Journal of Natural Products and Resources 2(2) (2016) 79–82

ISSN: 2455-0299

Contents List available at JACS Directory

Journal of Natural Products and Resources

journal homepage: http://www.jacsdirectory.com/jnpr

Chemical Characterization and Oral Toxicity of An Aqueous Extract of *Larrea tridentata*

S. López-Aguirre[1], J.C. García-López[2], J.M. Pinos-Rodriguez[3], L. Yáñez-Estrada[4], Y. López-Hernández[3,5,*]

[1]*Programas Multidisciplinarios de Posgrado en Ciencias Ambientales, Universidad Autónoma de San Luis Potosí, San Luis Potosí - 78210, México.*
[2]*Instituto de Investigación de Zonas Desérticas, Universidad Autónoma de San Luis Potosí, San Luis Potosí - 78377, México.*
[3]*Centro de Biociencias, Universidad Autónoma de San Luis Potosí, San Luis Potosí - 78321, México.*
[4]*Facultad de Medicina, Laboratorio de Género, Salud y Ambiente Universidad Autónoma de San Luis Potosí, San Luis Potosí - 78210, México.*
[5]*CONACyT-Universidad Autónoma de Zacatecas, Unidad Académica de Ciencias Biológicas, Laboratorio de Bioquímica Molecular e Inmunobiología, Zacatecas - 98000, México.*

ARTICLE DETAILS

Article history:
Received 16 October 2016
Accepted 03 November 2016
Available online 13 November 2016

Keywords:
Larrea tridentate
NDGA
Mass Spectrometry
Acute Toxicity
Chronic Toxicity

ABSTRACT

The use of antimicrobial compounds obtained from natural sources has been gaining impact in recent years. However, when planning to use these compounds in living beings, careful and exhaustive examination of potential toxicity is necessary. In the present work, we characterized the chemical composition of an aqueous extract of *Larrea tridentata* and evaluated the toxicity when administered orally to Wistar rats in both single and repeated dosing regimens. Spectrometric characterization of the aqueous extract was performed. Female Wistar rats were inoculated orally with single and repeated doses of the vegetable leaf extract and biochemical parameters and cytokine gene expression were evaluated. Mass spectra of the aqueous extract showed the presence of nordihydroguaiaretic acid (NDGA) and kaempferol along with other non-identified compounds. Rats treated with one dose of the aqueous extract did not show clinical manifestations of acute toxicity. However, treatment over four consecutive weeks, showed significant differences between the study groups in observed food and water consumption and hepatic enzyme levels ($P < 0.05$). Downregulation of genes coding for TNF-a, IL-1β and IL-6 with respect to the control animals was observed in liver and kidney of the animals receiving the aqueous extract.

1. Introduction

Larrea tridentata (Zygophyllum tridentatum), also known as creosote brush or chaparral, is a plant belonging to the family Zygophyllaceae and is closely related to the South American species *Larrea divaricada* [1]. Its popular name of Governor is given for its ability to inhibit the development of other plants in their immediate surroundings, a strategy for obtaining more water in these desert regions. This plant has been used for several years as natural remedy against a great number of diseases. Several studies have demonstrated that extracts obtained from *Larrea tridentata* have in vitro antifungal action against at least 17 pathogenic fungi [2]. Antibacterial and antiviral action against *M. tuberculosis* and Herpes Simplex Virus has also been reported [3, 4].

Reports of chaparral toxicity are inconsistent, as native populations in the southern United States have consumed it for decades without signs of toxicity. The main chemical compound of chaparral is NDGA. By 1943, NDGA at 0.02% (w/w) was used as an antioxidant in many foods to prevent fermentation. 25 years later, NDGA lost its previous status (generally recognized as safe) and the FDA required their removal from most foods due to reported cases of hepatotoxicity [1]. Animal studies evaluating NDGA did not show hepatotoxicity but did exhibit renal damage [5].

The combined effects of all the constituents in this plant could lead to a synergism that amplifies the effect of one primary active compound. The antimicrobial activity of an aqueous extract of *Larrea tridentata* has been in vitro and in vivo tested by our group (unpublished results). The aim of the present work is to verify the presence of NDGA in an aqueous extract from *Larrea tridentata* and to evaluate both the possible toxicity and anti-inflammatory effects when the extract is administered orally to Wistar rats.

