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RESEARCH ARTICLE

Assessment of the immunomodulatory effect of *Aloe vera* polysaccharides extracts on macrophages functions

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ABSTRACT

The present study evaluates the immunomodulatory effect of high molecular weight fractions of *Aloe vera* polysaccharides harvested during the dry season (March-April) and the rainy season (August-September). Peritoneal macrophages (MΦs) secluded from Balb/c mice underwent treatment with *A. vera* leaves extract and acemannan standard (the major component found in *A. vera)* and stimulated with *lipopolysaccharides* (LPS). Macrophage cell viability was assessed by the 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide method. Phagocytic activity was also evaluated in peritoneal macrophages, such as the production of nitric oxide and interleukin 6 (IL-6). In the results, found that the *A. vera* polysaccharides harvested during the rainy season stimulated the phagocytic activity with greater intensity than dry season and improvement NO and IL-6 production. No cytotoxic effect was found on cell viability and they cause a significant proliferative effect on macrophages in a concentration-dependent manner. It can be concluded that the *A. vera* polysaccharides harvested during the rainy season possessed a stronger immunostimulatory effect compared to the extracts from leaves obtained during dry seasons in a concentration-dependent manner without aff at the cell viability of macrophages.

Keywords: *Aloe vera*; Polysaccharides; Acemannan; Macrophages.

INTRODUCTION

Aloe vera (Liliaceae), also known as "*sábila*" has been used by Mayans in traditional medicine due to its curative and therapeutic properties. Genus Aloe contemplates about 360 species of tropical shrubby plants with succulent and elongated leaves even *Aloe vera, Aloe barbadensis, Aloe peyrii, Aloe chinensis, Aloe ferox* and *Aloe indica*. Due to their therapeutic properties, the most known, popular, and commercialized species of *Aloe* are *Aloe vera Linne* and *Aloe barbadensis Miller* (Reynolds, 2004; Sharma et al, 2014). *Aloe* leaf contains two parts, each producing different secondary metabolites with different composition and therapeutic properties, which will vary according to the season they are collected. The internal parenchymal tissues form a thin, transparent, gelatinous and tasteless material. (Tarro,1993). A bitter yellow exudate is produced by the external polycyclic tubules, which are located under the epidermis or external green bark of the leaves (Vogler and Ernst, 1999). *Aloe* gel is a mucilaginous pulp that is located in the center of the leaf. This parenchymal tissue gel of the inner leaf contains polysaccharides to which it has been attributed the medicinal effects of *Aloe*, however, those biological activities might be thanks to the synergistic action with other compounds, instead of the action of one chemical substance.

Several factors can affect the phenology of plants, the levels of nutrients, antioxidants, their secondary metabolites, as well as the biological activities of *A. vera*, such as the effect of high temperatures, levels of precipitation, edaphic factors, fertility. and the different levels of soil moisture. (Kumar et al, 2017). The biological activities attributed to *A. vera* extracts include the helps wound

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healing, fungicide activity, antidiabetic or hypoglycemic effects, anti-cancer anti-inflammatory, immunomodulatory and gastroprotective properties (Hamman, 2008). This biological activity has been attributed to various active components, such as amino acids, saccharides, minerals, vitamins, anthraquinones, salicylic acids, enzymes, lignins, and saponins, (Shelton,1991; Atherton, 1997; Atherton, 1998). Also, *Aloe vera* has products that are part of the isoprenoid pathway, such as steroids, carotenoids, phytosterols, and terpenes, and some essential and nonessential enzymes and amino acids. (Samman, 1998; Arshad et al, 2015).

The polysaccharides present in the *Aloe vera* gel are responsible for the healing of wounds due to their immunomodulatory properties. Most of these identified polysaccharides are glucomannan, mannans (acetylated), and pectin, all of them with a wide range of molecular weight (Pugh et al., 2001). Aloe vera has shown variations in the polysaccharides concentration, biomass, and gel production when subjected to water stress during irrigation. In 2014, the worldwide production of Aloe overcame 102,746.04 Ton (SAGARPA, 2012). Thanks to its natural factors, Yucatan has specific climatic and adequate soil conditions for the cultivation of Aloe; nevertheless, almost 37 % of the production is lost because of the climatic conditions during the rainy season (August-September) and the dry season (March-April).

