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Pharmacological Investigation of *Schwenckia americana* L. (Solanaceae) on Pain, Behavior and Inflammation in Rodents

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ABSTRACT

In order to verify the absence of *Schwenckia americana* extracts effect on motility and behavior in the rat, to evaluate the anti-inflammatory and confirm the analgesic activities, extracts sequentially prepared by maceration with DCM (SA1), ethanol (SA2), distilled water (SA3) and the traditional extract (SA4) were the subject of an acute toxicity studied up to a dose of 3000 mg / kg. Their analgesic effect was studied on abdominal cramps induced by acetic acid in mice and on the rat paw pressure test. The anti-inflammatory activity was studied on the model of edema of the rat paw induced by carrageenan. The photoelectric actimeter and the rotating stem were used to evaluate the effects of these extracts on the general behavior in mice. SA2, SA3 and SA4 are not toxic up to a dose of 3000 mg / kg. Only the SA1 extract is toxic with an estimated LD 50 of 750 mg / kg. On abdominal cramps induced by acetic acid, extracts showed a strong and dose-dependent analgesic activity. The SA1 extract showed the most important activity, significant from 62.5 mg / kg. The mean activity was recorded with the SA4 extract, the effect of which is significant from the dose of 125 mg / kg ($p < 0.05$). The SA2 and SA3 extracts were least active with a significant effect from the 250 mg / kg dose ($p < 0.05$). This activity was confirmed on all extracts at 250 mg / kg, using the paw pressure test. Up to a dose of 250 mg / kg, these extracts do not significantly modify the motility, the equilibrium reflexes and coordination of mouse movements. Extracts SA1 and SA2 from *Schwenckia americana* showed an anti-inflammatory effect at 250 mg / kg between 1h and 2h after treatment. These analgesic and anti-inflammatory properties are due to its numerous secondary metabolites.

Key words: *Schwenckia americana, analgesic, anti-inflammatory, motility, coordination, equilibration.*

INTRODUCTION

Pain is the main reason for medical consultation. It is estimated that out of three cases of consultations, are due to pain (Boureau and Sahmoud 1993). It is predominant in surgery and justifies by itself the presence in the operating room of the anesthesiologist. It is a major concern in postoperative patients. Because of its unpleasantness and complications, pain is one of the disabling factors, thus becoming a socio-economic scourge. When pain is not quickly relieved, it can evolve independently from the cause and become itself an evil, clinically called chronic pain. It is therefore compulsory to take care of it. Unfortunately, most analgesic drugs also induce many adverse effects (Nsonde Ntandou et al., 2015). Non-steroidal anti-inflammatory drugs, in chronic use, may cause gastrointestinal and kidney toxicity. Alpha-2 adrenoceptor agonists, such as xylazine, which are potent analgesics, also have a sedative effect and cause a drop in

blood pressure and heart rate. Narcotics that combine various molecules of the opioid family are the most powerful and effective analgesics against pain. But, they also cause drowsiness, nausea, vomiting, constipation, dry mouth, addiction and respiratory depression. There is a great need to discover and develop more and more specific drugs, very effective and better tolerated, without adverse effects. Nowadays, several works carried out in the field of ethnopharmacology, sufficiently show us that many plants used in traditional medicine are effective on different pharmacological models. Under traditional conditions of use, they have less toxicity than conventional drugs (Beaux et al., 1997). Plants are therefore promising to develop future phyto-medicines. The aim of this study is to evaluate the effects of *Schwenckia americana* extracts on pain, behavior and inflammation in rodents.

II. Theoretical basis

Previous studies have shown that *S. americana* (Solanaceae) is very used and for several pathologies in many countries of the world. Geographically *Schwenckia americana* is originates from Central and South America, but it has spread to tropical Africa and India as a weed. Among the pathologies treated in traditional medicine by this plant, many are related to pain and inflammation namely. Its decoction is used as a drink against stomach ache, edema, intercostal pain, swelling pain, rheumatism, osteoarthritis. The juice of the plant mixed with lemon juice or the decoction of the whole plant serves as eye drops and nasal drops in the treatments of headaches (Adjanohoun et al., 1989).

III. MATERIALS AND METHODS

Plant material

Schwenckia americana was collected from Pool Department (Congo) in May 2007. This harvest was carried out on the basis of information provided by traditional health practitioners. Immediately after collection, a sample was transported to the laboratory of Pharmacology of CERVE to be dried at room temperature protected from light, then ground before being sent to Institut de Chimie de Substances Naturelles in France for the extraction. A specimen was deposited in the national herbarium of the CERVE, under number MKC045.

