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Attenuation of allergic airways inflammation by an extract of *Hymenocardia acida*

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Tracheal hyperresponsiveness, airway mucus production and bronchoalveolar inflammation are the major components of asthma. Here, we aim to investigate the role in the control of asthma of a bioactive plant extracted from *Hymenocardia acida* in a physiological and pathophysiological model. The effect of *H. acida* crude extract (HACE) on total cellular components of bronchoalveolar (BAL) fluids was performed on ovalbumin (OVA) and lipopolysaccaride (LPS)-challenged Swiss mice for induction of allergic asthma and airways inflammation, respectively. Mice were pretreated with 0.9% sodium chloride (NaCl), HACE (oral doses at 100 mg/kg/body weight) for a week and then by intranasal instillation with OVA (0.5 mg/ml) + aluminium hydroxyde (20 mg/ml), during three days after intraperitoneally sensitization or with LPS (0.4 mg/ml) for a day (OVA or LPS + HACE). The BAL cells were collected in a mixed solution (0.9% NaCl and 2.6 mm Ethylenediaminetetraacetic acid EDTA) one day after the last challenge and total cells were numbered in a Neubauer chamber. The HACE: (i) significantly inhibited the airways inflammation induced by a single intranasal instillation of LPS or allergic asthma on mice challenged with 3 consecutive days intranasal instillation of OVA in comparison to control mice only instilled with 0.9% sterile. NaCl : (ii) significantly impaired the increased levels of total cells in OVA and LPS-treated mice, without changing the basal cellularity after NaCl or HACE treatment; (iii) and significantly inhibitshydroxyl radicalsandsuperoxideanions production. Taken together, these results suggest that HACE exposure induces a marked reduction of cellular component in the BAL fluid, which is only partially lymphocytes dependent.

Key words: Asthma, prevention, *hymenocardia acida*, mice.

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INTRODUCTION

Chronic respiratory diseases are public health priority because of their frequency and potential severity. Indeed, according to World Health Organisation estimates, 300 million people suffers from asthma and it could reach 400 million by 2025 with more than 200,000 deaths per year (Kouris-Blazos and Wahlqvist, 2007). The global strategy against chronic diseases, developed to reduce their negative impact, cited chronic respiratory diseases in four groups of diseases to be addressed as a priority (Warrington, 2014).

The main strategy is to reduce morbidity and premature death from chronic respiratory diseases. These diseases, including asthma, are characterized by bronchial hyper-responsiveness (BHR) and airway inflammation. For acute respiratory disease, it is well-documented that both tobacco smoking and air pollution are believed to be environmental factors affecting the prevalence of bronchial asthma (Majeed et al., 2008; Sasaki et al., 1998). Allergic disorders and asthma are often co-morbid, and allergic diseases has been shown to be a risk factor for asthma in adults and children (Inoue et al., 1983; Kamijo et al., 2013; Kitani et al., 1989; Kuruma et al., 2014). A relationship between BHR and infantile wheezing diseases has been reported. Infants with a genetic predisposition to atopy are more likely to wheeze with respiratory viral infection or bronchiolitis, and it is suspected that the continued BHR after the first attack of asthma may be induced or triggered by some viral infections (Mochizuki et al., 2000).

Many infections, including influenza infection, pulmonary cryptococcosis and pneumocystis carinii pneumonia, are known to be an exacerbating factor in the control of asthma (Kohno et al., 1992; Koshio et al., 2014; Suzuki et al., 1991). Gastroesophageal reflux disease (GERD) also, like other affections, is associated with a variety of extraesophageal symptoms including asthma (Sugawa et al., 2007). The research priorities, in addition to preventive strategies, should include the use of herbal component from traditional medicine. WHO encourages the inclusion of medicinal plants in health care programs in developing countries (Bielory, 2004; Ziment, 1997). Numerus studies in which informant consensus about plants are done was conducted by collecting ethnobotanical information during interviews with native knowledgeable people (Aaria-Kundalia et al., 2010; Alachkar et al., 2011; Kayani et al., 2014; Lee et al., 2008; Njoroge and Bussmann, 2006; Sargin et al., 2013). The most dominant medicinal plant species belonging to at least 50 plant families were utilized in west africa. These plants were of varying habits: herbs, shrubs, trees as well as some grasses and sedges. The traditional preparations were found to be made mainly from leaves, roots and barks. Anti-allergic activity of herbal plants on airway inflammation has been used for the treatment of allergic bronchial asthma (Costa et al., 2012; Lee et al., 2013; Li et al., 2006; Mahajan and Mehta, 2008; Nagai et al., 2004) and senegalese traditional medicine is rich in medicinal herbs for respiratory problems, including *H. acida (Euphorbiaceae). H. acida* is a small savannah tree or shrub about 9 m high. It is commonly found in an open woodland in association with other species.

