti-glycant activity, since this was not the only objective of this research. Our objective was to determine the anti-inflammatory and anti-glycation properties of the isolated compounds. In this sense, the concentrations that were tested were those that presented inhibition of TNF-α.

Disclosure statement

The authors declare no conflict of interest.
Funding
This work was supported by the Fundación de la Universidad Autónoma de Madrid (FUAM).

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