Vol. 14(10), pp. 555-558, October, 2020
DOI: 10.5897/JMPR2020.7016
Article Number: CE1F03E64813
ISSN 1996-0875

http://www.academicjournals.org/JMPR

ACADEMIC JOURNALS
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Journal of Medicinal Plants Research

Full Length Research Paper

Evaluation of antioxidant and protective effect against genotoxic damage of two extracts of chaya leaves (*Cnidoscolus aconitifolius*)

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Received 10 July, 2020; Accepted 25 August, 2020

Stress, excessive consumption of processed foods, pollutants and many other factors of today's life have caused oxidative stress in our body and damage to genetic material in our cells, leading to diseases such as diabetes, hypertension, cancer, metabolic syndrome, among others. Medicine has returned to take up traditional remedies mainly in plants. Chaya (*Cnidoscolus aconitifolius*) has been studied in this regard, mainly for its antidiabetic effects; however, there is little information about its antioxidant and protective properties against genotoxic damage. The present study focuses on the evaluation of the antioxidant and protective properties to genotoxic damage of aqueous and methanolic extracts of chaya leaves (*C. chayamansa*) in a model in mice which were induced to oxidative stress and genotoxic damage by exposure to arsenic and low doses of streptozotocin. Genotoxic damage was assessed by micronucleus count and antioxidant properties were assessed in the extracts and plasmas of the mice by the ABTS radical and using Trolox as standard. Additionally, the extracts were analyzed by HPLC-MS/MS. Mice treated with aqueous extract showed better recovery than those treated with methanolic extract; however, no significant statistical differences were found between both groups. HPLC analysis suggests the presence of some compounds of interest such as ferulic acid, protocatechonic acid, riboflavin, kaempferol and beta carotene. These could be associated with the effects of protection against genotoxic damage and as antioxidants found in the study. The aqueous extract seems have the best performance against genotoxic damage and have better oxidative properties, so the consumption of chaya can be recommended.

Key words: *Cnidoscolus aconitifolius,* Chaya, genotoxic damage, oxidative stress, micronucleus, High Performance Liquid Chromatography (HPLC).

INTRODUCTION

Chaya, (*Cnidoscolus aconitifolius*) is a large-leaved shrub native to the Mayan region of Mexico and Central

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America belonging to the Euphorbiaceae family and traditionally used as an ornamental plant and for medicinal purposes by the populations of these regions (Loarca et al., 2010). Traditionally, beneficial effects are attributed to it in the treatment of diabetes, improving blood circulation, treating varicose veins, anemias and protective properties against genotoxic damage. However, little has been published of its antioxidant and protective properties against genotoxic damage (Sánchez and Méndez, 2013). We currently know that some compounds can lead to the formation of reactive oxygen species (ROS) such as the OH radical and the peroxides, causing the alteration of the oxide-reduction homeostasis inside the cell (Sánchez and Méndez, 2013; García et al., 2013). Similarly, genotoxic damage caused by overprocessed food consumption, current life stress, pollution etc. cause serious damage to the genetic material of the cells, which can end in severe complications such as cancer development and serious mutations. The present study aimed to evaluate the antioxidant and protective effect against genotoxic damage in Long Evans rats by ingestion of aqueous or methanolic extracts of leaves of *C. aconitifolius*.

MATERIALS AND METHODS

Chaya leaves were collected from private crops in the city of Durango, Dgo., Mexico (24° 56 '05' 'N; 104° 54' 43 '' W) in June 2018 and dried by natural convection until loss 97% of its weight. The chaya has an ID Boucher from the CIIDIR-Dgo Herbarium number 53,591. Long Evans rats, male and female, weighing 230±34 g were used. They were kept at 25 ± 3°C with 12-h circadian cycles. They were fed with Purina ® Roden Chow pellets with free access to water or extract according to their group of experimentation. The ethical guidelines established by the Mexican standard NOM-062-ZOO-1999 (2001) that specifies the care of laboratory animals were respected throughout the whole experiment and were certified by the MVZ Gerardo del Campo Galindo (Reg SAGARPA 10-0006, Ced. Prof. 975133).

