v. 3, n. 6, 2023

International Journal of Biological and Natural Sciences

HEPATOPROTECTIVE ACTIVITY OF *MIMOSA LACERATA* AND *CROTON LECHLERI* ON THE HEPG2 CELL LINE

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INTRODUCTION

Liver diseases represent an important health problem around the world and in Mexico they represent the fifth cause of death in the economically active population (Torres-González et al., 2014).

In Mexico, approximately 60% of the population uses some medicine from plants to treat diseases (Torres-González et al., 2014). According to the 2004 WHO report; 60% of the Mexican population consumes plants for medicinal use and more than 80% of the world population uses botanical preparations as medicines (Salazar-Aranda et al., 2009).

The liver plays a fundamental role in the metabolism of most nutrients as it is a mainly metabolic organ and it carries out a series of physiological and biochemical processes related to protein and energy metabolism (García de Lorenzo and Rodriguez, 2008); This can be affected in numerous inflammatory processes such as viral infections, metabolopathies, autoimmune processes, different genetic defects, and toxicity from drugs and their metabolites (Tejada, 2010).

The body identifies almost all drugs as xenobiotics and subjects them to a series of processes to make them more soluble and facilitate their elimination through bile or plasma; it is the endoplasmic reticulum of the hepatocyte that is the site of metabolism of both endogenous and exogenous chemical substances. This mission of the liver in the biotransformation of drugs makes it the organ most susceptible to injury (Angosto et al., 2017).

Therefore, this work will have as objective to evaluate the effectiveness of the natural extracts of *Croton lechleri* and *Mimosa lacerata* as hepatoprotective agents by attenuating liver damage induced by CCl_4.

MATERIAL AND METHODS

Obtaining the sample

The vegetable samples were obtained by the Comprehensive Food Production Laboratory of the Faculty of Biological Chemical Sciences, they were subjected to the drying process in a microbiological oven at a temperature of 30°C for 96 hours. After this time, a total of 471 g of dry sample of *Croton lechleri* and 30 g of dry sample of *Mimosa lacerata* were weighed. The dry samples were crushed separately in a mill (GRINDOMIX GM 200), for their subsequent solid-liquid extraction (sequential maceration) using ethanol as a solvent with ethanolic washes for 48 hours with two repetitions for each sample, after time, the solvent was removed. Filtered and distilled in a rotary evaporator to obtain the extracts, which were transferred to glass containers where they were allowed to dry at room temperature.

To calculate the yield percentage of each extract obtained in this process, the following formula was used:

$$\% R = \frac{g\ of\ extract\ obtained}{\text{g of macerated vegetable material}}\ x\ 100$$

Once the dry extracts were obtained and the yield percentages calculated, these extracts were resuspended in 96% ethanol and homogenized in a vortex for 30 minutes, they were stored in amber containers for conservation and subsequent analysis in the administration in biological models.

Antioxidant activity

The antioxidant activity of the extracts was evaluated, according to the free radical 2,2-diphenyl-1-picrylhydrazil (DPPH) method described by Brand-Williams (1995) with some modifications, producing a colorimetric reaction.

For the analysis, aliquots of 0.025g of dry sample were taken, 5 mL of methanol were added, each sample tube previously labeled and centrifuged at 4000 rpm for 5 minutes.

Subsequently, different microvolumes of 5 µL, 10 µL and 20 µL of sample volumetric with DPPH at 2 mL were taken. The samples were kept in the dark.

The absorbances were read spectrophotometrically at a wavelength of 520 nm at 30 minute time intervals. Absorbance differences were expressed as inhibitory concentration IC_{50} (in mg mL^{-1}).

$$\%\ inhibition = \frac{Abs_{t=0} - Abs_{t=final}}{Abs_{t=0}}\ x\ 100$$

Thin layer chromatography

The hydrolyzed samples (0.5 µl) were analyzed by thin layer chromatography (TLC) in a stationary phase of silica gel 60, using ethyl acetate as solvent in a ratio of 15:1, visualization of the plates was carried out with a lamp. UV at 340 nm.

Cell culture

The vial was tempered with the aliquot of the HepG2 cell line in a frozen state at -80°C; for this, the cells were washed with phosphate buffer in saline solution (PBS).

