In vitro and *in silico* assessment of anti-inflammatory activity of cocoa powders

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Abstract: Plants are considered the major sources of biologically active compounds, which provide unlimited opportunities for their use either as medical treatments or as novel drug formulations. Cocoa powder is frequently used in nutrition and is known to have many benefits thanks to its wide range of biological activities. The presented study was focused on the evaluation of the anti-inflammatory potential of extracts obtained from cocoa powder. *In vitro* assays were employed to evaluate the level of inhibition of cyclooxygenases-1 and -2 activities (COX-1 and COX-2) by tested extracts. Molecular docking was used for *in silico* prediction of cyclooxygenase isoforms inhibition by selected cocoa powder constituents. The results showed that all tested extracts exerted much higher potential in inhibiting COX-2 activity and may be considered in use as selective inhibitors of COX-2 enzyme. On the other hand, *in silico* study shows quercetin and clovamide as the compounds with the highest potential to inhibit both COX-1 and COX-2.

Keywords: *Theobroma cacao* L., cocoa powder, inflammation, anti-inflammatory activity, *in vitro*, *in silico*

1. Introduction

The interest in the utilization of plants with outstanding nutritional properties, such as cocoa, is rapidly increasing and is in line with the recognition of the necessity to prevent and manage ubiquitous and numerous noncommunicable diseases. Cocoa powder, obtained from the seeds of *Theobroma cacao* L. (Malvaceae), is a rich source of compounds possessing a spectrum of biological activities. Among the most important bioactive compounds found in cocoa powder are methylxanthines and flavonoids [1, 2]. The alkalization process is optionally used to change the flavor and color of cocoa powder [3]. Cocoa powder has a specific and unique chemical composition of bioactive compounds and is therefore associated with various health benefits. In particular, some cocoa compounds are considered to be anti-inflammatory agents that can have a positive effect on general health and the prevention of chronic diseases.
This study aimed to evaluate the anti-inflammatory properties of cocoa powder extracts (CPE) towards the inhibition of cyclooxygenases-1 and -2 (COX-1 and COX-2) *in vitro* and *in silico* prediction of COX-1 and COX-2 inhibition by selected compounds contained in the CPE.

2. Materials and Methods

2.1 Preparation of the cocoa samples

Six natural and five alkalized cocoa powders were obtained from three companies: Gerkens, Netherlands; De Zaan Company, USA; Nederland, Spain. To obtain the CPE, 3 g of cocoa powder was suspended in 30 mL of 70% ethanol (v/v). The suspension was mixed for 24 h on a shaker at room temperature and centrifuged (4 000 rcf, 10 min, 25°C). The collected supernatants were lyophilized, and the extracts were stored as such and used for further analyses.

2.2 Anti-inflammatory activity in vitro

The anti-inflammatory effects of CPE were analyzed regarding the inhibition of the activity of cyclooxygenases-1 and -2 enzymes using *in vitro* assays. Inhibition assays for COX-1 and COX-2 were conducted per the modified methodology delineated in Mićović et al. [4]. The extracts were solubilized in DMSO, while indomethacin (purity ≥99%, MP Biochemicals, 190217) and celecoxib (purity ≥98%, Sigma, PZ 0008), serving as positive control substances, were dissolved in ethanol and DMSO respectively. The extract solutions were prepared at a concentration of 50 μg/mL; indomethacin at 1.25 μM concentration; and celecoxib at a concentration of 8.8 μM.

2.3 Anti-inflammatory activity in silico

For the *in silico* analysis of the selected compounds present in CPE, molecular docking simulations were applied. They were used for *in silico* prediction of the inhibitory potency of theobromine, caffeine, epicatechin, catechin, gallocatechin, procyanidin B1,

procyanidin C1, caffeoyl-aspartic acid, protocatechuic acid, clovamide, dideoxyclovamide, and quercetin toward COX-1 and COX-2 receptors. All simulations are performed according to the methodology from Katanić Stanković et al. [5].