Animal material

Male CDI mice (Charles River France), weighing between 18 and 20 g, and male Sprague-Dawley (Charles River France) rats weighing between 175 and 200 g were used. These animals were maintained under standard conditions (21 ° C, 40-70% RH, 12h / 12h light cycle / dark). They had access to water and food ad libitum. The recommendations of the ethics rules published by the International Association for the Study of Pain (IASP) were respected (Canadian Council 1980; Zimmermann 1983).

3. Methods

3.1. Preparation of extracts

7 kg of plant powder are placed in an extractor to be cold-macerated with mechanical stirring for 3 × 48 h in 3 × 60 liters of dichloromethane (DCM). After filtration on filter paper, the remaining marc is extracted twice again. The three extracts thus produced have the same chemical profile on TLC. They are then mixed and concentrated under reduced pressure with a rotavapor to give the final extract SA1 (283 g with an extraction yield of 4%). The residual Marc 1 is extracted again according to the same protocol in 95% ethanol to obtain the SA2 extract (213 g and 3.1% yield). The residual marc 2 obtained from the extraction with ethanol was extracted with water. This is a decoction prepared from 6 kg of the Marc 2 in 3x60 liters. The mixture is brought to boiling for 10 minutes and then filtered on cotton and then on filter paper (Nsonde Ntandou et al., 2010). The extract is then centrifuged for 20 minutes at 3000 rpm and then lyophilized to have a dry extract (SA3, 700 g, with a yield of 11.6%). The traditional extract was also prepared, for this purpose a sample of 600 g dry powder of vegetable material was boiled in

4 liters of water for 10 min. As before, the filtrate was centrifuged and then lyophilized under the same conditions to obtain a dry extract (SA4: 300 g, with a yield of 50%). These dry extracts (SA1, SA2, SA3 and SA4) were stored in the freezer - 4°C until their pharmacological evaluation. During the experiments, the SA1 extract was dissolved in 30% DMSO and the extracts SA2, SA3 and SA4 were dissolved in 2% DMSO.

3.2. Toxicity study

The study of the acute toxicity aimed to determine the 50 lethal dose (LD50) of the plant extracts and any macroscopic modification of the general condition of the animals subjected to an acute treatment. Eight (80) mice were divided into eight groups of ten mice each (n = 10). The first group considered as a control was treated with 10 ml / kg of distilled water administered by oral route. While the other seven groups were treated with increasing doses of the different extracts (SA1, SA2, SA3 and SA4), respectively, 250, 400, 600, 1400, 2000, 2600 and 3000 mg /kg observed over a 72-hours period.

3.3. Analgesic dose-effect relationship

This test was carried out according to the method of abdominal cramps induced by acetic acid described by Koster (1959). Five (5) mice per treatment were used. Two control groups were used and treated as follows: the first control group received 10 ml / kg of DMSO 30% and the second control group received 10 ml / kg of 2% DMSO. Control animals received the dissolution solvents of the administered products. Paracetamol (100 mg / kg) was used as a reference molecule. Extracts SA2, SA3 and SA4 were tested at doses of 62.5; 125; 250; 400 and 800 mg / kg. The extract SA1 was tested only with four (4) doses: 62.5; 125; 250; 400 mg / kg. The dose of 800 mg / kg was not considered with SA1 extract for toxicity reasons. All evaluated products were administered orally. 45 minutes after administration of the products, an injection of 0.6% acetic acid (0.1 ml / 10 g of weight) is carried out intraperitoneally (i.p.). This injection causes a painful symptom in the mouse, resulting in characteristic contortions (cramps): stretching of the hind legs and dorso-ventral musculature, digging of the flanks (Koster et al., 1959). The number of stretches is counted for 10 minutes from the injection of acetic acid. Analgesic substances are believed to cause a significant decrease in cramps.

3.4. Test of the pressure of the paw

This test involved only three extracts of *Schwenckia americana*; The extract with DC (SA1), the extract with ethanol (SA2) and the aqueous extract prepared in the laboratory (SA3). It is based on the application of a mechanical stimulus that induces pain. Increasing pressure is applied to the right hind leg until the pain threshold is reached. This threshold results in a deliberate withdrawal of the rat paw accompanied by a stereotyped thrill response. The maximum weight that can be applied is 750 g (Randall and Selitto, 1957). One hour (1 h) after administration of the product, the shrinkage threshold intensity of the right hind paw of the rat is measured and the latency time is deduced.

