International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2022; 10(4): 32-36
Received: 15-06-2022
Accepted: 25-07-2022

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Strychnos camptoneura Gilg & Busse (Loganiaceae) seeds: *In vivo* effect of aqueous and ethanolic extracts on a model of atopic dermatitis in mice

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Abstract
Atopic dermatitis is an inflammatory dermatosis whose consequences (morbidity and patient quality of life) make it a public health burden. The present study aimed to evalute the *in vivo* effect of aqueous and ethanolic extracts of *S. camptoneura* (Loganiaceae) seeds on a model of dermatitis in mice. Dermatitis was induced by application of 25 µl of oxazolone 7 days after sensibilization and the extracts effect estimated by measuring the variations in the thicknesses of edematous ears of the treated animals compared to the thicknesses before induction, and the control group. The extracts action was elucidated by the variation of the MPO and ALKP activities. The results revealed a significant (*p<0.05; **p<0.01) inhibition of oedema with aqueous, ethanol extracts and hydrocortisone 0.1% respectively compared to the control. A non-significant decrease (*p>0.05) of MPO activity with the aqueous extract against significant decrease (*p<0.05; **p<0.01)) with ethanolic extract and hydrocortisone respectively; non-significant decrease (*p>0.05) of ALKP activity with the aqueous and ethanolic extracts whereas it is significant (*p<0.05) with hydrocortisone 0.1% compared to the control. These results show that the extracts of *S. camptoneura* seeds endowed anti-inflammatory potentialities and act in inflammation like hydrocortisone cream by inhibiting the production of inflammation enzymes.

Keywords: *S. camptoneura*, atopic dermatitis, hydrocortisone, inflammation, oxazolone

1. Introduction
Atopic dermatitis (AD) or constitutional eczema is a chronic pruritic inflammatory dermatosis, which progresses through flare-ups and remissions [1]. It is a multifactorial condition associating a particular genetic background and the action of environmental factors. It most often begins in childhood but can persist or manifest itself in adulthood [2-3]. Although it primarily affects the skin, it is frequently associated with other pathologies such as asthma, rhinitis or allergic conjunctivitis in the affected subject or in his family [4-5]. It predisposes to abnormal immunological reactivity, mediated by immunoglobulin E (IgE), towards certain environmental antigens qualified as allergens [1]. In recent decades, its frequency has increased, especially in Western countries with a high standard of living. To date, it is estimated that 10 to 15% of infants in the world are affected and, in certain regions of Europe, statistics reach up to 30% of infants between 1 month and 2 years old [6]. This prevalence has consequences in terms of morbidity and quality of life for patients and their families and makes it a health burden for the public authorities. Currently, its therapy is based on the use of topical corticosteroids: creams with anti-inflammatory, immunosuppressive and antimitotic actions [7-8]. These widely used synthetic drugs have many side effects such as: toxicity on the renal and digestive systems and Mucocutaneous reactions (water and sodium retention, gastric ulceration, urticaria) generated by their long-term use [9]. Due not only to these harmful effects but also, and above all, to the difficulties of supply in developing countries, the quest for a better adapted treatment leads to medicinal plants with anti-inflammatory potential. *S. camptoneura* is a plant species of the Congolese pharmacopoeia having been the subject of studies involving inflammatory pathologies [10-11]. We proposed to evaluate the effects of aqueous and ethanolic extracts seeds of *S. camptoneura* in an experimental model of dermatitis (acute inflammation) induced by oxazolone in mice and to elucidate their modes of action.

2. Methodology
2.1 Plant material
The plant material consisted of seeds of *S. camptoneura*. The fruits were picked up after they had fallen to maturity in June 2021 in M'voula (village located 45 km from the Mbama sub-

prefecture in the Cuvette-Ouest department).The sample was authenticated at the National Institute for Research in Exact and Natural Sciences, ex ORSTOM of Brazzaville, in comparison with the reference sample registered under number 271. After collection, the fruits were cut, the seeds extracted (removed of the pulp) then dried at room temperature (25 ± 1 °C) in the Microbiology, Infectiology and Immunology laboratory of the Ecole Normale Supérieure.