The generic name *Hymenocardia* is derived from the Greek words ‘hymen’ - membrane and ‘kardia ’- heart, in reference to the heart-shaped fruits which have a transparent covering membrane (hymen). The specific epithet acida describes the sour taste of its fruits. Some authors consider the genus under the family Hymenocardiaceae. Tannins from *H. acida* stem bark are used to treat diarrhoea and dysentery and show good activity. In tradionnal medecine, the bark is used in concoctions as remedy for an unspecified disease condition. In East Africa, the plant is used in treating wounds. Root bark extracts exhibited cytotoxicity against the 60 human cell lines of the National Cancer Institute (NCI). *H. acida* extracts demonstrated a marked antibacterial activity against Klebsiella pneumoniae (Orwa et al., 2009). Findings show a mucociliary activity of *H. acida* ethanol extract, and justify its use in the treatment of airway disorders (Obidike et al., 2011). Other studies investigate the antioxidant activity of aqueous and methanolic extracts from *H. acida*. Qualitative thin layer chromatographic (TLC) of the extract was positive for flavonoids, phenols, steroids and triterpenoids. Thus, the alcoholic extract of the leaves of *H. acida* can be used as an easily accessible source of natural antioxidant (Sofidiya et al., 2009). In this present work, we aim to study the preventive effect of *H. acida*on an allergic airway inflammation experimental model.

MATERIALS AND METHODS

Animals

Male Swiss mice (20 to 26 g) were obtained from Institut Pasteur (Dakar, Senegal). They were housed in standard laboratory cages and allowed food and water ad libitum throughout the experiments. All animal procedures were approved and conducted in accordance with the guide for the care and use of laboratory animals as promulgated by the Senegalese Academic Bioethic Committee (Décret n° 2009 to 729).

Chemicals

All chemicals were of analytical grade and obtained from standard commercial sources. All reagent and extract were diluted in appropriate buffer solution before use.

Preparation of extracts

Crude extract of *H. acida* leaves were prepared as previously described (Sar et al., 2010). In brief, powdered material was subjected to extraction for 2 h using a 60% ethanol/water solution and macerated extract was then filtered in vacuum conditions and evaporated on a rotary evaporator.

Allergic airways inflammation model

To investigate the effects of *H. acida* leaves extract on airways pathology, we developped an acute model of airways inflammation by intranasal administration of LPS, as previously described (Puljic et al., 2007; Yingkun et al., 2013) and acute allergic response by OVA intranasal instillation (Nials and Uddin, 2008). During all experiments, the hygiene conditions (change of bedding every 48 h) as well as a regular diet were respected. Experiments were carried out as follow :

1. Preventive treatment by HACE (day 0 to 7): Mice (n= 18) received daily preventive treatment of *H. acida* Crude Extract (HACE : 100mg/kg/body weight) by gavage, while untreated (n=18) controls were receiving saline buffer (sterile 0.9% NaCl). The working concentration (100 mg/kg/body weight) was chosen on the basis of the *in vitro* maximum relaxation obtained with the *H.acida* crude extract at a concentration of 0.1mg/ml.
2. Airways inflammation induction (day 8 to 9): To induce airways inflammation, intranasal instillation technique was performed as previously described (Vernooy et al., 2001). At day 8, mice (n= 6) were anesthetized by intraperitoneal (IP) injection of 150 µl of a mixture of 83 µl xylazine and 257µl ketamin, dissolved in 1660 µl of sterile 0.9% NaCl and were challenged with a single intranasal instillation of 12.5 µl of LPS at a dose of 40 µg/ml per mouse. After intranasal treatment, the mice were kept in an upright position for 5 min to allow the fluid spread throughout the lungs. After 15 to 20 min the animals wakes up. Animals treated with LPS were presented in an isolated cage with food and water in sufficient quantity to isolate them 24 h before the bronchoalveolar lavage (BAL). Control mice were only instilled with sterile 0.9% NaCl. With this model, which rapidly recruits leukocytes to the lung airways, it is possible to quantify and characterize the cellular profile of recruited leukocytes.
3. Induction of allergic asthma (day 8 to 15): To assess the role of *H. acida*leaves extract in allergic asthma, at day 8, mice (n = 6 per group) were firstly sensitized by an intraperitoneal injection of an ovalbumin OVA (0.5 mg/ml) and aluminium hydroxide (AlOH) adjuvant (20mg/ml) mixture dissolved in sterile saline (NaCl 0.9%) for 3 days (8, 9, 10). They received on days 13, 14 and 15, intranasal instillation of 0.4 mg/ml ovalbumin solution dissolved in sterile saline (0.9% NaCl) to induce asthma crisis. After 15 days treatment, mice were sacrificed, and various biological samples were collected for analyses. Control mice were sensitized and challenged with sterile saline alone.

