Anti-inflammatory and antitussive activity of Asthpadose, a phytomedicine used in the treatment of asthma in Côte d'Ivoire.

abstract :

Introduction: The aim of this study is to contribute to the valorisation of an anti-asthmatic phytomedicine (Asthpadose) traditionally used in Côte d'Ivoire.
Aims: Evalute antiasthma's activity of the aqueous extract of Asthpadose (EAA) was.
Methodology: The antiasthma's activity of the aqueous extract of Asthpadose (EAA) was evaluated by the determining of its anti-inflammatory activity using the carrageenan oedema induction method. This anti-asthmatic activity was also evaluated by its antitussive action on the frequency of cough induced in rats exposed to liquid ammonia.
Results: The results revealed that EAA showed a very significant strong anti-inflammatory activity with an oedema inhibition rate ranging from 34.29 % to 70.9 5%. This activity was much better than that of diclofenac sodium (a reference molecule against inflammation) with an oedema inhibition rate ranging from 12.24 % to 58.39 %. As for its antitussive activity, the results also showed good antitussive activity of the extract. EAA presented a cough inhibition rate of 74.22 % compared to 58.76 % for dextrometaphan which is also a reference molecule against cough.
Conclusion: This observed activity could therefore justify the use of Asthpadose in the treatment of asthma which is a disease manifested by inflammation and cough. In view of the very promising results of this study, it would therefore be necessary to continue studies on this phytomedicine to understand its mechanism of action on asthma.

Keywords: *Asthpadose, Rats, dextromethorphan, sodium diclofenac, ammonia, carrageenan*

1. INTRODUCTION

Inflammation is a defense mechanism of the body against attacks of physical, chemical, biological or infectious origin [1;2]. This protective immune response can sometimes be harmful, linked to the pathogen and its persistence, to abnormalities in the regulation and production of pro-inflammatory cells [2-3]. As for coughing, it represents a means of sudden expulsion of air coming from the lungs thanks to the contraction of the expiratory muscles in order to rid the respiratory tract of foreign bodies and excessive secretions. Coughing also represents a defense reaction of the body and protection of the lung, which takes over when the muco-ciliary system which lines the respiratory tree no longer plays its role. It is a reflex act following irritation of the respiratory tract, the pleura or the diaphragm, which allows bronchial secretions (mucus) or a foreign body to be evacuated [4-6]. Inflammation and cough also appear during an asthma manifestation [7; 8]. Asthma is a chronic inflammatory disease of the airways representing a global public health problem. This disease affects around 350 million people worldwide [9],

Asthpadose is a phytomedicine used in Côte d'Ivoire in the traditional treatment of asthma, allergy, liver disease and constipation.

The objective of this study is to evaluate the anti-asthmatic activity of Asthpadose. For the realization of this present project, a preparation of the aqueous extract was made from the Asthpadose recipe (Aqueous Asthpadose) then the anti-inflammatory and antitussive activities of Asthpadose were carried out on *Wistars* rats.

2. MATERIAL AND METHODS

2.1. Material

2.1.1. Plant material

The plant material is a recipe called Asthpadose. This recipe is composed only of different parts (leaves, stems, flowers and roots) of medicinal plants. The plants that make up this recipe were harvested in Daloa. They were dried separately away from the sun for 2 weeks then sprayed individually. The different powders were weighed in proportional quantities then mixed to form Asthpadose.

2.1.2. Animal material

The animal material used in this study consists of male and female white rats of the *Wistar* albino strain. These animals come from the animal facility of the laboratory of the UFR of Pharmaceutical and Biological Sciences of the Félix HouphouëtBoigny University. These animals were acclimated to room temperature and fed twice a day with IVOGRAIN granules then hydrated with tap water. These adult male and female albino rats were aged 3 to 4 months and weighed between 120 and 190 g and were then used to study the anti-inflammatory and antitussive activity

2.2. Methods

2.2.1. Preparing the extract

The Asthpadose powder was used to prepare the aqueous extract of Asthpadose (EAA) which was obtained after pulverizing the different plants that constitute it. These plants were first collected in Daloa. They were washed, dried separately out of the sun for 2 weeks then sprayed individually. These different plants were then weighed in proportional

quantities and then mixed. The preparation of the extract used during this study (aqueous extract of Asthpadose) was obtained according to the method of Zirihi*and al*., [10;11]. For this preparation, 100 g grams of powder from the Asthpadose recipe was macerated in a liter of distilled water then homogenized in a blender. The homogenate obtained was successively filtered twice on hydrophilic cotton then on Whatman No. 3 filter paper. The filtrate obtained was dehydrated using an oven at a temperature of 55°C for 3 days. The dry evaporate was recovered on the 4th day in the form of a brown paste and constituted the aqueous extract of Asthpadose (EAA).

