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Antiinflammatory effect of *Cissus quadragularis* in asthma induced by ovalbumin in mice

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Abstract

Asthma is a disease characterized by difficulty breathing. According to the WHO, asthma affects approximately 300 million people worldwide. In Africa, its prevalence is between 1 and 12 % depending on the country. In Cameroon, about 5.7 % of the population suffers from asthma. The objective of our work was to evaluate the antiinflammatory effect of Cissus quadrangularis in asthma induced by ovalbumin in mice. For this purpose, thirty (30) female mice subdivided into 6 groups of 5 mice each were distributed. All animals (except normal control) were subjected to disease induction for 16 days. The animals were injected intraperitoneally on days 0 and 7 with 20 µg of ovalbumin mixed with 1 mg of aluminum hydroxide (as adjuvant) in a total volume of 200 µl of phosphate buffered saline (PH=7.4). Days 14, 15, and 16 after the initial injection with ovalbumin, mice were re-exposed by intranasal instillation with 20 µg ovalbumin in 20 µl phosphate saline once daily. The extract as well as dexametasone were administered daily to the mice for 17 days orally at doses of 75, 150, 300 and 1 mg/kg respectively to the test batches and to the positive control 1 h before the initial sensitization. The animals were sacrificed 24 h after the last instillation. Blood, liver, lungs, and pulmonary fluid were collected. Dosages of IgE, cytokines (IL-4, IL-5 and IL-13), and counting of cells in the pulmonary fluid were carried out. Our results showed that the pretreatment with the extract significantly reduced ($P < 0.001$) the level of total IgE, there is also a significant inhibition ($P < 0.001$) of the recruitment of inflammatory cells in the lungs and a significant decrease ($P < 0.001$) the level of inflammatory cytokines. Similarly, the administration of the extract at a dose of 150 mg/kg significantly reduced ($P < 0.001$) the level of MDA correlated with a significant increase ($P < 0.001$) in the level of SOD, CAT and GHS compared to the control negative. Furthermore, the histological analyzes of the lungs showed that the pretreatment with the extract leads to an improvement in the histopathological changes; these results suggest that the aqueous extract of Cissus quadrangularis improves inflammation in ovalbumin-induced asthma in mice.

Keywords: *Cissus quadrangularis*, Asthma, Ovalbumin, Antioxidant, Sensitization, Provocation.

INTRODUCTION

Asthma is a chronic inflammatory pathology of the airways combining hyperreactivity, remodeling and bronchial inflammation [11]. The typical asthma symptoms (attack) are characterized by the triad of shortness of breath (dyspnea), coughing and wheezing. However, symptoms may be limited to cough, shortness of breath from isolated wheezing, chest tightness or partial symptomatology [29]. Due to its high prevalence, particularly in children, the possible prevention of exacerbations and the costs generated by this disease, asthma is a public health priority [11]. For the WHO, asthma is not a public health problem reserved for rich countries but it is rampant in all countries, regardless of their level of development. Significant geographic variability in asthma prevalence has been demonstrated, ranging from 1.8 % in China to 21 % in Australia in 2000-2003 [10]. Globally, 250,000 deaths each year are attributable to asthma. This rate varies depending on the country due to inequalities in the management of the disease (1.3 deaths per 100,000 inhabitants in France) [5]. In Mali, its prevalence was 11.17 % in 2002; in Benin in 2005, the prevalence of asthma in schools in Cotonou was 7 % [1]. In Cameroon, the ISAAC (International Study of Asthma and Allergy in Childhood) estimates the prevalence of asthma at 5.7 % [2] . Asthma is a so-called multifactorial disease [16]. Host-related factors (genetics, obesity, sex) interact with behavioral (hygiene) and environmental (allergens) risk factors to develop the disease [10]. Many medications are used to fight asthma. Corticosteroids administered by inhalation (Salbutamol, terbutaline, etc.) are the basic treatment in asthma. However, in cases of severe asthma, this local corticosteroid therapy is not enough to reduce the symptoms. In recent years, several monoclonal antibodies targeting IgE, Th2

