Regulatory Toxicology and Pharmacology 86 (2017) 260–264

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Genotoxic and cytotoxic evaluation of *Jatropha dioica Sessé ex Cerv.* by the micronucleus test in mouse peripheral blood

Diana Isela Araujo-Espino [a], Ana Lourdes Zamora-Perez [b], Guillermo Moisés Zúñiga-González [c], Rosalinda Gutiérrez-Hernández [d], Gabriela Morales-Velazquez [b, e], Blanca Patricia Lazalde-Ramos [f, *]

[a] Maestría en Ciencias Biomédicas, Universidad Autónoma de Zacatecas, Zacatecas, Mexico
[b] Instituto de Investigación en Odontología, Centro Universitario de Ciencias de la Salud (CUCS), Universidad de Guadalajara, Guadalajara, Jalisco, Mexico
[c] Laboratorio de Mutagenesis, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, Mexico
[d] Doctorado en Farmacología Médica y Molecular, Unidad Académica de Medicina Humana, Universidad Autónoma de Zacatecas, Zacatecas, Mexico
[e] Doctorado en Farmacología, Centro Universitario de Ciencias de la Salud (CUCS), Universidad de Guadalajara, Guadalajara, Jalisco, Mexico
[f] Laboratorio de Etnofarmacología, Unidad Académica de Ciencias Químicas, Universidad Autónoma de Zacatecas, Zacatecas, Mexico

ARTICLE INFO

Article history:
Received 21 November 2016
Received in revised form
16 March 2017
Accepted 19 March 2017
Available online 23 March 2017

Keywords:
Jatropha dioica Sessé ex Cerv.
Genotoxicity
Cytotoxicity
Micronuclei
Erythrocytes
Mice
Peripheral blood

ABSTRACT

Jatropha dioica Sessé ex Cerv. is a medicinal plant credited with low cytotoxicity *in vitro*. Thus, the objective of this work was to evaluate the possible genotoxic and cytotoxic effect *in vivo* of the *J. dioica* aqueous extract by means of micronucleus assay in mouse peripheral blood. Four different *J. dioica* aqueous extract dose-units were evaluated (30, 60, 100, and 300 mg/kg). The extract was administered orally to male Balb-C-strain mice every 24 h during 5 days. Blood samples were taken at 0, 24, 48, 72, 96, and 120 h from the mouse's tail and were performed in duplicate extensions. The number of Polychromatic Erythrocytes (PCE), Polychromatic Micronucleus Erythrocytes (PCEMN), and Micronucleus Erythrocytes (MNE) was determined at the different sampling times in the different study groups. Our results showed that the group that received 60 mg/kg of cyclophosphamide (positive control) presented a significant decrease in the PCE ($p = 0.044$) proportion and a significant increase in MNE ($p = 0.032$, $p = 0.0001$). The groups that received the different *J. dioica* aqueous extract doses did not present either a PCE decrease or an increase in PCEMN and MNE. *J. dioica* exerts neither a genotoxic nor a cytotoxic effect on mouse peripheral blood at high doses.

1. Introduction

Jatropha dioica is native to Mexico and the U.S. state of Texas (Manzanero et al., 2009; Richardson, 2010; Fresnedo and Orozco, 2013) comprising one of the bush of the *Euphorbiaceae* spurge family that presents a colorless juice, which becomes darker when in contact with the air, hence its common name of "dragon's blood". There are works that describe its antiviral (Silva et al., 2013), antifungal (Alanís et al., 2007; Silva et al., 2014), and antimicrobial (Silva et al., 2014) activity, as well as its chemopreventive (Martínez, 2013) and hyperglycemic (Alarcon et al., 1998) effect. The medicinal effects of *J. dioica* are associated with its phytochemical components, which are credited with the curative properties of the plant; three diterpenes have been identified (citlalitrione, jatrophone, and riolozatrione) (Villarreal et al., 1988), a sterol, ellagic acid, and oxalic acid (Argueta et al., 1994).

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 (Alice et al., 1991; Badrie and Schauss, 2010).

