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**RESEARCH ARTICLE**

# Hepatoprotective effects and HSV-1 activity of the hydroethanolic extract of *Cecropia glaziovii* (embaúba-vermelha) against acyclovir-resistant strain

Fabrícia Petronilho[1,2], Felipe Dal-Pizzol[1], Geison Modesti Costa[3], Virgínia Demarchi Kappel[3], Simone Quintana de Oliveira[3], Jucélia Fortunato[4], Vanilde Cittadini-Zanette[4], José Cláudio Fonseca Moreira[5], Cláudia Maria Oliveira Simões[6], Felipe Dal-Pizzol[1], and Flávio Henrique Reginatto[3,6]

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**Abstract**

*Context*: *Cecropia glaziovii* Snethl. (Cecropiaceae), commonly known as "embaúba-vermelha", is widely distributed throughout Latin America and has been reported in Brazilian folk medicine to treat cough, asthma, high blood pressure and inflammation.

*Objective*: Investigate the hepatoprotective properties of crude hydroethanolic extract of *C. glaziovii* as well as its *in vitro* antioxidant and antiviral (HSV-1 acyclovir resistant strain) activities.

*Materials and methods*: The hepatoprotective effect, the antioxidant properties and antiviral activity of crude hydroethanol extract (RCE40) from *C. glaziovii* leaves were evaluated by carbon-tetrachloride ($CCl_4$)-induced hepatotoxicity, by TBARS (thiobarbituric acid reactive species) and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assays, respectively.

*Results*: The RCE40 extract (20 mg/kg) inhibited lipid peroxidation on liver in post injury treatment and decreased serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). In addition, in this protocol the RCE40 (20 mg/kg) enhanced the activity of hepatic enzymes (SOD/CAT) which are involved in combating reactive oxygen species (ROS), suggesting that it possesses the capacity to attenuate the $CCl_4$-induced liver damage. Moreover the RCE40 (20 mg/kg) inhibited TBARS formation induced by several different inductors of oxidative stress showing significant antioxidant activity, including physiologically relevant concentration, as low as 2 µg/mL. Concerning antiviral activity, the RCE40 was effective against herpes simplex virus type 1 replication (29R acyclovir resistant strain) with $EC_{50} = 40$ µg/mL and selective index (SI) = 50.

*Discussion and conclusion*: These results indicate that *C. glaziovii* could be a good source of antioxidant and anti-HSV-1 lead compounds.

**Keywords:** *Cecropia glaziovii*, Cecropiaceae, hepatoprotective effect, antioxidant activity, anti HSV-1 activity, acyclovir-resistant strain

## Introduction

*Cecropia glaziovii* Snethl. (Cecropiaceae), commonly known as "embaúba-vermelha", is widely distributed throughout Latin America and in the Southeast and South of Brazil (Berg & Rosseli, 2005). It has been reported in Brazilian folk medicine to treat cough, asthma, high blood pressure, inflammation, and is used as a diuretic (Lorenzi & Matos, 2008; Pio Correa, 1978).

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Several biological effects were described in the literature for *C. glaziovii* such as antihypertensive (Lima-Landmann et al., 2007; Ninahuaman et al., 2007), anxiolytic and antidepressant (Rocha et al., 2002; 2007), bronchodilator (Delarcina et al., 2007), antiacid secretion and antiulcer (Souccar et al., 2008).

Concerning their chemical composition, previous studies have reported phenolic compounds such as *C*-glycosylflavonoids, proanthocyanidins and phenolic acids in *Cecropia* species (Andrade-Cetto & Wiedenfeld, 2001; Tanae et al., 2007; Costa et al., 2011). These polyphenolic compounds are well known as inhibitors of herpes simplex virus type 1 (HSV-1) replication (Fortin et al., 2002; Bettega et al., 2004), antibacterial agents (Esquenazi et al., 2002) and as free radical scavengers and inhibitors of lipid peroxidation (Rice-Evans et al., 1996; De Oliveira et al., 2003).

As far as we are aware, there are no reports in the literature investigating the hepatoprotective effects and antioxidant activity of *C. glaziovii* with the exception of one anti HSV-1 research for this species (Silva et al., 2010). Thus, the aim of this study was to investigate the hepatoprotective properties of crude hydroethanolic extract of *C. glaziovii* as well as its *in vitro* antioxidant and antiviral (HSV-1 acyclovir resistant strain) activities.

## Materials and methods

### Plant material

The leaves of *C. glaziovii* Snethl. (Cecropiaceae) were collected in Criciúma, State of Santa Catarina, Brazil in August 2003. The plant material was identified by Dr. Vanilde C. Zanette and a voucher specimen (CRI 7371) was deposited in the Herbarium Pe Dr. Raulino Reitz (Universidade do Extremo Sul Catarinense).