cytokines or their receptors have been used to treat severe asthma [14] but the high cost of these antibodies and the fact of reinjection every six (6) months limit access to these bio-drugs. In addition, approximately 10% of patients are refractory to corticosteroids and many develop resistance to bronchodilator drugs [7]. High doses of corticosteroids in asthmatics have also been shown to increase the risk of osteoporosis and fractures; hence the search for other alternative treatment based on medicinal plants. In addition, several studies have demonstrated anti-inflammatory effects of different plants in the experimental treatment of asthma [24] . This is the case of Acacia gerrardii, Alchornea cordifolia, Pistacia lentiscus. Phytotherapy is an alternative therapy widely used in the world. The World Health Organization (WHO) estimates that 80 % of people worldwide use herbal medicines for their primary health care. It is therefore becoming essential to find new effective and ideally curative therapies to control or even eradicate the disease. With this in mind, it seemed interesting to us to test the antiasthmatic effects of the medicinal plant Cissus quadrangularis, whose anti-inflammatory effects have been demonstrated [21]. The objective of our work is to evaluate the antiasthmatic effects of the aqueous extract of aerial parts Cissus quadrangularis in mice. More specifically, it will be: Determine the qualitative phytochemical composition of the aqueous extract of the aerial parts of Cissus quadrangularis; Evaluate the in vivo anti-inflammatory potential of the aqueous extract of the aerial parts of Cissus quadrangularis; Evaluate the in vitro and in vivo antioxidant potential of the aqueous extract of the aerial parts of Cissus quadrangularis;

MATERIEL AND METHOD

Preparation of plante

The aerial parts of *C. quadrangularis* were harvested in Guider (department of Mayo-Louti) in the Far North region of Cameroon. The plant has been authenticated by comparison with the specimen deposited in the national herbarium of Cameroon under the number 36966HNC/Cam. Two hundred and fifty grams (250 g) of powdered aerial parts of *C. quadrangularis* was boiled in 1.6 liters of distilled water previously boiled. After cooling, the solution was filtered with a No. 4 coffee filter paper and the filtrate obtained was evaporated in an oven at 50°C for 24 h, which made it possible to obtain a mass of 42.57 g of dry extract of the aerial parts of *C. quadrangularis*.

Chemicals

Awareness was carried out using small quantities of OVA (1 mg/ml) (Sigma-
Aldrich, St. Louis, MO, USA) combined with an aluminum hydroxide (from Sigma-Aldrich) adjuvant dissolves at the rate of 1 mg/ml in a 9 ‰ saline solution**.** Control animal received orally distilled water and salbutamol (from Sigma-Aldrich) drugs used to reduce asthma.

Phytochemical analyses

Phytochemical analyses of the extract were tested using the following chemicals and reagents according to the methods of Trease et al. 1983. Saponin (frothing test), tannins (FeCl3), flavonoid (NaCl and HCl), phenol (FeCl3 and K3Fe (CN)6) and lipids (filter paper).

Animal and Experimental desing

Mice of both sexes weighing 25 ± 5g and aged 10 ± 2 weeks at the start of the experiment were used. Mice were kept in a room at room temperature in cages lined with litter before and during the period of the experiment. The mice were given free access to tap water and fed a standard diet.

Treatment and induction of ovalbumin asthma in mice

For this purpose, thirty (30) female mice subdivided into 6 groups of 5 mice each were distributed. Normal control group, negative control group, positive control group and 3 test groups. All animals (except normal control) were subjected to disease induction for 16 days. The animals were injected intraperitoneally on days 0 and 7 with 20 µg of ovalbumin mixed with 1 mg of aluminum hydroxide (as adjuvant) in a total volume of 200 µl of phosphate buffered saline (PH=7.4). On days 14, 15, and 16 after the initial injection with ovalbumin, mice were re-exposed by intranasal instillation with 20 µg ovalbumin in 20 µl phosphate saline once daily. The extract as well as dexametasone were administered daily to the mice for 17 days orally at doses of 75, 150, 300 and 1 mg/kg respectively to the test batches and to the positive control 1 h before the initial sensitization.

Sacrifice, organ and blood removal

After sacrifice, blood was immediately collected in a tube, containing an anticoagulant (EDTA), and intended for the determination of the complete blood count (CBC). Animals were dissected for organ harvesting, in this case the liver and lung. The organs were used for the preparation of the homogenates which were used for the determination of the parameters of oxidative stress. The lungs were fixed in a 10 % formalin solution in order to make histological sections.

Preparation of tissue homogenates

The liver was crushed and homogenized in 2 mL of phosphate buffer solution (TBS: 50 mM Tris, 150 mM NaCl, pH 7.4) at 1: 2 (W / V), and centrifuged (3500 rpm, 4 ° C, 35 min), the obtained supernatant was used for the assay. Blood count Total white blood cell count (WBC), hemoglobin concentration (Hb), mean blood cell volume (MCV), red blood cell distribution (RDW), platelets (Plt) and mean platelet volume (MPV)) are measured by an automatic hematology analyzer (Erma Coulter, Inc., model: PCE - 210N).