3.5. Study of anti-inflammatory activity

The anti-inflammatory activity on the edema of the paw induced by carrageenin was studied according to the method described by Winter et al., (1962). This method allows the study of acute inflammation. Inflammation is induced by sub-plantar injection of 20 µl of 2% carrageenin (diluted in 0.9% NaCl buffered to pH 7) in the right hind paw of the rat (Elion Itou et al., 2014); One (1) hour after administration of the product, the edema is monitored by measuring the volume of the paw using an Oditest micrometer (Kroeplin). The measurements are carried out blind. The volume of the leg was measured up to the level of the tibiotarsal joint. Measurements were made at t_0 (0 mn

before carrageenin injection) and at t_1 (t_0+ 30 mn), t_2 (t_0+ 60 mn), t_3 (t_0+ 90 mn), after injection of carrageenan. Anti-inflammatory substances reduce the volume of the paw. Groups of five (5) mice were formed and the dose of 250 mg / kg was used to evaluate the effect of the four (4) extracts. The aspirin used as a reference molecule was administered at a dose of 300 mg / kg. The 1st and 2nd control groups received 10 ml / kg of DMSO30% and DMSO2%, respectively. All products were administered orally.

3.6. Behavioral testing

Lots of five (5) mice consist of animals deprived of food 18 hours before the test. The 1st and 2nd control groups received DMSO 30% and DMSO 2%, respectively, at a dose of 10 ml / kg. The extracts were each administered at a dose of 250 mg / kg. All products were administered orally.

3.6.1. Photoelectric Actimeter Test

The substances are administered preventively and their influences are appreciated by comparison with control mice. The actimeter is a device in the form of a closed cabinet containing 6 transparent Plexiglas cages. Each cage is equipped with photoelectric cells providing 2 infrared photoelectric rays crossed at right angles. Each passage of the mouse through the light beam is recorded and the number of passes is automatically summed for 5 minutes. Recording times are short (5 minutes) to prevent the curiosity of the animal from dulling and leads to a reduction in motility.

3.6.2. Test of rotating stem or Rota-Rod

The technique used is that of Boissier (1962). It consists in looking for the length of time that the mice hold on an axis rotating at slow speed. A series of animal tests was carried out before the actual test, under the following conditions: the observation time was limited to 60 seconds, the tests were spaced 10 minutes. At the first test the speed per minute (rpm) was zero, while in the other two tests, it was 4 rpm. 30 min after the third test, we measure the basal threshold by adjusting the apparatus to a maximum duration of 300 seconds (cut-off) with a rotation speed between 4 and 40 rpm. After 15 minutes and knowing the basal threshold which will constitute our control, the animal is then treated by a per os administration of the product. One hour after treatment animals were tested.

4. Statistical analyzes

The results are expressed as mean ± standard error. The statistical significance was calculated using an ANOVA. Significant differences were determined using the Duncan's multiple-range test. Values of $p < 0.05$ were considered significant.

IV. RESULTS

1. Acute Toxicity

In this study, mice were treated with SA2, SA3, and SA4 at doses of 250, 400, 600, 1400, 2000, 2600 and 3000 mg /Kg. Extracts SA2, SA3 and SA4 are not toxic up to 3000 mg / kg. Only the SA1 extract is highly toxic with an estimated lethal dose of 750 mg / kg.

2. Dose effect relationship

All extracts administered orally in mice reduce acetic acid abdominal cramps. These extracts showed a strong and dose dependent analgesic activity. The extract SA1 showed the most significant activity from 62.5 mg / kg (figure 1a).

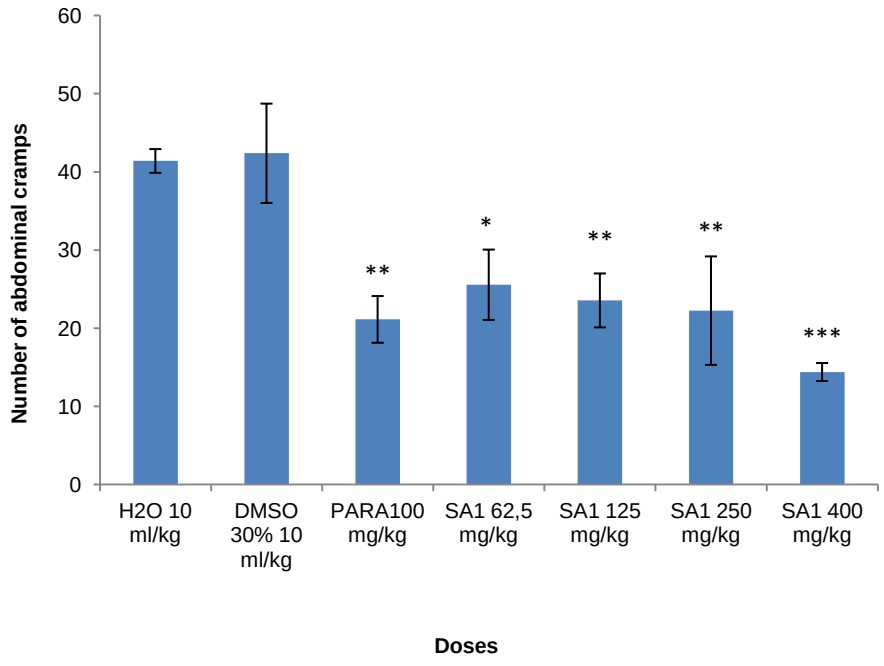

Figure 1a: Effec of *Schwenckia americana* DCM extract (SA1) on abdominal cramps induced by acid acetic n= 5; *p < 0,05; **p < 0,01; ***p < 0,001; significative difference compared to control. DCM: dichloromethane; Para: paracetamol