2.2 Animal material

The animal material consisted of 4-week-old male Swiss race mice (20-25 g) supplied by the company Janvier LABS (Route du Genest, 53940 Le Genest-Saint-Isle, France).They were fed in a standard way with free access to water and a night-day lighting rhythm, with a photoperiod of 12/24 hours.

2.3 Preparation of aqueous and ethanolic extracts of *S. camptoneura* seeds

The aqueous and ethanolic extracts of the seeds of *S. camptoneura* were prepared by maceration of 100 g of seeds in 1000 ml of different solvents (distilled water and 95° ethanol) for 7 days with magnetic stirring. The macerates obtained were filtered three times with absorbent cotton and the filtrates concentrated at 60 °C. with a Buchi Switzerland Rotavapor. Yields determined and dry extracts kept in airtight bottles away from humidity for testing.

2.4 *In vivo* anti-inflammatory effect of *S. camptoneura* seed extracts

It was estimated by measuring the variations in the thicknesses of the edematous ears of the groups of treated animals compared to their starting thicknesses (before induction of the oedema) and to the control group, during the treatment. One week after acclimatization of the animals, a 3% oxazolone solution in acetone was prepared. A delimited surface of 1 cm² on the upper part of the mice's ears is shaved. 100 µl of oxazolone solution are then applied for one week to the shaved surface of mouse ears to sensitize them to the allergen (oxazolone). Oxazolone is a reference product, known internationally for its use in experimental studies of A.D. (atopic dermatitis).The animals are divided into five (5) groups of six (6) mice each and treated as follows: group 1 (negative control) healthy untreated; group 2 (positive control) inflamed untreated; group 3 animals inflamed and treated with the aqueous extract; group 4 animals inflamed and treated with ethanolic extract and group 5 animals inflamed and treated with 0.1% hydrocortisone. One week after sensitization, 25 µl of the oxazolone solution is applied to the ear of the animals in groups 2 to 5 and the animals in the healthy control group receive 25 µl of acetone.24 hours after induction, inflammation appears and is characterized by the presence of erythema and edema in the ears of the mice.

2.4.1 Application of treatment

Twenty-four hours (24h) after the onset of inflammation, groups 3, 4 and 5 are treated daily for five (5) days respectively with the aqueous, ethanolic and cream extract of hydrocortisone 0.1% by applying 15µl of each formulation to each mouse ear. The application of the cream is done using 1/5 of the knuckle unit. Before each treatment, the thickness of each mouse ear is measured using an electronic micrometer.

2.5 Probable mode of action of aqueous and ethanolic extracts of *S. camptoneura* seeds

The probable mechanism of action of the extracts is elucidated by measuring the variation in the enzymatic activities of MPO and ALKP, activities which are proportional to the extent of the inflammation. Five (5) days after administration of the various treatments, the animals are sacrificed using the cervical dislocation technique, their ears removed, weighed and then stored in 5ml hemolysis tubes containing 1ml of the specific extraction solution for each quantified enzyme: exadecylmethylamonium bromide solution for MPO and DPBS (Dulbeco's phosphate buffer solution) for alkaline phosphatase (ALKP). The tubes are then stored in the freezer at -20 °C.

2.5.1 Myeloperoxidase and alkaline phosphatase assay

Myeloperoxidase (MPO) and alkaline phosphatase (ALKP) are enzymes present in large quantities in neutrophil granulocytes. In inflamed tissues, their quantity is proportional to the intensity of the inflammation. The tubes containing the mouse ears are thawed and the ears crushed using the ultraturax at the maximum speed of 40% for 1 min then frozen again for 20 min after thawing, they are then ground. Three (3) freezing-thawing-grinding cycles are carried out. The ground material is centrifuged (10,000 rpm) for 10 min, the supernatant recovered and then frozen until the MPO and ALKP assay.