ELISA Analysis

Proinflammatory cytokines IL-4, IL-5,IL-13 and Ig E were measured using the ELISA technique (Stat Fax 303 Plus Microstrip Reader, Palm City, FL, USA). Proinflammatory cytokines detection and quantification were performed using commercially ABTS ELISA development kits (PeproTech EC, Ltd., London, UK).

Evaluation of the antioxidant activity in vivo of the aqueous extracts of *Cissus quadrangularis*

The antioxidant potential of the extract was assessed by estimating in the liver homogenate of Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) according to the methods of Wilbur et al in 1949; Misra and Fridovish in 1972; Sinha in 1972 and Ellman in 1959 respectively.

Histological study

It was carried out according to the technique described by Smith and Bruton (1977). It comprises the following stages: fixing, coating and obtaining the blocks, making the sections, coloring and mounting.

Statistical analyzes

Results were expressed as the mean ± standard error of the mean (ESM) for each group. Number per group = 5. The one way analysis of variance test (Anova) was used followed by the Student Newman Kells post test to compare the values with each other. The results were considered to be significantly different for $p < 0.05$.

RESULTS

Table 1: Distribution of Animal

Groups	Treatment	Route
Normal	Eau distillée	Orale
Positif control	Dexametason (2mg/kg)	Orale
Negative control	Eau distillée	Orale
Test 1	*C. quadrangularis* (75mg/kg)	Orale

Test 2	*C. quadrangularis* (150mg/kg)	Orale
Test 3	*C. quadrangularis* (300mg/kg)	Orale

Qualitative Phytochemistry

The phytochemical screening of the aqueous extract of *of Cissus quadrangularis* revealed the presence of certain classes of bioactive compounds such as alkaloids, flavonoids, coumarins, terpenoids and tannins (Table 2).

Table 2: Result of the qualitative Phytochemistry of the aqueous extract of *Cissus quadrangularis*

Class of compound	Observations
Phenolic compound	+
Flavonoids	+
Tannins	+
Saponins	+
Terpenoids	+
Sugars	+
Quinones	-
Coumarins	+

-Absence ; + presence

In vitro antioxidant activity of *Cissus quadrangularis* aqueous extract

The antioxidant potential of the aqueous extract of *C. Quadrangularis* was evaluated by tests of the DPPH and FRAP (Figure 1). The results of these two tests revealed that the extract has a percentage of inhibition in DPPH of 67.03 % and the strike of 74.12 % compared to the positive witness (BHT) which has a percentage of inhibition of 50 , 68 %.

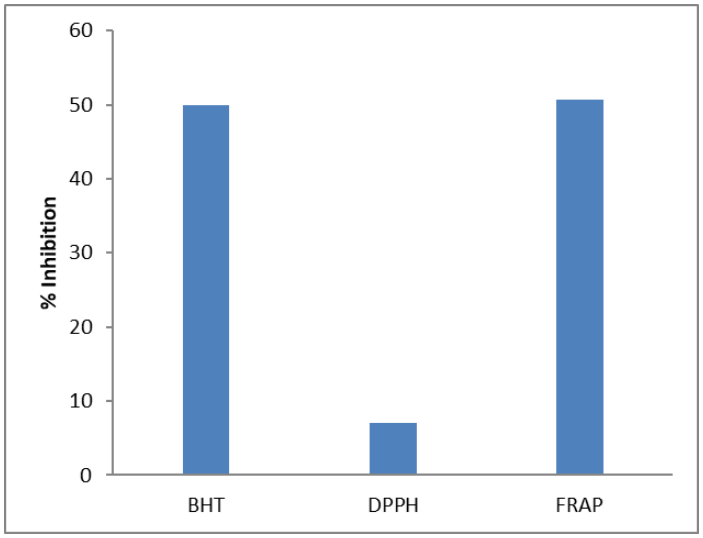

Figure 1: *In vitro* antioxidant power of the aqueous extract of *C. quadrangularis* BHT: Butylhydroxytoluene DPPH:(2,2-Diphenyl-2 Picrylhydrazyl) FRAP: reducing antioxidant power of iron

Effect of *Cissus quadrangularis* aqueous extract on body weight

Figure 2 shows that the difference is significant ($p < 0.01$) between the relative weights of the lungs of the negative controls compared to the normal controls. Moreover, the difference is significant ($p < 0.01$) between the weights of the lungs of the mice treated with the extract compared to the negative controls.