The toxicity of the species *J. dioica* has been evaluated *in vitro* through the colorimetric MTT reduction assay (3-(4,5-Dimethyl-2-thiozol-2,yl)-2.5-diphenolterazolium Bromide). Low cytotoxicity was reported in the ethanolic and aqueous extracts of *J. dioica* leaves and roots in mouse fibroblast cells (3T3/NIH) (Oliveira et al., 2013) and null cytotoxicity in the hexane extract of the *J. dioica* root in Chang cellular lines (human hepatocyte cells), OK (Opossum

Abbrevations: PCE, Polychromatic Erythrocytes; PCEMN, Polychromatic Micronucleus Erythrocytes; MNE, Micronucleus Erythrocytes; TE, Total Erythrocytes; CP, Cyclophosphamide.

* Corresponding author. Campus UAZ Siglo XXI, Edif. L1, 3er piso, Carretera Zacatecas-Guadalajara Km 6, Ejido la Escondida, 98160 Zacatecas, Mexico.
E-mail address: blancalazalde@gmail.com (B.P. Lazalde-Ramos).

http://dx.doi.org/10.1016/j.yrtph.2017.03.017
0273-2300/

Kidney epithelial cells), and LLCPK-1 (pig epithelial cells) (Silva et al., 2014). However, reports evaluating the cytotoxic and genotoxic effect of the *J. dioica* aqueous extract *in vivo* were not found in the literature. It has been described that information generated by *in vitro* models frequently does not fully explain the observed phenomena within the complexity of an organism. Therefore, there is a need to corroborate *in vivo* such observations on order to be able to integrate the information, in this way drawing unmistakable conclusions about the studied phenomenon. The advantage of *in vivo* models is that these show the studied phenomenon precisely as the phenomenon would occur in an organism, including all the variables exerting an effect on it (Hayashi et al., 2007).

To evaluate the possible genotoxic effect of a compound and or substance, there is a wide variety of tests, including the micronucleus (MN) test. Micronuclei are chromosomal fragments or complete chromosomes that, either spontaneously or due to clastogenic (chromosomes are broken) or to aneuploidogenic (mitotic spindle is affected) effects, are excluded from the nucleus during mitosis (Schmi, 1975; Hayashi et al., 2000). 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 (Zúñiga et al., 2003a and b; Gómez et al., 2004; Zamora-Perez et al., 2011). Based on what has been previously established, the objective of this work was to evaluate the possible genotoxic and cytotoxic effect of the *J. dioica* aqueous root extract in mouse peripheral blood.

2. Materials and methods

2.1. Plant material

The *J. dioica* root used in this study was provided by Zacateca's Laboratories DEMIR S.A. de C.V. in Zacatecas, Mexico. The plant was authenticated and the voucher specimen with the number 30036 was deposited at the CIIDIR herbarium from the National Polytechnic Institute of Durango, Mexico (SEMARNAT: DGO-FLO-174-0405).

2.2. Preparation of the aqueous extract of the plant

The dry roots of the plant were ground into a fine powder. The powder was dissolved at a proportion of 1 g per 10 ml of water. The mixture was placed under mechanical stirring during 2 h at a temperature of 65 ± 5 °C, and then it was filtered. The filtrate was taken to dryness by means of lyophilization. The samples were stored at a refrigeration temperature of 4 °C. The *Jatropha dioica*

Table 1
Proportion MNE, PCMNE and PCE in the groups of studied.