2.2.2.Preparation of animals

The animals were chosen at random and then marked to allow individual identification. Then, they were kept in their cages for acclimatization to laboratory conditions for 7 days before the experiment.

2.2.3. Anti-inflammatory activity of Asthpadose aqueous extract (EAA)

The anti-inflammatory activity test was carried out according to the method of *Winter et al*. [2;12;13] with some modifications. It was carried out by induction of oedema in the right hind leg of the rat using carrageenan (1%).30 rats weighing between 120 g and 190 g were fasted for 12 h. These rats were divided into 5 groups of 6 rats each. The diameter of the leg at time T0 of each animal was determined using a caliper. The rats were distributed as follows:batch 1 (control batch) received distilled water, batch 2 received distilled water at a dose of 1ml/100g bw. Rats from batch 3 (reference control) received Sodium Diclofenac (25 mg/kg bw). As for batches 4 and 5 (test batches), they received EAA at respective doses of 250 mg and 500 mg/kg bw 1 hour after administration of distilled water, Diclofenac and EAA by gavage, the 1% carrageenan, was injected cutaneously into the plantar aponeurosis of the right hind paw of each rat at a volume of 0.1 ml. The evolution of the oedema of the right paw was then determined at 1 h, 2 h, 3 h, 4 h, and 5 h [14] then the increase in oedema was assessed by determining the average percentage increase (%AUG) in the volume of the rat paw according to the formula:

$$\%AUG = ((Vt - V0) / V0) \times 100$$

% AUG: Percentage increase of the paw
Vt: Volume of the paw at time t
V0: Initial volume of the paw
[2 ;14]

The anti-inflammatory activity was finally evaluated by calculating the percentage of inhibition (% INH) of oedema according to the formula:

$$\%\ INH = ((\%\ AUGTM - \%\ AUGTT)/\%\ AUGTM) \times 100$$

% INH: Percentage of oedema inhibition
$\%AUG_{TM}$: percentage increase in cookies
$\%AUG_{TT}$: percentage increase in Treaties
[2 ; 14]

2.2.4. *Effect of Asthpadose extract on cough*

The experiment was carried out according to the method described by [13 ; 15 ; 16] with some modifications. To make it, a grid under which a bowl has been placed will be placed in a cage. The grid served as a platform for the movement of animals exposed to the ammonia contained in the bowl. 3 mL of liquid ammonia (25% NH_4OH) are taken using a syringe then poured into the bowl. 30 seconds later, the animal was exposed to liquid ammonia for 2 minutes on this platform in the hermetically closed cage. The rat was taken out of this cage and placed in a second cage (observation cage). Each cough was detected by visual observation of the animal. The number of times the animal coughed was recorded over 5 minutes. Observation time was measured using a stopwatch.

2.2.4.1. *Animal behavior*

The detection of cough in animals placed in observation cages after exposure to liquid ammonia was described by Morice *et al*., [13 ; 17]. Cough was detected by the following symptoms: mouth opening, characteristic sound and postural changes (stretching of the front legs and forward stretching of the neck) accompanied by inhalation and exhalation.

2.2.4.2. *Inhibition of cough frequency*

The animals exposed to ammonia were randomly divided into 5 groups of 6 animals (3 males and 3 females). The animals from batch 1 (control batch) were untreated. The second batch (batch 2) received 1 mL/100 g bw of distilled water. As for batch 3 (reference control), the rats in this batch received Dextromethorphan (20 mg/kg bw). The rats in batches 4 and 5 received the Asthpadose extract at respective doses of 250 mg and 500 mg/kg of body weight with a quantity of 1 mL/100 g of body weight. The animals were then placed back in the cage. experiment (cage containing liquid ammonia 25 % NH4OH) 1 hour after administration of extract and distilled water to be exposed again for 2 minutes. The number of coughs was again determined during the first 5 minutes.
The percentage of cough frequency was calculated by the following formula:

$$\%FT = (1 - T / C) \times 100$$

%FT: *percentage of cough frequency*
T: *number of coughs recorded after treatment of animals with Asthpadose*
DC: *number of coughs emitted by the animals in the control batch (Lot 1)*
[13 ; 18]

