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New Formulation of Curcumin: Study of Oral Absorption and Bioavailability

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ABSTRACT

Turmeric or Curcuma longa has been used for centuries in both Ayurveda and Chinese traditional medicines. The present study aims at evaluating the oral bioavailability of curcuminoids released from the HGC-C® complex, contained in Curcumin-gel 95+® formulation, and the systemic anti-inflammatory activity of bioavailable fraction. HGC-C® complex is mainly composed of two different types of *Curcuma longa* rhizome extract: the first, hydrodispersible, with a 50% curcuminoid content expressed as curcumin, while the second, organized in double-layered liposomes with amphoteric properties, containing 20% of curcuminoids expressed as curcumin.

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INTRODUCTION:

The Curcumin a yellow powder, is used in Traditional Chinese and Ayurvedic medicine for its anti-inflammatory properties (1). The root Turmeric has different names in Sanskrit, each one referring to specific properties. The main biologically active component of turmeric is curcumin. Many scientific studies and clinical works have focused on the potential of curcumin for treating various pathological conditions (2). This spice is used as a herbal remedy in the treatment of colic, jaundice, hemorrhage, toothache, chest pain, urinary bleeding and cancer (5-6).

In Western Countries, curcumin is increasingly studied for its properties: as a matter of fact, it has been demonstrated that this substance has antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, and anticancer actions and that, consequently, it owns a great potential against various malignant diseases, diabetes, allergies, arthritis, Alzheimer's disease, and other chronic conditions.

Curcumin was investigated mainly for its anti-inflammatory and anti-oxidant potency. Inflammation and oxidative damage are now known to be a root cause of cancer and neurodegenerative diseases (3-4). There are over 30 molecular targets modulated by curcumin. NF-kB is thought to be one of the primary targets of curcumin activity (5). Despite the ingestion of gram level doses of curcumin, plasma curcumin levels remain at low levels in patients, demonstrated, however, that naturally, curcumin cannot achieve its optimum therapeutic outcomes *in-vivo* due to its low solubility and poor gastrointestinal absorption and systemic bioavailability. Only such type of curcumin like nanotechnologic formulation could be used (7).

The present study aims at evaluating the oral bioavailability of curcuminoids contained in Curcumin-gel 95+® formulation and the systemic anti-inflammatory activity of the bioavailable fraction.

MATERIALS AND METHODS

Curcumin-gel® complex is mainly composed of two different types of Curcuma longa rhizome extract: the first, hydrodispersible, with a 50% curcuminoid content expressed as curcumin, while the second, organized in double-layered liposomes with amphoteric properties, containing 20% of curcuminoids expressed as curcumin.

All experiments were performed by ECSIN-ECAMRICERT SRL Laboratory, while active principle (curcuminoids contained in the Curcumin-gel® complex) quantification was performed by ECAMRICERT SRL laboratory.

Oral absorption

The formulation was exposed to saliva, and oral absorption was evaluated using the *in-vitro* model of human reconstructed oral mucosa (HOE, SkinEthic). Briefly, the system is composed of two compartments, apical or oral cavity and basolateral or serosal part, separated by a semipermeable membrane. The *in-vitro* model employed for the present study is endowed with the typical morpho-functional features of oral mucosa cells (**Figure 1**). At the end of the absorption process, the bioavailability of the active principle (curcuminoids contained in the HGC-C® complex) was evaluated via HPLC.

Figure 1. Schematic organization of oral epithelium *in-vitro* model

Determination of active principle bioavailability in the oral epithelium *in-vitro* model

The formulation was added to the apical compartment (oral cavity), while HBSS (Hanks' Balanced Salt Solution) was added to the basolateral compartment (serosal). After 30 seconds, 1 and 3 minutes exposure, and relative transit time, both apical and basolateral fractions were collected and curcuminoid content determined by HPLC. Curcuminoids titer was determined through liquid chromatography coupled with a UV detector, following Pharmacopoeia USP36-NF31 "Powdered Turmeric Extract" method. Chromatographic separation was performed with a C18 reverse-phase column in isocratic elution with mobile phase tetrahydrofuran and water 4:6 + citric acid 1 mg/mL. For detection, the UV detector was set at 420 nm. Finally, curcuminoids quantification was obtained through calibration with external standards.