### Preparation of the extracts

Air-dried leaves (50 g) of *C. glaziovii* were crushed and extracted under reflux (90°C) with 500 ml of ethanol 40° GL (Gay-Lussac – alcohol concentration unit) for 30 min. After cooling, the extract was filtered and then evaporated under reduced pressure to dryness yielding the crude hydroethanolic extract (RCE40).

### Chemical characterization of the RCE40 extract by high-performance liquid chromatography (HPLC)

The qualitative analysis of phenolic compounds was performed on methodology previously described by Costa et al. (2011). Briefly, a PerkinElmer Brownlee Choice C18 column (150 × 4.6 mm i.d.; 5 mm) was employed and a gradient combining solvent A (acetonitrile) and solvent B (acetic acid 1%, adjusted to pH 3.0) as follows: 0–30 min, linear change from A–B (5:95 v/v) to A–B (20:80 v/v); 30–40 min, isocratic A–B (20:80 v/v), with a flow rate of 1.0 mL/min, detection at 340 nm and UV spectra monitored over a range of 450–200 nm. The injection volume was 10 μL and the peaks were characterized by comparing the retention time, UV spectra and by the co-injection of the sample with the reference standards.

### Antioxidant activity

#### In vitro *assay*

The antioxidant activity of *C. glaziovii* crude extract (RCE40) was estimated by the inhibition of thiobarbituric acid reactive substances (TBARS) formation induced by three different free radical generators in a lipid rich substrate (Polydoro et al., 2004). Briefly, 500 μl of 0.1% (w/v) lipid homogenate in phosphate buffer (pH 7.4) was mixed with 500 μl of trichloroacetic acid (10%) and centrifuged at 1,200*g* (10 min). The RCE40 extract at different concentrations (2, 20, 200 μg/mL) was added to a test tube together with 2,2-azobis (2-amidino-propane) dichloride (AAPH-0.5 M), iron sulfate ($FeSO_4$-0.145 mM) or hydrogen peroxide ($H_2O_2$-0.4 M) solutions to induce lipid peroxidation. The tubes were incubated at room temperature for 15 min and 500 μL of thiobarbituric acid (TBA-0.67%) was added and heated at 100°C for 30 min. After cooling, the absorbances were measured by a spectrophotometer (Micronal®) at 532 nm, and the results were expressed as nmol malondialdehyde equivalents (MDA)/mL of substrate. Controls were run simultaneously.

#### In vivo *assay*

*Animals*. Male Wistar rats (250–350 g, 2–3 months old) from our own breeding colony were housed in five per cage under 12-h light/dark cycle (lights on 7:00 a.m.) at constant temperature of 23° ± 1°C. Animals ($n = 60$) were maintained with food (Nuvilab® chow) and water *ad libitum*. *In vivo* studies were performed in accordance with the National Institutes of Health guidelines and with the approval of the local ethics committee.

*Treatment and liver injury induction*. In the post injury protocol an acute liver injury was induced by an i.p. administration of $CCl_4$ (5 mg/kg, dissolved in soy oil 1:1, v/v %) as previously described (Lee et al., 2003). The animals were divided into five groups, in six animals per group: 1) control (ethanol 1% p.o.); 2) $CCl_4$ (5 mg/kg i.p.); 3) $CCl_4$ + RCE40 (20 mg/kg in ethanol 1%, p.o.); 4) $CCl_4$ + RCE40 (40 mg/kg in ethanol 1%, p.o.); 5) $CCl_4$ + NAC (*N*-acetylcysteine) (100 mg/kg i.p.). All treatments were administered for 3 h after $CCl_4$ administration and continued every 12 h for 2 consecutive days. Plasma obtained from the tail vein was collected from each rat, 12 h after $CCl_4$ administration for the determination of enzymes ALT and AST. 12 h after, rats were killed by decapitation, the liver was isolated and fixed in a 4% formalin solution for histological analyses or immediately stored at –70°C until TBARS assay. In the prophylactic protocol (Ilavarasan et al., 2003) rats were divided into five groups as described above. In this protocol all groups were treated once, during seven days, and on the seventh day, $CCl_4$ (5 mg/kg, dissolved in soy oil 1:1, v/v % i.p.) was administered except in the group 1. Plasma obtained from the tail vein was collected from each rat, 6 h

after $CCl_4$ administration for the determination of serum ALT and AST. After 24 h, rats were killed by decapitation, the liver isolated and fixed in a 4% formalin solution for histological analyses or immediately stored at –70°C until TBARS assay.