The percentage of cough inhibition was calculated by the following formula:

$$\%IT = (1 - Ta / Ca) \times 100$$

%IT: *percentage of cough inhibition*
Your: *percentage of cough frequency of animals from batches treated with Asthpadose*
That: *Average diameter of the legs of animals from the control batch (Lot 1)*
[13 ; 18]

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Action of Asthpadose on inflammation

3.1.1.1. *Measurement of oedema induced by carrageenan*

The EAA results on the increase in paw oedema in rats treated with carrageenan are recorded in Table 1. These results showed an increase in paw diameter (oedema) over time in control animals as well as those of treated animals. During this study, no side effects or mortality were observed in rats given carrageenan by injection.

The diameter of the paw of batch 1 animals (control batch) and batch 2 (treated with 2 ml/100g of distilled water) gradually increased by 0.20 ± 0.0 cm for batch 1 and by 0.19 ± 0.0 cm for batch 2 from the first hour to reach a maximum diameter at the fifth hour with a diameter increase of 0.30 ± 0.03 cm for batch 1 and 0.25 ± 0.03 cm for batch 2 while the paw diameter of rats treated with sodium diclofenac reaches its maximum increase in the first hour 0.19 ± 0.03 cm then gradually decreases from the second hour to the fifth hour 0.14 ± 0.07 cm.

However, in rats from batch 4, the batch having received 250 mg/kg bw of EAA, the oedema reached its maximum in the first hour with an increase in diameter of more than 0.17 ± 0.02 cm then was reduced from the second hour with a diameter of 0.16 ± 0.01 cm to reach a constant diameter of edema which was 0.16 ± 0.07 cm from the fourth hour until the fifth hour.

As for the rats treated with the dose of 500 mg/kg bw of EAA (batch 5), the oedemas in these rats reached their maximum increase at the first hour 0.15 ± 0.0 cm then decreased gradually from the second hour to fifth hour 0.10 ± 0.06 cm.

Table 1: effect of Asthpadose extract on carrageenan-induced paw oedema

Treatment	0 hours	1 hour	2 hours	3 hours	4 hours	5 hours
Batch 1 (Control batch)	0	0.20 ± 0.0	0.24 ± 0.01	0.25 ± 0.06	0.28 ± 0.03	0.30 ± 0.03
Batch 2 (ED 2ml/100g)	0	0.190 ± 0.0	0.237 ± 0.04	0.25 ± 0.05	0.25 ± 0.03	0.25 ± 0.03
Batch3 (DFS 25)	0	0.19 ± 0.030	0.13 ± 0.027	0.13 ± 0.084	0.15 ± 0.072	0.14 ± 0.070
Batch4 (EA 250)	0	0.17 ± 0.02	0.16 ± 0.01	0.17 ± 0.06	0.16 ± 0.07	0.16 ± 0.02
Batch5 (EA 500)	0	0.15 ± 0.0	0.12 ± 0.05	0.12 ± 0.07	0.11 ± 0.07	0.10 ± 0.06

***Batch 1**: untreated control batch; **batch 2** batch treated with distilled water; **batch 3**: batch treated with sodium diclofenac at a dose of 25 mg/kg bw; **Batch 4** batch treated with Asthpadose extract at a dose of 250 mg/kg bw; **batch 5** batch treated with Asthpadose extract at a dose of 500 mg/kg bw; ED: distilled water; DFS: diclofenac sodium; EA: Asthpadose extract*

3.1.1.2. *Percentage increase in oedema*

The percentages of increase in paw oedema in rats are represented by Figure 1. Indeed, until the 5th hour, the smallest value of the rate of increase was obtained in rats from batch 5 (treated batch with the dose 500 mg/kg bw of EAA). This value was 2.96 %. The rate of increase of batch 5 is followed by that of batch 3 (batch treated with 25 mg/kg bw of sodium diclofenac) with a slightly higher rate of increase. This rate is also followed by that of batch 4 (batch treated with the dose 250 mg/kg bw of EAA) with respective values of 4.24% and 7.24%. As for batch 2, its rate of increase remains the highest with a rate of 10.19 compared to the control batch but lower than those treated with diclofenac and EAA. The percentages of increase in oedema in batches 1 and batch 2 were statistically identical to each other.($p > 0.05$). On the other hand, the percentage increases in batches 3, 4 and 5 were statistically identical but lower than those of the control (batch 1) and batch 2. This difference in frequency compared to the control is very significant ($p \leq 0.01$).