Bioavailability is reported as percentual absorption, the amount of absorbed active principle per unit of active principle and apparent permeability (Papp).

Cell viability and barrier integrity

Formulation impact on employed *in-vitro* model was evaluated via cell viability determination with MTS assay, based on viable cell ability to metabolize yellow tetrazolium salt with mitochondrial succinate dehydrogenase. Barrier properties were analyzed through 1) monolayer trans-epithelial electrical resistance (TEER) determination and 2) paracellular transport of Lucifer Yellow marker. Apparent permeability (Papp, cm/min) was calculated using the following formula: Papp = (ΔC V)/(Δt A C0) where ΔC/Δt is the flux of the amount of molecule transported during incubation through the monolayer (mM/s), V is the basolateral compartment volume (cm3), A is the semipermeable membrane superficial area (cm2), Co is the initial concentration of the molecule in the apical compartment.

Systemic anti-inflammatory activity

The systemic anti-inflammatory activity of the bioavailable fraction of curcuminoids released from the HGC-C® complex was evaluated in monocytic leukemia cells (THP-1) differentiated towards a macrophage phenotype with phorbol 12-myristate 13-acetate (PMA; 0.5 µM).

To evaluate anti-inflammatory activity of absorbed curcuminoids, differentiated THP-1 cells were pre-treated for 2 hours with the previously identified bioavailable concentration of curcuminoids (5 µg/mL) and then exposed 4 hours with the same curcuminoids concentration either in the presence or absence of pro-inflammatory compound lipopolysaccharide (LPS; 0.5 ng/mL). Macrophages in cell culture medium and then treated with LPS were used as negative and positive controls, respectively.

Anti-inflammatory activity was determined by measuring the release in cell culture medium of pro-inflammatory cytokines interleukin-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-α). IL-1β and TNF-α were quantified by commercial ELISA (Enzyme-Linked Immunosorbent Assay) kits (R&D Systems; Peprotech), following the manufacturer's instructions.

Anti-inflammatory effect of absorbed curcuminoids was confirmed measuring cyclooxygenase (COX) enzyme 1 and 2 activity. Total COX and COX-2 activities were assessed by using a commercial COX activity assay kit (Cayman Chemical), following the manufacturer's instructions.

RESULTS AND DISCUSSION:

Oral absorption

1. Oral absorption of curcuminoids

Oral absorption was evaluated by monitoring the passage of curcuminoids through the oral epithelium following 30 seconds, 1 and 3-minute exposure. To determine the bioavailability of the active principle, apical (oral cavity) and basolateral (serosal) fluids were collected and curcuminoids titer quantified via HPLC. Absorption is reported as percentual absorption, the amount of absorbed active principle per unit of active principle and apparent permeability (P_{app}). As evidenced in **Figure 2** and **Table 1**, curcuminoids oral absorption from the formulation is higher after 30-second exposure (0.17% equivalent to 1.71 µg of absorbed/ mg of active principle and to a permeability P_{app} of 0.00012 cm/min).

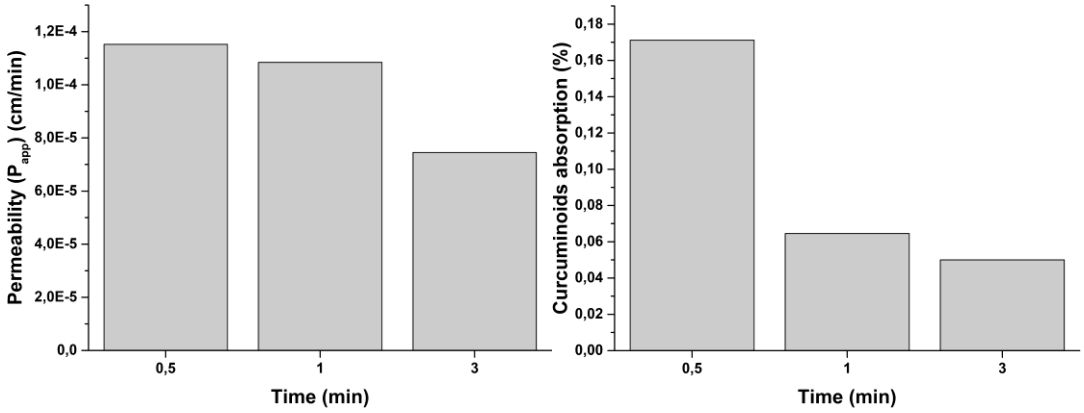


Fig. 2 (A) **Fig. 2 (B)**

Figure 2. Percentual oral absorption (A) and permeability (B) of curcuminoids.

