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Evaluation of the Genotoxicity and Cytotoxicity of *Opuntia ficus-indica* Mucilage Through the Micronuclei Test *In Vivo* in the Mouse Blood

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Abstract

Background: The use of medicinal plants has increased, as well as the reports of suspicion of toxicity and adverse events associated with its secondary metabolites. *Opuntia ficus-indica* the species with the highest economic importance worldwide, it is used in the elaboration of food, cosmetologically and industrial products and has a great number of curative effects, but only in México, their young cladodes are consumed as vegetables.

The safety of herbal medicines has become an issue for the regulatory authorities, as serious effects have been reported, including genotoxic and cytotoxic effects. The objective was to evaluate *in vivo* the genotoxic and cytotoxic effect of *O. ficus-indica* mucilage in mouse peripheral blood.

Methods: Four doses of *O. ficus-indica* (15, 60, 120 and 240 mg / Kg) were administered orally to Balb-C mice for 5 days. The mice were sampled every 24 hours at 5 days. The frequency of Micro Nucleated Erythrocytes (MNE), Micro Nucleated Polychromatic Erythrocytes (MNPCE) and Polychromatic Erythrocytes (PCE) in peripheral blood samples of mice at different sampling times in the different study groups were evaluated.

Results: The groups that received the different doses of *O. ficus - indica* mucilage showed neither a decrease in PCE nor an increase in MNPCE and MNE.

Conclusion: No genotoxic and cytotoxic effects were observed after oral administration of the four different doses of *O. ficus - indica* mucilage in a mouse.

Keywords: Cytotoxicity; Genotoxicity; Micronuclei; Mouse; *Opuntia ficus-indica* Mucilage

Abbreviations

CP	:	Cyclophosphamide
MNE	:	Micronucleus Erythrocytes
PCE	:	Polychromatic Erythrocytes
MNPCE	:	Polychromatic Micronucleus Erythrocytes
TE	:	Total Erythrocytes

Introduction

The traditional medicine has played an important role in the treatment of diverse pathologies and has formed the basis of the systems of traditional medicine for the health of the population [1]. *Opuntia ficus-indica* is the cactus species with the highest economic importance worldwide, is is a succulent plant arborescent, shrubby or creeping characterized by its remarkable adaptation to arid and semi-arid climates in tropical and subtropical regions of the globe [2].

Traditionally, In Mexico, young cladodes are produced for consumption as vegetables [3,4].

It has been described that the cactus has anti-inflammatory, anti-neoplastic, antioxidant, healing, antiulcer, hypoglycemic, hepatoprotective, hypolipidemic and hypocholesterolemic effects [2,5-9]. The cactus when suffering mechanical damage begins to exude a "mucilaginous compound", this mucilage is an acid polysaccharide (pH = 4.5 to 5.7) with a broadly branched structure, which is made up of neutral sugars and an acid sugar [10].

The mucilage is constituted by sugars such as arabinose in 47%, xylose in 23%, galactose in 18%, rhamnose in 7% and galacturonic acid in 5% with respect to its molecular weight [11]. Currently, the mucilage of *Opuntia ficus-indica* has many uses because it has many properties, the human being uses them to make flours, juices, cosmetics, shampoo, gums, fresh vegetables and others [2,4,5]. In folk medicine *O. ficus-indica*, it has been used for the treatment of different conditions such as gastritis, fatigue, diabetes, hypertension, hypercholesterolemia, gastric mucosal diseases, asthma, liver damage and whooping cough [2,6]. The safety of herbal medicines has become an issue for the regulatory authorities, as serious effects have been reported, including genotoxic and cytotoxic effects [12].

The antigenotoxic activity of *O. ficus-indica* has been evaluated from the cladodes *in vivo* using the micronucleus assay in bone marrow cells at doses of 25, 50 and 100 mg/kg against the mycotoxin zearalenone in Balb-C mice, the results showed no genotoxic and cytotoxic effect [13]. On the other hand, the chemoprotective effect of *O. ficus-indica* in a dose of 50 mg / kg, on oxidative stress and Geno toxicity of Aflatoxin B1 (AFB1), where a total reduction of markers of oxidative damage and genotoxic effect [14].