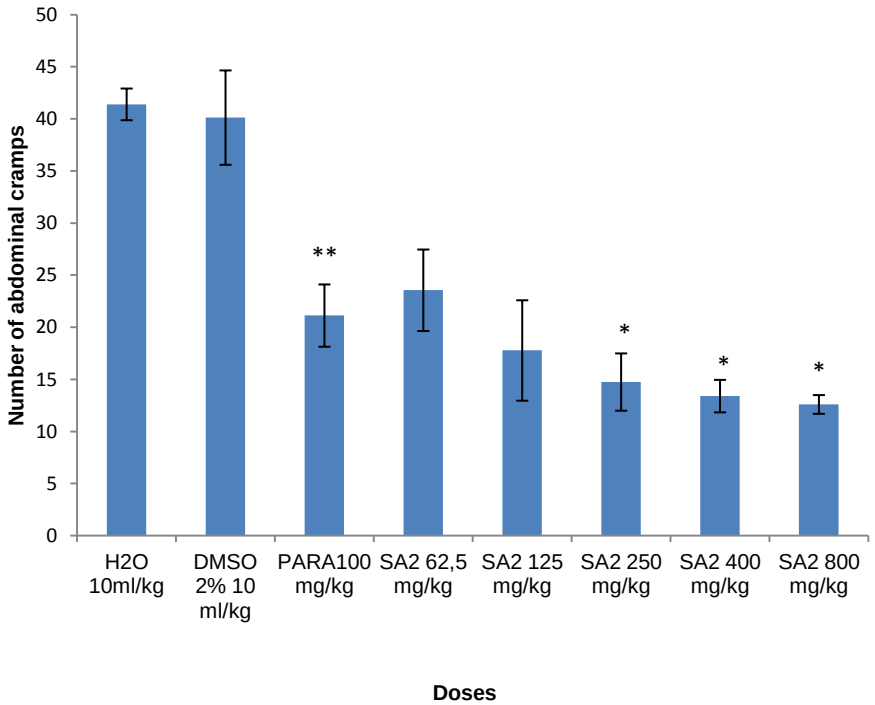

Figure 1b: Effec of *Schwenckia americana* ethanolic extract (SA2) on abdominal cramps induced by acid acetic n= 5; *p < 0,05; **p < 0,01 significative difference compared to control. Para: paracetamol

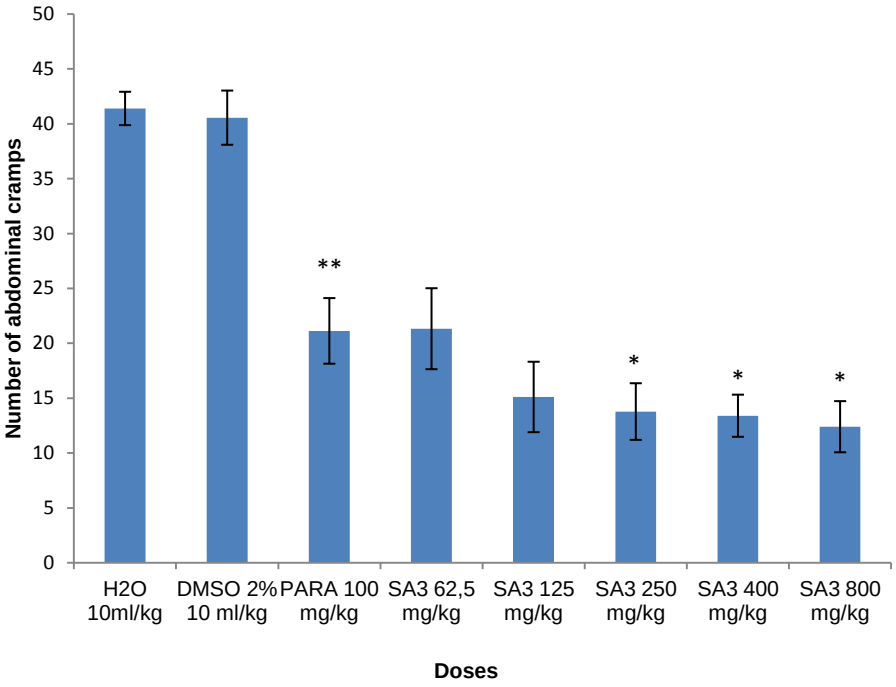

Figure 1c: Effec of *Schwenckia americana* aqueous extract (SA3) on abdominal cramps induced by acid acetic n= 5; *p < 0,05; **p < 0,001 significative difference compared to control. Para: paracetamol