*Determination of hepatic lipid peroxidation*. TBARS in liver homogenate was determined by reaction with TBA and by an index of lipid peroxidation (Draper & Hadley 1990). Briefly, the samples were mixed with 1 ml of trichloroacetic acid 10% and 1 ml of TBA 0.67%; this mixture was then heated in a boiling water bath for 30 min and the malondialdehyde (MDA) equivalents were determined by the absorbance at 532 nm using 1,1,3,3-tetramethoxypropane as an external standard. The results were expressed as nmol MDA equivalents per milligram of protein (nmol/mg) (Lowry et al., 1951).

*Antioxidant enzyme activities*. Superoxide dismutase (SOD) activity was measured spectrophotometrically by the inhibition rate of autocatalytic adrenochrome formation in a reaction buffer containing 1 mM adrenaline/50 mM glycine-NaOH (pH = 10.2) as previously described (Bannister & Calaberese 1987). The method of Aebi (1984) was used to analyse catalase (CAT) activity. Homogenates were sonicated in 50 mM phosphate buffer (pH 7.0) and the resulting suspension was centrifuged at $3000 \times g$ for 10 min. The supernatant was used for enzyme assay. CAT activity was assayed by measuring the rate of decrease in $H_2O_2$ absorbance at 240 nm. Enzyme activities were reported as units/mg of protein.

*Determination of serum biochemical parameters*. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined routinely by commercial available kits (Labtest, Brazil).

*Histological examination of liver*. For histopathological analyses after fixation, excised liver tissues were soaked in paraffin and then routinely stained with hematoxylin and eosin. A blinded experienced pathologist performed histopathological analyses.

*Antiviral assay and cytotoxicity evaluation–Cell viability test*. The cytotoxicity evaluation was performed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] method, according to Takeuchi et al. (1991) and Sieuwerts et al. (1995) with minor modifications. Briefly, VERO cell cultures ($2 \times 10^5$ cells/mL) were prepared in 96-well tissue culture plates (Corning®). After a 24 h period of incubation at 37°C under a humidified 5% $CO_2$ atmosphere, the cell monolayers were confluent, the medium was removed from the wells, and 200 µL of each extract/fraction dilutions (1:2 – ranging from 2000 to 15.6 µg/mL prepared in cell culture medium) was added to each well. As a cell control, only 200 µL of medium was added to the cells. The plates were incubated under the same conditions cited above. After 4 days, the medium was removed by suction from all wells and 50 µL of MTT (Sigma®, 1 mg/mL) solution prepared in cell culture medium was added to each well and the plates were incubated once more for 4 h. After the MTT solution was removed without disturbing the cells, 100 µL of DMSO was added to each well to dissolve the formazan crystals. After gently shaking the plates, the crystals were completely dissolved, and the absorbances were read on a multiwell spectrophotometer (Bio-Tek®, Elx 800) at 540 nm. The $CC_{50}$ was defined as the cytotoxic concentration of sample that reduced the absorbance of treated cells to 50% when compared with that of the cell control.

*Antiherpes assay*. VERO cell cultures ($2 \times 10^5$ cells/mL) were prepared in the same way as described above and, when the cell monolayers were confluent, the medium was removed from the wells and 100 µL/well of non-cytotoxic concentrations ($\leq CC_{50}$ values) of the extracts and 100 µL/well of HSV-1 (29-R acyclovir resistant strain – University of Rennes, France) at 0.5 of multiplicity of infection (MOI) were added simultaneously to the cells. Cell and viral controls were performed by adding only 200 µL of minimum essential media (MEM) or 200 µL of viral suspension, respectively. The plates were incubated for 96 h. The same MTT method used to evaluate cell viability was followed. The percentages of protection were calculated as $[(A–B) \times 100/(C–B)]$, where A, B and C indicate the absorbances of the extracts, virus and cell controls, respectively. Each obtained $EC_{50}$ value was defined as the effective concentration that reduced the absorbance of infected cells to 50% when compared with cell and virus controls. The selective index was calculated as $CC_{50}/EC_{50}$.

*Statistical analysis*. For antioxidant activity, the results were expressed as means ± S.D. and $p$ values were considered significant when $p < 0.05$. Differences in experimental groups were determined by ANOVA. Comparison between means was carried out using a Newman–Keuls test. For antiviral activity the 50% cytotoxic ($CC_{50}$) and 50% effective ($EC_{50}$) concentrations were calculated from concentration-effect curves after linear regression analysis. The results represent the mean ± standard error (S.E.) of the mean values of three different experiments.

## Results and discussion

The antioxidant properties, hepatoprotective effect and antiviral activity of crude hydroethanol extract (RCE40) from *C. glaziovii* leaves were evaluated, respectively, by using TBARS, carbon-tetrachloride ($CCl_4$) induced hepatotoxicity and MTT assays.