2.5.1.1 Myeloperoxidase assay protocol

The assay is carried out by spectrophotometry at 460 nm. The tissues are ground in a 50 mM phosphate buffer at pH 6 containing 0.5% hexadecyl methyl ammonium bromide (HTCAB). 1.8 ml of the 0.167 mg/ml (S1) O-dianisidine dihydrochloride solution previously prepared are placed in a hemolysis tube. Then, 200 µl of supernatant obtained after grinding the ears and centrifugation are added. The mixture is homogenized using a vortex for 1 min and 100 µl of 0.005% hydrogen peroxide are added; the mixture and quickly homogenize with a vortex (5 s).Two measurements of absorbance (OD) with a spectrometer at 460 nm are carried out: a first measurement immediately after homogenization with the vortex t0 and a second measurement 1 min after the first measurement t1.The difference in D.O between t1 and t0 is then calculated.

2.5.1.2 Alkaline phosphatase assay protocol

For the determination of alkaline phosphatase (ALKP), the “Sensolytep NPP phosphatase Assay kit, colorimetric” assay kit from the company MoBiTec (Goettingen, Germany) is used.

2.6 Statistical analysis

Statistical analysis was performed using Microsoft Excel (Microsoft Office Canto 2016) and Graph Pad version 5 statistical software. The results are expressed as mean ± standard deviation. Differences between two treatment groups were analyzed by Student's t-test and comparisons between three or four groups were made by one-way analysis of variance (ANOVA). A value of $p<0.05$ was considered statistically significant.

3. Results

3.1 Extracts and hydrocortisone effect on animal ear volumes

Figure 2 represents the effects of extracts and hydrocortisone on the thicknesses of the ears of the different groups of animals after application of 15 µl of each formulation. Group 1 negative control, group 2 positive control with untreated edematous ears. Groups 3, 4 and 5 (edematous ears) inflamed treated with aqueous, ethanolic and hydrocortisone extracts respectively. Groups 3, 4 and 5 (edematous ears) inflamed treated with aqueous, ethanolic and hydrocortisone extracts respectively. A significant decrease (*p<0.05) in the volumes of the ears of the animals is observed from the 3rd day in groups 3, 4 and 5 treated respectively with the aqueous and ethanolic extract of the seeds of S. camptoneura and with hydrocortisone (reference molecule) compared to group 1 (negative control) and group 2 (positive control).This decrease becomes more significant (**p<0.01) on the 4th day in groups 4 and 5 treated with the ethanolic extract and hydrocortisone compared to the controls (group 1 and 2) where the volume of the ears remains unchanged and do not decrease, testifying to the strong inflammation. Whereas in group 3, the significance of the decrease in ear volume remains at *p<0.05.

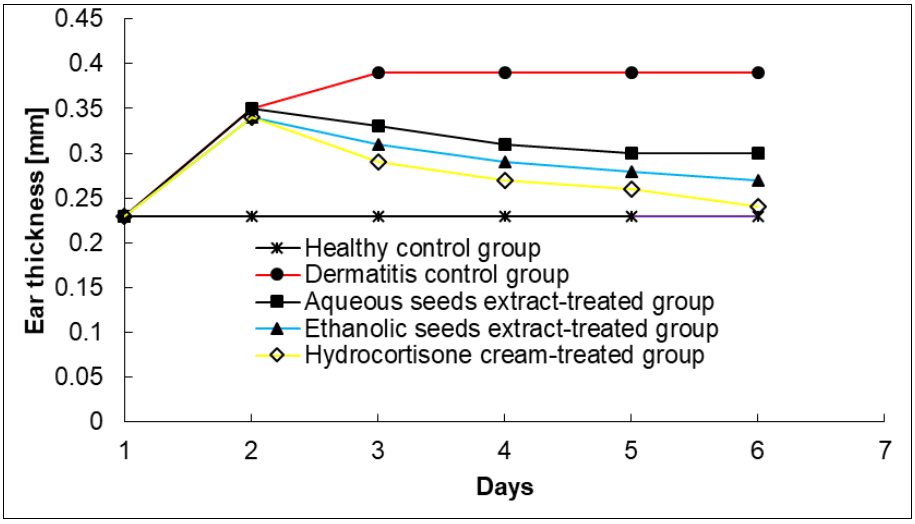

Fig 1: Ears thickness of each group of treated mice compared to positive control. Values are represented as means ± standard deviation. Significative *p<0,05and **p<0,01; no significative (*p>0,05) et n=6.

