Antimutagenic and antiherpetic activities of different preparations from *Uncaria tomentosa* (cat's claw)

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ABSTRACT

Uncaria tomentosa have been used to treat viral diseases such as herpes due to multiple pharmacological effects, but its therapeutic efficacy against this virus have not been reported yet. Thus, *in vitro* antiherpetic activity of hydroethanolic extract from barks, purified fractions of quinovic acid glycosides and oxindole alkaloids was evaluated by plaque reduction assay, including mechanistic studies (virucidal, attachment and penetration action). Once exposure to physical agents might lead to reactivation of the herpetic infection, antimutagenic effect (pre-, simultaneous and post-treatment protocols) was also evaluated by Comet assay. The antiherpetic activity from the samples under investigation seemed to be associated with the presence of polyphenols or their synergistic effect with oxindole alkaloids or quinovic acid glycosides, once both purified fractions did not present activity when evaluated alone. Inhibition of viral attachment in the host cells was the main mechanism of antiviral activity. Although both purified fractions displayed the lowest antimutagenic activity in pre and simultaneous treatment, they provided a similar effect to that of cat's claw hydroethanolic extract in post-treatment. Given that purified fractions may result in a reduced antiherpetic activity, the use of cat's claw hydroethanolic extract from barks should be prioritized in order to obtain a synergistic effect.

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1. Introduction

Uncaria tomentosa (Willd.) DC. (Rubiaceae) (UT), commonly known as cat's claw, is a woody vine native to the Peruvian Amazon widely used in folk medicine for many purposes, including the treatment of viral infections (Keplinger et al., 1999). Its bark extract is mainly composed of polyphenols, quinovic acid glycosides, tetracyclic (TOA) and pentacyclic oxindole alkaloids (POA) (Heitzman et al., 2005). *Uncaria guianensis* (Aubl.) Gmel is the main adulterant of *U. tomentosa* given the botanical similarity and their same habitat occurrence (Heitzman et al., 2005). However, *U. guianensis* differs from cat's claw presenting a lower content of oxindole alkaloids and quinovic acid glycosides, but has a similar content of polyphenols (Heitzman et al., 2005).

The anti-inflammatory response assigned to cat's claw seems to be related to the effect of quinovic acid glycosides, oxindole alkaloids and polyphenols acting alone or in a synergistic combination (Aquino et al., 1991; Reis et al., 2008). Cat's claw bark extracts have displayed immunomodulatory and anti-inflammatory properties, which are related to stimulation of normal B and T lymphocyte, IL production by macrophages, phagocytosis, suppression of TNF-α synthesis (Sandoval et al., 2002; Heitzman et al., 2005) and inhibition of NF-kβ (Zeng et al., 2009). Thus, it is possible to modulate more than one system simultaneously acting on the same target. In addition to the pharmacological activities mentioned above, NF-kβ inhibition has an antimutagenic effect because it suppresses exacerbated inflammation, acting on proinflammatory cytokines, which are endogenous sources of free radical production (Mammone et al., 2006).

In view that *U. tomentosa* preparations have previously demonstrated immunomodulatory and anti-inflammatory effects, both desirable for an antiherpetic therapy, we decided to evaluate the *in vitro* antiherpetic activity of different UT preparations (hydroethanolic extracts from its barks, as well as the purified fractions of quinovic acid glycosides and oxindole alkaloids, which were very well-characterized chemically) as well

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as the synergistic or antagonistic effect of *U. guianensis*. Although UT extracts standardized to 5% of mitraphylline have been proposed for use in topical treatment of herpes labialis due to their secondary pharmacological effects (Caldas et al., 2010), *in vitro* antiherpetic activity and mechanistic studies have not been carried out yet.

The protective effect of these cat's claw preparations on UV-induced DNA damage was also evaluated since latent herpes simplex virus (HSV) infections can be reactivated by UV-B light (Rooney et al., 1992). UV-C and UV-B mutagenesis can be characterized by the high frequency of transition mutations at dipyrimidine sequences containing cytosine (Pfeifer et al., 2005). Both lesion types are usually repaired by enzymes involved in the nucleotide excision repair pathway (Chazal et al., 2004). Although Mammone et al. (2006) have evaluated the protective effect of cat's claw preparations previously, skin cell culture was used and the incubation time of cells after UV exposure was relatively high (repair mechanisms could have occurred in this interval). If a considerable repair effect on UV-induced DNA damage is shown, a product with topical application could be proposed.