*Corresponding Author
Email Address: ylopezher@conacyt.mx (Y. López-Hernández)

2. Experimental Methods

2.1 Plant Material and Chemicals

The vegetal material was collected in the autumn in Matehuala, San Luis Potosi. Plants were dried under mild sunlight to constant weight and leaves were separated from tails. A voucher specimen was identified and deposited at Isidro Palacios Herbarium from Instituto de Investigación de Zonas Desérticas de la Universidad Autónoma de San Luis Potosí. Air dried leaves were ground in a grinding machine until a particle size of 1 mm was obtained. This material was kept from light at 22 °C for their subsequent use. NDGA was purchased from Sigma Aldrich (St. Louis, MO, USA). Ethanol, acetonitrile and methanol were purchased from Honeywell (MI, USA). Ultrapure water from a Milli-Q-System (Millipore Inc., USA) was employed.

2.2 Extraction Methodology

Infusion process with deionized water was used for the extraction. The proportion of 30 mL of solvent per gram of poured and dry leaves was selected for the extraction procedure. Extraction was performed at 65 °C for 40 minutes. After extraction, tubes were cooled for 30 min. 1 mL from each tube was transferred to one dried cresol, previously weighted and incubated at 96 °C until constant weight. The obtained suspension was filtered through Whatman paper and concentrated in a rotary evaporator at reduced pressure and a temperature of 55 °C. Total dry was achieved by lyophilisation. The process was carried out by triplicate.

2.3 Spectrometric Characterization

At Centro de Biociencias, Universidad Autónoma de San Luis Potosí México, the separation and detection of the components was performed on a Waters ACQUITY UPLC system (Waters Corp., Milford, MA, USA) coupled with a Waters Synapt™ High Definition TOF Mass system (Waters Corp., Milford, USA) equipped with the electrospray as ionization source. Chromatographic separations were achieved on an ACQUITY UPLC™BEH

Cite this Article as: S. López-Aguirre, J.C. García-López, J.M. Pinos-Rodriguez, L. Yáñez-Estrada, Y. López-Hernández, Chemical characterization and oral toxicity of an aqueous extract of *Larrea tridentata*, J. Nat. Prod. Resour. 2(2) (2016) 79-82.

C18 column (50 mm × 2.1 mm i.d., 1.7 μm, Waters Corp.) at 30 °C and the flow rate of the mobile phase was 0.20 mL/min. Mobile phase A was acetonitrile while mobile phase B consisted of 0.1% formic acid in water. The column was eluted with a linear gradient of 10-90% A over initial 7.0 min, 90-10% A over 1.5 min, 10-90% A over 30 s. The mass spectrometric full-scan data were acquired in the negative ion by Z mode from 50 to 1200 Da with a 0.5 s scan time. Other conditions were as follows: Capillary voltage of 2.5 kV, sample cone voltage of 20-50 V, desolvation temperature of 150 °C, source temperature of 80 °C, cone gas flow of zero L/h and desolvation gas flow of 600 L/h for negative ion mode. Data were continuum and mass was corrected during the acquisition using an external reference (Lock-Spray™) consisting of a 200 pg/mL solution of leucine enkephalin infused at a flow rate of 0.01 mL/min via a lockspray interface, generating a reference ion for negative ion mode ([M-H] −= 554.2615 Da) to ensure accuracy during the MS analysis. Collision induced dissociation (CID) experiments were carried out using Argon as collision gas and collision energies were in the range of 20-45 V. Data were analysed by MassLynx 3Æ5 software (Micromass, UK).

2.4 In Vivo Studies

2.4.1 Animals

The study was developed at animal facilities of the Centro de Biociencias, Universidad Autónoma de San Luis Potosí, México. Animal procedures were reviewed and supervised by a Bioethics committee in accordance with the Official Mexican regulations on technical specifications in the production, maintenance and use of laboratory animals (NOM-062-ZOO-1999). Wistar rats were housed under normal conditions of temperature and humidity, and maintained on a free access diet of rodent chow and fresh water.