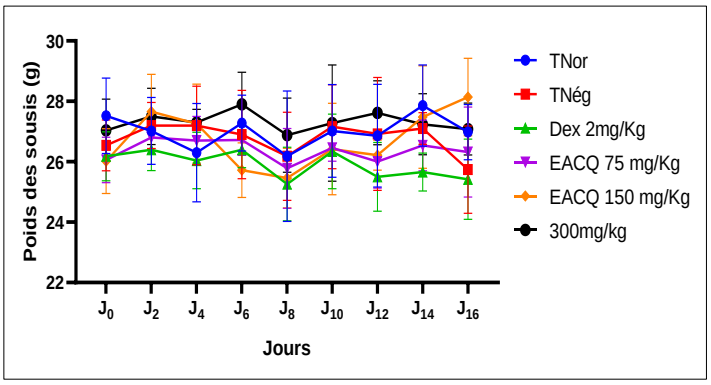

Figure 2: Change in absolute weight of control mice, mice treated with aqueous extract of *Cissus quadrangularis* and mice sensitized to ovalbumin (OVA).

Each of these values is expressed as the mean ± SEM. Significant differences: * compared to the negative control ($p * \leq 0.05$; $p ** \leq 0.01$; $p *** \leq 0.001$). θ compared to the normal control ($p \theta \leq 0.05$; $p \theta\theta \leq 0.01$; $p \theta\theta\theta \leq 0.01$)

Effect of aqueous extract of *Cissus quadrangularis* on body weight, relative and absolute organ weight

The variations in body weight of mice and in the relative and absolute weights of the liver and lungs are shown in figure 3. Sensitization to OVA causes a significant decrease ($p < 0.05$) in body weight as well as a significant increase ($p < 0.01$) in absolute and relative lung weights compared to the normal batch. Furthermore, a significant decrease in the absolute weight of these organs was observed in the positive groups, tests at 75 mg / kg, 150 mg / kg and 300 mg / kg respectively ($p < 0.01$; $p < 0.001$; $p < 0.01$; $p < 0.01$) compared to the negative control. Likewise, a significant decrease ($p < 0.001$) in the relative weights of the lungs is noted in these different batches.

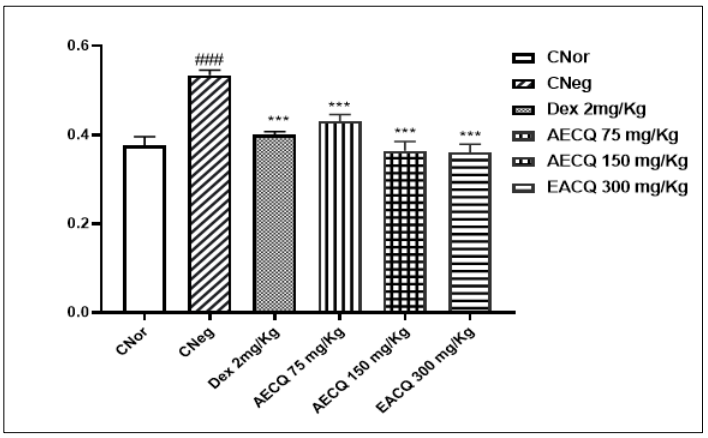

Figure 3: Effect of aqueous extract of *C. quadrangularis* on relative lung weights

Effect of aqueous extract of *Cissus quadrangularis* on haematological parameters

Table 3 shows the level of blood cells. Based on these results, we observed a significant decrease in white blood cells in the positive batches, test at 75 mg / kg, 150 mg / kg, 300 mg / kg ($p < 0.05$) compared to the negative control. Likewise, a significant decrease in mean corpuscular volume (MCV), blood platelets and hemoglobin distribution was recorded in these same mice ($p < 0.01$); ($p < 0.001$) respectively compared to the negative group (Table 3). On the other hand, there is a significant increase ($p < 0.05$) in hemoglobin (HGB) and in red blood cells compared to the negative group.

Table 3: Effect of aqueous extract of *Cissus quadrangularis* on the blood cell count

	Expérimental lots					
Parameters	**Normal control**	**Negative control**	**Positive control**	**AECG 75mg/kg**	**AECG 150mg/kg**	**AECG 300mg/kg**
WBC	3.34±0.17	4.73±0.55	3.66±0.55*	2.10±0.17**	3.55±0.34*	3.70±0.21*
RDW	7.16±0.41	6.24±1.29	7.90±0.49*****	7.10±0.25*****	6.92±0.25*****	7.04±0.25*****
HCT	37.40±0.32	34.50±3.37	48.73±0.77	37.63±1.15	38.06±1.59	39.88±0.99
HGB	110.33±2.84	95.33±12.71	113.33±3.84*	106.00±1.52*	116.00±3.21*	104.00±2.08*
PLT	347.00±31.6	511.00±9.81	281.67±10.08***	380.00±28.05***	316.67±10.17***	279.67±9.52***
VGM	50.86±1.45	56.70±2.69	50.33±2.57	41.56±2.88**	49.26±1.18*	43.70±2.60**
VMP	7.20±0.32	8.33±0.66$^{\theta\theta}$	6.73±0.37	7.13±0.63	7.83±0.37	6.79±0.49