With the thawed cells, cultures were carried out in T-75 and T-25 flaks using the DMEM Advanced culture medium supplemented with 10% fetal bovine serum (SFB), 1% antibiotics (penicillin and streptomycin) and 1% L- Glutamine.

Once the culture reached a confluence of 70-80% in an approximate time of 48 hrs, the medium was removed and several washes were carried out with phosphate buffer in saline solution (PBS), 1 mL of trypsin was added, and incubated for 5 minutes at 37°C with 5% CO_2. After that time, 6 mL of DMEM culture medium with 10% FBS were added to neutralize the trypsin activity; This solution was collected in a 15 mL Falcon tube and

centrifuged for 5 minutes at 2000 rpm and 25 °C. Once centrifuged, the supernatant was decanted and the cell button was resuspended in 1 mL of culture medium.

Damage model with CCl_4

The trial was performed according to the methodology described by Delgado 2022 with some modifications. The necessary dose to induce the damage was established as 8 µL of CCl^4 in a determined time of 2 hours. In 6-well plates, 1 million cells/well were added, 12 hrs after adding the cells, the medium was removed and they were washed with PBS. Solutions were tested at a concentration of 0.4% CCl_4 in PBS with 0.05% DMSO. After 2 hours of exposure of the cells with CCl_4, the supernatant was removed and immediately placed in an ice bath to quantify the AST enzyme.

Hepatoprotection model

The evaluation of the hepatoprotective activity was carried out as reported by Delgado 2022 with small modifications: 1 million cells were seeded in 6-well plates, the medium was removed 12 hours later and washed with PBS. After this, the solutions of the extracts to be evaluated dissolved in PBS at a concentration of 10 µg/mL^{-1} were added and kept under incubation conditions for one hour (37 °C and 5% CO_2).

After this time, the solutions were withdrawn to add the xenobiotic; it was allowed to act under incubation conditions, the supernatant was removed and immediately placed in an ice bath to quantify the AST enzymatic activity.

The AST determination was carried out in the Ilab 300 plus equipment with the Instrumentation laboratory brand kit at an absorbance of 340 nm.

RESULTS

Extract yield percentage.

An 11.28% yield was obtained from the macerated sample of M. *lacerata* and a 5.31% yield of C. *lechleri* (Table 1).

	g macerated sample	g of extract	% performance
Croton lechleri	471	25.02	5.31
Mimosa lacerata	25	2.82	11.28

Table 1. Percentage yield of the extracts

Antioxidant activity

An increase in the percentage of antioxidant activity is observed with respect to the increase in the concentration of the samples. The antioxidant activity in both samples above 50% was observed from a concentration of 10 µg/mL^{-1} (Table 2).

Concentration µg/mL^{-1}	*M. lacerata*	*C. lechleri*
5	67.3 %	23.7 %
10	*74.5 %*	51.8 %
20	*85.3 %*	58.5 %

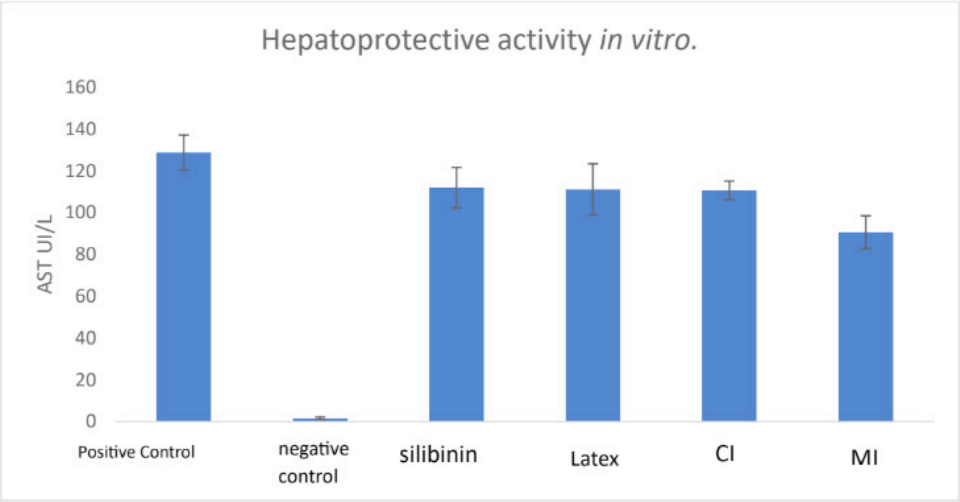
Table 2. Percentage of antioxidant activity of the extract of M. *lacerata* and C. *lechleri*.