2.4.2 Acute Toxicity

Wistar rats (n = 35, 8-9 weeks old) 226 g (±18.26) body weight, were randomly distributed in four separated groups and were orally inoculated with a single dose of the aqueous extract at 0.5, 1 and 1.5 g/Kg (of body weight) of the aqueous extract. Control treatment was a single dose of phosphate buffered saline solution (PBS). Intra-gastric cannula was used for the inoculation. Aliment was retired 4 h prior inoculation. Rats were observed for 15 days in order to determine the toxicity of the given doses and were weighed weekly. Water and feed intake was also recorded on a weekly basis. Blood was extracted by retro orbital puncture under light anaesthesia at days 7 and 14. Blood samples were centrifuged at 4,000 x g for 15 min at 4 °C and the recovered serum samples were stored at -80 °C until analysed.

2.4.3 Chronic Toxicity

A group of ten Wistar rats 8-9 weeks old (245.30 ± 19.08 body weight) was daily inoculated with 1.5 g/Kg of body weight of vegetal extract by intra-gastric route over four weeks. Blood samples were collected under light ether anaesthesia from the medial cantus of the eye at days 0 and 28 and serum was obtained and properly stored. Water and food intake as well as body weight was monitored weekly. At day 28, rats were ethically sacrificed by diethyl ether inhalation. Liver and kidney were excised immediately, rinsed with PBS, weighed, frozen in liquid nitrogen, and stored at -80 °C until utilized.

2.4.4 Determination of Biochemical Parameters in Sera

Commercial kits (Spinreact, Spain) were used for determination of alanine transaminase (ALT), glutamic piruvic transaminase (TGP), glutamic oxalacetate transaminase (TGO), aspartate amine transferase (ASAT), alkaline phosphatase (ALP) and triglycerides, glucose in sera. Determinations were performed following manufacturer instructions.

2.4.5 RNA Isolation and Real Time PCR

For evaluation of gene expression of TNF-a, IL-6 and IL-1β, total RNA was extracted from frozen liver and kidney tissues using TRIzol Reagent following manufacturer instructions (Sigma-Aldrich, St. Louis, MO, USA). Reverse transcription was carried as follows: 10 min at room temperature, 37 °C for 90 min and 94 °C for 5 min (one cycle). Real-time PCR was performed in a 10 μL of reaction solution containing 5 μL of 2X SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA), 200 nM primers and cDNAs. The cycles for PCR were as follows: 95 °C for 5 min, 39 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s. Melting curves were determined by heat-denaturing PCR products with a temperature gradient. All samples were normalized by the corresponding expression of 18S. The expression level of the gene of interest in the extract-treated group relative to the control group expression level was calculated by using the formula $2^{-\Delta\Delta Ct}$.

2.5 Statistical Analysis

Evaluation of the biochemical parameters between Larrea tridentata-treated groups and control group were performed using Student´s t-test or Mann-Whitney rank sum test. Differences in the cytokines mRNA expression levels were analysed using one way analysis of variance on ranks coupled to Dunn´s Method. Sigma Plot v11 (Systat Software Inc., San Jose, CA) and Graph-Pad Prism 5 v5.03 (Graph-Pad Software, San Diego, California, USA) software were used to perform the statistical analysis. A significance level of 0.05 was considered.

3. Results and Discussion

3.1 Spectrometric Identification

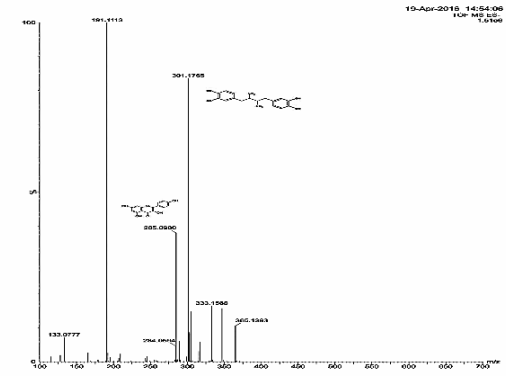

Fig. 1 Mass spectra of the aqueous extract of Larrea tridentata. Direct infusion (ESI/MS) in negative mode revealed the presence of two peaks of m/z 301.1765 and 285.0980, corresponding to the monoisotopic mass of NDGA and kaempferol respectively

The identification of active compounds in the vegetable extract was performed by Liquid Chromatography coupled to mass spectrometry (LC-ESI/MS). Fig. 1 shows the mass spectra of the vegetal extract. Analysis of the aqueous extract by direct infusion revealed good ESI ionization efficiency in the negative ion mode, with molecular base ion at m/z 301.1765 and m/z 285.0980 for protonated $[M- H]^-$. These masses correspond to the monoisotopic masses reported for NDGA [6] and kaempferol [7] respectively.