Each of these values is expressed as the mean ± SEM. The significant differences: * compared to the negative control (p * ≤ 0.05; p ** ≤ 0.01; p *** ≤ 0.001). θ compared to the normal control (p θ ≤ 0.05; p θθ ≤ 0.01; p θθθ ≤ 0.01)

Effect of *Cissus quadrangularis* Extracts on the Production of Pro-Inflammatory Cytokines

To further check the anti-inflammatory potential of CQ in the asthma induce in rat model, the levels of pro-inflammatory serum cytokines TNF-α, IL-1β, and IL-6 were measured. As observed in Figure 4, the levels of serum IG E, IL-4, and IL-65, IL-13 identified in the control group were significantly higher than those identified in the normal group. Administration of dexametasone and CQ extracts in the ovalbumin-induced inflammation groups significantly reduced the levels of IL-4, IL-5 and IL-13. The same tendency was observed for IGE as well, but with no statistical significance (Figure 4).

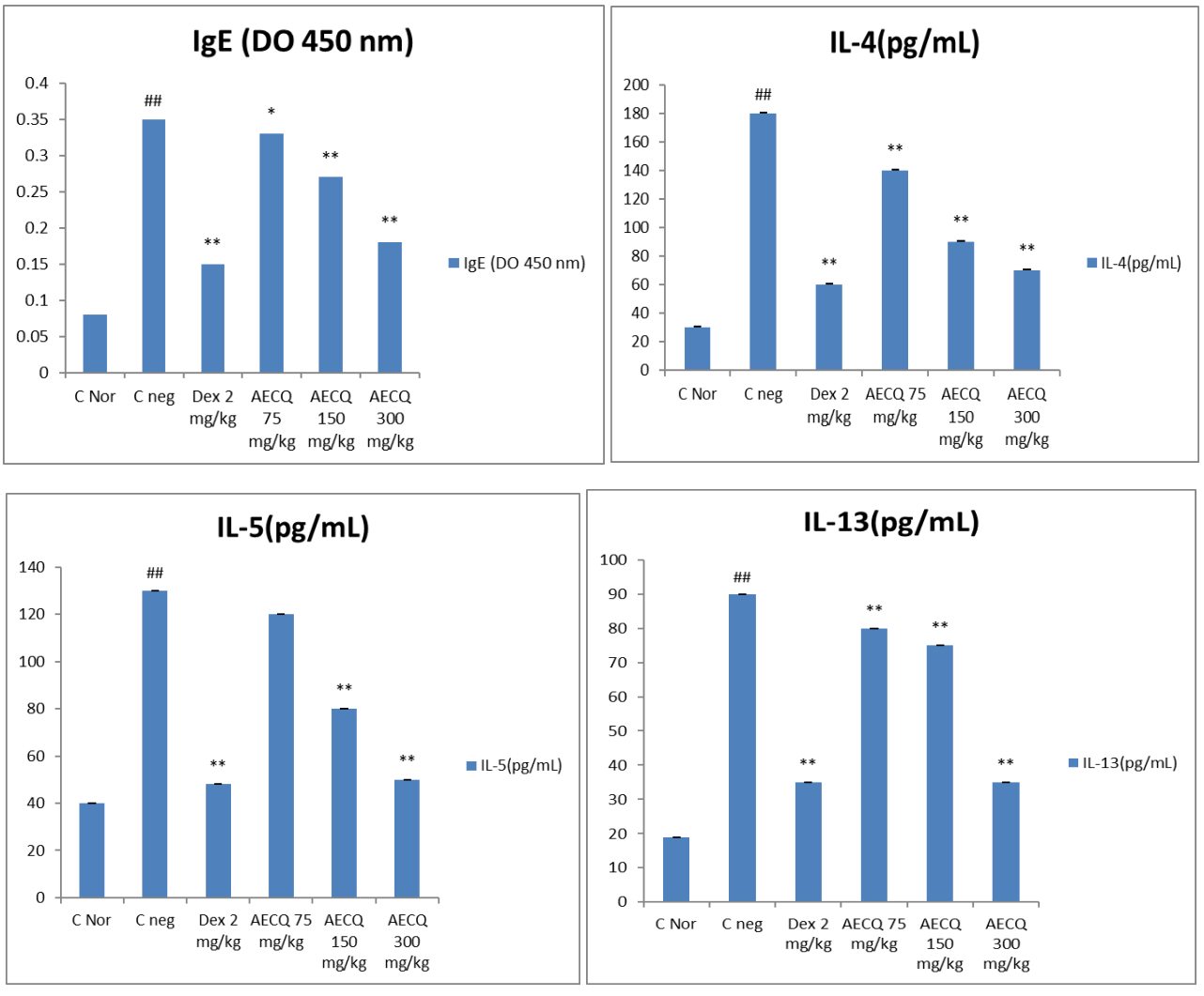

Figure 4: Effect of *Cissus quadrangularis* extracts on the inflammatory cytokines IGE, IL-4, and IL-5, IL-13 in asthmas induce in rats