Sampling time (hours)						
	0	24	48	72	96	120
PCE/1000 TE						
Sterile water	9.08 ± 3.74	12.4 ± 3.15	15.4 ± 3.33	15.6 ± 4.41	15 ± 3.09	15.8 ± 2.7
P-Value		*NS*	*NS*	*NS*	*NS*	*NS*
CP (60 mg/kg)	16.4 ± 3.74	7.6 ± 3.15	6.8 ± 3.39	2.0 ± 4.41	2.8 ± 3.09	3.8 ± 3.42
P-Value		*NS*	*NS*	*NS*	*0.035*	*NS*
JD (30 mg/kg)	15 ± 3.74	18.4 ± 3.15	19.2 ± 3.33	24.8 ± 4.41	20.2 ± 3.09	16.2 ± 3.42
P-Value		*NS*	*NS*	*NS*	*NS*	*NS*
JD (60 mg/kg)	21.0 ± 3.62	16.6 ± 3.15	17.6 ± 3.33	14.6 ± 4.41	18.6 ± 3.09	17 ± 3.42
P-Value		*NS*	*NS*	*NS*	*NS*	*NS*
JD (100 mg/kg)	11.8 ± 3.74	9 ± 3.15	13.6 ± 3.33	14.8 ± 4.41	15.4 ± 3.09	14.6 ± 3.42
P-Value		*NS*	*NS*	*NS*	*NS*	*NS*
300 mg/kg de *JD*	17.2 ± 3.74	20.6 ± 3.15	14.4 ± 3.39	18 ± 4.41	23 ± 3.09	20.4 ± 3.42
P-Value		*NS*	*NS*	*NS*	*NS*	*NS*
PCMNE/1000 PCE						
Sterile water	4.4 ± 3.52	7.4 ± 4.32	10.4 ± 5.2	9.4 ± 3.23	10 ± 4.54	13.6 ± 4.00
P-Value		*NS*	*NS*	*NS*	*NS*	*NS*
CP (60 mg/kg)	10.8 ± 3.30	13.2 ± 4.32	28 ± 5.24	15.8 ± 3.23	8.0 ± 4.54	15.6 ± 4.00
P-Value		*NS*	*0.014*	*NS*	*NS*	*NS*
JD (30 mg/kg)	8.8 ± 3.52	7.4 ± 4.30	10.6 ± 5.24	10.0 ± 3.23	10.0 ± 4.54	2.2 ± 4.00
P-Value		*NS*	*NS*	*NS*	*NS*	*NS*
JD (60 mg/kg)	11.2 ± 3.52	15.8 ± 4.32	21.2 ± 5.24	15.6 ± 3.23	13.4 ± 2.09	23.4 ± 4.00
P-Value		*NS*	*NS*	*NS*	*NS*	*NS*
JD (100 mg/kg)	16 ± 3.52	7.4 ± 4.32	11.2 ± 5.24	7.6 ± 3.23	5.4 ± 2.07	2.6 ± 4.00
P-Value		*NS*	*NS*	*NS*	*NS*	*NS*
300 mg/kg de *JD*	17.6 ± 3.52	19.8 ± 4.32	18.2 ± 5.24	21.2 ± 3.23	25 ± 4.54	26.8 ± 4.00
P-Value		*NS*	*NS*	*NS*	*NS*	*NS*
MNE/10,000 TE						
Sterile water	21.6 ± 4.89	17.4 ± 4.82	24.2 ± 4.18	19.4 ± 3.79	24.6 ± 4.57	30.6 ± 6.20
P-Value	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
CP (60 mg/kg)	28.2 ± 4.89	39 ± 4.82	41.2 ± 4.03	43.4 ± 3.79	50.4 ± 4.57	58 ± 6.20
P-Value	*NS*	*NS*	*NS*	*NS*	*0.032*	*0.0001*
JD (30 mg/kg)	27.8 ± 4.89	28.6 ± 4.82	24.4 ± 4.18	25.4 ± 3.79	21.8 ± 4.57	15 ± 6.20
P-Value	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
JD (60 mg/kg)	33.8 ± 4.89	31.8 ± 4.82	30.2 ± 4.18	27.6 ± 3.79	33.4 ± 4.57	32.4 ± 6.20
P-Value	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
JD (100 mg/kg)	35.4 ± 4.89	26.4 ± 4.82	21.4 ± 4.18	24.8 ± 3.79	22.8 ± 4.57	22.2 ± 6.20
P-Value	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
300 mg/kg de *JD*	35.8 ± 4.89	42.2 ± 4.82	36.2 ± 4.18	39.6 ± 3.79	45 ± 4.57	41.2 ± 6.20
P-Value	*NS*	*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 (PCE); PCMNE: Micronucleus Polychromatic Erythrocytes; MNE: Micronucleated Erythrocytes TE: Total Erythrocytes; CP: Cyclophosphamide; JD: *Jatropha dioica*; NS: not significant.