Extracts

Two extracts were tested, one aqueous and the other methanolic. For the aqueous extract, 2.2 g of dry leaf were weighed and boiled in 300 ml of water for two minutes. This is the way the population has traditionally consumed chaya in Mexico. They were cooled and filtered through Whatman No. 40 paper. The solution was then graduated to 1 L with cold water. The methanolic extract was obtained by weighing 5.7 g of the leaf with 1 L of HPLC grade methanol and treated with ultrasound for 15 min at 14 kHz. Thereafter, it was left to rest for 8 days sheltered from light. At the end of this period, 100 mL were filtered through Whatman No. 40 paper and roto-evaporated at 40°C and the precipitate was reconstituted in 1 L of water. Both extracts were supplied *ad libitum* according to the experimental group.

Experimental design

Rats were randomly divided into three groups of six rats each. The first served as a healthy control group and received no treatment.

To induce damage by oxidative and genotoxic stress, Groups 2 and 3 were exposed, for two months, to concentrations of Arsenic in drinking water of 50 µg/l, (this concentration is 5 times more than that specified by the World Health Organization (2017), which is10 ug/ml, but it is the average concentration of As in the water of the population of Durango. Additionally, at the end of this period, rats in these groups were induced oxidative stress by administration of 40 mg/kg weight of streptozotocin. Two days after the administration of streptozotocin, treatments were started.

Treatments

Group 1 (control) was fed normally and rats had access to water *ad libitum*. Group 2 was treated with aqueous extracts and group 3 was treated with methanolic extract, both supplied as drinking water *ad libitum*.

Antioxidant power and genotoxic damage

Peripheral blood samples were taken from all groups at 5 times: before induction of oxidative stress and genotoxic damage (t1), after stress induction but before starting treatments (t2) and 2, 4 and 6 weeks of treatment (t3, t4 and t5 respectively). The antioxidant power of both extracts (aqueous and methanolic) and plasma of rats was evaluated by generating the ABTS radical (Marecek et al., 2017). Trolox as the reference substance was used too. The results are expressed as Trolox equivalent antioxidant capacity (TEAC). Genotoxic damage was assessed by the micronucleus test (Carranza, 2011). The micronucleus assay has been widely used as the most reliable assay to assess the induction of chromosomal aberrations (Hayashi, 2016). The results are expressed as micro-nucleated red-cell frequencies for every 10,000 red cells counted (MNRF).

Analysis of extracts by HPLC-MS

Both the aqueous and methanolic extracts were analyzed in an Agilent 1200 chromatograph equipment with binary pump and auto sampler. The separation of the compounds was carried out on a C18 column, 4.6 x 100 mm, 3.5 um. The mobile phase was composed of Acetonitrile and a 0.1% aqueous formic acid solution. The flow was maintained in isocratic form at 0.8 ml/min. An aliquot of 5 µl was injected for each extract and for each solvent. The chromatograph was coupled to an Agilent 6410 QQQ-MS/MS triple quadrupole mass detector with ESI ionization chamber. The drying gas was maintained at 200°C at a flow of 13 L/min and a pressure of 30 psi. A power of fragmentation at 135 V and mass interval from 100 to 550 with a positive polarity were used. In order to compare the effects, ANOVA tests were applied using the IBM-SPSS statistics v.22 software.

RESULTS

The antioxidant capacity of the aqueous extract was greater than the methanolic extract; the TEAC was 321.74 ± 2.4 µM/ml for the first and 234.35 ± 27.1 µM/ml for the second. The plasmas of the rats of the different groups did not show differences before induction of damage; however, once the damage was induced, the antioxidant power of the plasma was significantly decreased. Plasmas from rats treated with aqueous

Table 1. Antioxidant activity of plasma expressed in TEAC ± S.D.[1] (μM /ml).

Group	t1[2]	t2	t3	t4	t5
1	331 ± 5	299 ± 17	314 ± 18	318 ± 15	336 ± 13
2	330 ± 2	237 ± 2	290 ± 16	295 ± 15	289 ± 5
3	343 ± 7	237 ± 4	236 ± 4	248 ± 18	287 ± 12

[1] Trolox equivalents antioxidant capacity ± standard deviation. [2] Treatments.

Table 2. Frequency of micro-nucleated red cell for every 10,000 red cells counted ± S.D. at the different times of the experiment (t).