Different studies have shown that *A. vera* has an immunostimulatory effect. In 2012, Akhtar et al., managed an ethanolic extract from *A. vera* to chickens with coccidiosis and observed that the levels of total IgG and IgM increased as well as the lymphoproliferative responses. Likewise, studies in rainbow trout and common carp nourished with different concentrations indicated that the IgM levels and phagocytic activity of macrophages increased (Haghighi, et al 2014). Macrophage because of its importance on performing defense mechanisms against pathogen microorganisms and inflammatory responses has been target of several strategies in order to enhance its effector mechanisms (Flannagan et al, 2015). Among the three main functions of macrophages are phagocytosis, antigen presentation, and immunomodulation through the release of proinflammatory cytokines such as interleukins 6 (IL-6) and 1β (IL-1β) as well as tumor necrosis factor. α (TNF-α), and various inflammatory mediators, involving nitric oxide and hydrogen peroxide. Additionally to their functions in removal of dying cells and tissue homeostasis, tissue-resident MΦs patrol epithelia of barrier organs, which represent putative entry and colonization sites for pathogens and the first location for controlling infectious invaders (Weiss and Schaible, 2015). This present study focuses on study immunomodulatory effects of *Aloe vera* polysaccharides extracts collected in rainy season (August-September) and the dry season (March-April) of Yucatán, Mexico. These vast climatic variations may cause a difference in the phytoconstituents of the plant species. Thus, the study carried out to investigate the immunomodulatory effects of polysaccharides of *Aloe vera* leaves extracts harvested in two different seasons on macrophages functions.

MATERIALS AND METHODS

Reagents

Standard Acemannan Verapol Premium® was kindly donated by Natural Aloe from Costa Rica (S. A. 4 km Sur Liberia-Guanacaste). Silica gel 60 G (Merck), fetal bovine serum (FBS), Dulbecco's modified eagle medium (DMEM) and penicillin-streptomycin were obtained from Gibco-BRL® (Grand Island, NY, USA). Ethylenediaminetetraacetic acid (EDTA), Bovine serum albumin (BSA), dimethyl sulfoxide (DMSO), trypan-blue dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), LPS (lipopolysaccharides of *Escherichia coli* 0111: B4), sodium nitrite, propidium iodide (PI) and Griess reagent were acquired from Sigma Aldrich® (St. Louis, MO, USA). Murine IL-6 enzyme-linked immunosorbent assay (ELISA) development kit, were acquired from Peprotech® (London, UK).

Plant material

The leaves of *A. vera* were acquired from "Sabileros del Mayab" in Yucatan, Mexico during two climate seasons. To evaluate the polysaccharides concentration under extreme conditions and water shortage, the first harvest occurred during the rainy season from August-September, and the second harvest occurred during drought season from March-April.

Preparation of *A. vera* gel extract

Samples of *A. vera* leaves (450 g) were washed and sanitized with chlorinated water for 20 minutes and allowed to dry. Then, two transverse cuts were made in the leaves, the first one approximately 15 cm from the apex, and the second one 5 cm from the leaf base; afterward, they were left to drain for eliminating the acíbar.

Extraction of *A. vera* gel

Both lateral edges were cut beginning from the apex to the base, detaching the epidermis of both sides with a knife. *A. vera* filets were homogenized with a blender to obtain the juice. To remove coarse solids, *A. vera* gel was filtered through four layers of gauze and through a Whatman filter paper no. 1. The filtrate passed through a membrane of 0.2 μm (to remove smaller impurities). An ultrafiltration

device Amicon® Ultra-0.5 (Millipore, Sigma-Aldrich Co., St. Louis, MO) was used to obtain two fractions of *A. vera* gel nominal molecular weight limit (NMWL), 100 kDa), The first fraction had components which were larger than 100 kDa (concentrate) and the second fraction had components which were smaller than 100 kDa (filtrate).

Quantification of acemannan by HPLC

The 100 kDa fraction was used to extract polysaccharides by size-exclusion chromatography (SEC). A 95 cm long (1 cm of diameter) chromatographic column packed with Sephacryl S-300 (Sigma-Aldrich) was also used. A total of 80 fractions of 1 mL were stockpiled by utilizing sterile bi-deionized water as mobile phase. Dextran blue was used as a Molecular weight markers to approximate the size of the polysaccharides contained in the fractions. Fractions are estimated by thin layer chromatography. (TLC) and n-butanol: pyridine, water (6: 4: 3) was used as the mobile phase. The TLC revelation was performed with sulfuric acid (H_2SO_4) and water (H_2O) (2:1) with consequent heating at 105 °C (Cuevas et al., 2016).