Telles-López et al., 2014 [15], evaluated the toxic activity of the methanolic extract of *O. ficus indica* at doses of 50 to 1000 µg/mL on *Artemia salina nauplii*, using potassium dichromate as a positive control at a concentration of 400ppm, finding that the methanolic extract of *O. ficus-indica* at the doses tested showed no toxic effects. The plants present therapeutic effects, but they may also exhibit toxic and lethal effects; thus, toxicological tests must be conducted on the plants, including genotoxicity tests [16,17]. Micronuclei (MN) are chromosomal fragments or entire chromosomes that were not included into the daughter cell nuclei at mitosis, and thus represent a loss of DNA to the cell's nucleus [18]. Determination of MN in mouse peripheral blood allows for the evaluation of both the genotoxicity and the cytotoxicity of a compound in an easy, simple, rapid, and a convincing manner [19,20]. Based on what has been previously established, the objective of this work was to evaluate *in vivo* the genotoxic and cytotoxic effect of the *Opuntia ficus-indica* mucilage in mouse peripheral blood by assessing the MN test to determine its biosecurity.

Materials and Methods

Plant Material

The *O. ficus-indica* mucilage was obtained from cactus collected in the community of Los Rodarte in the municipality of Jerez, Zacatecas, Mexico. The harvest was made on June 27, 2014 from a single shrub.

Preparation of *O. ficus-indica* Mucilage

The mucilage was extracted from the species *Opuntia ficus-indica* and was based on reported by Domínguez-Canales et al., (2011) [21]. The prickly pear cactus was removed by collecting the hearts, which were cut into squares of about 2 cm, then liquefied and cooked at 90°C for 1 hour. It was left to cool and then precipitated with 96% ethyl alcohol in a 1: 1 ratio in glass jars, allowed to stand at refrigeration temperature (4 ± 2°C) for 48 h. After 48 hours of rest, the precipitate was collected and left to dry in the sun, to be pulverized later, obtaining a yield of 0.745%.

Animals

Thirty male Balb-C mice 3 months of age (average weight 22.16 ± 2.13g) were used. The animals were kept in plastic boxes in windowless rooms, with automatic controls for temperature (22 ± 2°C), under a 12-hour light-dark cycle, and relative humidity maintenance (50 ± 10%).

All animals were healthy and were provided by the animal facilities of the bioterium Claude Bernard, UAZ-Siglo XXI, Zacatecas, Zacatecas, Mexico. Animals received standard laboratory pellet food (Purina, Mexico) and tap water *ad libitum*. The study was approved by ethics committee on research of the Autonomous University of Zacatecas, Mexico, with number CICUALACS/UAZ PROY002D/2014.

All experiments were performed according to the guidelines for the use and care of research animals in the regulations and national norms (Official Mexican Standard NOM-062- ZOO-1999) and of the International Institutes of Health for the humane treatment of research animals [22,23]. At the end of the experimental period, the mice were anaesthetized by chloroform inhalation followed by cervical dislocation and sacrificed as per the guidelines of Institutional Animal Ethics Committee (IAEC).

Study Groups and Micronuclei Induction in Mouse

Mice were randomly distributed into six groups (5 mice/ group/ per cage): Group 1 received sterile water (negative control); Group 2 received 60 mg/kg of Ciclofosfamide (CP) divided in two doses (positive control); Group 3 received *O. ficus-indica* mucilage 15 mg/kg; Group 4 received *O. ficus-indica* mucilage 60 mg/kg; Group 5 received *O. ficus-indica* mucilage 120 mg/kg; Group 6 received *O. ficus-indica* mucilage 240 mg/kg. The *O. ficus-indica* mucilage doses selected was based on previous reports [9,14].

The doses were administered orally with esophageal cannula to each mouse once daily for 5 days. All doses were administered at a volume of 0.1 ml/10 g of weight.

Sample Preparation and Micronucleus Analysis in Mouse

A drop of peripheral blood was taken from the tip of the tail of each mouse immediately before the treatment (0 hours or basal value) and 24, 48, 72, 96, and 120 hours later. Two smears were made on clean, pre-coded microscope slides. These were dried at ambient temperature and fixed in absolute ethanol during 10 min and stained with acridine orange (CAS No. 10127023; Sigma-Aldrich) (Zúñiga-González 2003), specific staining for nucleic acids [24,25]. The MN in each sample was scored using an Olympus CX31 (Center Valley, Pennsylvania, USA) equipped with epifluorescence and an oil-immersion objective (100X). Genotoxicity was determined through the increase in the proportion of the number of MNPCE and MNE; the number of MNE in 10,000 Total Erythrocytes (TE) was counted and MNPCE in 1000 PCE. Cytotoxicity was determined by means of counting the PCE in 1000 TE [26].

Statistical Analysis

The results were expressed as mean ± Standard Deviation (DE) per group. Comparisons were performed in each group and its own background value (0h) through repeated measures Analysis of Variance (ANOVA), and the Bonferroni goodness-of-fit test was implemented for post hoc comparisons.