### Antioxidant activity

#### In vitro *assay*

The *in vitro* antioxidant activity assay employed three different oxidant systems to induce TBARS formation [AAPH (0.5 M), $FeSO_4$ (0.145 mM) or $H_2O_2$ (0.4 M) solutions]. The extract (RCE40) in all concentrations tested

(2, 20 and 200 μg/mL, respectively) inhibited TBARS formation induced by all inductors of oxidative stress, showing a significant antioxidant activity, including physiologically relevant concentration (as low as 2 μg/mL) (Table 1). In addition, these results showed that RCE40 could act as a lipid peroxidation chain breaker, direct scavengers of peroxyl/hydroxyl/hydrogen peroxide and as an iron chelator.

The most valuable exogenous reactive oxygen species (ROS) generator in biological systems is $H_2O_2$. In particular, $H_2O_2$ is a potential source for hydroxyl radical (·OH), one of the most dangerous radicals formed through the Fenton reaction in the presence of transition metal ions, as iron. Additionally, azo-initiators like AAPH induce the formation of peroxyl radicals, capable of initiating the chain reaction of lipid peroxidation (Cao et al., 1997; Martini & Termini 1997; Kanno et al., 2003).

### *In vivo* assay

In this work, we showed the effect of RCE40 on hepatic oxidative damage through four parameters: lipid peroxidation (TBARS), hepatic antioxidant enzymes (SOD/CAT), serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity and by the degree of inflammatory response and hepatic necrosis. These treatments with RCE40 (20 and 40 mg/kg) on $CCl_4$-induced liver injury were compared to the effect of *N*-acetylcysteine (NAC) as the antioxidant standard. Additionally, we used two protocols: injury protocol (after $CCl_4$ administration) and prophylactic protocol (before $CCl_4$ administration).

In order to determine the antioxidant effects of RCE40, we determined TBARS levels in liver homogenate. Administration of $CCl_4$ increased TBARS content in comparison to the control group as expected. All treatments in the post injury, inhibited lipid peroxidation on liver (Figure 1A). On the other hand, in the prophylactic protocol liver protection was not detected against oxidative damage induced by $CCl_4$. In contrast RCE40 (20 mg/kg) potentiated the oxidative damage induced by $CCl_4$ administration (Figure 1B).

The thiobarbituric acid reactive substances (TBARS) is an indirect measurement of lipid peroxidation (Nan et al., 2003). Lipid peroxide levels in a liver tissue were found to be significant in $CCl_4$-challenged rats (Halliwell et al., 1995). These free radicals trigger cell damage through two mechanisms namely covalent binding to cellular macromolecules and lipid peroxidation, which affect the ionic permeability of the membrane structure (Basu, 2003). The RCE40 effect in the post injury protocol may be attributed to the antioxidant activity of the extract which is exhibited by scavenging the $CCl_3$ radical

Table 1. Inhibition of lipid peroxidation by hydroethanolic extracts from *C. glaziovii* leaves induced by $H_2O_2$, $FeSO_4$, AAPH.

| Extracts and free radical generators | MDA (nmol/mL substrate) | | | |
|---|---|---|---|---|
| | Control | 2 μg/mL | 20 μg/mL | 200 μg/mL |
| RCE40 | 0.087 ± 0.009 | | | |
| $H_2O_2$ | 0.133 ± 0.008[a] | | | |
| RCE40 + $H_2O_2$ | | 0.073 ± 0.01[b] | 0.073 ± 0.010[b] | 0.083 ± 0.013[b] |
| $FeSO_4$ | 0.150 ± 0.016[a] | | | |
| RCE40 + $FeSO_4$ | | 0.063 ± 0.013[b] | 0.109 ± 0.008[b] | 0.078 ± 0.011[b] |
| AAPH | 0.399 ± 0.020[a] | | | |
| RCE40 + AAPH | | 0.206 ± 0.029[b] | 0.226 ± 0.067[b] | 0.267 ± 0.053[b] |

Each value represents mean ± SD.
[a]Statistically different from control ($p < 0.05$) by ANOVA test.
[b]From $H_2O_2$, $FeSO_4$, AAPH groups ($p < 0.05$) by Newman–Keuls test.