The HPLC determination was carried out through an isocratic chromatography with Acetonitrile-Water (65:35 ratio) as mobile phase at 0.5 mL/min on a Prevail carbohydrate ES column (250 x 4.6mm) at 20 °C. Detection was simultaneously carried out with a Light Scattering Detector (ELSD). The calibration curve for quantification was made by using the acemannan standard at concentrations ranged from 20 ppm to 200 ppm.

Animals

Male Balb/c mice (20 ± 5 g weight) from Hideyo Noguchi Regional Research Center of Autonomous University of Yucatán were kept in standard laboratory conditions, stress-free and pathogen with 12 h light/dark cycle, a temperature approximate of 22 ± 2 °C, with purified water *ad libitum* and special food. Mice were preserved in accordance with the principles and guidelines of the National Institutes of Health (NIH) Guide for Treatment and Care for Laboratory Animals and by The Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999).

Isolation and treatment of peritoneal murine macrophages

According to the method described by Arana-Argáez et al. (2017) isolation and treatment of peritoneal murine MΦs were performed. Briefly, peritoneal murine MΦs were removed from the peritoneal cavity of mice through lavages by using a cold phosphate buffered saline (PBS). The lavage fluid was gathered and centrifuged for 15 min (150 x *g*, 4 °C). MΦ cells were re-suspended in RPMI-1640 culture media supplemented with 10 % FBS and 1 % of penicillin-streptomycin. Cells were counted in a hemocytometer and viability was determined by trypan blue dye exclusion and was found typically ≥ 95 %. About $1x10^4$ cell/mL were placed in each well of a 96-well plate (Costar, Cambridge, MA) to determine cell viability assay. Additionally, $1x10^5$ cell/mL were placed in each well of a 24-well plate (Costar, Cambridge, MA) for the analysis of phagocytic activity, and supernatants obtained to determinate the levels of IL-6 and NO. The plates were incubated (95% air atmosphere, 5% CO_2) at 37 ºC during 48 h (Zhang et al., 2008). After removing non-adhered cells, MΦs were treated with polysaccharides extracts obtained from *A. vera* gel and acemannan (1, 10, 100, 200, 500 and 1000 μg/mL) respectively. All plates were incubated (5 % CO_2, 95 % air atmosphere) at 37 ºC for 24 h. To activate the MΦs, E. coli LPS (1 μg/mL) in supplemented RPMI-1640 media was added and incubated for 48 h under the previously described conditions. In all assays, MΦs activated (LPS) in supplemented RPMI-1640 media without treatment were considered as positive control and cells in supplemented RPMI-1640 media were considered as negative control.

Cell viability

Using MMT assay, cell viability was determined as described before by Abdullah et al, 2017. Macrophages of 96-well plates (1×10^4 cells/well) were treated with polysaccharides obtained from *A. vera* gel and acemannan standard (1, 10, 100, 200, 500 and 1000 μg/mL).

Phagocytic activity

The phagocytic activity was determined according to the protocol described by Alonso-Castro et al. (2012). Murine peritoneal MΦs were seeded in 24-well plates at concentrations of $5x10^5$ cells/well and incubated overnight. The acemannan standard and polysaccharides obtained from *A. vera* gel were added to the final concentrations per well of 1, 10, 100, 200, 500 and 1000 μg/mL., was measured by the cellular fluorescent intensity emitted from the PI using a Cell Lab Quanta SC flow cytometer (Beckman Coulter, USA).

Nitric oxide production

For nitrites production as indicator of NO synthesis through the Griess reaction described by Zamani et al. (2014). The plate was incubated for 10 min in a dark room and absorbance was measured at 490 nm in a Microplate Reader (iMark, Bio-Rad, USA). Nitrite concentration was determined by comparing it with a $NaNO_2$ standard curve (0-50 μM).

Determination of IL-6 production

The determination of IL-6 production was done according to the manufacturer's instructions for commercial ELISA kits (Peprotech®, London, UK). A

capture antibody was used at 2 μg/mL and serial dilutions of recombinant IL-6 were used as standard curve. By last, cytokines present in the supernatants of the macrophages were incubated with a detection antibody (0.5 μg/mL) and avidin-peroxidase. The absorbances were measured at 490nm using the microplate reader. Cytokines concentration was determined by comparison with the standard curve.

Statistical analysis

All experiments were done in triplicate and the results expressed in means ± SD. One-way ANOVA and the Dunnett *post hoc* tests were utilized for data analysis ($p < 0.05$) by using the GraphPad Prism® V5.03 software (GraphPad Software Inc., California, United States of America).