Bronchoalveolar lavage (BAL)

BAL was made with the mixture of physiological saline solution (0.9% NaCl) and 2.6 M EDTA. HACE-treated mice and challenged with LPS or OVA asleep after surgical anesthesia with a mixture of ketamine/zylazine dissolved in physiological saline. The bronchoalveolar lavage was performed after insertion of a cannula in the trachea and injection of 500µl saline / EDTA. After energic massage of chest for 10 to 15 seconds, the bronchoalveolar fluid was aspirated. This was repeated 10 times, and the lavages were pooled (5ml mean volume). Thereafter, proceeded to centrifugation at 1200 rpm/min for 5 min at 21°C followed by hemolysis by a mixture of water and KCl (0.6 M) for a total volume of 1.5 ml followed by another centrifugation. After centrifugation, the cell pellets were resuspended in 1 ml of NaCl plus EDTA solution. BAL cells were collected and total cells were counted as described in the protocol.

Counting and cell differentiation

Total count:Two drops of collected BAL fluid were filed between slide and coverslip. The cells were counted under a microscope in a neubauer counting chamber ($1mm^3$ volume, magnification 10).

Cell differential count: Population of eosinophils, neutrophils, lymphocytes and macrophages in BAL fluids (200 cells/slide) from individual mice were determined using morphologic criteria under a light microscope after trichrome stain with May Grünwald Giemsa (Life Technologies, Auckland, New Zealand). Results are expressed as percentage of cells.

Hydroxyl radical scavenging: Spontaneous hydroxyl radical release by Fenton's reaction in the presence of an iron-chelating agent (Cobalt-EDTA complex) was measured by luminol-enhanced chemiluminescence in a luminometer (430 nm, Wallac Trilux 1450 Micro bêta liquid scintillation and luminescence counter) at 37°C. This fluorescence generally decreases when an antioxidant is present in the medium. Thus, the hydroxyl radical scavenging effect of the *H. acida* crude extract was examined at a concentration of 2 and 10 µg/ml in triplicate using a 96 well microplates (Greiner Bio-one 650 201). Results are expressed as a percentage of the decrease in absorbance at 430 nm using a microplate reader (Greiner Bio-one 655 209), compared to those of Quercetol (0.5µg/ml), a well-known radical scavenger.

Superoxide anion scavenging: The superoxide anion radical scavenging effect test of the *H. acida* crude extract was assessed according to the method of Chaabi et al., 2008. The superoxide radicals were generated *in vitro* by the hypoxanthine/xanthine oxidase system. The scavenging activity of the extract (at a concentration of 25 and 100 µg/ml) was determined by the nitro blue tetrazolium (NBT) reduction method. In this method, O_2^- reduces the yellow dye NBT^{2+} to produce the blue formazan, whose absorbance was measured spectrophotometrically at 560 nm (VERSA Max Microplate Reader, Concord, Canada). Antioxidants are able to inhibit the formation of purple NBT. The xanthine oxidase system is sensitive to temperature, the tests should be performed at 25°C. Each sample was tested in triplicate using a 96 Well Microplates (Greiner Bio-one 650 201). Quercetol (1 µg/ml) was used as a positive control that maximally inhibits the production of superoxide free radical. The results are expressed as the percentage inhibition of the NBT reduction with respect to the reaction mixture without a sample (buffer only).

Preliminary phytochemical screening: In order to quantify and correlate the biological acitivity with the molecules present in the studied extracts, we carried out an initial phytochemical screening by high performance liquid chromatography (HPLC) method, the most commonly used separation technique for nonvolatile molecules. The HPLC equipment consisted of an integrated system (Varian 920 LC Liquid chromatograph) with a multiwavelenght diode array detector (DAD). The chromatographic separation was achieved with an pre-packed HPLC column (EC 150/3 Nucleodur