A p value of <0.05 was considered significant. All statistical tests were performed using the Statistical Package for Social Sciences (SPSS v. 20, IBM Co., Armonk, NY, USA).

Results

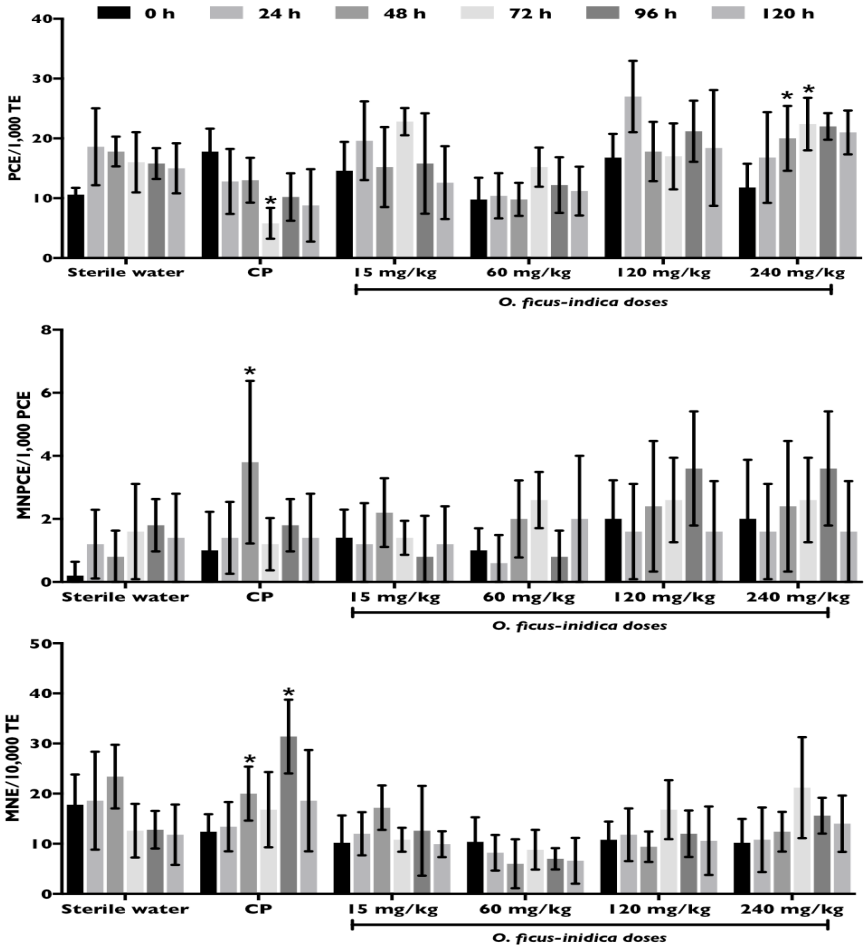
The statistical significance of MNPCEs/1000 PCEs, MNEs/ 10,000 TE, and PCEs/1000 TE for the different groups are shown in (Table 1and figure 1).

Sampling time (hours)	0h	24h	48h	72h	96h	120h
PCE/1,000 TE						
Sterile wáter	10.60 ± 1.14	18.60 ± 6.42	17.80 ± 2.48	16.00 ± 5.04	15.80 ± 2.58	15.00 ± 4.18
P-value		NS	NS	NS	NS	NS
CP (60 mg/kg)	17.80 ± 3.834	12.80 ± 5.44	13.00 ± 3.74	5.80 ± 2.58	10.20 ± 3.96	8.80 ± 6.05
P-value		NS	NS	**0.0001**	NS	NS
O. ficus- inidica (15 mg/kg)	14.60 ± 4.82	19.60 ± 6.58	15.20 ± 6.68	22.80 ± 2.28	15.80 ± 8.40	12.60 ± 6.10
P-value		NS	NS	**0.026**	NS	NS
O. ficus- inidica (60 mg/kg)	9.80 ± 3.63	10.40 ± 3.78	9.80 ± 2.77	15.20 ± 3.27	12.20 ± 4.65	11.20 ± 4.08
P-value		NS	NS	NS	NS	NS
O. ficus- inidica (120 mg/kg)	16.80 ± 3.96	27.00 ± 5.96	17.80 ± 4.96	17.00 ± 5.52	21.20 ± 5.11	18.40 ± 9.68
P-value		**0.024**	NS	NS	NS	NS
O. ficus- inidica (240 mg/kg)	11.80 ± 3.96	16.80 ± 7.59	20.00 ± 5.43	22.40 ± 4.39	22.00 ± 2.23	21.00 ± 3.67
P-value		NS	NS	**0.002**	**0.036**	NS
MNPCE/1,000 PCE						
Sterile water	0.20 ± 0.44	1.2 ± 1.09	0.80 ± 0.83	1.60 ± 1.51	1.80 ± 0.83666	1.40 ± 1.40