Group	t1	t2	t3	t4	t5
1	0.31 ± 0.12	0.33 ± 0.09	1 ± 0.08	0.28 ± 0.097	0.32 ± 0.11
2	0.25 ± 0.5	4.6 ± 2	2.5 ± 1.2	1 ± 0.8	1.2 ± 0.3
3	0.3 ± 0.15	4.51 ± 2.1	2.4 ± 1.8	2.2 ± 1.09	1.6 ± 0.8

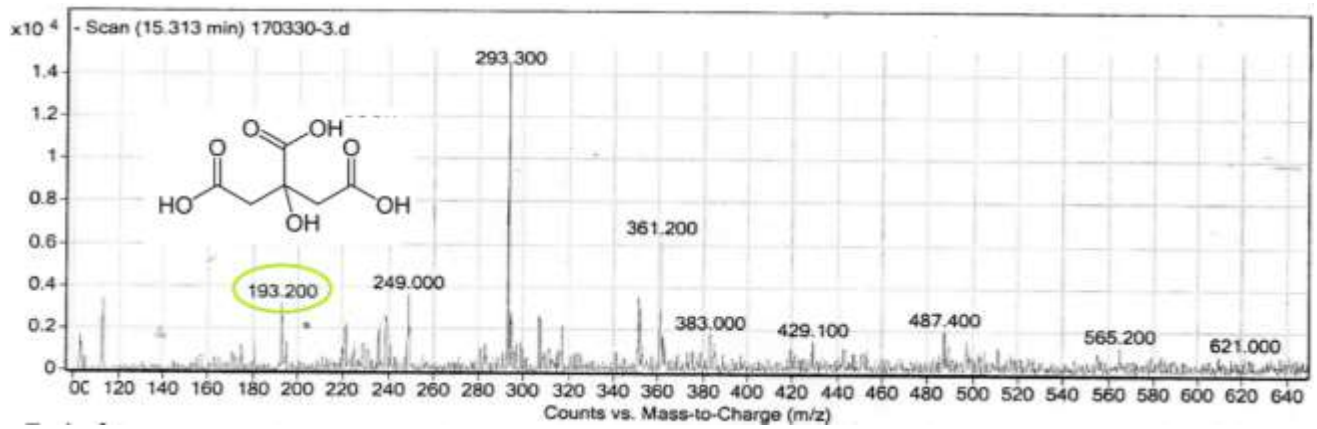

Figure 1. Citric acid elution at retention time 15.31 min.

extracts showed an average recovery of 88% after 6 weeks of treatment, whereas plasmas from rats treated with methanolic extracts showed a recovery of 75%. Statistical tests did not show significant differences between both treatments (p> 0.05). Table 1 show the antioxidant power expressed as Trolox equivalents antioxidant capacity (TEAC).

Both extracts showed a protective effect against genotoxic damage. The number of MNRF decreased by 78% with respect to the time of stress induction for the aqueous extracts and decreased by 64% for the methanolic extract in the same period. Table 2 shows these results. No significant differences were found between both extracts (p> 0.05).

HPLC analysis

The analysis of both extracts by HPLC showed the presence of numerous compounds, but among the most abundant, we can mention ferulic acid, protocatechonic acid, citric acid, astragalin, caffeic acid, myristic acid, palmitic acid, riboflavin, kaempferol and beta carotene. Figure 1 shows an example of citric acid identification.

DISCUSSION

The compounds found in the extracts can explain the antioxidant and protective properties found. Ferulic acid is widely used in sunscreens since it has properties against damage by UV radiation. This could be associated with protection against genotoxic damage, which is the main consequence of prolonged exposure to these rays. The pharmaceutical industry also investigates its applicability in oncological and anti-inflammatory treatments (Santiago, 2020). Protocatechic acid has been associated, in numerous studies, with antioxidant activity,

antidiabetic activity, anti-inflammatory and anti-cancer activity, possibly due to the inhibition of the generation, and elimination, of free radicals and the strengthening of regulatory antioxidant enzymes (Kakkar and Bais, 2014). Routine is a flavonoid-type antioxidant to which anti-inflammatory and vasodilatory properties are attributed; it is also said to have a role as an inhibitor of certain types of cancer and as a protector of the liver. Naringenin is a flavonoid-type antioxidant which has been shown to improve insulin sensitivity and glucose tolerance. It is said to have anti-inflammatory and preventive properties against liver cancer; and has also been seen to reduce the oxidative stress of DNA (Erlund, 2014). It is difficult to attribute the antioxidant and geno-protective properties to a single compound. We must think that the synergistic combination of all substances provides this effect. Either way, it is suggested to split the extracts and study the properties of each particular fraction.

Conclusion

The aqueous and methanolic extracts of chaya leaves seem to provide protection against genotoxic damage and have a good antioxidant effect. Aqueous extracts seem to have the best effect. Further studies are required to demonstrate the effect of these compounds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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