Despite that aqueous extracts from cat's claw have been the most frequently preparations administered to patients, hydroethanolic extracts and fractions purified from them were considered in view that they have shown higher anti-inflammatory (Aguilar et al., 2002) and antioxidant activity (Pilarski et al., 2006) than aqueous extracts. Both extracts have not yet been compared in terms of their immunomodulatory effect.

2. Materials and methods

2.1. Plant material

Stem bark samples of *U. tomentosa* (Willd.) DC. and *U. guianensis* were kindly gifted by Quimer Ervas e Especiarias (São Paulo, Brazil). A voucher specimen of *U. tomentosa* and *U. guinanensis* were deposited at the Herbarium of Universidad Nacional Mayor de San Marcos (Lima, Peru/N° 10500) and at the Central Herbarium of the Universidade Federal do Mato Grosso (Mato Grosso, Brazil/Nº 24715), respectively.

2.2. Extraction procedures

2.2.1. Maceration extraction

The extractive solutions of the *U. tomentosa* (UT mac) and *U. guianensis* (UG mac) were prepared by 4-day maceration, using a hydroethanolic solution 40% (v/v) and a drug: solvent ratio of 1:10 (w/v). Each mixture was processed separately, filtered, and concentrated under vacuum at 50 °C up to half of their original weights. The concentrates were freeze-dried immediately (Modulyo 4 L, USA). The *U. tomentosa* plus *U. guianensis* mixture (UT + UG mac) was performed by weighted ratio of 1:1 (w/w).

2.2.2. Reflux extraction

The extractive solutions of the *U. tomentosa* (UT ref) and *U. guianensis* (UG ref) were prepared by 45-min reflux using a hydroethanolic solution 40% (v/v) and a drug: solvent ratio of 1:10 (w/v). Each mixture was pressed separately, filtered, and concentrated under vacuum at 50 °C up to half of their original weights. The concentrates were immediately freeze-dried. The *U. tomentosa* plus *U. guianensis* mixture (UT + UG ref) was performed by weighted ratio of 1:1 (w/w).

2.3. Obtention of oxindole alkaloids purified fraction (OAPF) and quinovic acid glycosides purified fraction (QAPF)

The UT mac was pre-purified with polyvinyl-polypyrrolidone (PVPP, BASF, Germany), filtrated under vacuum and the filtrate was then acidified with formic acid to pH 3.0. The pre-purified extract was subjected to an ion-exchange process in column previously filled with strong anionic resin (Dowex Marathon, Sigma Aldrich, USA). The column was eluted with 40% v/v hydroethanolic solution (step 1), ammonium acetate buffer 0.3 M (pH 7.0) (step 2) and 80% v/v hydroethanolic solution (step 3) at flow of 5.0 mL/min. The eluates obtained in the first (Anionic resin eluate – ARE) and third steps (OAPF) were concentrated under vacuum at 50 °C up to half of their original weights and freeze-dried immediately. ARE was properly solubilized in ultra-pure water and subjected to solid-phase extraction in column previously filled with macroporous polystyrene resin (Diaion HP-20, Supelco, USA). The column was eluted with methanol: water solutions in decreased polarity

gradient at flow of 2.5 mL/min. The eluates obtained from methanol: water (90:10, v/v) and methanol were pooled (QAPF), concentrated under vacuum at 50 °C up to a quarter of their original weights and immediately freeze-dried.