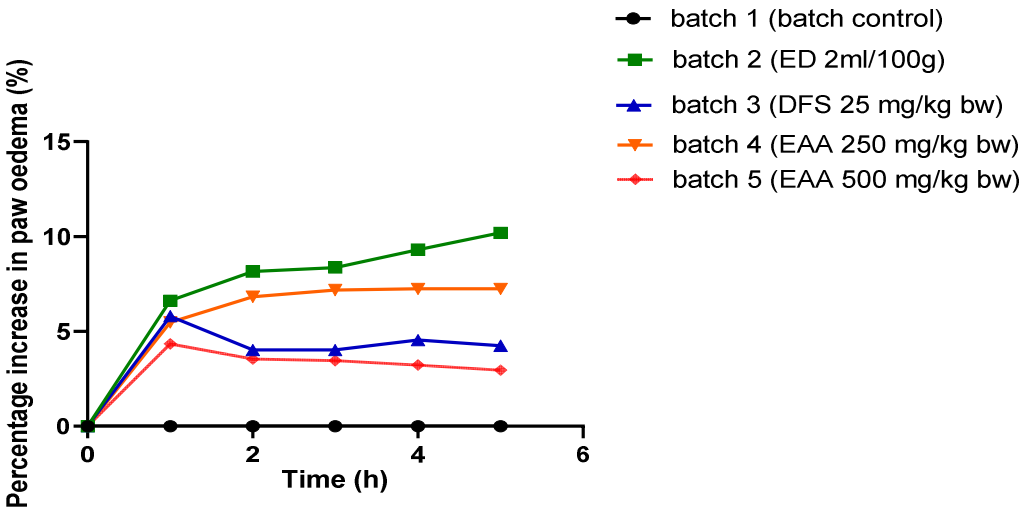


Figure 1: percentage increase in paw oedema induced by carrageenan

batch 1*: untreated control batch;* ***batch 2****: batch treated with distilled water;* ***batch 3****: batch treated with sodium diclofenac at a dose of 25 mg/kg bw;* ***batch 4****: batch treated with Asthpadose extract at a dose of 250 mg/kg bw;* ***batch 5****: batch treated with Asthpadose extract at a dose of 500 mg/kg bw;* ***ED****: distilled water;* ***DFS****: diclofenac sodium;* ***EAA****: Asthpadose extract*

3.1.1.3. *Percentage inhibition of edema*

The percentages of inhibition of paw oedema in rats 1 hour after injection of carrageenan are presented in Figure 2. This result revealed that from the first hour to the fifth hour, EAA at a dose of 500 mg /kg bw gave the highest percentage of inhibition of carrageenan-induced oedema with values of 34.29 % up to 70.95 %. This activity is followed by that of sodium diclofenac with a percentage of inhibition ranging from 12.24% to 58.39% then by EAA at a dose of 250 mg/kg bw which presented an inhibition rate of 24.32 % to 55.64 %. The oedema inhibition percentages of batches 1 and 2 were statistically identical to each other ($p > 0.05$). Furthermore, those of batches 3, 4 and 5 were statistically identical ($p > 0.05$) but higher than those of the control (batch 1) and batch 2. This difference in frequency compared to the control was very significant ($p \leq 0.01$). At a dose of 500 mg/kg, EAA (unpurified extract) seemed to be more effective than sodium diclofenac, which is a reference molecule.