Table 1. Oral bioavailability of active principles reported as: (i) percentual absorption; (ii) the amount of absorbed active principle per unit of active principle; (iii) apparent permeability (P_{app}).

Curcuminoids			
Time (min)	0.5	1	3
absorption (%)	0.17	0.06	0.05
µg absorbed/mg active principle	1.71	0.64	0.5
Permeability (cm/min)	0.00012	0.00011	0.000075

2. Formulation impact on membrane integrity and vitality of the oral epithelium *in-vitro* model

The impact of the formulation on barrier integrity and cell vitality was evaluated, at all considered time points, through apparent permeability (P_{app}) determination, transepithelial electrical resistance (TEER) measurement and MTS assay (Figure 3 and 4). Curcumin gel-95+® formulation does not induce significant alterations of both barrier properties and cell vitality.

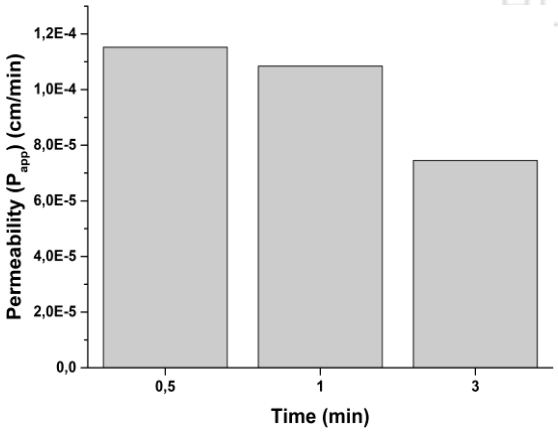


Fig. 3 (A)

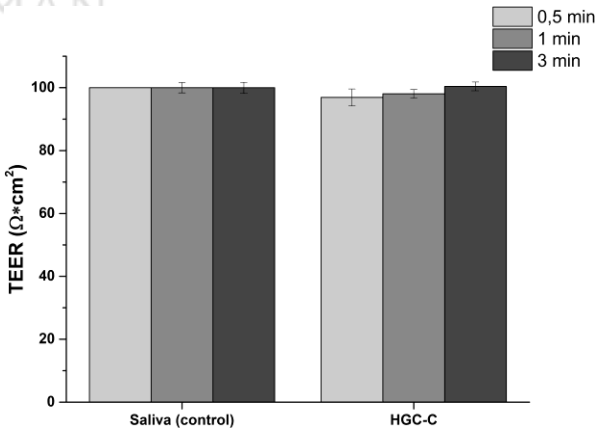


Fig. 3 (B)

Figure 3. Formulation impact on P_{app} (A) and trans-epithelial electrical resistance (TEER) (B) of the oral epithelium *in-vitro* model. *p<0.05

Even if oral epithelium *in-vitro* model exposure to Curcumin-gel 95+® formulation induces a

small increase in membrane permeability, as evidenced from the apparent permeability of Lucifer Yellow marker (Figure 3A), barrier properties are preserved and no variations in TEER values were observed (Figure 3B). Moreover, the treatment with the formulation does not alter oral epithelium vitality (Figure 4).

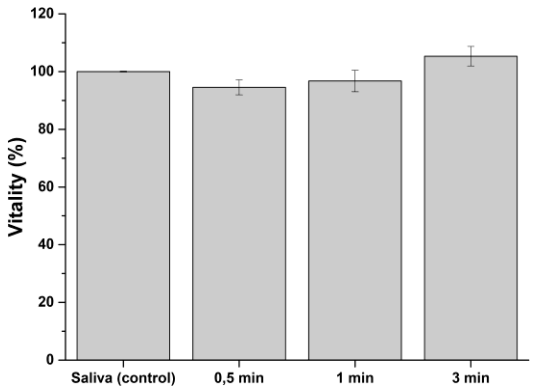
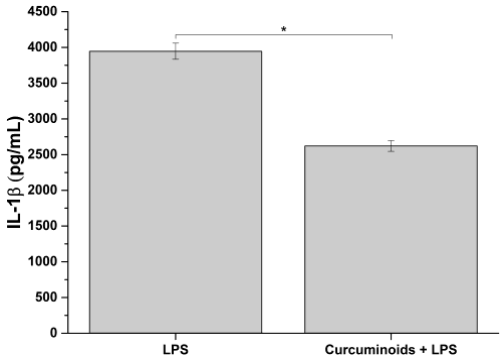