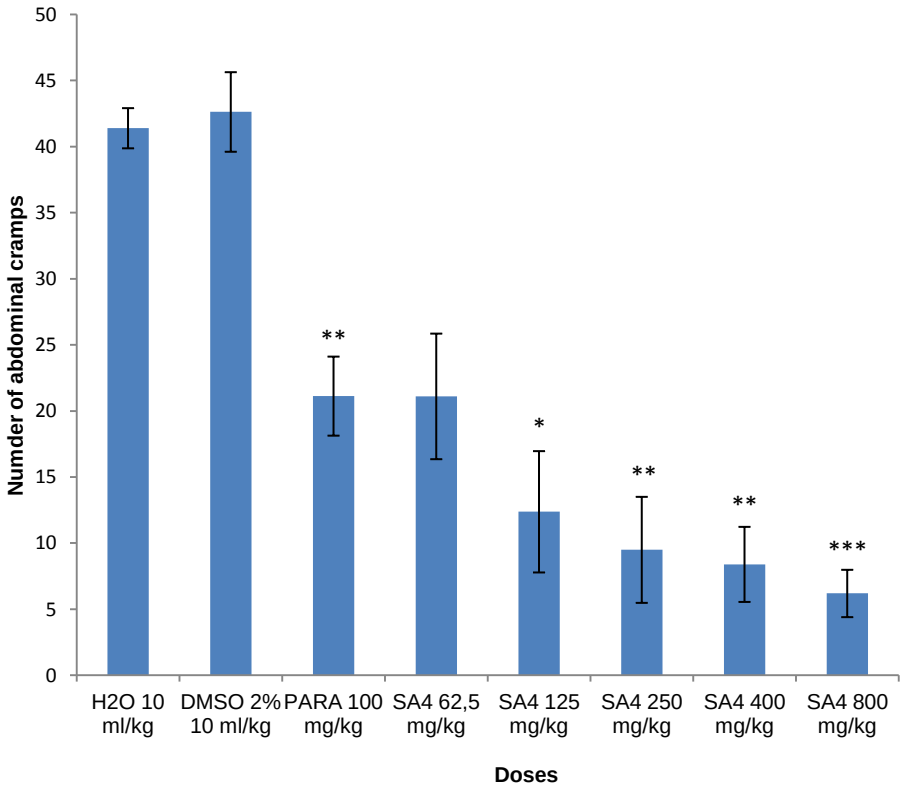

Figure 1d: Effec of *Schwenckia americana* traditional aqueous extract (SA1) on abdominal cramps induced by acid acetic n= 5, *p < 0,05; **p < 0,01; ***p < 0,001 significative difference compared to control. Para: paracetamol

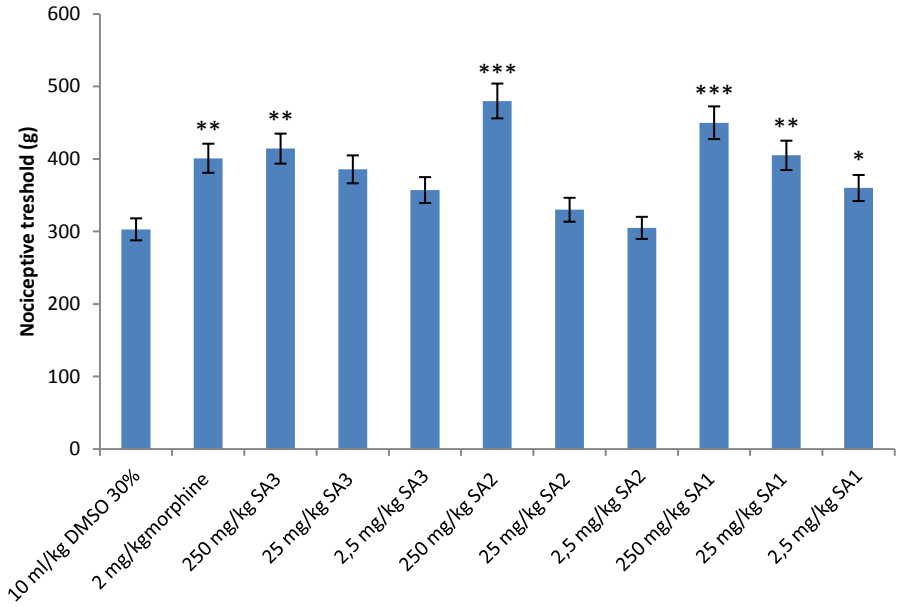

Figure 2: Analgesic effect of *Schwenckia americana* (SA) extracts on the rat paw pressure expressed in nociceptive tresholdn=5, results are expressed as mean± SE, ***p<0,001; *p<0,5. 1: DCM extract, 2: ethanolic extract, 1: aqueous extract.