2.4. Assay of polyphenols, oxindole alkaloids and quinovic acid glycosides

2.4.1. Polyphenols by Folin–Ciocalteu

The experimental conditions were performed in accordance with Kaiser et al. (2013a). Freeze-dried samples of 20 mg were solubilized separately in 10 mL of 40% v/v hydroethanolic solution. Aliquots (4 mL) were mixed with 2 mL of Folin–Ciocalteu reagent 1N (Proton, Brazil) and 10 mL of water, and the final volume diluted up to 25 mL with 20% w/v Na_2CO_3 solution. A blank solution was prepared to 4 mL of 40% v/v hydroethanolic solution instead of sample aliquot. Absorbance measurements were obtained in a spectrophotometer (Hewlett-Packard, USA) at 760 nm, 30 min after the addition of Na_2CO_3 solution. The total polyphenol content was expressed as µg/mg of freeze-dried sample by mean value of three determinations using the epicatechin (Sigma–Aldrich, batch 45H2643, USA) as external standard.

2.4.2. LC analysis for low molecular weight polyphenols, oxindole alkaloids and quinovic acid glycosides

The analyses were performed employing a HPLC-PDA method (Shimadzu, Kyoto, Japan) previously validated for precision, accuracy, linearity, specificity and recovery (Pavei et al., 2010; Pavei et al., 2012; Kaiser et al., 2013b). Specific analytical conditions were considered for each compound group as shown in Table 1.

2.5. Virus and cells

Vero cells (ATCC, CCL 81) were grown in minimum essential medium (MEM), supplemented with 10% fetal bovine serum, penicillin (100 U/mL), streptomycin (100 µg mL^{-1}), and amphotericin B (25 µg mL^{-1}). Cell cultures were maintained at 37 °C in a humidified 5% CO_2 atmosphere chamber. The virus used was HSV-1 KOS strain (Faculty of Pharmacy, University of Rennes, France), whose titer was determined by plaque assay and expressed as plaque forming units (PFU/mL) (Burleson et al., 1992).

2.6. Cytotoxicity and antiherpes screening

The cytotoxicity of the samples was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983). Briefly, confluent Vero cells were exposed to different sample concentrations (0.98–500 µg mL^{-1}) for 72 h The medium was then replaced by MTT solution and incubated for 4 h. After dissolution of formazan crystals with DMSO (Merk, Germany), optical densities were read at 540 nm (Infinite 1200 TECAN, Austraia) and the concentration of each sample that reduced cell viability by 50% (CC_{50}) was calculated based on untreated controls. Subsequently, the potential antiherpetic activity was evaluated by the plaque reduction assay as previously described (Silva et al., 2010). Monolayers of Vero cells grown in 24-well plates were infected with 100 PFU per well of each virus for 1 h at 37 °C. Treatments were performed by adding samples simultaneously with the virus (simultaneous treatment) or after the virus infection (post-infection treatment). Cells were subsequently covered with CMC medium (MEM containing 1.5% carboxymethylcellulose) and incubated for 72 h. Cells were then fixed and stained with naphthol blue black and viral plaques were counted. The concentration of each sample that reduced the plaque number by 50% (IC_{50}) was calculated by standard method (Burleson et al., 1992). Acyclovir (ACV) was used as positive control. IC_{50} and CC_{50} values were estimated by non-linear regression of concentration–response curves generated from the data. Cytotoxicity and antiviral activity results were used to calculate the selectivity index of each sample (SI = CC_{50}/IC_{50}).

2.7. Mechanism of antiherpes activity

Results from preliminary antiherpes evaluation showed a significant activity in the first steps of viral replication since only in simultaneous treatment significant HSV-1 inhibition was detected. Therefore, virucidal activity, attachment and penetration assays were performed.

The virucidal assay was carried out as described by Ekblad et al. (2010), with minor modifications. Mixtures of equal sample volumes (0.5 µg mL^{-1}) and 4×10^5 PFU of HSV-1 (KOS) in serum-free MEM were co-incubated for 20 min at 4 or 37 °C. Samples were then diluted to non-inhibitory concentrations (1:1000) to determine the residual infectivity by plaque reduction assay as described above (item 2.6). Citrate-buffer (pH 3.0) was used as positive control.