Bars represent means ±SEM, N=5 mice per group. ###p<0.001 Significant compared to normal control; ***p<0.001: very significant, **p<0.01: significant compared to negative controls. TNor: normal control; TN: negative control; Dex: Dexametason; AECQ75mg/kg; AECQ150 mg/kg; AECQ 300 mg/kg

AECQ: Aqueous Extract of Cissus quadrangularis at a dose

Effect of *Cissus quadrangularis* Extracts on Oxidative Stress Parameters

The antioxidant potential of the extract was assessed by estimating in the liver homogenate of Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) according to the methods of Wilbur et al. In 1949; Misra and Fridovish in 1972; Sinha in 1972 and Ellman in 1959 respectively (figure 5).

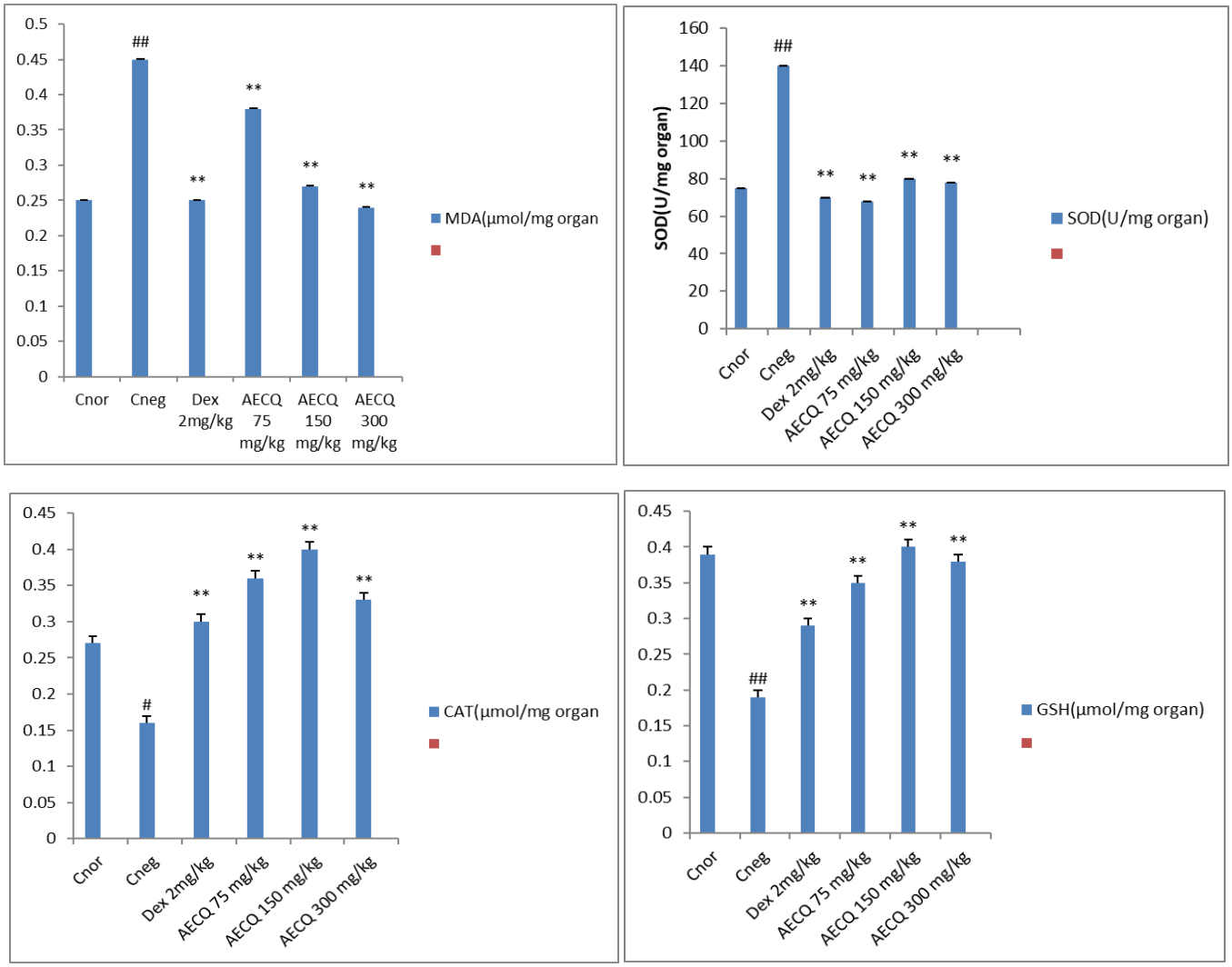

Figure 5: Effect of *Cissus quadrangularis* Extracts on Oxidative Stress Parameters