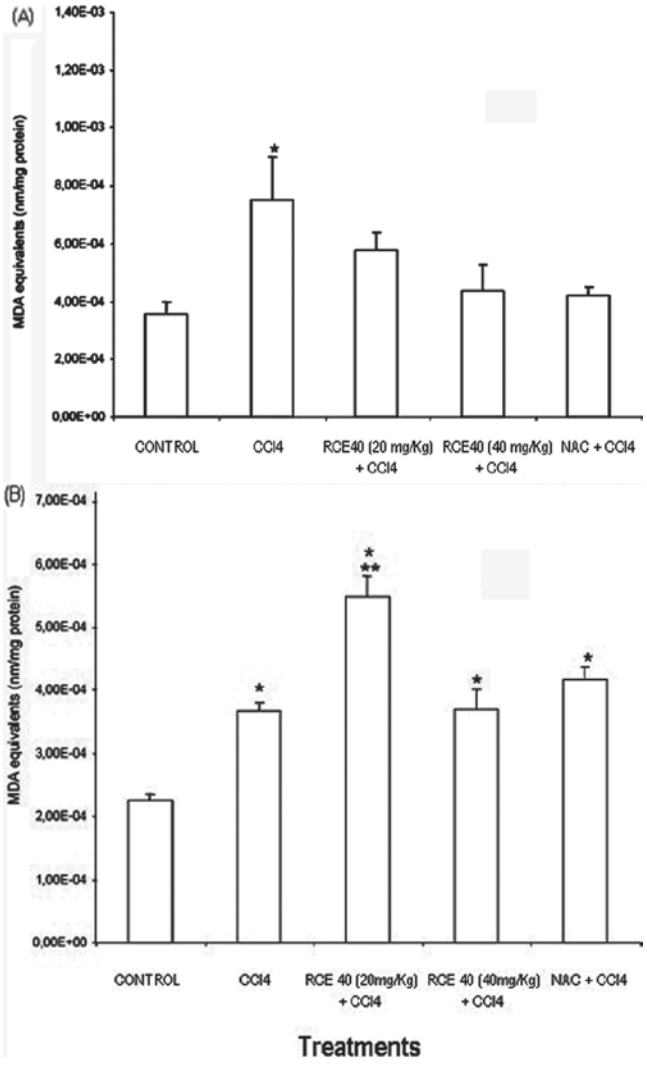


Figure 1. (A) TBARS values in carbon tetrachloride intoxicated rat liver treated with an RCE40 extract of *C. glaziovii* orally and NAC intraperitoneally in two protocols. In the post injury protocol, rats were pretreated with carbon tetrachloride ($CCl_4$) (5 mg/kg). Three hours later, rats were treated with RCE40 (20 and 40 mg/kg p.o.) or NAC (100 mg/kg i.p.) twice daily. Normal rats were given in ethanol 1%.Data are mean ± SD *Different from control, $p < 0.05$; **different from rats receiving $CCl_4$ alone, $p < 0.05$. (B) TBARS values in carbon tetrachloride intoxicated rat liver treated with an RCE40 extract of *C. glaziovii* orally and NAC intraperitoneally in two protocols. In the prophylactic protocol rats were pretreated with ethanol 1%, RCE40 or NAC in the same doses that the post injury protocol, once a day, for seven days, on the seventh day, a single dose of the mixture of $CCl_4$ (5 mg/kg) and soy oil (1:1, v/v%,5 ml/kg i.p.) was administered. Data are mean ± SD *Different from control, $p < 0.05$; **different from rats receiving $CCl_4$ alone, $p < 0.05$.

generated due to the metabolic transformation of $CCl_4$ in the liver or by the interruption of the lipid peroxidation progression (Ahmed & Urooj, 2010). This protection is of similar magnitude to the effects of NAC, a well-known and potent antioxidant. The higher protection exhibited by the post injury when compared to the prophylactic protocol could be associated to different factors. Probably the antioxidant compounds do not accumulate or are metabolized to inactive, non- toxic compounds (Skibola & Smith 2000; Galat & O'Brien 2004), and these are in accordance with our results.

Concerning to hepatic antioxidant enzyme activities, there was a significant decrease of the SOD/CAT activity with $CCl_4$ administration in the post injury protocols. The decrease in antioxidant enzymes activities induced by $CCl_4$ in post injury protocols may be secondary to the inactivation caused by free radicals (Silk-Lesiuk et al., 2003). The superoxide dismutase enzyme (SOD) is considered the most effective antioxidant defense that the body has against superoxide free radicals. However, in the process of removing superoxide free radicals, SOD rarely operates alone, and requires the catalase enzyme (CAT) to remove hydrogen peroxide molecules which are by-products of the reactions created by SOD (Halliwell & Gutteridge, 1999). In this protocol, RCE40 (20 and 40 mg/kg) increased the activity of antioxidant liver enzymes (Figure 2A), as well as increased SOD/CAT relation in the prophylactic protocols (Figure 2B).

The analyses of serum biochemical parameters showed that the serum levels of ALT and AST in both prophylactic and post injury protocols were significantly increased in comparison to the control, suggesting hepatocellular death in this model. In the post injury protocol, RCE40 (20 and 40 mg/kg) decreased serum levels of ALT and AST, while in the prophylactic treatment these protective effects were less pronounced and the high doses of RCE40 (40 mg/kg) increased ALT and AST plasmatic levels (Table 2).