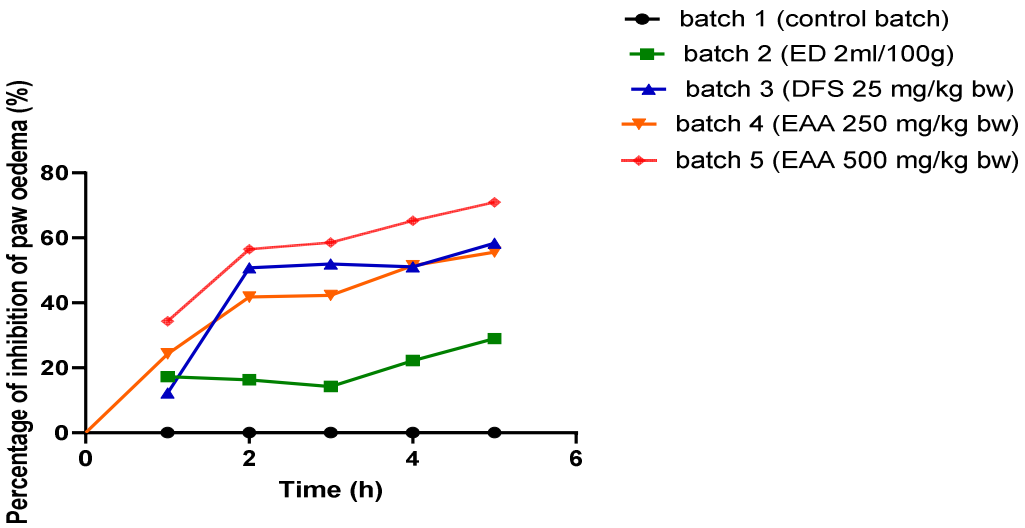


Figure 2: percentage of inhibition of paw oedema induced by carrageenan

Batch 1*: untreated control batch;* ***batch2****: batch treated with distilled water;* ***batch 3****: batch treated with sodium diclofenac at a dose of 25 mg/kg bw;* ***batch 4****: batch treated with Asthpadose extract at a dose of 250 mg/kg bw;* ***batch 5****: batch treated with Asthpadose extract at a dose of 500 mg/kg bw;* ***ED****: distilled water;* ***DFS****: diclofenac sodium;* ***EA****: Asthpadose extract*

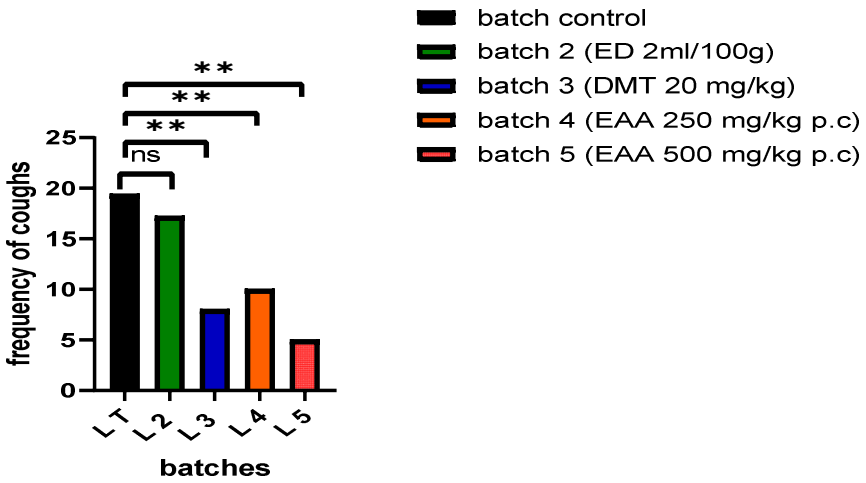
3.1.2. Result of the action of Asthpadose on cough

3.1.2.1. *Influence of Asthpadose aqueous extract on the frequency of coughs caused by ammonia*

The in vivo study of the effect of EAA on cough gave the results illustrated in Figures 3. The results present the influence of EAA on the frequency of coughs caused by ammonia in rats.

Untreated batch 1 indicated a cough frequency of 19.40 ± 0.93. As for batch 2 (batch treated with distilled water), the frequency of coughing was 17.20 ± 0.86; while batch 3 which received 20 mg/kg bw of dextromethorphan revealed a cough frequency of8.0 ± 0.71. The cough frequencies provided by the animals in batches 4 and 5 having received the respective doses of 250 and 500 mg/kg bw, were respectively 10.0 ± 1.41 and 5.0 ± 0.71.