The attachment and penetration assays followed the procedures described by Piret et al. (2002) and modified by Silva et al. (2010). In the attachment assay, pre-chilled Vero cell monolayers were exposed to viruses (100 PFU per well), in the presence or absence of the samples. After incubation at 4 °C for 2 h, samples and unabsorbed viruses were removed by washing with cold phosphate-buffered saline (PBS) and cells were overlaid with CMC medium. Further procedures were the same as the described above (item 2.6). For the penetration assay, viruses (100 PFU per

Table 1
HPLC conditions for the quantification of low molecular weight polyphenols, oxindole alkaloids and quinovic acid glycosides from UT different preparations.

Parameter	Low molecular weight polyphenols (Pavei et al., 2010)	Oxindole alkaloids[*] (Kaiser et al., 2013b)	Quinovic acid glycosides[**] (Pavei et al., 2012)
Column	Gemini RP-18 column (250 × 4.6 mm, 5 µm) (Phenomenex, USA)	Gemini-NX RP-18 column (250 × 4.6 mm, 5 µm) (Phenomenex, USA)	Synergy Fusion RP-18 column (150 × 4.6 mm, 4 µm) (Phenomenex, USA)
Mobile phase	0.1% v/v trifluoroacetic acid (A) and methanol: TFA (99.9:0.1, v/v) (B) in a linear gradient program	Ammonium acetate buffer 10 mM (pH 7.0) (A) and acetonitrile (B) in a linear gradient program	0.01% v/v formic acid (A) and acetonitrile: formic acid 0.01% (90:10, v/v) (B) in a linear gradient program
Flow rate (mL/min)	0.9	1.0	1.0
UV detection (nm)	325	245	205
Temperature (°C)	23 ± 1	23 ± 1	35 ± 1

Results were expressed as µg/mg of freeze-dried sample by mean of three determinations using chlorogenic acid (Fluka, batch 455159/1, Switzerland), caffeic acid (Extrasynthèse, batch 0381024, France) and rutin (Sigma, batch 128K1177, USA) as external standards for low molecular weight polyphenols; mitraphylline (Phytolab, batch 2946, Germany) for Oxindole alkaloids and α-hederin (Extrasynthèse, batch 08040314, France) for Quinovic acid glycosides.
[*] The total POA and TOA contents were calculated by the sum of individual alkaloid contents (speciophylline, uncarine F, mitraphylline, isomitraphylline, pteropodine, and isopteropodine for POA; rhyncophylline and isorhyncophylline for TOA), previously characterized through UV and MS–MS data (Kaiser et al., 2013b)
[**] The total quinovic acid glycosides content was calculated by the sum of individual contents of the seven major peaks previously characterized as quinovic acid glycosides through UV and MS–MS data (Pavei et al., 2012).

well) were adsorbed for 2 h at 4 °C on pre-chilled cells. After the removal of un-bound viruses, the temperature was shifted to 37 °C to allow penetration. Then, the cells were treated with different concentrations of pre-warmed samples, and incubated at 37 °C for 1 h. Unpenetrated viruses were inactivated with citrate-buffer (pH 3.0). Cells were washed with PBS and covered with CMC medium. The percentage of inhibition was calculated based on the reduction of plaque number as mentioned previously (item 2.6).

2.8. Antimutagenicity testing

The protective effect of samples at 1000 µg mL^{-1} (non-cytotoxic concentration) on UV-induced DNA damage was assessed using the Comet assay with minor modifications (Tice et al., 2000). Three different approaches were used to evaluate the antimutagenic mechanism of tested samples: pre-, simultaneous and post-treatment. Different UV-C doses and exposure times were tested in order to obtain different fragmentation patterns in fibroblasts. However, a UV-C irradiation dosage of approximately 50 mJ/cm^2 for 5 min provided satisfactory levels of DNA damage. One-way ANOVA with Tukey's post test was performed using GraphPad InStat (GraphPad Prism 5 Software, San Diego, CA), and $p < 0.05$ values were considered significant.

For antimutagenicity tests confluent cell monolayers (2.5×10^5 cells/well) were incubated at 37 °C and 5% CO$_2$ for 2 h with different samples added before (pre-treatment assay), immediately (simultaneous assay) and after (post-treatment) the irradiation process. Afterwards, cells were maintained under same incubation conditions for 90 min.