3. Results

According to the obtained results of the *in vitro* tests (Figure 1), all tested CPE showed quite low inhibition of COX-1 enzyme activity. The values ranged from 2.29% of COX-1 inhibition for extract 2 to 10.66% for extract 8. In comparison with the positive control indomethacin with 40.25% of COX-1 inhibition, the COX-1 inhibitory effects of the CPE are negligible. On the other hand, the results of COX-2 inhibition were much more prominent. Extracts 1, 2, 9, 10, and 11 stood out in particular with COX-2 inhibitory activity of 88.91, 58.19, 60.02, 62.09, and 61.17%, respectively. These results are even higher than those of Celecoxib as a positive control (46.09%). Generally, non-selective COX inhibitors (non-steroidal anti-inflammatory drugs-NSAIDs) are known for their adverse effects, particularly present in the gastrointestinal tract. In this case, the cocoa powder extracts showed much higher inhibition towards COX-2 activity in comparison to COX-1 inhibition, which actually represents a great advantage in terms of protection of the gastrointestinal tract. Based on the obtained results, it can be concluded that CPE may be used for the inhibition of COX-2 activity particularly.

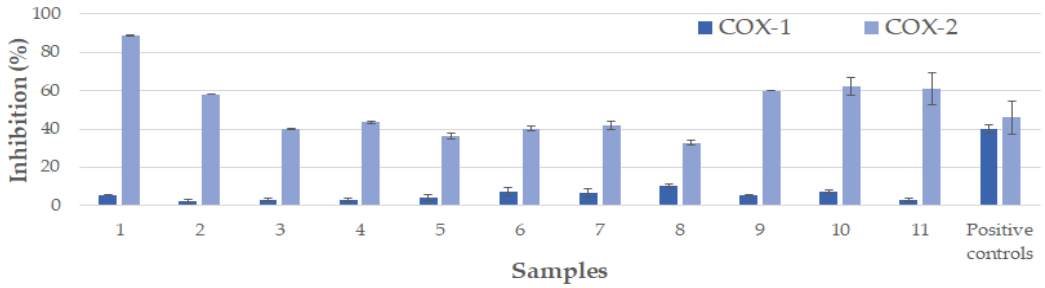

Figure 1. The inhibition of cocoa extract (50 μg/mL) towards COX-1 and COX-2 activities. The results are from two independent experiments (n = 4, mean ± SD). Positive controls: indomethacin (1.25 μM) for COX-1 and celecoxib (8.8 μM) for COX-2.

The influence of the selected compounds from the cocoa extracts (theobromine, caffeine, epicatechin, catechin, gallocatechin, procyanidin B1, procyanidin C1, caffeoyl-aspartic acid, protocatechuic acid, clovamide, dideoxyclovamide, and quercetin) on the activity of COX-1 and COX-2 enzymes was tested *in silico*. A careful inspection of the recognized interactions revealed the inhibitory potency of the tested compounds treated as ligands in molecular docking simulations towards COX-1 and COX-2. The obtained results indicate quercetin and clovamide as the most important compounds. Of all tested compounds, quercetin possessed the lowest values of the ΔG_{bind} and K_i and it has the highest inhibitory potency toward COX-1 and COX-2 (ΔG_{bind} = -9.02 kcal/mol and K_i =

0.24 μM, and ΔG_{bind} = -9.85 kcal/mol and K_i = 0.06 μM, respectively). Clovamide followed the quercetin results and the obtained values of ΔG_{bind} and K_i were almost the same as those of quercetin ((ΔG_{bind} = -8.91 kcal/mol and K_i = 0.29 μM towards COX-1 and ΔG_{bind} = -9.82 kcal/mol and K_i = 0.06 μM towards COX-2). In addition, it seems that both quercetin and clovamide have the same inhibitory potency against COX-2. In the case of inhibition of COX-1, quercetin showed a slightly higher inhibition potency than clovamide.

4. Conclusions

The *in vitro* assessment of the anti-inflammatory activity of the extracts from six natural and five alkalized cocoa powders showed much higher inhibitory potential towards COX-2 activity. There was no clear difference in the activity of alkalized and non-alkalized compounds. Based on the results of the *in silico* study, quercetin and clovamide stand out significantly for their inhibitory potential of both isoforms of cyclooxygenase. Further qualitative and quantitative evaluation of the phytochemical composition of tested extracts is needed in order to define active principles and propose the mechanism of anti-inflammatory action.

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