Bars represent means ±SEM, N=5 mice per group. ##p<0.01 Significant compared to normal control; **p<0.01: significant compared to negative controls. TNor: normal control; TN: negative control; Dex: Dexametason; AECQ75mg/kg; AECQ150 mg/kg; AECQ 300 mg/kg

AECQ: Aqueous Extract of Cissus quadrangularis at a dose

Effects of the aqueous extract of the leaves of *Cissus quadrangularis* on the histology of the lungs

Histological analysis of the lung tissue revealed in the normal control normal wall architecture indicating that the interalveolar area is very thin due to the absence of numerous inflammatory cells. In the negative control group, pathological changes were observed showing significant inflammatory infiltration in the lungs with an interalveolar area filled with exudates. The batches treated with the extract at different doses and with the reference substance showed a restructuring of the bronchial wall close to that of the normal control group.

DISCUSSION

The present study aimed to evaluate the antiasthmatic activity of the aqueous extract of the of *Cissus quadrangularis* in a model of ovalbumin-induced asthma in mice. The antioxidant potential of the aqueous extract of *C. quadrangularis* was evaluated by DPPH and FRAP tests. The results of these two tests revealed that the extract possesses DPPH and FRAP inhibition capacity. This reducing activity would be attributed to the phytochemicals such as

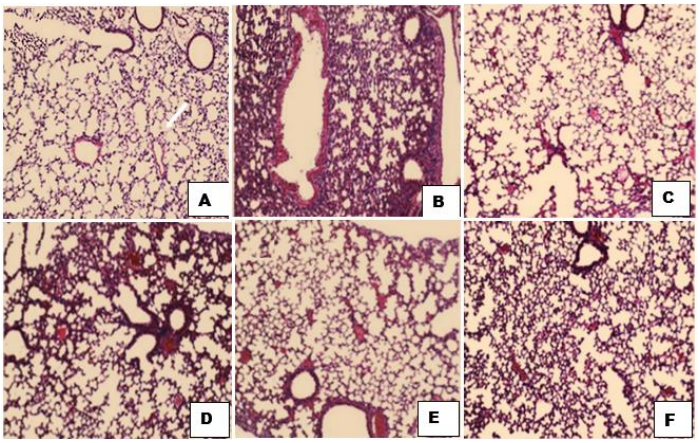

Figure 6: Histological sections of mouse lungs from the six experimental batches (x40). (Hematoxiline-eosin staining**)**

A: normal control; B: positive control; C: negative control; D, E, F: Test batches treated with extract at different doses. AV: the alveoli; CI: inflammatory cells.

DISCUSSION

flavonoids, steroids, tannins that the extract contains. . Work has demonstrated that certain chemical groups such as flavonoids, reducing compounds, saponins, triterpenes, tannins and coumarins have antioxidant properties [3, 22]. This property would be of interest in the management of diseases with an inflammatory component, including