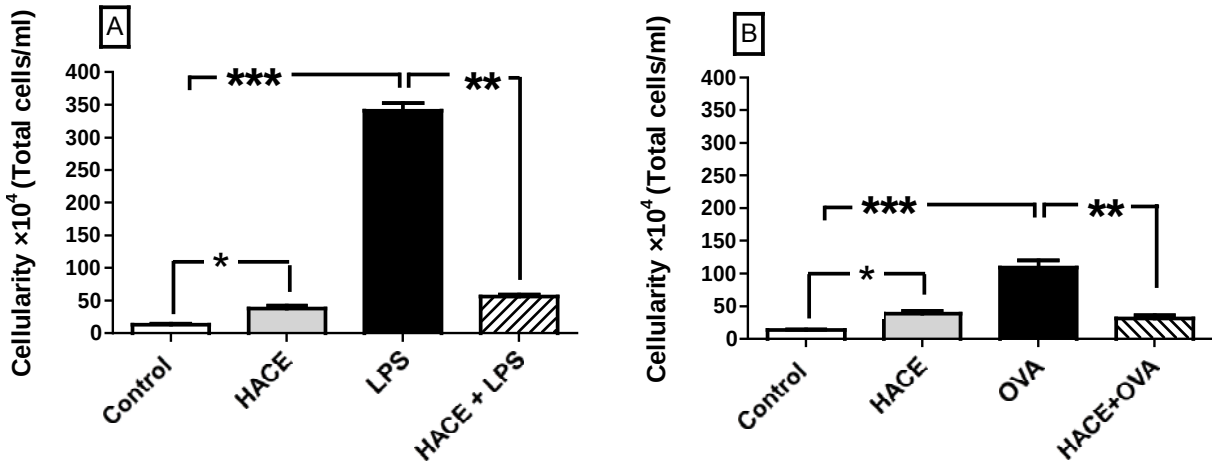

Figure 1. Effect of *Hymenocardia acida* leaves crude extract (100mg/kg/body weight) on (A) airway inflammation induced by a single intranasal instillation of LPS (40µg/ml per mouse) or on (B) allergic asthma on mice challenged with 3 consecutive days intranasal instillation of 0.4 mg/ml ovalbumin (OVA) in comparison to control mice only instilled with 0.9% (LPS or OVA)-free sterile NaCl. Results areexpressed as meanof total number of counted cells ± SEMfor at leastsixexperimentsobtained fromdifferentmice. ns =not significant, * = p <0.05, ** = p < 0,01 and *** = p < 0,001(Unpaired t-test).

Sphinx RP, 3 µm-Macherey-Nagel) operating at room temperature. The mobile phase used was a mixture of water and methanol (95 : 5, v/v) at a flow rate of 0.7ml/min and the injection volume was 10µl. By this method, we analyzed the crude extracts of *H. acida*, preparated at 5mg/ml, filtered and 200 µl of the solution were placed in vials for HPLC analysis. The compounds were identified by chromatographic comparisons with authentic standards by their UV spectra. Quantification was based on the fluorescence signal response at 280 nm.

Statistical analysis

Values are expressed as mean ± SEM. Statistical evaluation was performed with student's t test for independent data. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Effect of *Hymenocardia acida* crude extract on the inflammatory asthma component

In order to determine the preventive effect of *H. acida* on airway inflammation, we compared the overall cellularity in BAL fluid from LPS-challenged mice and those who received HACE before. As shown in Figure 1, we observed a basal cellularity of 13.5±0.46 $x10^4$ cells/ml on BAL fluid from Saline-exposed control mice. HACE treatment alone (34.67±3.22 $x10^4$ cells/mL) did not modify the basal cellularity. Treatment with LPS induced a strong airway inflammation objectified by a significantly increase of cell population influx (365.42±9.83 $x10^4$ cells/ml) in comparison to those noted in control saline-exposed mice. Finally, preventive treatment with the *H. acida* (HA) extract significantly reduced the cellular influx induced by LPS (from 365.42±9.83 $x10^4$ to 55.75±2.03 $x10^4$ cells/ml).

Effect of *Hymenocardia acida* crude extract on the asthma allergic component

Similarly, the overall cellularity was assessed in OVA-exposed mice treated with the HA extract in order to investigate the preventive role of that plant in the development of allergic asthma induced by OVA. Thus, as shown in Figure 1, intranasal instillation of OVA significantly increases bronchoalveolar cellularity with a cellular influx of 109.17±11.08 $x10^4$ cells/ml compared to the basal cellularity of control mice (13.85±0.62 $x10^4$ cells/ml)and consistent with an allergic asthma. Aqueous extract administration alters the BAL fluid basal cellularity (control) on mice, which increases to 38.55±4.36 $x10^4$cells/ml compared to NaCl-exposed controls, but significantly reduced cellular influx induced by OVA (31.54±4.67 $x10^4$).