Cells were visually scored according to tail length into five classes: (1) class 0: undamaged, with no tail; (2) class 1: with tail shorter than the diameter of the head (nucleus); (3) class 2: with tail length $1 - 2\times$ the diameter of the head; (4) class 3: with tail longer than $2\times$ the diameter of the head and (5) class 4: comets with no heads. The final score represent the damage index, which may range from 0 (completely undamaged: 100 cells × 0) to 200 (with maximum damage: 100 cells × 4) (Tice et al., 2000; Pereira et al., 2009). The protective effect (expressed in percentage) may then be calculated by comparing the final score of each UT preparation and positive control (cells irradiated with 50 mJ/cm^2 of UV-C for 5 min).

3. Results

3.1. Content of polyphenols, oxindole alkaloids and quinovic acid glycosides in different Uncaria spp. preparations

UT mac, UT + UG mac and UT + UG ref showed higher total polyphenol content in all preparations. It is worth noting that only 5% of these fractions were composed of low molecular polyphenols, such as phenolic acids and flavonoids (Table 2). In addition, these preparations showed similar oxindole alkaloid constitution (only speciophylline, uncarine F, mitraphylline, isomitraphylline, pteropodine, and isopteropodine were detected), but different quinovic acid glycosides content. Adulteration of *U. tomentosa* with *U. guianensis* (UT + UG mac and UT + UG ref) caused signifi-

cant change only in the quinovic acid glycosides content in relation to UT mac.

In contrast, only quinovic acid glycosides and POA were detected in QAPF and OAPF, respectively, since polyphenols were removed of UT mac by complexation with PVPP. Additionally, the content of quinovic acid glycosides and POA was four times higher in the QAPF and OAPF, respectively, as compared to that of UT mac.

3.2. Antiherpetic activity

Preliminary antiherpetic activity was evaluated by simultaneous and post-treatment of cells with samples and virus. QAPF and OAPF did not present antiviral activity, whereas UT mac, UT + UG mac and UT + UG ref inhibited HSV-1 replication when added to cells in simultaneous treatment, but not after virus infection (Table 3). Once simultaneous treatment was more efficient than post-infection, a direct inactivation of viral particles or inhibition of virus replication at the initial phases of the viral replication cycle could be involved. To evaluate these hypotheses, virucidal, viral adsorption and penetration assays were performed with UT mac, UT + UG mac and UT + UG ref. Pre-incubation of virus suspensions at 4 and 37 °C with these preparations had no significant inactivating effects on HSV-1 KOS (data not shown) at the tested concentrations (1.5–50 µg mL^{-1}). These results indicate that the virucidal effect does not seem to be involved in the antiviral activity of cat's claw extracts.

The adsorption assays results demonstrated that three evaluated extracts of cat's claw strongly inhibited attachment of HSV-1 (Table 3). However, these preparations were not able to prevent HSV-1 penetration into cells (data not shown).

3.3. Antimutagenic effect

The different approaches used in this study provide evidence of antimutagenic mechanisms of the tested materials. Overall, the simultaneous treatment provided greater protective effect on UV-induced DNA damage (reaching protection levels of 75%) than the other two experimental protocols (Fig. 1) and this effect clearly showed to be dependent on sample concentration (250–1000 µg mL^{-1}, data not shown). Both purified fractions (QAPF and OAPF) showed lower protective effect on UV-induced DNA damage than UT mac ($p < 0.05$) after pre- and simultaneous treatment, and similar protective effect in post-treatment ($p > 0.05$).

Table 2
Assay of polyphenols, oxindole alkaloids and quinovic acid glycosides in the different preparations.