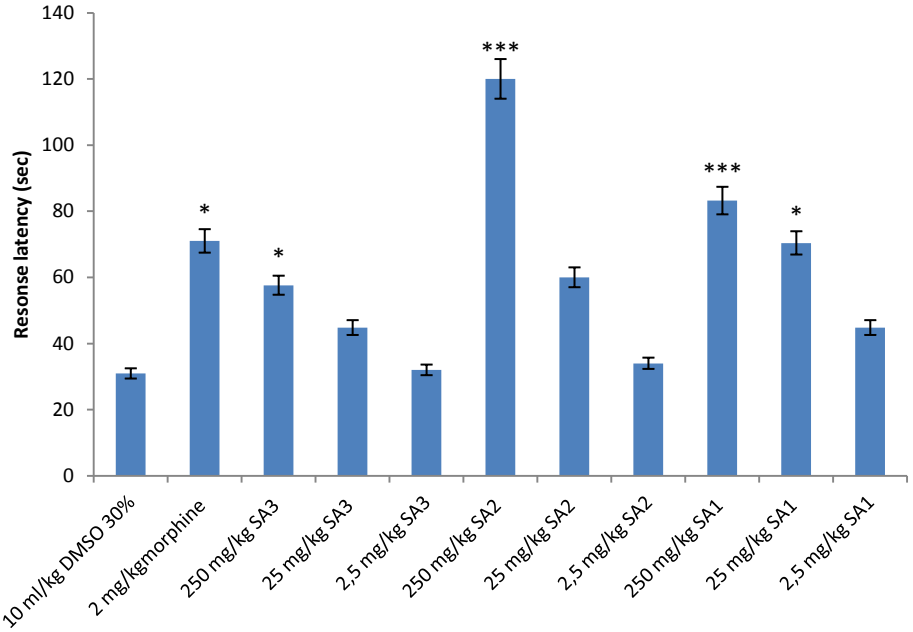

Figure 3: Analgesic effect of *Schwenckia americana* (SA) extracts on the rat paw pressure expressed in latency time n=5, results are expressed as mean± SE; ***p < 0,001; *p < 0,5.
1: DCM extract, 2: ethanolic extract, 3: aqueous extract.

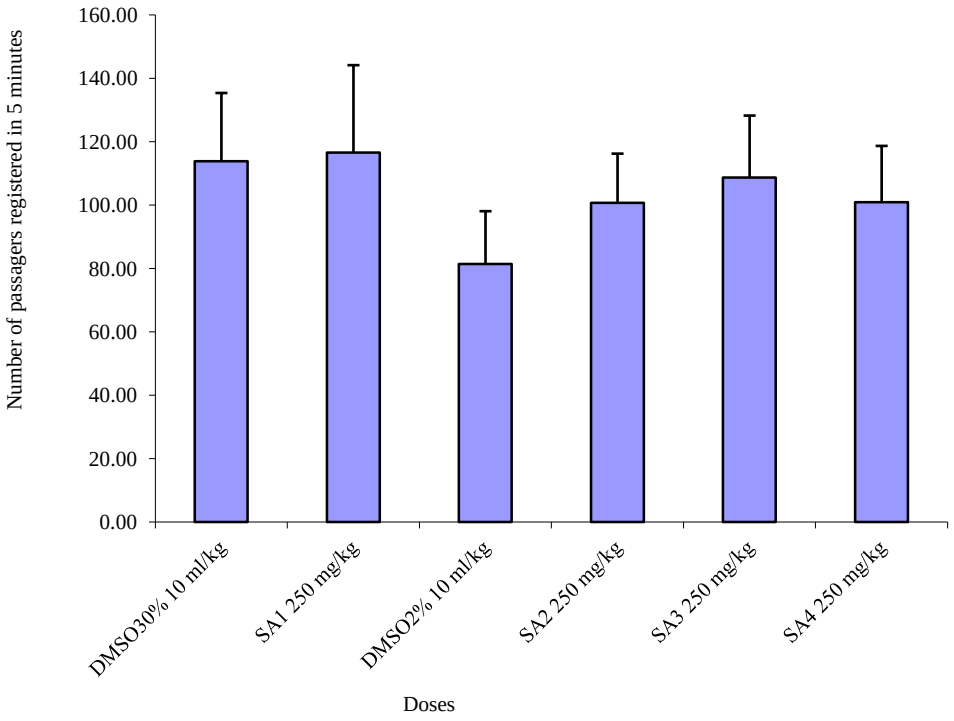

Figure 4: Motility of mice treated with Schwenckia americana (SA) extracts. Results expressed as mean±SE , n=5, 1: DCM extract, 2: ethanolic extract, 3: aqueous extract.