3.2 Enzymatic activities of MPO and ALKP

3.2.1 MPO activity

Figure 2 shows the enzymatic activity of MPO in the ears of each treated inflamed group compared to group 2 (positive control).A non-significant decrease (*p>0.05) in the concentrations of MPO is observed in group 3 treated with the aqueous extract of S. camptoneura whereas it is significant (*p<0.05) with the group 4 treated with ethanolic extract of the same seeds and, with group 5 treated with hydrocortisone, the concentrations decreased significantly (**p<0.01) compared to the control group (group 2).

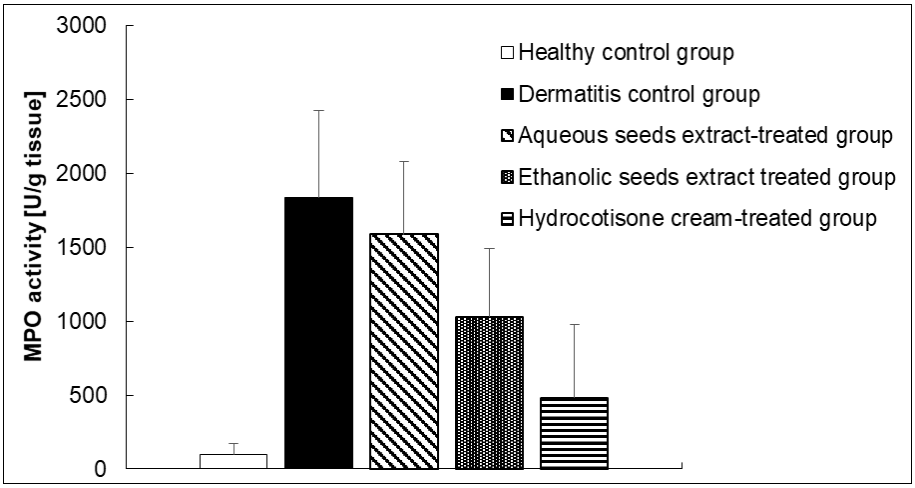

Fig 2: MPO activity of each inflamed and treated group against positif control. Means±standard deviation. Significative (*p<0,05; **p<0,01); nosignificative (*p>0,05) n=6.

3.2.2 ALKP activity

Figure 3 shows the calibration curve of alkaline phosphatase (ALKP) which allows the reading of MPO concentrations.