Effect of *Hymenocardia acida* crude extract on different cell populations in inflammatory asthma

In order to study the effect of HACE treatment on inflammatory asthma, we evaluated changes in cell populations of the BAL fluid of LPS-treated mice. The main results (Figure 2 and Table 1) showed, in saline-

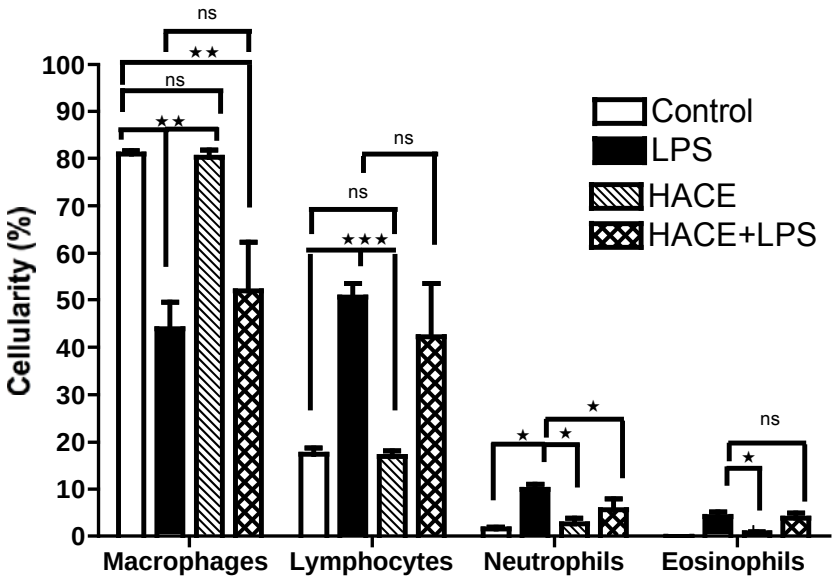

Figure 2. Changes of bronchoalveolar lavage fluid cell populations from LPS-exposed mice (40µg/ml per mouse) by a single intranasal instillation after *Hymenocardia acida* leaves crude extract (100mg/kg/body weight) treatment.Results areexpressed as meanof total number of counted cells ± SEMfor at leastsixexperimentsobtained fromdifferentmice compared with control mice only instilled with 0.9% (LPS)-free sterile NaCl. ns =not significant, * = p <0.05, ** = p < 0,01 and *** = p < 0,001(Unpaired t-test).

Table 1. Maximum effects of *Hymenocardia acida* crude extract (100mg/kg/body weight) ondifferent cell populationsofbronchoalveolar lavage fluidinallergicandinflammatorymodels of asthma. Results are expressed as percentage of cells.

Cell types (%)	Control	HACE	OVA	LPS	HACE+OVA	HACE +LPS
Macrophages	81,0±0,71	80,33±1,52	74,33 ± 4,16	44,00±5,63	71,00 ± 3,00	52,00±10,39
Lymphocytes	17,5 ±1,22	17,00 ± 1,15	18,66 ± 0,88	50,66 ± 2,96	20,33 ± 2,43	42,33 ± 11,35
Neutrophils	1,50 ± 0,41	2,66 ±1,17	3,00 ± 1,15	10,00 ± 1,15	4,66 ± 2,03	5,66 ± 2,18
Eosinophils	00,00 ± 00,00	0,66 ± 0,33	5,33 ± 2,41	4,21 ± 0,88	3,00 ± 0,58	3,78 ± 1,09

exposed controls mice, a majority of macrophages (80%), followed by lymphocytes (about 20%) and neutrophils oreosinophils(less than 5%), under basal conditions. HACE treatment, in comparison to control, does not alter the macrophages, and lymphocytes or neutrophils and eosinophils populations. It significantly reduces the increase of lymphocytes and polynuclear induced by LPS. Indeed, the lymphocytes (50.66±2.96% after LPS-treatment) are reduced at 42.33±11.35% when preventively treated with HACE. Similarly, polynuclear vary from 10.00±1.15% (after LPS-treatment) to 5.66±2.18% (after LPS+HACE-treatment). However, the macrophage populations, although reduced after LPS-treatment, are not changed following treatment with HACE.

Effect of *Hymenocardia acida* crude extract on different cell populations in allergic asthma

In order to study the effect of the administration of HA extraction the recruitment of different cell types in allergic asthma induced by OVA, we measured cell populations of macrophages, lymphocytes, neutrophils and eosinophils in BAL fluid before and after treatment of mice with HA aqueous extract. Results (Figure 3 and Table 1) showed that macrophages represent at least 80% of cells in the BAL fluid. They are followed by lymphocytes (20 to 25%), neutrophils and eosinophils (less than 10%). Treatment of mice with HACE did not reduced the macrophages population in allergic asthma (from 74.33±4.16% in OVA-exposed controls to 71.00±3.00% in