P-value		NS	NS	NS	NS	NS
CP (60 mg/kg)	1.00 ± 1.22	1.40 ± 1.14	3.80 ± 2.58	1.20 ± 0.83	1.80 ± 0.83	1.40 ± 1.40
P-value		NS	**0.033**	NS	NS	NS
O. ficus- inidica (15 mg/kg)	1.40 ± 0.89443	1.20 ± 1.30384	2.20 ± 1.09	1.40 ± 0.54	0.80 ± 1.30	1.20 ± 1.20
P-value		NS	NS	NS	NS	NS
O. ficus- inidica (60 mg/kg)	1.00 ± 0.70	0.60 ± 0.89	2.0 ± 1.22	2.60 ± 0.89	0.80 ± 0.83	2.00 ± 2.00
P-value		NS	NS	NS	NS	NS
O. ficus- inidica (120 mg/kg)	2.00 ± 1.22	2.8 ± 2.16	1.00 ± 1.00	0.80 ± 0.83	2.80 ± 2.38	1.60 ± 1.60
P-value		NS	NS	NS	NS	NS
O. ficus- inidica (240 mg/kg)	2.00 ± 1.87	1.60 ± 1.51	2.40 ± 2.07	2.60 ± 1.34	3.60 ± 1.81	1.60 ± 1.60
P-value		NS	NS	NS	NS	NS
MNE/10,000 TE						
Sterile water	17.80 ± 6.01	18.60 ± 9.78	23.40 ± 6.34	12.60 ± 5.36	12.80 ± 3.76	11.80 ± 6.01
P-value		NS	NS	NS	NS	NS
CP (60 mg/kg)	12.40 ± 3.50	13.40 ± 4.92	20.00 ± 5.38	16.80 ± 7.52	31.40±7.36	18.60 ± 10.11
P-value		NS	0.031	NS	0.0001	NS
O. ficus- inidica (15 mg/kg)	10.20 ± 5.44	12.00 ± 4.30	17.20 ± 4.43	10.80 ± 2.38	12.60 ± 8.96	9.920 ± 2.58
P-value		NS	NS	NS	NS	NS
O. ficus- inidica (60 mg/kg)	10.40 ± 4.87	8.20 ± 3.56	6.00 ± 4.89	8.80 ± 3.96	7.00 ± 2.12	6.60 ± 4.56
P-value		NS	NS	NS	NS	NS
O. ficus- inidica (120 mg/kg)	10.80 ± 3.63	11.80 ± 5.26	9.40 ± 3.04	16.80 ± 5.89	12.00 ± 4.63	10.60 ± 6.84
P-value		NS	NS	NS	NS	NS
O. ficus- inidica (240 mg/kg)	10.20 ± 4.76	10.80 ± 6.45	12.40 ± 3.97	21.20 ± 10.08	15.60 ± 3.57	14.00 ± 5.61
P-value		NS	NS	NS	NS	NS

Data are expressed as mean ± standard deviation per group. Comparisons were made between each group and their respective baseline number (0 h), by Analysis of Variance (ANOVA) for repeated measures and Bonferroni test post hoc for multiple comparisons. Were considered statistically significant when $P < 0.05$. PCE: Polychromatic Erythrocytes; MNPCE: Micronucleus Polychromatic Erythrocytes; MNE: Micro nucleated Erythrocytes TE: Total Erythrocytes; CP: Cyclophosphamide; *O. ficus-inidica*: *Opuntia ficus-indica*; NS: not significant.

Table 1: PCE, MNPCE and MNE frequencies in the study groups.



Mean values are expressed as columns and error bars represent standard deviation. Intragroup comparisons were performed between baseline samples (0 h) against the following sampling times: 24; 48; 72; 96, and 120 h. Statistically significant was considered with a p value < 0.05. *: Statistically significant differences; CP: Cyclophosphamide; PCE: Polychromatic erythrocytes; MNPCE: Micronucleus polychromatic erythrocytes; MNE: Micronucleus erythrocytes; TE: Total erythrocytes.

Figure 1: PCE, MNPCE and MNE values in mouse peripheral blood in the study groups.