Assay	Content (µg/mg of freeze-dried sample)				
	UT mac	UT + UG mac	UT + UG ref	OAPF	QAPF
Total low molecular weight polyphenols[a]	8.75 ± 0.22	10.46 ± 0.54	9.82 ± 0.30	ND	ND
Total polyphenols[b]	183.81 ± 0.13	170.95 ± 0.20	188.41 ± 0.33	ND	ND
Total TOA[c]	ND	ND	ND	ND	ND
Total POA[d]	15.35 ± 0.19	16.86 ± 0.21	15.55 ± 0.07	62.73 ± 0.16	ND
Total quinovic acid glycosides[e]	67.76 ± 1.38	41.79 ± 0.55	35.07 ± 0.09	ND	217.80 ± 0.46

UT mac: *U. tomentosa* maceration extract; UT + UG mac: *U. tomentosa* plus *U. guianensis* maceration extract; UT + UG ref: *U. tomentosa* plus *U. guianensis* reflux extract; OAPF: oxindole alkaloid purified fraction; QAPF: quinovic acid purified fraction; ND: not detected.
[a] Obtained by Folin–Ciocalteu and expressed as epicatechin.
[b] Sum of individual contents expressed as chlorogenic acid, caffeic acid and rutin.
[c] Sum of individual tetracyclic oxindole alkaloids contents expressed as mitraphylline.
[d] Sum of individual pentacyclic oxindole alkaloids contents expressed as mitraphylline.
[e] Sum of individual contents expressed as α-hederin. These results represent the mean ± SD of three determinations.

Table 3
Cytotoxicity and antiherpetic activities of the different preparations.

Preparations	CC_{50} (µg/mL) Vero cells	IC_{50} (µg/mL) HSV-1 (KOS)		
		Simultaneous treatment	Pos-infection treatment	Adsorption
UT mac	>500	<15.75	NI	1.05 ± 0.21 (SI > 476.19)
UT + UG mac	255.60 ± 50.37	<15.75	NI	1.00 ± 0.20 (SI = 255.60)
UT + UG ref	>500	<15.75	NI	1.17 ± 0.23 (SI = 427.35)
OAPF	>500	<15.75	NI	NT
QAPF	>500	<15.75	NI	NT
Aciclovir (control)	>2500	NI	0.49 ± 0.1 (SI > 5100)	NI

UT mac: *U. tomentosa* maceration extract; UT + UG mac: *U. tomentosa* plus *U. guianensis* maceration extract; UT + UG ref: *U. tomentosa* plus *U. guianensis* reflux extract; OAPF: oxindole alkaloid purified fraction; QAPF: quinovic acid purified fraction; CC_{50}: 50% cytotoxic concentration for Vero cells (µg/mL); IC_{50}: 50% inhibitory concentration (µg/mL); selectivity index values (SI = CC_{50}/IC_{50}) are presented between parenthesis; NI: no inhibitory activity; NT: not tested. These results represent the mean ± SD of four independent experiments.
No statistically significant differences were observed among IC_{50} values of UT mac, UT + UG mac and UT + UG ref in adsorption studies (ANOVA, Tukey's test).
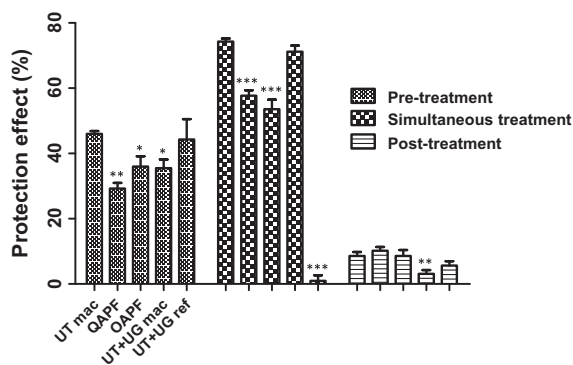
Fig. 1. Protective effect (%) of different cat's claw preparations (UT mac: *U. tomentosa* maceration extract; UT + UG mac: *U. tomentosa* plus *U. guianensis* maceration extract; UT + UG ref: *U. tomentosa* plus *U. guianensis* reflux extract; OAPF: oxindole alkaloid purified fraction; QAPF: quinovic acid purified fraction) on UV-induced DNA damage after pre-, simultaneous and post-treatment. Treatments with cat's claw preparations and exposed to UV-C were compared to cells only exposed to UV-C. Data were expressed as mean ± standard deviation (SD) of the three independent experiments. ***$p < 0.0001$, **$p < 0.001$, *$p < 0.05$ indicate statistically significant differences between each treatment and UT mac (ANOVA, Tukey's test). Each treatment protocol (pre-, simultaneous and post-treatment) has been analyzed separately.