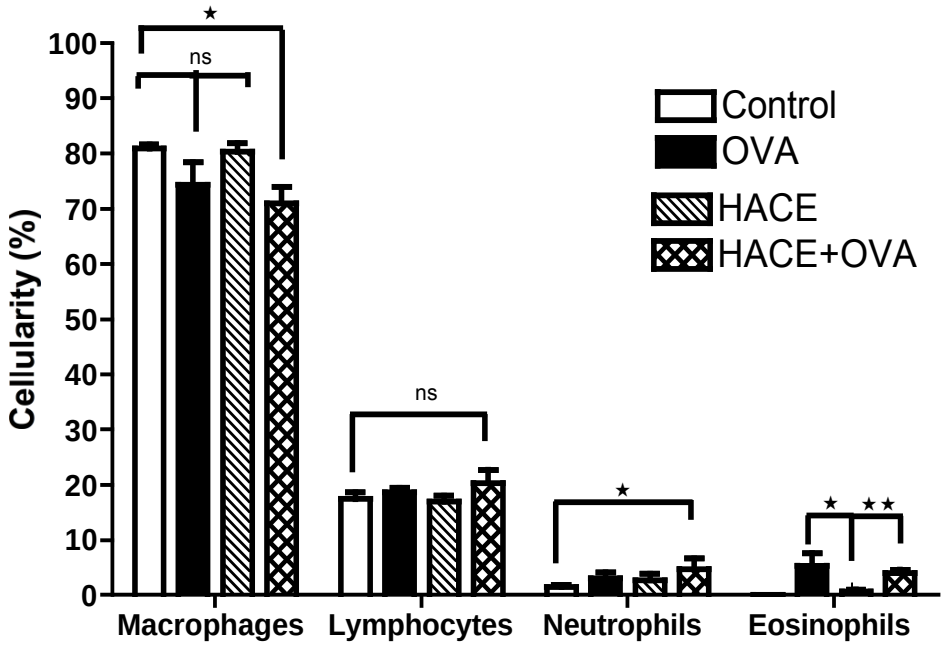

Figure 3. Changes of bronchoalveolar lavage fluid cell populations from OVA-exposed mice (0.4 mg/ml ovalbumin) by an intranasal instillation after *H. acida* leaves crude extract (100mg/kg/body weight) treatment. Results are expressed as mean of total number of counted cells ± SEM for at least six experiments obtained from different mice compared with control mice only instilled with 0.9% (OVA)-free sterile NaCl. ns =not significant, * = p <0.05 and ** = p < 0,01(Unpaired t-test).

(HACE+OVA)-treated mice); nor the neutrophils population (3.00±1.15% in OVA-exposed controls and 4.66±2.03% in (HACE+OVA)-treated mice. OVA exposure significantly increases the population of eosinophils (from any cells in NaCl-exposed controls to5.33±2.41% in OVA-challenged mice). HACE treatment does not alter the basal cellularity (0.66±0.33%), but significantly reduces the cellular influx induced by OVA (from 5.33±2.41 to 3.00±0.58 %) in the eosinophils population. For the lymphocytes population, any significaly changes was observed.

Effect of *Hymenocardia acida* crude extract onfree radicals production

Results shown in Figure 4 indicated that the *H. acida* crude extract significantly inhibits hydroxyl radicals and superoxide anions production. Indeed, we note a significant increasing inhibition depending on the extract concentration. At 2 µg/ml, an inhibition of 40.13%±6.09 for hydroxyl radicals was obtained which is significantly lower than that obtained with10 µg/ml,66.77±3.21%. Similar results were obtained with the extraction of the superoxide anions inhibition, but with higher concentrations. At 25 µg/ml, we measured an inhibition of 45.51% ±5.98and at 100µg/ml, a significantly greater inhibition of 67.21%±3.70 was obtained. However, for the various concentrations tested, the percentage inhibition of the extract remains below that of the reference quercetol, which inhibits the radicals to 100% at a standard concentration of 1 µg/ml.

Phytochemical constituents of *Hymenocardia acida* crude extract

Phytochemical analysis by HPLC, as indicated in Figure 5, revealed about thirty compounds whose majority has not been identified (Table 2). Among the compounds identified (Table 2), it is found mainly in glycosides, phenolic acids, alkaloids, glucosylflavonoids, benzopyrans and polycyclicaromatic derivatives.