RESULTS AND DISCUSSION

Extraction was performed using a Sephacryl S-300 with a bed volume of 50.86 cm3. According to the results, fractions with compounds greater than 100 kDa were pooled (Data not reported)

Quantification of acemannan by HPLC

High molecular weight fraction (>500 000 Da) of *Aloe vera* gel was determined by HPLC. The major component in *Aloe vera* gel from both seasons was acemannan (Fig. 1). Acemannan fractions presented concentrations of 2407.60757 μg/L (rainy season) and 1162.07526 μg/L (dry season).

The obtained chromatogram showed the most important peak from 5.0 to 5.5 min (estimated molecular weight between 4 and 7 million Daltons) which was similar to the results reported by Pugh et al. (2001).

Effect of the polysaccharides obtained from *Aloe vera* gel over the cell viability

The results demonstrated that polysaccharides from *Aloe vera* gel and acemannan standard did not affect the viability of peritoneal murine macrophages at different concentrations (Fig. 2). Furthermore, the percentages did not show significant differences in comparison to negative control composed by macrophages without treatment or stimuli (100.00%). The percentage means of three independent experiments were compared to a control group or C (-), supplemented (RPMI-1640) without treatment.

Effect of the polysaccharides obtained from *A. vera* gel on MΦ phagocytic activity

The effect of polysaccharides obtained from *A. vera* gel and acemannan standard over the phagocytic activity was determined by internalizing *Saccharomyces cerevisiae*, labeled with PI and then, determined by using a flow cytometer (Fig. 3). Treatments with *A. vera* extracts harvested during the rainy season, significantly increased the phagocytosis activity, and reflected as phagocytosis percentages, as follows: 5.49 % (1 μg/mL), 8.53 % (10 μg/mL), 39.40 % (100 μg/mL), 64.20 % (200 μg/mL), 69.49 % (500 μg/mL) and 54.70 % (1000 μg/mL). The results obtained by using *A. vera* extracts from the dry season although it stimulated phagocytic activity, it was lower than that obtained with treatment with *A. vera* extracts from the rainy season: 3.95 % (1 μg/mL), 5.48 % (10 μg/mL), 32.70 % (100 μg/mL), 56.39 % (200 μg/mL), 58.86 % (500 μg/mL) and 49.14 % (1000 μg/mL). The treatment with acemannan standard showed the highest phagocytic activity than that obtained with treatments with *A. vera* extracts from the rainy and dry season: 6.32 % (1 μg/mL), 10.24 % (10 μg/mL), 45.12 % (100 μg/mL), 68.90 % (200 μg/mL), 73.20 % (500 μg/mL) and 63.19 % (1000 μg/mL). The observed basal phagocytic activity (8.33%) was considered as a negative control or C

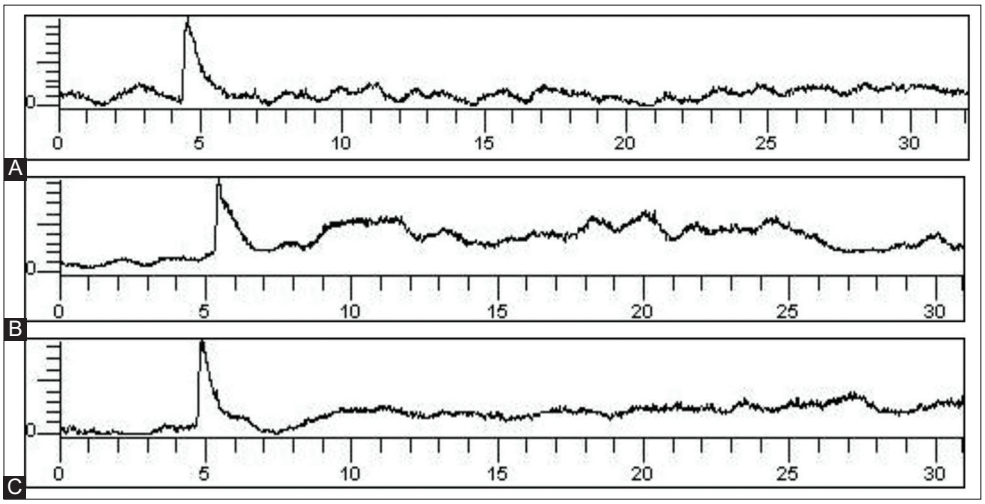

Fig 1. Identification by HPLC of acemannan. Mobile phase acetonitrile-water at a ratio of 65:35 (A). Acemannan 25 ppm (B). Rainy season (C). Dry season.