When LC was performed, a major peak eluted with a retention time of 4.30 min and other minor fraction eluted at 6.41 min (Fig. 2a). Analysis of the major peak by ESI/MS, revealed the presence of a molecular base ion at m/z 301.2109, corresponding to the molecular mass of NDGA (Fig. 2b).

To validate the identity of the NDGA, MS/MS analysis was performed and ions with molecular mass of 301. 2069 were fragmented into daughter ions (collision energy 28). As a result, in both the standard and aqueous extract samples an identical fragmentation pattern was obtained, consisting of one fragment with m/z 122.1174 (Fig. 3a) for the extract and a fragment of m/z 122.1017 for the standard (Fig. 3b).

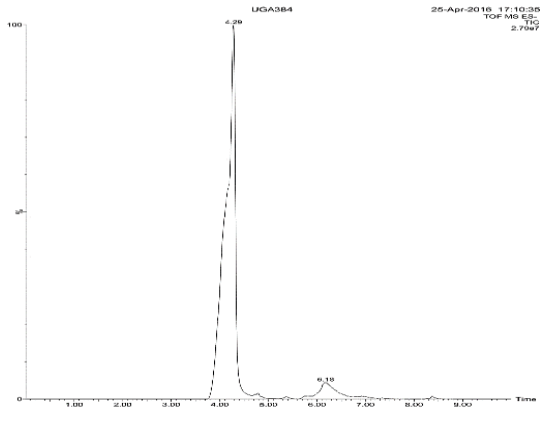

Fig. 2a Chromatographic profile of the aqueous extract

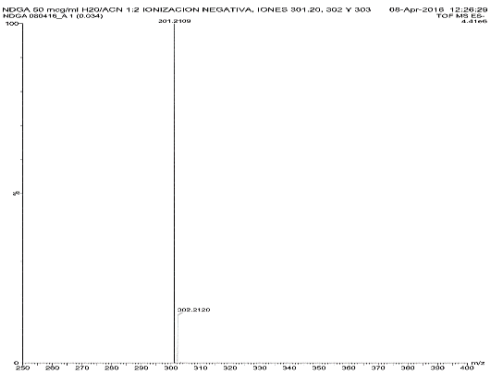

Fig. 2b MS of the first eluted peak. The presence of the peak of m/z 301.2109 correspond to the molecular mass of NDGA

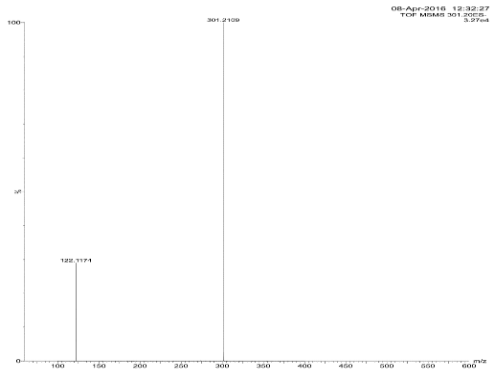

Fig. 3a Fragmentation of peak m/z 301.2109 from the first eluted peak of the aqueous extract rendering a fragment of m/z 122.1174

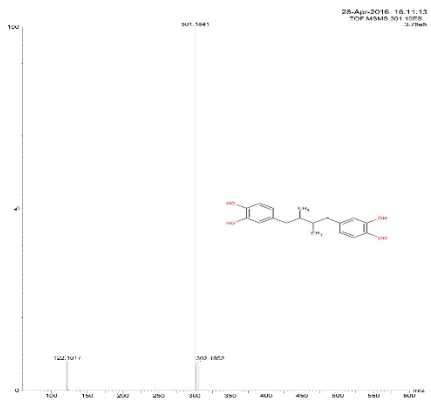

Fig. 3b Fragmentation of the peak m/z 301.1841 from the NDGA standard rendering a fragment of 122.1017. Product ions observed were consistent with predicted fragmentation patterns NDGA with a base peak at m/z 122.1 Da

Table 1 Biochemical parameters evaluated in the acute toxicity experiment at 7 and 14 days post-inoculation with different doses of Larrea tridentata