DISCUSSION

This work was initiated in order to study the effects of *H.acida* on the allergic component of asthma, oxidative stress and inflammation. This plant from the african

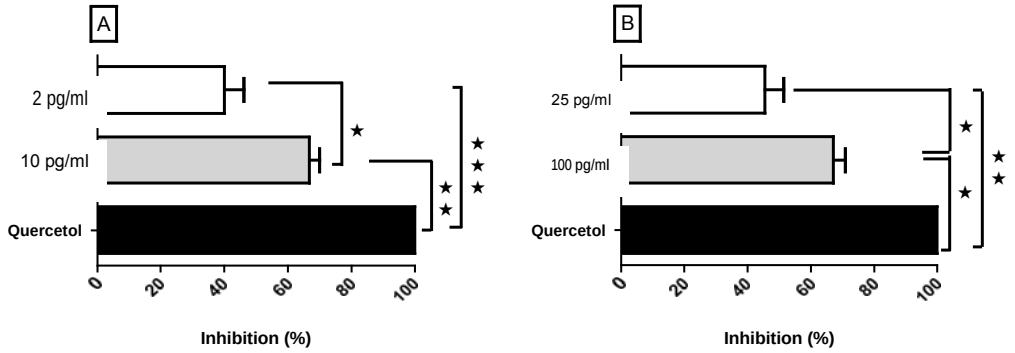

Figure 4. Percentage inhibition of hydroxyl radical (A) and superoxide anion (B) after *hymenocardia acida* crude extract treatment. The percentage scavenging effects were calculated from the decrease in absorbance against Quercetol, a well-known radical scavenger, as positive control. Results areexpressed as mean ± SEMof triplicate determinations.

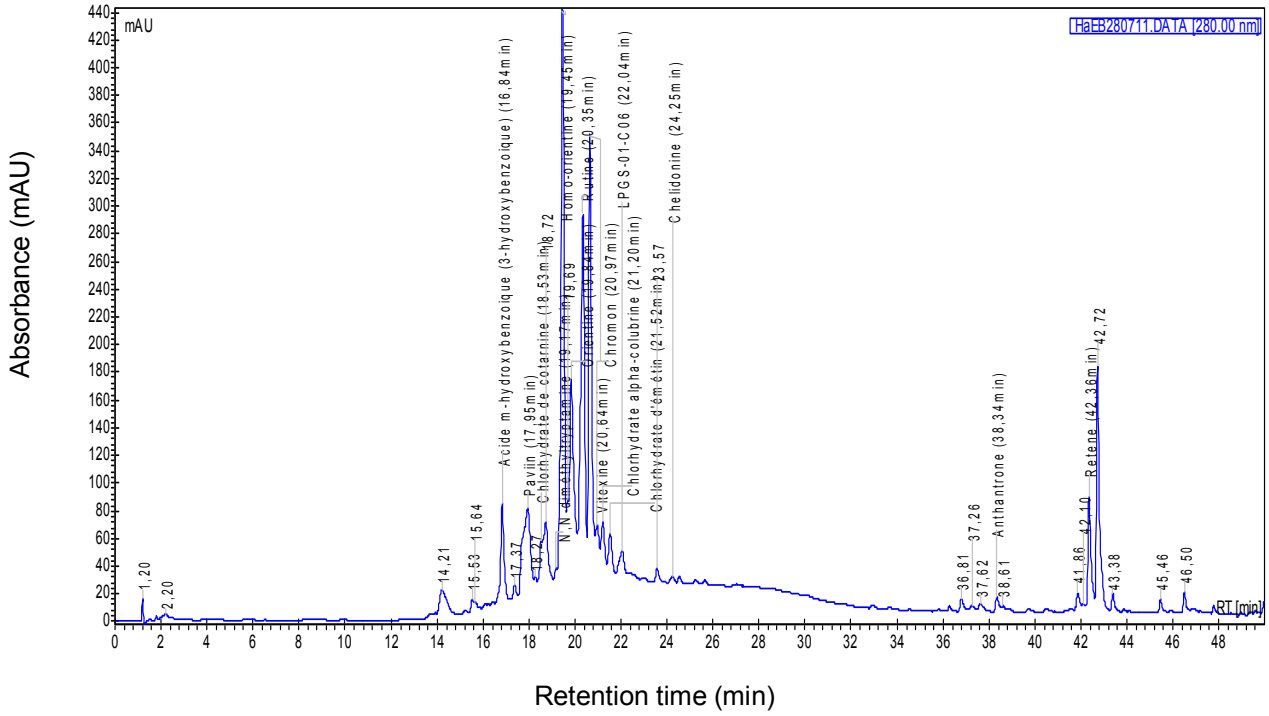

Figure 5. HPLC chromatogram of the *H. acida* leaves crude extracts (5 mg/ml) based on the fluorescence signal response at 280 nm. Compounds were identified by chromatographic comparisons with authentic standards and by their UV spectra.

pharmacopoeia, whose preventive effect on airways contraction had already been demonstrated *in vitro* (Sar et al., 2010), showed an inhibitory effect on airways inflammation induced by LPS and allergic asthma induced by ovalbumin. Indeed, based on cell morphology, our results suggest that preventive treatment with *H.acida* crude extract significantly reduced the cellular influx induced by LPS in a neutrophils and eosiniphils dependant manner. Also, a significant reduction was observed on cellular influx induced by ovalbumin when treated with *H. acida* crude extract, which is only eosinophils-dependent. The inflammation induction by LPS intranasal instillation and the bronchoalveolar lavage technique method have been carried out by Frossard and Fajac (1995) and this allowed us to have an adequate cellularity in eight groups of mice under different conditions.