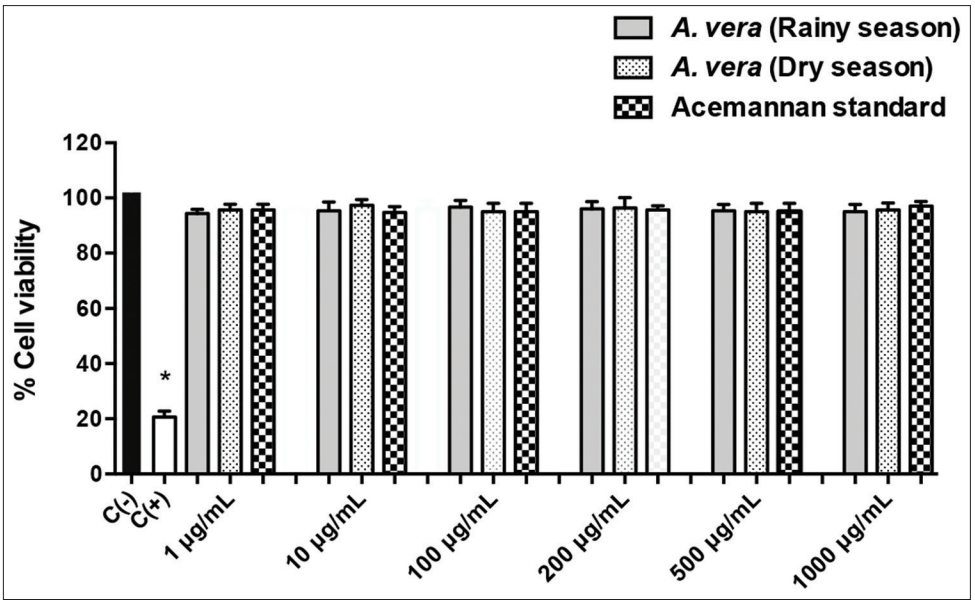

Fig 2. Effect of *A. vera* extract obtained in rainy season, dry season and acemannan standard on cell viability. The results represent the mean ± SD of three independent experiments in triplicate. Data were analyzed using one-way ANOVA followed by Dunnett's *post hoc* test. * Levels of $p < 0.05$ were considered as statistically significant in contrast to C(−). C(−): negative control, C(+): positive control.