In relation to the effect of RCE40 on histological changes in the liver, we observed that liver histopathology after $CCl_4$ administration showed lobular disarray, fatty degeneration, potent inflammatory cell infiltration, and severe necrosis of hepatocytes (Figure 3A). In the post injury protocol, RCE 40 mg/kg (Figure 3B) reduced $CCl_4$-induced necrosis and inflammation. In the prophylactic treatment the lower doses minimally reduced the damage induced by $CCl_4$ (data not shown). In contrast, the highest dose of both extracts increased the hepatotoxicity of $CCl_4$ in the prophylactic protocol (Figure 3C).

In the present study, we found that the post injury treatment with the RCE40 inhibits $CCl_4$-induced damage as evidenced by decreased serum activities of AST and ALT, decreased hepatic lipid peroxidation, and by the histological observations. The decrease in antioxidant enzymes activities induced by $CCl_4$ administration may be also resulting from inactivation caused by lipid peroxides or enhanced free radical concentration in oxidative stress conditions (Silk-Lesiuk et al., 2003).

The chemical analysis by HPLC-UV/DAD, RCE40 showed isovitexin (rt = 28.4 min) as the major compound. Furthermore chlorogenic acid (rt = 13.7 min), isoorientin

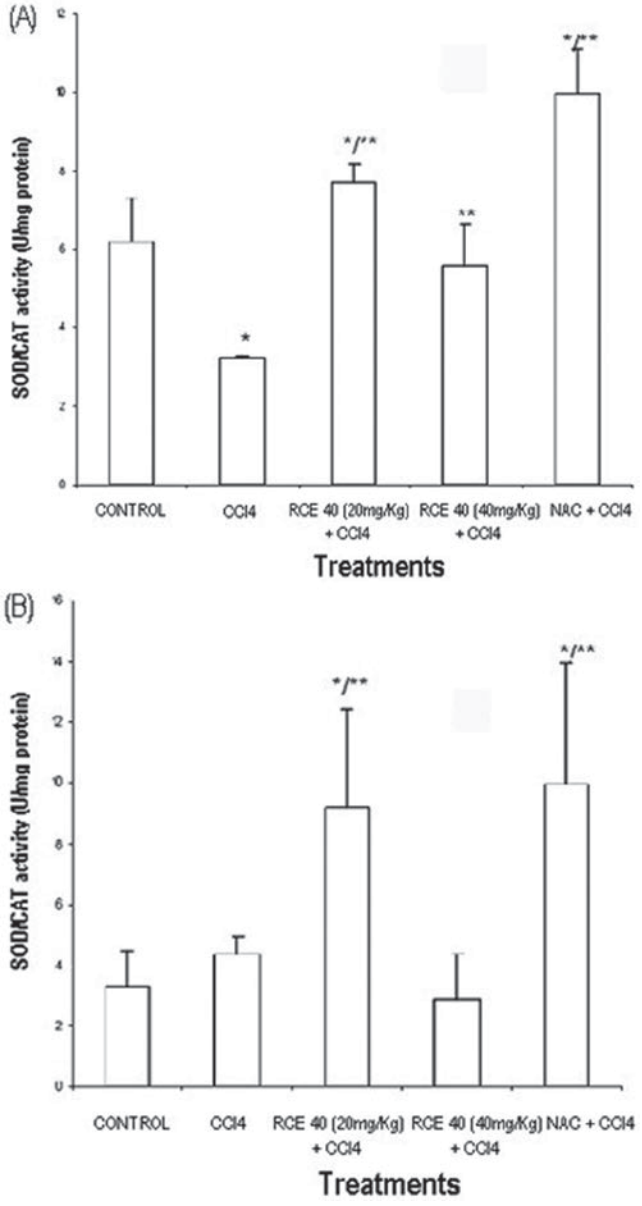


Figure 2. (A) SOD/CAT values in carbon tetrachloride intoxicated rat liver treated with a RCE40 extract of *C. glaziovii* orally and NAC intraperitoneally in two protocols. In the post injury protocol, rats were pretreated with carbon tetrachloride (5 mg/kg). Three hours later, rats were treated with RCE40 (20 and 40 mg/kg p.o.) or NAC (100 mg/kg i.p.) twice daily. Normal rats were given in ethanol 1%. Data are mean ± SD *Different from control, $p < 0.05$; **different from rats receiving carbon tetrachloride alone, $p < 0.05$. (B) SOD/CAT values in carbon tetrachloride intoxicated rat liver treated with a RCE40 extract of *C. glaziovii* orally and NAC intraperitoneally in two protocols. In the prophylactic protocol rats were pretreated with ethanol 1%, RCE40 or NAC in the same doses that the post injury protocol, once a day, for seven days, on the seventh day, a single dose of the mixture of $CCl_4$ and soy oil (1:1, v/v%, 5 ml/kg i.p.) was administered. Data are mean ± SD *Different from control, $p < 0.05$; **different from rats receivingcarbon tetrachloride alone, $p < 0.05$.