Hepatoprotection model

The biological activity of the extracts analyzed at a concentration did not show statistical significance in the reduction of the damage caused by CCl_4. The ethanolic extract of M. *lacerata* showed a lower decrease in cell damage compared to the ethanolic extract of C. *lechleri* and the latex of C. *lechleri* (Graph 1).

DISCUSSION

No statistically significant differences were found in the reduction of AST enzymatic activity between the groups treated with the extract and the CCl_4 damage control group. This may be due to the lack of standardization



Graph 1. Quantification of AST enzymatic activity in the CCl_4-induced damage model. Positive Control (0.4% CCl_4), Negative Control (0.05% DMSO), Sibillin (10 µg/mL^{-1}), Latex (10 µg/mL^{-1}), Cl (C. *lechleri* 10 µg/mL^{-1}) y Ml (*M. lacerata* 10 µg/mL^{-1}).

of the *in vitro* assay technique and the low concentration of the extracts analyzed. As reported by Carrión et al (2009), the antioxidant activity of *C. lechleri* is optimal at a concentration greater than 10 µg mL^{1}, in this study that same concentration showed an activity of 51.8%, however, at this same concentration concentration M. *lacerata* showed a better activity with 74.5% in the ethanolic extract and this may be due to the different amount of metabolites present in each sample and the type of solvent used for their extraction. For the methanolic extract of M. *lacerata*, this activity is related to the presence of compounds such as flavonoids, phenols, and mainly tannins with the structure of polymersprofisetidine/ prorobonetidine, which at a concentration of 100 µg mL^{-1} present good antioxidant activity (Vargas-Álvarez et al. al., 2022). In addition, the presence of terpenic compounds obtained from the bark has demonstrated antioxidant and antimicrobial activity (Mayo et al., 2018).

Lock and Rojas (2004) describe the antioxidant activity of C. *lechleri* latex with a value of 935.4 ± 141 µM, mentioning that it contains a high concentration of antioxidant compounds, so this compound could be related to the biological activity of *C. lechleri* in the *in vitro* study, since the activity shown by the ethanolic extract and the latex is similar.

For C. *lechleri*, it has been found that there is no cytotoxicity of the extracts at concentrations of 900 to 187 µg/mL^{-1} (Chen et al., 1994). Likewise, C. *lechleri* latex does not cause deaths or signs of toxicity at doses of 2000 mg/kg^{-1} (Ceballos et al., 2016), so the doses used in this study could be high to increase biological activity. Of these compounds without reaching the already proven cytotoxic concentrations.

Torres-Gonzáles et al, (2014) in Mexico, evaluated the hepatoprotective activity of silymarin (SLM), silibinin (SLB) and silifos (SLP) against liver damage induced by CCl_4 in an in vitro model and have shown that silibinin a A dose of 150 µg/mL^{-1} is the best positive control for antioxidant activity. For this study, a concentration of 10 µg/mL^{-1} was used, therefore it was not possible to observe a significant decrease in the damage caused by CCl_4.

The CCl_4 damage induction assay reduced the antioxidant activity with a value of statistical significance ($p<0.05$), at a concentration of 10 mM in each extract. It was observed that PBS with 0.05% DMSO does not cause cell damage.

The best performance of the compounds is M. *lacerata* which presented a yield of 11.28% against 5.31% of *C. lechleri*, however, these yields could improve in both species worked.

Due to the above, it is important to search for the correct concentrations of the compounds, as well as to test various solvents and standardize the best extraction method with the best performance.

CONCLUSION

The extract with the highest yield was that of *Mimosa lacerata*, which showed a better yield with 11.28% in the extract. Due to the positive antioxidant capacity of the plant specimens studied in relation to their concentration of compounds, it is possible to improve the biological activity in vitro, fulfilling the hepatoprotective function, given the results expressed in the decrease in the enzymatic activity of AST. In addition, it is important to identify the metabolites that cause this activity.

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