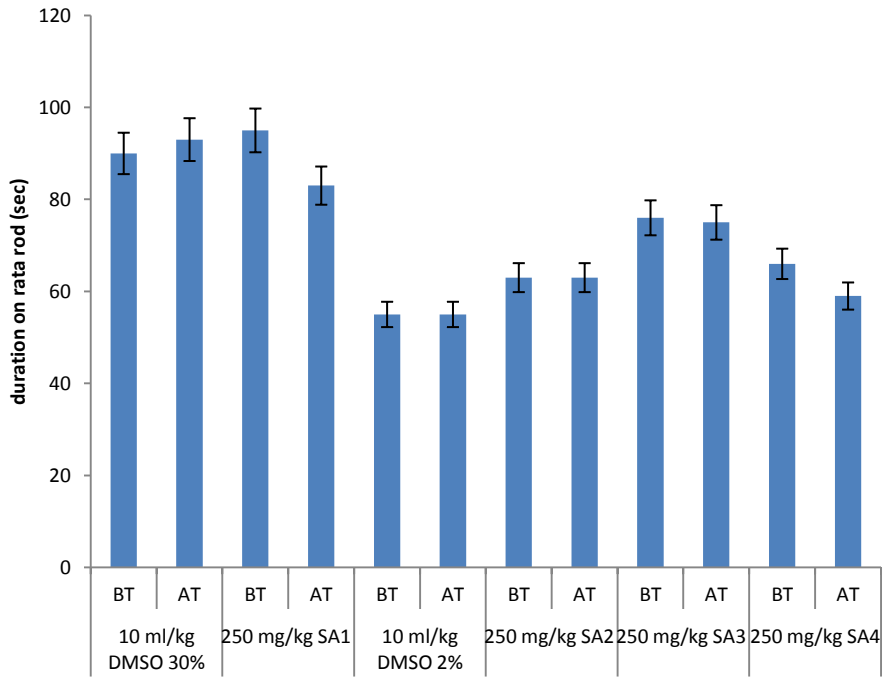

Figure 5: Effect of *Schwenckia americana* extracts on equilibration and movement coordination reflex, p=5, results expressed as mean±SE , BT: before treatment, AT:after treatment, 1: DCM extract, 2: ethanolic extract, 3: aqueous extract.

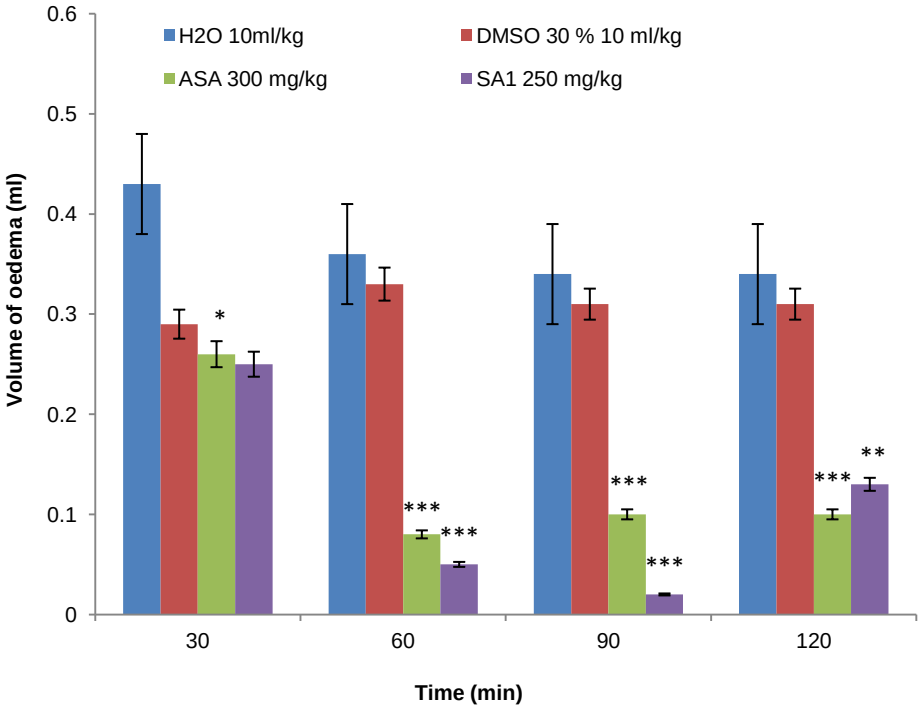

Figure 6a: Antiinflammatory effect of *Schwenckia americana* (SA1) DCM extract on the carrageenan induced hind paw oedema n= 5, *p< 0,05; **p< 0,01; ***p< 0,001 significant difference compared to control group. ASA : aspirine