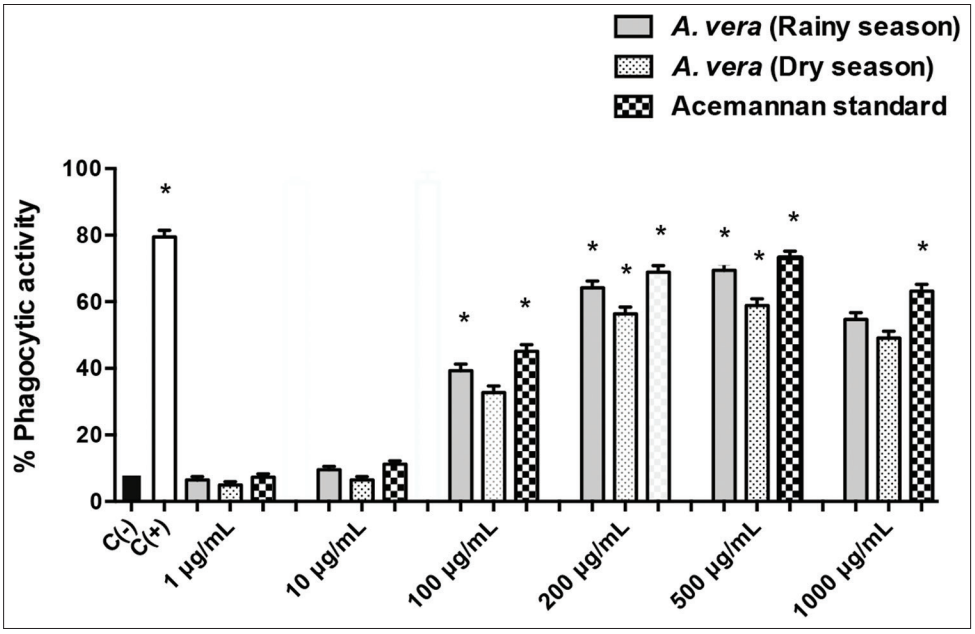

Fig 3. *Aloe vera* extracts and acemannan standard increased the phagocytosis activity *in vitro*. Murine peritoneal macrophages co-cultured with *Saccharomyces cerevisiae* yeast were treated with RPM1-1640 (negative control), LPS 1 µg/ml (positive control) or indicated concentrations of *Aloe vera* extracts and acemannan for 24 h. The amount of *Saccharomyces cerevisiae* phagocytosed was measured by flow cytometry. The results represent the mean ± SD of three independent experiments in triplicate. Data were analyzed using one-way ANOVA followed by Dunnett's *post hoc* test. * Levels of $p < 0.05$ were considered as statistically significant in contrast to C(−). C(−): negative control, C(+): positive control.

(-). MΦs stimulated with LPS (1 µg/mL) were considered as a positive control or C (+), at the highest percentage of phagocytic activity (79.43%).

Effect of the polysaccharides obtained from *Aloe vera* gel and the acemannan standard over NO release

Treatments with *A. vera* extracts harvested during the rainy season, significantly increased the NO production, and reflected as nitrites levels, as follows: 4.18 µM/mL (1 µg/mL), 16.34 µM/mL (10 µg/mL), 20.13 µM/mL (100 µg/mL), 27.19 µM/mL (200 µg/mL), 38.21 µM/mL (500 µg/mL) and 36.13 µM/mL (1000 µg/mL). The results obtained by using *A. vera* extracts from the dry season although it stimulated NO production, it was lower than that obtained with treatment with *A. vera* extracts from the rainy season: 3.15 µM/mL (1 µg/mL),

12.42 μM/mL (10 μg/mL), 17.12 μM/mL (100 μg/mL), 25.15 μM/mL (200 μg/mL), 34.12 μM/mL (500 μg/mL) and 31.19 μM/mL (1000 μg/mL). The treatment with acemannan standard showed the highest NO production than that obtained with treatments with *A. vera* extracts from the rainy and dry season: 5.19 μM/mL (1 μg/mL), 18.51 μM/mL (10 μg/mL), 22.26 μM/mL (100 μg/mL), 31.14 μM/mL (200 μg/mL), 40.30 μM/mL (500 μg/mL) and 38.21 μM/mL (1000 μg/mL). The basal NO production found (2.57 μM/mL) was considered as a negative control or C (-). MΦs stimulated with LPS (1 μg/mL) were considered as a positive control or C (+), at the highest percentage of NO production (45.32 μM/mL) (Fig. 4).

Effect of the extract of polysaccharides obtained from *Aloe vera* gel on MΦ-related cytokines production

The effects of *A. vera* polysaccharides and the acemannan on IL-6 production by activated MΦs was evaluated. MΦs cells were incubated at different concentrations of *A. vera* and cytokines secreted were measured by ELISA by analyzing culture supernatant. Treatments with *A. vera* extracts harvested during the rainy season, significantly increased the IL-6 production as follows: 72.17 pg/mL (1 μg/mL), 124.31 pg/mL (10 μg/mL), 235.73 pg/mL (100 μg/mL), 372.98 pg/mL (200 μg/mL), 784.92 pg/mL (500 μg/mL) and 719.45 pg/mL (1000 μg/mL). The results obtained by using *A. vera* extracts from the dry season although it stimulated IL-6 production, it was lower than that obtained with treatment with *A. vera* extracts from the rainy season: 68.54 pg/mL (1 μg/mL), 108.63 pg/mL (10 μg/mL), 193.82 pg/mL (100 μg/mL), 309.92 pg/mL (200 μg/mL), 717.83 pg/mL (500 μg/mL) and 673.32 pg/mL (1000 μg/mL). The treatment with acemannan standard showed the highest IL-6 production than that obtained with treatments with *A. vera* extracts from the rainy and dry season: 79.43 pg/mL (1 μg/mL), 139.92 pg/mL (10 μg/mL), 267.41 pg/mL (100 μg/mL), 412.83 pg/mL (200 μg/mL), 832.89 pg/mL (500 μg/mL) and 781.34 pg/mL (1000 μg/mL). The basal IL-6 production found (65. 72 pg/mL) was considered as a negative control or C (-). MΦs stimulated with LPS (1 μg/mL) were considered as a positive control or C (+), at the highest percentage of NO production (921.71 pg/mL) (Fig. 5).