those of the respiratory system such as asthma. In this study, we also measured the weights of the animals and the relative weights of the lungs, but there was no significant difference between the body weights of these animals. On the other hand, the relative weights of the lungs of the negative controls increased significantly ($p< 0.05$) compared to the normal controls. The extract significantly ($p < 0.01$) decreased the lung weights of extract-treated animals compared to negative controls. This increase in relative lung weights in asthmatic mice may be associated with the immunogenicity of ovalbumin. In addition, OVA represents a sensitizing allergenic molecule also playing the role of a xenobiotic responsible for significant tissue damage in batches treated with ovalbumin [29], resulting in significant cellular infiltration at the level lung leading to airway remodeling [20]. These results are in line with those of Mauser et al (2013) and suggested that provocation with the allergen (ovalbumin) induces increased plasma exudation and edema, therefore swelling of the inflamed organ. Moreover, this inflammatory phenomenon continues with the migration of inflammatory cells from the vascular compartment to the lungs (diapedesis). In this work, cell counting showed that exposure to ovalbumin increased leukocyte count in the negative control group compared to the normal control group. On the other hand, the pretreatment with the extract significantly reduced the level of leukocytes in the treated batches compared to the negative controls. We also quantified IgE and cytokines (IL-4, IL-5 and IL-13). Our results showed that exposure to ovalbumin (immunogen) induces an increase in the level of IgE, the level of leukocytes, the production of mucus, the level of Th2 type cytokines (IL-4, -5, and - 13) compared to the negative control. Indeed, [26] have shown that there is a good correlation between the levels of TH2 cytokines produced during the activation of T lymphocytes and serum IgE levels and the relative risk of asthma. Th2-type cytokines play an essential role in the development and maintenance of asthma. Indeed, IL-4 regulates allergic inflammation by promoting the differentiation of Th2 cells, the synthesis of IgE and the hypersecretion of mucus [27]. These IgEs are found on the one hand in the bloodstream, the others are fixed by their Fc fragment on the FcεRIs of mast cells and basophils, these cells are then said to be sensitized [15]. IL-5 promotes eosinophilic inflammation and infiltration in the airways (Foster et al. 1996) and IL-13 induces bronchial hyperresponsiveness [8]. The concept of oxidative stress is widely found in allergic reactions; the link between oxidative stress and allergic diseases, in particular asthma, has variable results; several of them have suggested an active involvement of reactive oxygen species (ROS) in the pathogenesis of allergic diseases including asthma. Indeed, exposure to ovalbumin, which represents a pro-oxidant factor, induced the production of reactive oxygen species (ROS). On the other hand, the administration of the extract decreased production of ROS while increasing the capacity of endogenous free radical sensors such as GSH, CAT and SOD is implemented [18, 32]. The extract caused a significant decrease in MDA content in the test batches. Furthermore, Ben Anes et al., 2015 claimed that the MDA level is higher in people with allergic asthma. This decrease was correlated with the increase in the level of GSH and the activities of CAT and SOD in the liver. Indeed, SODs are metalloproteins with an enzymatic activity that catalyzes the disproportionation of superoxide anions into dioxygen and hydrogen peroxide [28]. CAT is a heme oxidoreductase that catalyzes the dismutation of hydrogen peroxide into water and oxygen [28]. GSH is vital for detoxifying heavy metals; the thiol group reacts with the salts of these heavy metals by creating a very strong sulphur-metal bond with them so that they are then excreted without causing harm to the body [17]. The observed effect could lie in the radical scavenging properties of bioactive compounds such as flavonoids. The present results therefore demonstrated the correlation between the antiasthmatic effects of the aqueous extract of *C. quadrangularis* against asthma induced by ovalbumin and its antioxidant capacity. These results are in line with the work of [31] who demonstrated the correlation between the antioxidant and antiasthmatic effects of Utica duoica extract in an experimental asthma model in mice. Histological analysis of the lung tissues showed, by hematoxylin and eosin A (H&E) staining, a normal microstructure of the organ, in animals belonging to the normal control group. In the negative control group, ovalbumin induced tissue changes characterized by leukocyte infiltration, peribronchial leukocyte infiltration, in pulmonary vessels, alveolar ducts, and entire pulmonary alveoli. There is a lot of leukocyte infiltration in the negative batches compared to the normal control. Histology therefore confirms the anti-asthmatic effect of the extract [12].

CONCLUSION

At the end of this work, the objective of which was to evaluate the anti-asthmatic activity of the aqueous extract of aerial parts of *Cissus quadrangularis*, we can say that our extract exerts its anti-asthmatic effect by decrease in the infiltration of inflammatory cells; By reduction of Th2 type inflammatory cytokines and IgE; decrease in the MDA level correlated with the increase in the activity of SOD, CAT and the level of GSH.

Credit authorship contribution statement

Atsang À Kiki Gisele, Ousmaila Hamadou conceived and designed the experiments and carried out the major part of experiments. Egre Finsia, Takvou Francis: Havested the plant and prepared the extract. Dzeufiet Djomeni Paul Désiré: Performed histopathological examination. Atsang À Kiki Gisele: Analyzed data and wrote the paper. Takvou Francis, Egre Finsia, Ernest Nogma Sombié, Dzeufiet Djomeni Paul Désiré: Made provision of reagent and proof read the paper. All authors read and approved the final manuscript.

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Conflict of interest statement

The authors declare that they have no conflict of interest to disclose.

Ethical considerations

The study was approved by ethic Committee of the Faculty of Sciences of the University of Maroua (Ref N°14/0261/Uma/D/FS/VD-RC), according to the guidelines of Cameroonian bioethics committee (Reg N.° FWA-IRB00001954).

Disclosure statement

The authors declare that they have no conflict of interest to disclose.

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