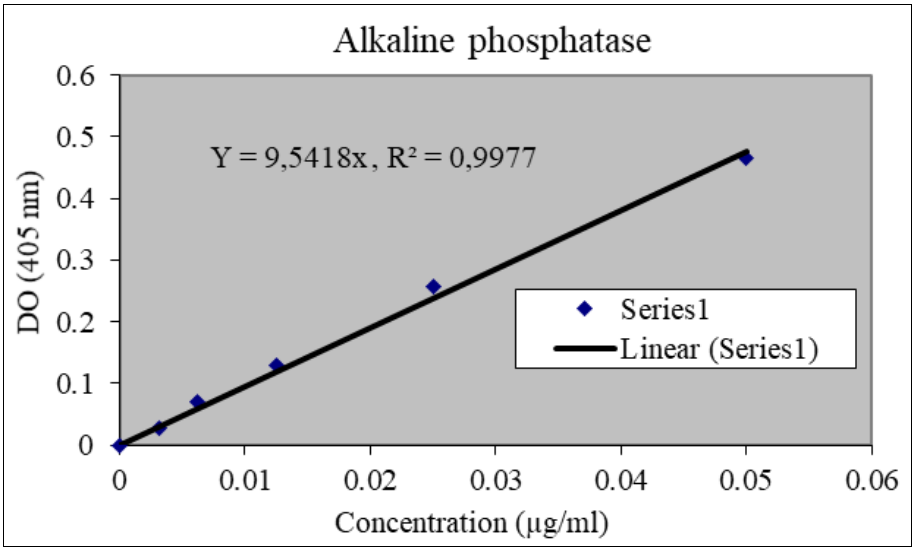

Fig 3: Courbe d'étalonnage de l'ALKP

Figure 4 represents the enzymatic activity of ALKP in the ears of each treated inflamed group compared to group 2 (control).A non-significant decrease (*p>0.05) in ALKP concentrations is observed in groups 3 and 4 treated respectively with aqueous and ethanolic extracts of S. camptoneura. Whereas with group 5 treated with hydrocortisone, the decrease in ALKP concentrations is significant (*p<0.05) compared to group 2 (positive control).

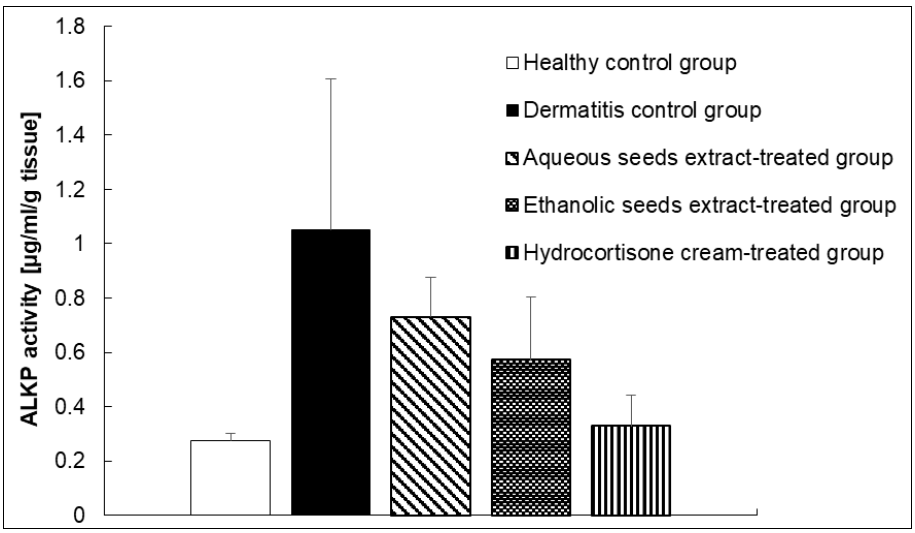

Fig 4: ALKP activity of each inflamed and treated group against positif control. Means±standard deviation. Significative (*p<0,05; **p<0,01); nosignificative (*p>0,05) n=6.

4. Discussions

Inflammation is the set of organism defense hhowever, the inflammatory reaction sometimes exceeds and causing deleterious effects. Such is the case of A.D, a chronic pruritic inflammatory dermatosis, linked to a predisposing condition to abnormal immunological reactivity, mediated by class E immunoglobulins (IgE) towards allergens [12].

From this experiment it appears that, after treatment of different groups of animals with oxazolone, the volumes of the ears increase significantly in all the animals of all the inflamed groups, compared to group 1 (negative control) not inflamed or not treated with oxazolone. This increase testifies to the irritating nature of oxazolone which would act as an irritant agent by inducing the accumulation of a liquid which leads to the formation of an edema characteristic of an acute inflammation. Indeed, one week after sensitization of the animals, the topical application of oxazolone provokes an acute inflammatory reaction, characterized by vasodilatation and a slowing down of the circulatory current leading to the passage of the exudates into the interstitial connective tissue, the infiltration leukocytes in the lesion focus and the formation of edema. The volume of the edema reaches its maximum three (3) days after the application of the oxazolone. However, after treatment of inflamed animals with aqueous and ethanolic extracts of *S. camptoneura* seeds and 0.1% hydrocortisone (reference anti-inflammatory), a significant decrease in edemais observed respectively (*p< 0.05) with the aqueous extract, (**p<0.01) with the ethanolic extract and 0.1% hydrocortisone, whereas the volume of the ears remains at a high level in the animals of group 2 (inflamed but untreated group). Treatments with extracts in duce a significant reduction in edema, as with hydrocortisone cream, which seems more pronounced in relation to its significance. This result demonstrates an inhibition of inflammation or an alleviation of inflammatory symptoms. These results suggest that the seed extracts have an anti-inflammatory potential, thus corroborating the results obtained with the aqueous extract of the stem bark of the same plant [10]. If the extracts inhibit inflammation, this suggests that the extracts have inhibitory potential on the