Figure 4. Formulation impact on the vitality of the oral epithelium *in-vitro* model. *p<0.05

The systemic anti-inflammatory activity of absorbed curcuminoids

Systemic anti-inflammatory effects of the bioavailable fraction of curcuminoids were evaluated on inflamed monocytic/macrophage THP-1 model by measuring pro-inflammatory cytokine IL-1β and TNF-α levels released in cell culture medium in the presence or absence of the formulation.

After a single dose administration, the bioavailable fraction significantly reduced IL-1β level (approximately 30%) (Figure 5A), while no significant changes in TNF-α level occurred (Figure 5B).



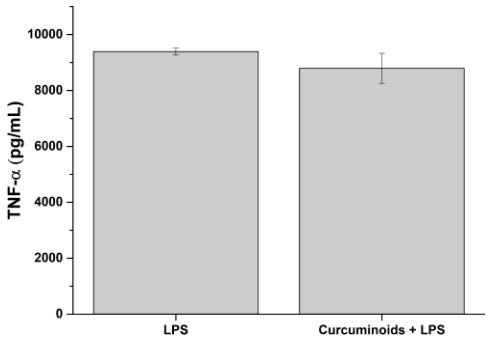
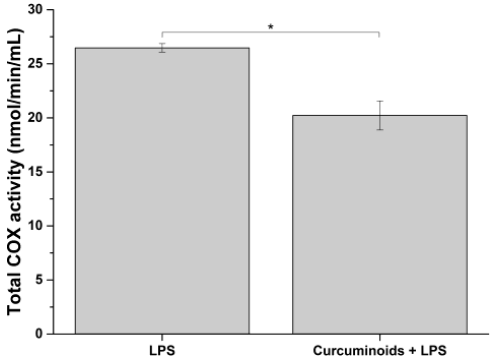


Fig. 5 (A) **Fig. 5 (B)**

Figure 5. Profile release of IL-1β (A) and TNF-α (B) in inflamed macrophages. LPS is a positive control and curcuminoids + LPS refers to inflamed cells treated with the bioavailable fraction of curcuminoids. *p<0.05

Anti-inflammatory effect of the bioavailable fraction was also evaluated measuring total COX activity and specific COX-2 activity. In the presence of curcuminoid absorbed fraction, a significant reduction in total COX activity (approximately 20%) was observed (Figure 6A). A non-significant decreasing trend was also highlighted for specific COX-2 activity (Figure 6B).



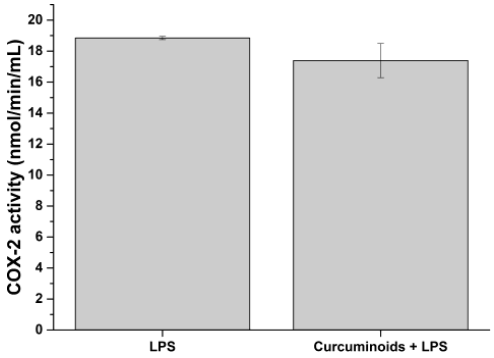


Fig. 6 (A) **Fig. 6 (B)**

Figure 6. Total COX activity (A) and specific COX-2 activity (B) in inflamed macrophages (LPS) and cells treated with the bioavailable fraction of curcuminoids (Curcuminoids + LPS). *p<0.05

CONCLUSION:

The Curcumin is one of the most investigated natural substances as anti-inflammatory action. But the problem is its poor bioavailability. Considering the overall results from oral absorption and systemic anti-inflammatory activity, we can affirm that the formulation is endowed with a fast and significative absorption at the oral epithelium level and the bioavailable fraction ensures an effective systemic anti-inflammatory activity even after a single administration.

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