		0.5 mg/kg	1 mg/kg	1.5 mg/kg	Control	P value
7 days	Glucose	155.4 ± 4.4	148.8± 3.9	149.0 ± 6.3	150.6± 8.9	0.334
	Triglycerides	168.4 ± 9.2	172.2 ± 17.9	164.8 ± 9.2	166.0 ± 11.3	0.804
	Cholesterol	119.2 ± 37.6	126.8 ± 34.9	125.8 ± 39.	111.5 ± 40.0	0.808
	ALT	40.8 ± 4.6	39.8 ± 4.0	40.4 ± 6.5	42.6 ± 7.4	0.886
	AST	86.2 ± 2.7	82.0 ± 4.9	81.0 ± 2.7	77.2 ± 3.2	0.015*
	ALP	110.2 ± 3.8	109.6 ± 9.8	111.4 ± 5.3	113.5 ± 5.2	0.823
14 days	Glucose	149.4± 4.3	142.8 ± 3.8	143.0 ± 6.3	150.6 ± 2.0	0.051
	Triglycerides	165.4± 9.2	169.2 ± 17.9	161.8 ± 9.2	102.6 ± 26.5	0.056
	Cholesterol	116.2± 37.6	123.8 ± 34.9	122.8 ± 39.	96.0 ± 19.0	0.61
	ALT	37.8 ± 4.6	36.8 ± 4.0	37.4 ± 6.5	52.6 ± 9.2	0.01**
	AST	83.2 ± 2.7	79.0 ± 4.9	78.0 ± 2.7	79.3 ± 3.2	0.158
	ALP	107.2 ± 3.8	106.6 ± 9.8	108.4 ± 5.3	119.0 ± 4.0	0.083

*Differences between group treated with 0.5 mg/Kg and control group
**Differences between all treated- groups and control group

3.2 Acute Toxicity

For determining the possible toxicity of the aqueous extract, Wistar rats were inoculated orally with a sole dose of the extract. Rats were divided in four groups of five rats each, receiving 0.5, 1, 1.5 mg/Kg or PBS. After 15 days, no deaths were recorded. Rats did not present diarrhoea, pain, weight loss or changes in behaviour during the evaluated period. Table 1 shows the results of biochemical parameters determined in sera. At day seven, the group inoculated with 0.5 mg/Kg showed significant differences in the AST values when compared with control group (P = 0.015). At day 14, all the groups treated with the extract showed significant diminution of ALT (P = 0.01). At the moment of sacrifice, no macroscopic alterations in kidney, liver, intestine and spleen were observed.

3.3 Chronic Toxicity

For determining the effect of repeated administration of the aqueous extract, the selected dose of 1 mg/kg was inoculated orally during 4 weeks. We observed a significant decrease in the level of ALT (P = 0.004), AST (P < 0.001) and ALP (P = 0.021) respect to the control group (Table 2). In the first week there was significant difference in food intake (P = 0.002) and water consumption (P = 0.002). There were not significant differences in the body weight between control and treated animals. There were not macroscopic alterations in kidney, liver, intestine and spleen at the moment of sacrifice.

Table 2 Parameters evaluated in the experiment of chronic toxicity with repeated administration of 1 mg/Kg of the aqueous extract

Parameter	Control	Extract 1 mg/kg	P value
Body Weight			
Initial	245.300 ± 19.085	244.400 ± 19.552	0.918
Week 1	254.000 ± 17.920	250.778 ± 20.030	0.716
Week 2	263.200 ± 17.268	258.000 ± 20.609	0.557
Week 3	272.600 ± 16.229	266.333 ± 21.101	0.475
Week 4	278.100 ± 17.698	272.889 ± 21.734	0.572
Feed intake			
Week 1	125.000	116.667 ± 10.735	0.002
Week 2	141.600 ± 9.766	113.222 ± 12.488	< 0.001
Week 3	137.400 ± 9.834	122.222 ± 11.289	0.019
Week 4	109.200 ± 6.973	104.444 ± 5.318	0.116
Water intake			
Week 1	339.600 ± 66.858	251.556 ± 32.454	0.002
Week 2	391.400 ± 91.869	325.333 ± 47.342	0.07
Week 3	401.000 ± 52.957	343.111 ± 104.349	0.128
Week 4	320.000 ± 67.869	286.667 ± 111.627	0.129
Hepatic enzymes*			
ALT	53.400 ± 7.877	43.600 ± 5.125	0.004
AST	146.700 ± 26.816	85.000 ± 5.657	< 0.001
ALP	119.700 ± 3.683	114.000 ± 6.092	0.021