The proportion of PCE in the positive Control Group (CF) exhibited a significant decrease at 72h (p= 0.0001). The negative control group (water) and the groups administered with the four doses of *O. ficus-indica* mucilage did not demonstrate a significant decrease in this proportion. Nevertheless, O. *ficus-indica* mucilage increase this proportion with respect to the basal value.

In relation to the proportion of MNPCE, the groups that received water and the different doses of *O. ficus-indica* mucilage did not present a significant increase in this proportion in relation to the initial time; in contrast, the group that received CF showed a significant increase in this proportion at 48h (p = 0.033). The proportion of MNE increased statistically in the group that received CF at 48h (p = 0.031) and 96h (p = 0.0001) with respect to time zero (baseline number). The groups that received water and doses of *O. ficus -indica* mucilage (15, 60, 120 and 240 mg/kg) did not show significant increase in this proportion.

Discussion

The therapeutic properties of medicinal plants are defined by their active compounds; however, they are also responsible for intoxications and adverse effects if they are used in inadequate doses or for prolonged periods [16, 27].

The lack of information on the possible risks and benefits of the consumption of medicinal plants is one of the main factors that lead the population to make use of them, without considering the possible toxic effects just because they are natural [28]. For this reason, toxicological tests are carried out, including genotoxicity tests [19,29]. The genotoxic effect of *O. ficus-indica* mucilage has

not been described, which is why in this work the genotoxic and cytotoxic effect of four doses of mucilage of *O. ficus-indica* was evaluated administered orally by the MN assay in mouse peripheral blood erythrocytes.

The cytotoxic damage was evaluated by counting the number of PCE/1000 TE. The decrease in PCE number indicates that the compound used is cytotoxic [30]. The genotoxic damage was evaluated by counting MNPCE/1,000 PCE, which indicates the damage occurred during the 24 to 48 h after the administration of the new phytodrug and the number of MNE / 10,000 TE, which allows us to determine the damage accumulated [30,31].

As a micronucleogenic agent CF was used (Krishna 2000) [32], which has a genotoxic and cytotoxic effect due to its alkylating and clastogenic activity and is therefore used as a positive control in genotoxicity test [33]. *Opuntia ficus- indica* mucilage showed no significant increase in the number of MNE and PCMNE in the tested concentrations in relation to time zero (before the administration of *O. ficus- indica* mucilage) or decrease in the number of PCE.

Our results show that the mucilage of *O. ficus indica* in the concentrations tested does not present genotoxic or cytotoxic effects (Figure 2), this is in agreement with the results shown by Zorgui et al., (2008) [13], where the authors evaluated the antigenotoxic activity of the *Opuntia ficus-indica* cladodes against the mycotoxin zearalenone in Balb-C mice by the micronucleus assay in bone marrow cells in doses of 25, 50 and 100 mg / kg.

In the same way, our results match those shown by Brahmi et al., (2011) [14] who evaluated the chemo protective effect of *O. ficus-indica* on oxidative stress and the genotoxicity of Aflatoxin B1 (AFB1), *in vivo* a dose of 50 mg / kg intraperitoneally for 2 weeks, which showed reduction of markers of oxidative damage and an antigenotoxic effect.

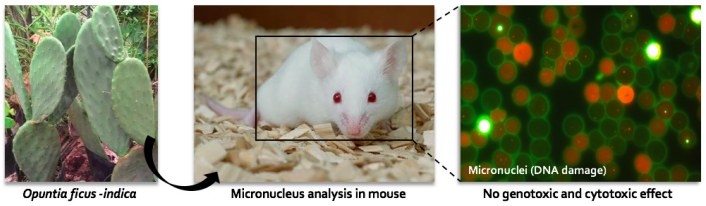


Figure 2: No genotoxic and cytotoxic effect of *Opuntia ficus-indica* by the micronucleus assay.

The genotoxic and cytotoxic evaluation of medicinal plants by means of mouse model, including *O. ficus-indica*, might be considered in medicinal plants, it not only present curative effects but also may present toxic effects associated with their phytochemical compounds [16].

The present work showed that *Opuntia ficus-indica* mucilage does not present genotoxic and cytotoxic effects in the exposed doses, which contributes to the scientific knowledge in the use of traditional herbal medicine.

Conflicts of Interest

All the authors declare that there are no conflicts of interest

Ethics Approval

This study was approved (Approval number CICUALACS/ UAZ PROY002D/2014) by the ethics committee on research of the Autonomous University of Zacatecas, Mexico. All experiments were performed in accordance with the guidelines for the use and care of research animals in the regulations and national norms (Official Mexican Standard NOM-062-ZOO-1999) and of the International Institutes of Health for the humane treatment of research animals [22,23].

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