Table 2. Precision accuracy and recovery data for identified and non identified compounds after HPLC *H. crude* extract analysis. NI : non identified; Rt, min: retention time, minute; mAU: milli Absorbance Unit; CV: RSD: Relative standard deviation.

Compound	Rt, min	mAU	RSD, n=3
m-hydroxybenzoiq acid (3-hydroxybenzoiq)	16.84	82	0.88
Paviin	17.95	82	0.81
Cotarnin chrlohydrate	18.53	62	0.76
Homo orientin	19.45	420	0.47
Orientin	19.84	280	0.89
Rutin	20.35	360	0.64
Vitexin	20.64	60	0.72
Chromon	20.97	54	0.33
Alpha-colubrin	21.20	44	0.52
Emetin chlorhydrate	21.52	40	0.43
Chelidonin	24.25	24	0.51
Anthatrone	38,34	24	0.78
Retene	42.36	32	0.39
NI	1.20	19	0.23
NI	2.20	08	0.43
NI	14.21	24	0.51
NI	15.53	20	0.66
NI	15.64	78	0.43
NI	17.37	80	0.67
NI	18.27	38	0.57
NI	36.81	22	0.22
NI	37.26	20	0.42
NI	37.62	18	0.54
NI	38.61	22	1.02
NI	41.86	26	0.48
NI	42.10	56	0.57
NI	42.72	76	0.98
NI	43.38	120	0.56
NI	45.46	24	0.77
NI	46.50	26	0.49

Bronchoalveolar lavage is also interesting in asthma to highlight the recruitment and activation of immune cells during this condition in which it finds an important cellular infiltrate (Chang and Crapo, 2002; Delayre-Orthez et al., 2005). Using murine tracheal mucus exudation and mucociliary motility in pigeons as experimental models, Obidike et al. (2011) showed that the ethanol leaf extract of Hymenocardia acidaled to increased ciliary activity effect and mucociliary velocity was dose-dependent (Obidike et al., 2011).

It is also generally accepted that allergy orin flammation are underpinned by oxidativestress (Auerbach and Hernandez, 2012; Berair et al., 2013; Holguin, 2013). Anti radical tests allowed us to correlate the inhibitory effects of the *H.acida* extract on allergy and inflammation through its antioxidant potential. This has only been shown *in vitro* and requires additional work. However, antioxidant properties of this plant extracts have been reported. Indeed, the inhibition values of the extracts and quercetin were found to be very close, with no significant differences in their ability to inhibit 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sofidiya et al., 2009). The diversity of compounds recovered after HPLC analysis explain the wide use of this medicinal plant in tropical Africa. Results of HPLC and UV absorption spectroscopy at 280 nm (not shown) showed also the presence of unidentified compounds in the *H. acida* crude extract and that these compounds may have pharmacological properties in various pathologies such as asthma and other respiratory diseases. At the phytochemical level, qualitative TLC of the extract was positive for flavonoids, phenols, steroids and triterpenoids (Sofidiya et al., 2009)

and another phytochemical screening revealed also the presence of carbohydrates, tannins, flavonoids, saponins, alkaloids, cardiac glycosides, resins, steroids and terpenes (Ibrahim et al., 2007). This could explain their use in traditional medicine and if they are isolated, lead to new and effective avalilable treatment.

Indeed, the powder leaves of this plant is used in the treatment of sickle cells deseases (Ibrahim et al., 2007; Mpiana et al., 2007), diabetis (Ezeigbo and Asuzu, 2012) and other pharmacological such as vasorelaxant and antihypertensive effects (Nsuadi et al., 2013) antitrypanosomal (Hoet et al., 2004) , anti plasmodial and cytotoxic (Sowemimo et al., 2007) and antimicrobial (Starks et al., 2014).

Conclusion

Traditional treatments although widely used often lack scientific argument to justify their use. Similarly, the understanding of their mechanisms of action remains worth documented. This work opens up very interesting perspectives about the preventive treatment of allergic and inflammatory components of asthma by certain plants of traditional Senegalese pharmacopoeia including *H. acida*.

AKNOWLEGMENTS

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Conflict of interest

Authors declare that there are no conflicts of interest.

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