Table 2. Effect of RCE40 extract in plasmatic ALT and AST levels in rats with liver injury induced by carbon tetrachloride.

| Group | ALT (UI/L) | AST (UI/L) |
|---|---|---|
| Control | 40 ± 3 | 20 ± 2.3 |
| $CCl_4$ | 901 ± 56[a] | 569 ± 43[a] |
| $CCl_4$ + RCE40 (20 mg/kg)[b] | 210 ± 23[c] | 140 ± 19[c] |
| $CCl_4$ + RCE40 (40 mg/kg)[b] | 168 ± 35[c] | 123 ± 36[c] |
| RCE40 (20 mg/kg) + $CCl_4$[d] | 506 ± 34[c] | 445 ± 43 |
| RCE40 (40 mg/kg) + $CCl_4$[d] | 1590 ± 98[c] | 750 ± 53[c] |

Each value represents mean ± SD.
[a]different from control ($p < 0.05$). [b]Post injury group;
[c]different from CCl4 ($p < 0.05$). [d]Prophylactic group.

(rt = 24.7 min) and orientin (rt = 25.3 min) (Figure 4) were also identified. Since the ability of plant extract to scavenge ROS and/or metal chelating seems to be related to the chemical structure of phenolic compounds (Rice-Evans et al., 1996; Halliwell & Gutteridge, 1999), the biological effects herein described could be related to flavonoids present in the RCE40 extract, which are involved in free radical-scavenging and metal ion-chelating (Heras et al., 1998; Higdon & Frei 2003; Kaneko et al., 2003; Velázquez et al., 2003).

## Antiviral activity

The RCE40 extract from the leaves of *C. glaziovii* showed low cytotoxicity against VERO cell cultures ($CC_{50}$ > 2.000

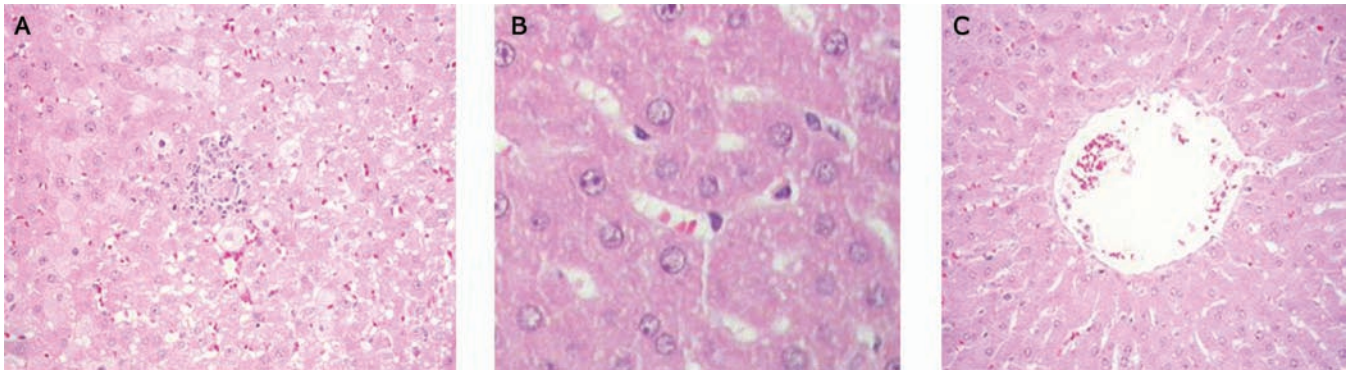


Figure 3. (A) Effects of *C. glaziovii* in post injury or prophylactic protocols on $CCl_4$-induced liver damage in rats. Liver tissue sections from rat receiving $CCl_4$. HE × 400, representative of three animals/group. (B) Effects of *C. glaziovii* in post injury or prophylactic protocols on $CCl_4$-induced liver damage in rats. Liver tissue sections from rat with $CCl_4$ and RCE40 (40 mg/kg) post injury protocol. HE × 400,representative of three animals/group. (C) Effects of *C. glaziovii* in post injury or prophylactic protocols on $CCl_4$-induced liver damage in rats. Liver tissue sections from rat with $CCl_4$ and RCE (40 mg/kg) prophylactic protocol. HE × 400, representative of three animals/group