A wide variety of biological activities have been attributed to the isolated polysaccharides of *A. vera*. Among the carbohydrates that make up the *A. vera* gel, the largest fraction is acemannan, which is a mixture of polymers of various lengths with β (1-4) bonds of acetylated mannan (Manna and McAnalley, 1993), which has been known for having several immunomodulatory activities, especially as an immunopotentiator, *in vivo* as well as *in vitro*.

In this study, the high molecular weight polysaccharides that are present in the *A. barbadensis Miller* (*Aloe vera*) gel were extracted during the rainy and dry seasons. In the determination and identification of acemannan through HPLC, there was an increase during the rainy season in relation to the drought. Some authors assert that acemannan can undergo degradation due to changes in temperature and pH, or the presence of bacterial contamination (Waller et al., 1978; McAnalley, 1990; Pelley et al., 1993; Diehl and Teichmüller, 1998; Djeraba and Quere, 2000; Suárez-Luque et al., 2002; Davis and Goux,

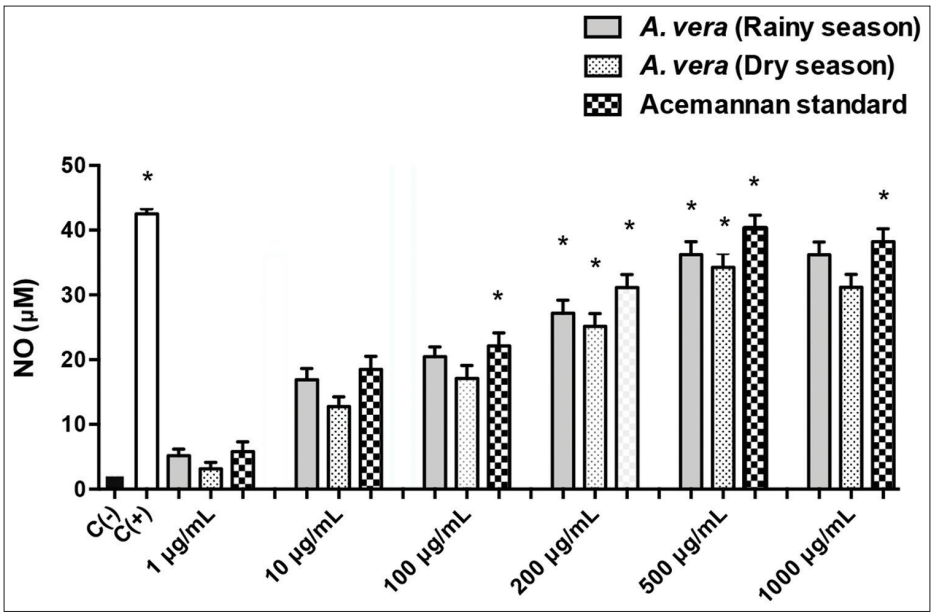

Fig 4. Effect of *Aloe vera* extracts and acemannan standard on NO production. The results represent the mean ± SD of three independent experiments in triplicate. Data were analyzed using one-way ANOVA followed by Dunnett's *post hoc* test. * Levels of $p < 0.05$ were considered as statistically significant in contrast to C(+). C(+): positive control, C(−): negative control.