production of inflammation factors such as MPO and ALKP, two enzymes involved in the inflammatory process. That,s why we research the mode of action by measuring the variation in the enzymatic activities of MPO and ALKP activities that would be proportional to the extent of the inflammation. A decrease or increase in the concentrations of the set wo enzymes would provide information on the mode of action of the extracts in comparison with that of the hydrocortisone cream. The dosage of MPO with the aqueous extract shows a non-significant decrease (*p>0.05) in its concentrations in group 3 compared to group 2 (control) where as it is significant (* p<0.05) with groups 4 treated with ethanolic extract and more significant (**p<0.01) in group 5 treated with hydrocortisone. With ALKP, the reduction in concentrations remains non-significant (*p>0.05) in groups 3 and 4 treated respectively with aqueous and ethanolic extracts of *S. camptoneura*. Whereas, with group 5 treated with hydrocortisone, the decrease in ALKP concentrations is significant (*p<0.05) compared to group 2 (control). These results, although not significant, suggest that the extracts are endowed with inhibitory potential on the two enzymes, just like hydrocortisone (reference product). The ethanolic extract appears more active in inhibiting MPO than that of ALKP compared to the reference product which significantly inhibits both enzymes. Oxazolone (irritant) in fact, promotes an increase in the activity of phosphatase A2 (PLA2) which catalyzes the hydrolysis of membrane phospholipids into arachidonic acid. The latter is involved in the synthesis of prostaglandins, leukotrienes and thromboxane A2 (lipid mediators) which constitutes the first stage of the inflammatory reaction. Also, the cellular and molecular mechanism by which oxazolone induces inflammation mobilizes resident cells (macrophages and dendriticcells) which, when activated, secrete soluble messengers such as TNF-α, IL-1 and IL-6: cytokines pro –inflammatory [13]. Hydrocortisone has immunosuppressive, antimitotic and anti-inflammatory properties. It also acts on transcriptional regulation proteins (NF-kB and AP-1) and then inhibits the production of pro-inflammatory cytokines [14]. It induces a humoral-mediated immune response by activating L. This which subsequently differentiate into LTh2 secreting anti-inflammatory cytokines such as IL-4, IL-5 and IL-13 which stimulate LBs which are transformed into cells secreting antibodies of particular isotypes (IgG1, IgA, IgE) specific for the antigen [15]. The analysis of the modes of action of oxazolone and hydrocortisone seems to corroborate our results. Indeed, our extracts would probably act like hydrocortisone by inhibiting pro-inflammatory cytokines and other inflammation factors such as the enzymes evaluated. They would probably induce the production of anti-inflammatory cytokines which would inhibit inflammation. These extracts (aqueous and ethanolic) would inhibit the formation of inflammatory mediators, especially the production of anti-inflammatory cytokines. The therapeutic efficacy of plant extracts being a function of their content of bioactive substances; these results suggest that the aqueous and ethanolic extracts of the seeds of *S. camptoneura* would contain phytochemical compounds with anti-inflammatory potential that need to be identified.

5. Conclusion

The goal of this work was to evaluate the anti-inflammatory activity of aqueous and ethanolic extracts of *S. camptoneura* seeds on a model of atopic dermatitis in mice and to elucidate the extracts mechanism of action. From the results, it appears that the aqueous and ethanolic extracts of *S. camptoneura* are endowed with anti-inflammatory potential in a model of atopic dermatitis in mice. The extracts act in inflammation like hydrocortisone cream by promoting the inhibition of the production of inflammation factors. These preliminary results open up a perspective in the management of inflammatory pathologies.

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