UT + UG mac decreased the protective effect on DNA damage compared to UT mac after pre-treatment ($p < 0.05$) while UT + UG ref after simultaneous treatment ($p < 0.001$). Furthermore, the addition of *U. guianensis* in *U. tomentosa* extracts also provided a substantial reduction of this parameter in post-treatment, particularly for samples subjected to maceration ($p < 0.01$). Although purified fractions (QAPF and OAPF) have provided lower protective effect in pre- and simultaneous treatment, a positive effect was observed in post-treatment.

4. Discussion

Herpes symptoms are caused predominantly by the lytic replication of the virus in epithelial cells. In addition to that, alterations in the immune system might be observed. Melchjorsen et al. (2006) reported that human monocyte-derived macrophages and dendritic cells express both type I and III interferons [IFN-α, IFN-β and IL-28, IL-29, respectively], TNF-α, CCL5 and CXCL10 chemokines after HSV-1 infection. Given that these cytokines have been associated with herpes pathogenesis, additional effects on them would be expected with antiviral candidates. Reis et al. (2008) have reported that cat's claw pentacyclic oxindole alkaloids are able to reduce cytokines expression such as TNF-α and INF-α, which would result in an immunomodulatory effect on the herpetic infection.

The spectrum of symptoms in HSV-1-infected patients also suggests that an induction of inflammatory cytokines in response to viruses plays a significant role in the pathogenesis of the disease, especially in cases in which complications as encephalitis may be observed (Kurt-Jones et al., 2004). This further reinforces the need for investigations that take into consideration the antiviral candidates activity on different physiological systems.

Phosphorylation/recruitment/activation events lead to the activation and translocation of NF-κβ to the nucleus and the transcription of inflammatory and anti-inflammatory cytokine genes. Activation of Toll-like receptors by LPS in response to virus also represents another mechanism to induce the production of

inflammatory cytokines. In this sense, some activity on these mediators is expected in order to reduce inflammation as well as the severity of pathology (encephalitis, pneumonia, and hepatitis). Studies have already been carried out to identity the anti-inflammatory potential of cat's claw preparations, focusing on their applications in rheumatoid arthritis (Mur et al., 2002). Sandoval et al. (2000, 2002) have reported that *U. tomentosa* preparations may also inhibit LPS-induced proinflammatory cytokines, mainly TNF-α, besides iNOS gene expression, nitrite formation and activation of the transcription factor NF-κβ, which would contribute to an anti-inflammatory effect.

Given that the anti-inflammatory and immunomodulatory properties of cat's claw extracts have been relatively well studied, we therefore focused in the evaluation of the antiherpetic and antimutagenic activities.

Previous antiviral activity studies regarding the combination of drugs with immunomodulatory properties such as topical corticorteroids and antiviral agents have provided additional clinical benefit compared to antiviral treatment alone (Evans et al., 2002). Thus, it is relevant to find a candidate presenting combined biological activities as well as low toxicity.

The antiherpetic activity from the tested samples seems to be associated with the polyphenols or to their synergistic effect with pentacyclic oxindole alkaloids or quinovic acid glycosides since both purified fractions (QAPF and OAPF) did not present activity. The addition of *U. guianensis* decreased the total quinovic acid glycosides content; however, significant differences in antiviral activity among these samples were not observed (UT mac vs. UT + UG mac or UT + TG ref). These findings support the hypothesis that polyphenols may be related to antiherpetic activity, given that both plants present similar contents of these compounds, but differ in pentacyclic oxindole alkaloid or quinovic acid glycosides content (*U. guianensis* has a reduced content of the last one). The major constituents of polyphenolic fraction seem to be condensed tannins, composed of catechin and epicatechin monomeric units (Sandoval et al., 2000; Gonçalves et al., 2005; Heitzman et al., 2005). After virus penetration into cells, cat's claw extracts were not effective in suppressing the virus replication due to the fact that viral proteins may have already been synthetized when the extract is added (post-infection treatment). Given that *U. tomentosa* hydroethanolic extracts demonstrated a significant *in vitro* inhibitory effect on the replication of herpes simplex virus type 1 after a simultaneous treatment protocol, the effect of these samples at the initial phases of the viral replication cycle was evaluated. Inhibition of viral attachment in the host cells by cat's claw hydroethanolic extracts was characterized as the main mechanism of antiviral activity. Gescher et al. (2011) have also reported from analysis of literature reviews that plant-derived polyphenols, especially tannins, present anti-HSV activity usually by acting on the early phases of infection, similar to that found in our study. According to these authors, polyphenols would avoid a specific interaction between host cells and virus, reducing infection. The inhibitory potency or affinity of the polyphenols for specific proteins may be associated with the amino acid composition and hydrophilicity of the target proteins.