The cough frequencies of batches 1 and batch 2 were statistically identical to each other ($p > 0.05$). Furthermore, the cough frequencies of batches 3, 4 and 5 were statistically identical ($p > 0.05$) but lower than those of the control (batch 1) and batch 2. This difference in frequency compared to the control was very significant ($p \leq 0.01$). However, the cough frequency value of batch 5 remained the lowest.



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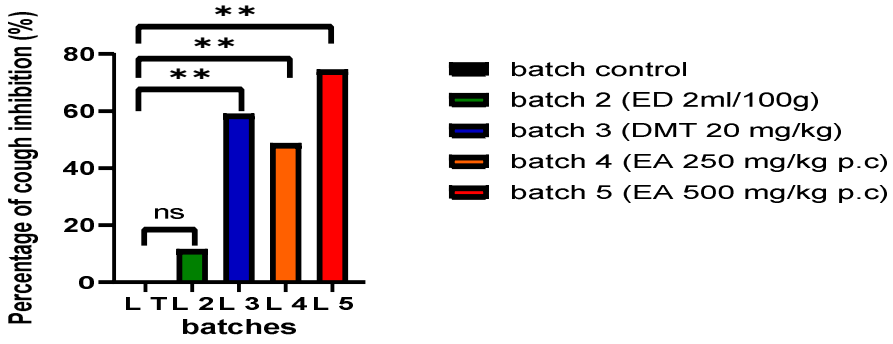
233 **Figure 3: Frequency of ammonia-induced cough**
234 *ED: distilled water; EAA: aqueous extract of Asthpadose; DMT: dextrometrophan L1: batch 1; L2:*
235 *batch 2; L3: batch 3 L4: batch 4 and L5: batch 5*
236 *Values were expressed as means ± SEM (n = 6).*
237 *The mention ns and ** represents statistical significance.*
238 *The urea values of the animals from the milked batches (L3, L4 and L5) compared to that of batch 1*
239 *were statistically very significant (p ≤ 0.01).*

240 **3.1.2.2. C*ough inhibition percentage***

241 The evaluation of the antitussive activity of EAA is represented by Figure 4. The result
242 obtained with the animals of batch 2 to which distilled water was administered was 11.34 %.
243 While the cough inhibition percentage of batch 3, batch having received 20 mg/kg bw of
244 dextromethorphan, was 58.76 %. As for that of batch 4 (lot which received a dose of 250
245 mg/kg bw of EAA), it was 48.45 %. Finally, batch 5 which received the 500 mg/kg bw dose of
246 the same extract gave an inhibition percentage of 74.22 %. Comparison of cough inhibition
247 percentages showed statistically identical inhibition percentages (p > 0.05) between batch 3,
248 batch 4 and batch 5. However, comparing batch 3, batch 4 and batch 5 to the control batch,
249 the difference in the percentage of inhibition was statistically very significant (p ≤
250 0.01).Generally speaking, the percentage of inhibition of EAA at the dose 500 mg/kg bw
251 remained the highest followed bydextromethorphan which is a reference molecule at 20 mg.
252 /kg bw then EAA at the dose 250 mg/kg bw to finish with distilled water.



253
254 **Figure 4: Percentage of cough inhibition**

ED: distilled water; EAA: aqueous extract of Asthpadose; DMT: dextrometrophan L1: batch 1; L2: batch 2; L3: batch 3 L4: batch 4; and L5: batch 5
Values were expressed as means ± SEM (n = 6).
*The mention ns and ** represents statistical significance.*
The urea values of the animals from the milked batches (L3, L4 and L5) compared to that of batch 1 were statistically very significant (p ≤ 0.01).

3.2. Discussion

Inflammation is a reaction phenomenon implemented by the organism whenever the integrity of its morphological and biological constants is threatened [19]. The injection of carrageenan into animals causes local inflammation caused by tissue damage which results from the action of the prostaglandins and histamine produced. These mediators increase the permeability of capillaries in the region. As a result, exudate escapes from the bloodstream into the interstitial space. This exudate is the cause of localized oedema, which in turn compresses the nerve endings and thus causes a sensation of pain [20 ; 21]. Carrageenan-induced paw oedema occurs in two phases. A phase involving the release of pro-inflammatory compounds such as histamine, serotonin and kinins which takes place in the first hour (T = 1 h) and a late phase due to an increase in COX-2 and release of PGE2 manifesting after the first hour (T> 1 h) [22 ; 23]. According to Ghedira [24], the reduction in oedema of the aqueous extract of Asthpadose could be explained by a probable blockade of the excitation of the nociceptive afferent nerve endings of the extract. The activity anti-inflammatories linked to the presence of flavonoids in Asthpadosis was shown by Baumann *and al*., in [25 ; 26]. The aqueous extract of asthpadose acts on inflammation by reducing prostaglandin. This result could be justified by the study carried out by Morimoto *and al*., [27] who showed that flavonoids acted on prostaglandins, which are involved in the final phase of acute inflammation and pain. Uguru [28] and Hajjaj [29] have shown the effect of flavonoids in inhibiting the synthesis of prostaglandins and the effect of tannins in inhibiting arachidonic acid from anti-inflammation.