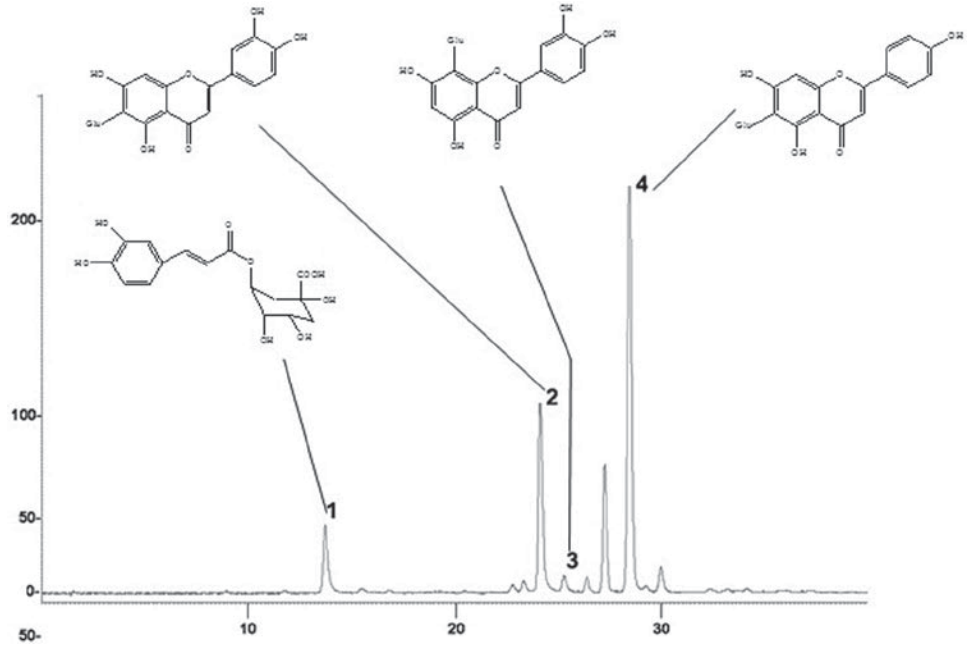


Figure 4. HPLC chromatogram of crude hydroethanol extract (RCE40) from *C. glaziovii* leaves, with respectively UV-spectra (340 nm) of identified compounds: 1. Chlorogenic acid, 2. Isoorientin, 3. Orientinand 4. Isovitexin. For chromatographic conditions, see Material and methods section.

μg/mL) and inhibited significantly the replication of HSV-1 (29R strain), ($EC_{50}$ = 40 μg/mL) yielding a good selectivity index (SI = 50). Recently, our research group showed the *in vitro* anti HSV-1 (KOS strain–acyclovir sensitive) and HSV-2 (333 strain) of the crude extract obtained from *C. glaziovii* leaves and their *C*-glycosylflavonoid-enriched fraction (Silva et al., 2010). Herein, we demonstrate that RCE40 extract is active against HSV-1 (29R strain–acyclovir resistant).

There are several reports of antiviral activity of phenolic compounds (Okuda et al, 2005; Orhan et al., 2010). Chlorogenic acid and its related compounds are abundant plant polyphenols that have anti HSV-1 activity (Chiang et al., 2002; Wang et al., 2009; Nolkemper et al., 2010). In the same way, the anti-HSV activity of naturally occurring flavonoids has also been described (Lyu et al, 2005; Schnitzler et al., 2010). Therefore, our findings suggest that at least part of the anti HSV-1 effects of the RCE40 extract might be attributable to its phenolic composition.

Since the efficacy of acyclovir is limited by the recent increase in the resistance of virus and recurrence of latent virus, particularly from immunocompromised patients (Cheng et al., 2002), the data presented herein indicate that the *C. glaziovii* leaf extracts have HSV-1 activity against acyclovir resistant strain and should be considered as a potential new sources of antiviral agents. However, further studies to elucidate the active principles and the underlying mechanism responsible for the antiherpes effects of phenolic compounds present in this extract as well as the mechanism responsible for the protective effects are needed.

## Conclusion

The results showed that *C. glaziovii* leaves RCE40 extract possess *in vitro* and *in vivo* capacities of inhibiting lipid peroxidation and attenuating the $CCl_4$-induced hepatic damage respectively. This extract was also effective against herpes simplex virus type 1 replication (29R strain, acyclovir-resistant). Finally, the HPLC fingerprint of RCE40 showed the presence of isoorientin, orientin, isovitexin and chlorogenic acid as major compounds. Further studies are in progress to determine the exact mechanism associated with pharmacological activities detected, their possible synergistic effect, as well as the toxic effects observed in the prophylactic treatment group.

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## Declaration of interest

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