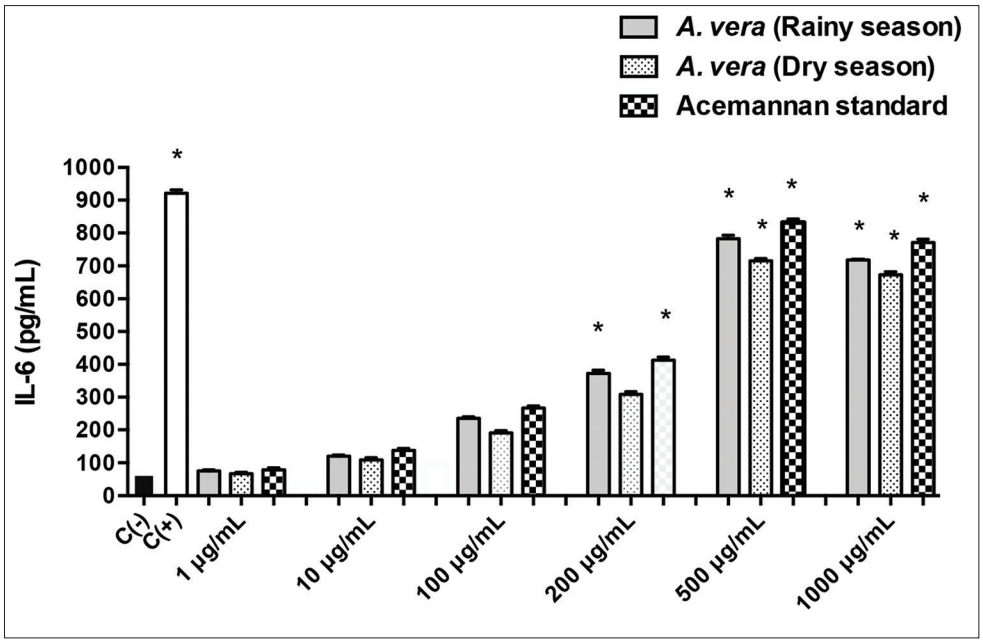

Fig 5. Effect of *Aloe vera* extracts and acemannan standard on IL-6 release. The results represent the mean ± SD of three independent experiments in triplicate. Data were analyzed using one-way ANOVA followed by Dunnett's *post hoc* test. * Levels of $p < 0.05$ were considered as statistically significant in contrast to C(+). C(+): positive control, C(−): negative control.

2009; Jawadul et al., 2014). In the same way, recent studies show that the deficit of water reduces the mannose of acemannan (Minjares-Fuentes et al., 2017). Cells treated with the acemannan standard and *Aloe vera* extracts at 1, 100, 200, 500 and 1000 µg/ml induced the proliferation of macrophage cells, the acemannan standard grew by 94 % and in both seasons increased by 95 %. In the phagocytosis tests, the highest macrophage stimulation was obtained from 72.30 % for the acemannan standard, 69.49 % for the rainy season and 58.86 % for the drought. In the production of nitric oxide, values of 38.21 µM/mL were obtained for the rainy season, 34.12 µM/mL for the dry season and 40.30 µM/mL for acemannan. Through a inflammation or infection, proinflammatory cytokine secretion occurs through innate activation of macrophages, such as IL-6 and TNF-α , these aid in the acute phase response by acting on a variety of cells (Wynn et al., 2013). In our study, the extract of A. vera leaves increased the levels of IL-6 in a concentration-dependent manner. The amount of IL-6 was measured by ELISA: 784.92 pg/mL for the season rainy, 717.83 pg/mL for the dry season and 832.89 pg/mL for the acemannan.

Some of the immunopotentiating activities of acemannan appear to be mediated through the activation of macrophages (Womble et al., 1988; Womble et al., 1992). A proposed mechanism involves macrophages and dendritic cells because they possess receptors for glucans and mannans and induce phagocytosis and the secretion of cytokines, leukotrienes, and prostaglandins (Pens et al., 1991). Similarly, Lujan et al. (2008), reported that phagocytic activity *in vivo* where the number of bacteria phagocytosed by macrophages was higher in mice inoculated with aqueous extract of *Aloe vera* gel, in which a greater number of macrophages was observed per field and that the majority shows phagocytosis. There is an increase in phagocytic activities in the rainy season compared to the drought, possibly due to the water deficit, since studies carried out by Minjares et al. (2017), suggest that acemannan does not suffer deacetylation because of deficits in applied water; however, this is the most affected bioactive polymeric compound by water deficit since there was a reduction of 41% in mannose.

CONCLUSIONS

The results allow us to affirm that the extraction method of polysaccharides from the *Aloe vera* leaf collected in the rainy season or drought was effective since the immunostimulant effect in peritoneal macrophages was determined efficiently, and there were differences in both seasons. Studies show that the composition of acemannan depends on the methods used to isolate the polysaccharide, the time of harvest and the growth conditions. Understanding the influence of water deficit on the main characteristics of *Aloe vera* polysaccharides could be a useful tool for the design, development, and control of biologically active ingredients based on the *A. vera* plant. For conclusion, in this work we observed that the extract from *A. vera* gel showed an immunostimulatory effect on phagocytic activity, beginning with the overproduction

of NO and IL-6 in peritoneal murine macrophages. Various polysaccharides are present in *A. vera* gel and it is possible that these metabolites are associated in the immunostimulatory effects. Notwithstanding these results, more phytochemical and pharmacological investigations of this plant are still required.

Authors' contributions

CHHM and VAA conceptualized and designed the research; BAC and JTC carried out the in vitro study and cell culture analysis; LVV and LCG accrued out the HPLC; IIF and SSP wrote the manuscript. All authors read and approved the submission of the manuscript.

CONFLICT OF INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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