*Hepatic enzimes were determined in the sera at day 28

3.4 Gene Expression of IL-6, IL-1β and TNF-a

In order to evaluate the effect of repeated administration of *Larrea tridentata* in the expression of genes coding for pro-inflammatory cytokines, real-time PCR was performed, amplifying mRNA from liver and kidneys of rats treated with the aqueous extract. Fig. 4 shows the results from relative gene expression of IL-6, IL-1β and TNF-a in liver (Fig. 4a) and kidney (Fig. 4b). In both organs, the expression level of the cytokines was significantly down-regulated (P < 0.05) with respect to that in control rats.

Although several authors have reported the increase of liver enzyme levels and hepatotoxicity associated with the consumption of chaparral, in those reported cases, the ingested doses have been elevated for long periods of time [8]. In our study, the dose employed for the experiment of chronic toxicity was 1 mg/Kg during four weeks, which is substantially lower than the equivalent doses employed in humans suffering from hepatitis. Release of liver mitochondrial enzymes is associated with increased levels of reactive oxygen species (ROS) produced in states of oxidative stress [9]. The reduction of the level in hepatic enzymes could be related to the antioxidant character of NDGA and other polyphenols present in the extract, although the demonstration of the antioxidant level of NDGA was not conducted in our study. Several studies conducted in animals and humans have demonstrated this antioxidant property [10]. It has been reported that NDGA is a potent peroxynitrite, singlet oxygen, hydroxyl radical, superoxide anion and hypochlorous acid scavenger, due to the presence of two catechol groups, in which, hydrogen atoms of the four phenolic hydroxyl groups react with reactive oxygen species [11]. It has been also reported that NDGA has the potential to act both as a pro- and anti-oxidant, depending on its concentration and biological environment [12].

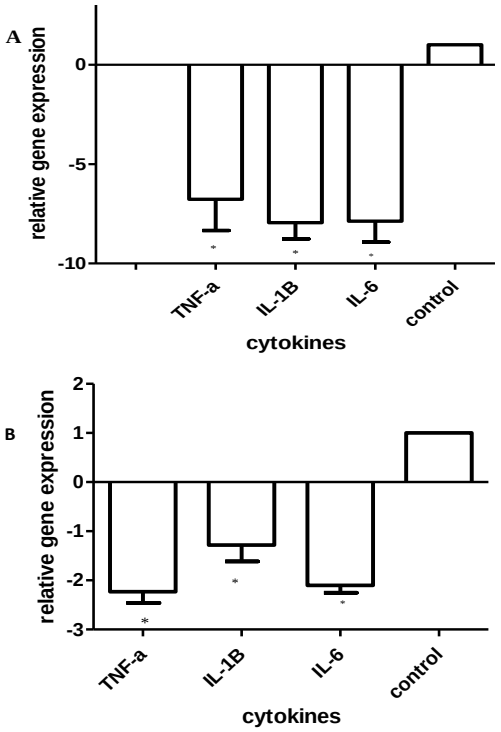

Fig. 4 Relative gene expression of TNF-a, IL-6 and IL-1B mRNA in liver (A) and kidneys (B) of rats administered with repeated dose of the aqueous extract at day 28. Expression of TNF-a, IL-1 and IL-6 was evaluated by quantitative RT-PCR using the 18S as gene control. Results are expressed as the percentage of the relative mRNA intensity with respect to untreated cells. The data are shown as the mean +/- S.E.M. *P < 0.05 compared to the untreated cells

The relative gene expression of those cytokines showed a down-regulation pattern when rats were treated with the aqueous extract. These cytokines are involved in inflammatory responses during acute or chronic rat liver injury. In the extract, due to the confirmed presence of NDGA and kaempferol, it is possible to suggest that both bioactive compounds could act synergistically reducing hepatotoxicity and inflammation. NDGA has been proven to selectively inhibit arachidonic acid 5-lipoxygenase activity, which reduces leukotriene and prostaglandin synthesis, thus leading to a reduction of inflammatory pathways [13]. A recent study demonstrated that not only the antioxidant activity of NDGA but also its high hydrophobicity is a determinant in the pharmacological properties of this molecule [11]. Further studies with this extract are needed to demonstrate the anti-inflammatory effect in an in vivo model of induced inflammation.