Considering the protective reinforcement effect against UV exposure on the skin for cat's claw preparations previously observed by Mammone et al. (2006), experiments evaluating this effect in other materials (hydroethanolic extract from its barks, purified fractions of quinovic acid glycosides and oxindole alkaloids) were carried out, as well as assays to identify the protection mechanism on UV-induced DNA damage. UV irradiation appears to reduce the effectiveness of the immune system, promoting the re-activation of the herpetic infection (Rooney et al., 1992).

Desmutagenesis would be detected with pre- and simultaneous treatment, whereas bio-antimutagenicity with post-treatment (Kuroda et al., 1992; Kojima et al., 1992). The presence of UV-absorbing compounds in cat's claw preparations may reduce the exposure of cells to this physical agent and, consequently, DNA damage. In regards to that, antioxidant effects are able to prevent reactive oxygen and nitrogen species formation or to scavenge these species before they cause oxidative damage to biomolecules as DNA. Previous studies on antioxidant effect of cat's claw extracts performed by Pilarski et al. (2006) have suggested higher activity for alcoholic extracts compared to aqueous, which has been associated with phenolic constituents, especially proanthocyanidins. In our study, it is suggested that cat's claw polyphenols might have contributed to the pronounced desmutagenic activity since purified fractions displayed lower protective effect on DNA damage in both pre- and simultaneous treatment. Moreover, Falkiewicz and Łukasiak (2001) have reported that cat's claw non-phenolic compounds such as ursolic and oleanoic acid might be considered efficient protectors against lipid peroxidation, which may also justify the higher desmutagenic activity of UT mac compared to the purified fractions.

A possible mechanism for the bio-mutagenic action of *U. tomentosa* would be its trigger initiation in DNA repair systems. This repair effect has been previously shown in studies evaluating cat's claw extracts as well as isolated compounds. Sheng et al. (2001) showed that oral consumption of C-Med-100 commercial extract enhances DNA repair in humans after chemotherapy-induced damage. In another study, the pteropodine (a cat's claw POA) reduced the frequency of sister-chromatid exchanges and micronucleated polychromatic erythrocytes in mice and thus, this compound may be considered an effective antimutagen (Paniagua-Pérez et al., 2009).

Inasmuch as the purified fractions (QAPF and OAPF) and UT mac displayed similar antimutagenic effect in post-treatment, oxindole alkaloids (including the pteropodine) and/or quinovic acid glycosides may be responsible for increased effect in this step.

Adulteration with *U. guianensis* influenced negatively the bioantimutagenesis activity provided by UT mac (an antagonistic action might be shown). This emphasizes the need to establish quality control testing in order to prevent adulteration of raw materials since different biological effects can be obtained.

Overall, the reduction observed in oxidative DNA damage is possibly the result of enhanced base excision repair and an inherent antioxidant effect, being these biological processes mediated by different compounds.

5. Conclusion

Cat's claw bark hydroethanolic extracts may be considered promising for antiherpetic therapy due to their anti-inflammatory, immunomodulatory, antiherpetic and antimutagenic effect. In view of the fact that both alkaloid-enriched fractions may result in reduction or even loss of antiherpetic activity, the use of crude cat́s claw barks extracts should be prioritized in order to obtain a synergistic effect, promoting improvement in the clinical presentation of herpes. *In vivo* studies should be carried out in order to confirm the *in vitro* results.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

The Transparency document associated with this article can be found in the online version.

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