The study of the antitussive activity of Asthpadose consisted of inducing a dry cough by exposing rats to the vapor of a liquid ammonia solution (NH2OH, 25%) and treating them with different doses of EAA in comparison with dextromethorphan, a reference cough suppressant. Liquid ammonia is a cough inducer which causes a reduction in respiratory amplitudes, this is bronchoconstriction. [30]. Irritation of RARS (Rapidly Adapting Receptors or irritant receptors) [31] causes contractions of the intercostal muscles. This irritation would be due to the release of histamine and the formation of other mediators [31 ; 32]. Histamine causes broncho constriction, vasodilation and increased capillary permeability [33]. During this study, a significant reduction in the number of coughs in animals was observed after treatment with dextromethorphan as well as aqueous extracts of Asthpadose. The aqueous extract of Asthpadose could therefore have an action on the central nervous system, particularly at the level of the brainstem since, according to studies carried out by Cantekin *and al*. [34] and Gavliakova *and al.* [35] dextromethorphan would have a central action on the central nervous system at the brainstem level. It stimulates mu and kappa opioid receptors by depolarization of the vagus nerve. This depolarization results in an increase in the cough threshold, leading to a reduction in cough frequency. The aqueous extract of Asthpadose therefore demonstrated potential antitussive power in vivo. Also, Smith [36] showed that dextromethorphan acts on N-methyl-D-aspartate (NMDA) receptors in the central nervous system. These results would be comparable to those obtained in the study conducted by Jain *and al.* [18] on *Caesalpinia bonducella* in mice. These authors showed that extracts of this plant have antitussive activity. These results are also in agreement with those of Agnero [13] by evaluating the antitussive activity of *chrysophyllum welwitschii*engl.in rats and comparing it to that of dextromethorphan. Also, just like asthma, cough is caused by a stimulus and this stimulus would be inflammatory (allergens), mechanical, chemical (inhalation of gas, smoke) or thermal [37]. It is therefore probable that the aqueous extract of

Asthpadose reduces the number of coughs by inhibiting either the inflammatory process or the nerve impulse responsible for triggering the cough [38]. Also, according to some researchers, cough is linked to mixed airway inflammation involving interactions between eosinophils and other cells such as neutrophils [39 ; 40].

4. CONCLUSION

This study is part of the valorisation of medicinal plants and especially the search for new molecules from plant extracts. During this work, the aqueous extract of Asthpadose (EAA) used showed therapeutic action on inflammation and cough. Indeed, it would act on inflammation by inhibiting prostaglandins, histamine and arachidonic acid from inflammation. Also, its antitussive activity would be linked by its action on the central nervous system at the level of the brain stem. The beneficial effect of the Asthpadose tested would probably be attributable to the bioactive compounds it contains. These beneficial effects could also result from its combined action on prostaglandins, histamine and arachidonic acid in inflammation. However, additional studies will be necessary to understand the cellular and molecular mechanisms linked to these anti-inflammatory and antitussive activities.

AUTHORS' CONTRIBUTIONS

BGL and AJAAB initiated this work. BGL carried out the manipulations, the results and wrote the manuscript. AJAAB supervised the analysis of the results and the writing of the article. BKKR, YYG and OAP participated in writing of the manuscript.

CONSENT (WHEREEVER APPLICABLE)

As per international standard or university standand written ethical approval has been collected and preserved by the author (s)

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

The study wa sapproved by the Institutional Ethics Committee.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

EAA: ExtraitAqueuxd'Asthpadose
DFS: diclofenac sodium
ED: distilled water
DMT: dextrometrophan