4. Conclusion

The aqueous extract from Larrea tridentata obtained in this work, contains NDGA and kaempferol, among other bioactive components. We demonstrated that the repeated administration of low doses of the aqueous extract did not produce acute or chronic toxicity in Wistar rats. Significant reduction in the level of hepatic enzymes was also observed after repeated oral administration. Moreover, the extract downregulates the expression of proinflammatory cytokines such as TNF-a, IL-1β and IL-6. These results allow to suggest the safe use of the aqueous extract of *Larrea tridentata* in animal facilities.

Acknowledgements

The present research was supported by Nutrición y Genética Saludable, S.A. de C.V. León, Guanajuato, México through the Project FINNOVA-2012-02, registration 190601 (CONACyT, México). Authors thankful to Dr. Mariana Salgado, Dr. Claudia Castillo and MSc. Sandra Orta for their valuable support in laboratory analysis and manuscript revision.

References

[1] S. Arteaga, A. Andrade-Cetto, R. Cardenas, *Larrea tridentata* (Creosote bush), an abundant plant of Mexican and US-American deserts and its metabolite nordihydroguaiaretic acid, J. Ethnopharmacol. 98 (2005) 231-239.
[2] M.A. Verastegui, C.A. Sanchez, N.L. Heredia, J.S. Garcia-Alvarado, Antimicrobial activity of extracts of three major plants from the Chihuahuan desert, J. Ethnopharmacol. 52 (1996) 175-177.
[3] H. Chen, L. Teng, J.N. Li, R. Park, D.E. Mold, et al, Antiviral activities of methylated nordihydroguaiaretic acids, Targeting herpes simplex virus replication by the mutation insensitive transcription inhibitor tetra-O-methyl-NDGA, J. Med. Chem. 41(1998) 3001-3007.
[4] S. Guzman-Beltran M.A. Rubio-Badillo, E. Juarez, F. Hernandez-Sanchez, M. Torres, Nordihydroguaiaretic acid (NDGA) and alpha-mangostin inhibit the growth of mycobacterium tuberculosis by inducing autophagy, Int. Immunopharmacol. 31 (2016) 149-157.
[5] T. Goodman, H.C. Grice, G.C. Becking, F.A. Salem, A cystic nephropathy induced by nordihydroguaiaretic acid in the rat, Light and electron microscopic investigations, Lab Invest. 23 (1970) 93-107.
[6] L. Xiu-Qin, J. Chao, S. Yan-Yan, Y. Min-Li, C. Xiao-Gang, Analysis of synthetic antioxidants and preservatives in edible vegetable oil by HPLC/TOF-MS, Food Chem. 113 (2009) 692-700.
[7] A.Y. Chen, Y.C. Chen, A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention, Food Chem. 138 (2013) 2099-2107.
[8] N.M. Sheikh, R.M. Philen, L.A. Love, Chaparral-associated hepatotoxicity, Arch. Intern. Med. 157 (1997) 913-919.
[9] H. Cichoz-Lach, A. Michalak, Oxidative stress as a crucial factor in liver diseases, World J. Gastroenterol 20 (2014) 8082-8091.
[10] S. Guzman-Beltran, J. Pedraza-Chaverri, S. Gonzalez-Reyes, F. Hernandez-Sanchez, U.E. Juarez-Figueroa, et al, Nordihydroguaiaretic acid attenuates the oxidative stress-induced decrease of CD33 expression in human monocytes, Oxid Med. Cell Longev. 2013 (2013) 1-14.
[11] L.C. Paracatu, C.M. de Faria, M.L. Zeraik, C. Quinello, C. Renno, et al, Hydrophobicity and antioxidant activity acting together for the beneficial health properties of nordihydroguaiaretic acid, Food Funct. 6 (2015) 1818-1831.
[12] J. Hernandez-Damian, A.C. Anderica-Romero, J. Pedraza-Chaverri, Paradoxical cellular effects and biological role of the multifaceted compound nordihydroguaiaretic acid, Arch. Pharm. (Weinheim) 347 (2014) 685-697.
[13] P. Bhattacherjee, N.K. Boughtonsmith, R.L. Follenfant, L.G. Garland, G.A. Higgs, et al, The Effects of a novel series of selective inhibitors of arachidonate 5-lipoxygenase on anaphylactic and inflammatory responses, Ann. NY Acad. Sci. 524 (1988) 307-320.