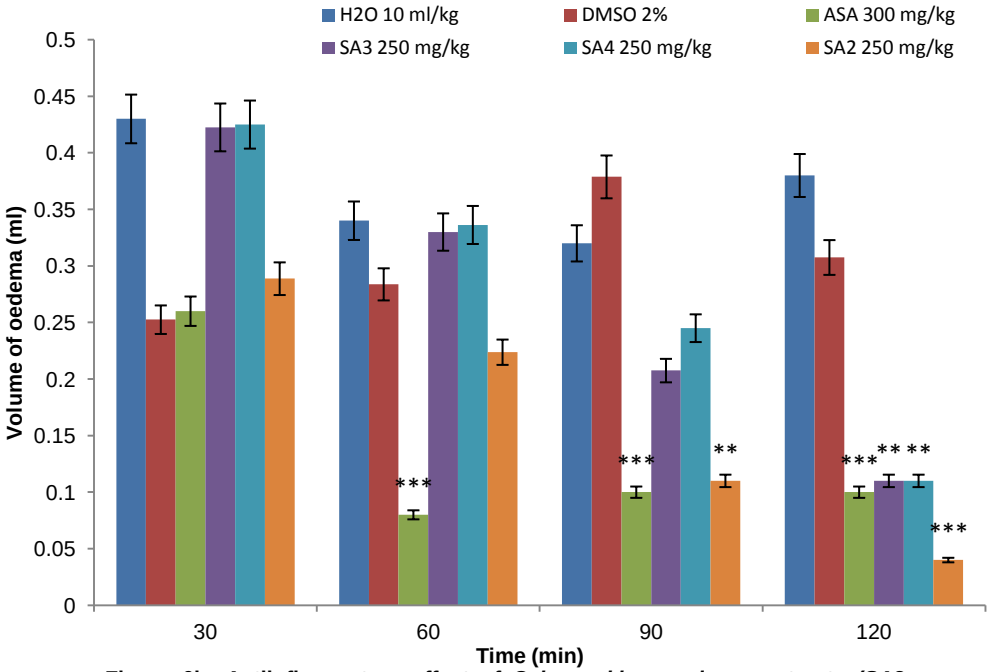

Figure 6b: Antiinflammatory effect of *Schwenckia americana* extracts (SA2, SA3 and SA4) on carrageenan induced hind paw oedema n= 5, **p< 0,01; ***p< 0,001 significant difference compared to control group. ASA: aspirine, 2: ethanolic extract, 3: aqueous

3. Pressure test

Figures 2 and 3 show a significant analgesic activity at the dose of 250 mg / kg with the three extracts ($p < 0.05$) compared to the control.

4. Behavior

4.1. Photoelectric actimeter test

Figure 4 shows the effects of the extracts of *Schwenckia americana* (SA1, SA2, SA3, SA4) on the motility of the animals.

4.2. Testing the rotating rod or Rota-Rod

Figure 5 shows the effects of the *Schwenckia americana* extracts on the reflexes of equilibration and coordination of the movements of the mice.

5. Anti-inflammatory activity

Schwenckia americana extracts SA1 and SA2 showed an anti-inflammatory effect at 250 mg / kg between 1h and 2h after treatment (Figures 6a and 6b).

V. DISCUSSION

The objective of this study was on the one hand to evaluate the safety of our extracts in order to be able to determine the doses to be used during the pharmacological study and on the other hand, to validate the use of this plant in traditional medicine against pain and inflammation. The acute toxicity study did not reveal signs of toxicity up to the maximum dose of 3000 mg / kg for ethanol extracts (SA2) and water (SA3 and SA4) of *Schwenckia americana*. Only the extract of *Schwenchia Americana* with DCM (SA1) is very toxic with an estimated lethal dose fifty (LD50) at 750 mg / kg. This explains why the maximal dose of SA1 at 400 mg / kg was fixed for pharmacological studies, while the other extracts were tested up to 800 mg / kg.

The results obtained show that under our experimental conditions, all extracts prepared are active against pain. The test of acetic acid makes it possible to demonstrate the effect of a product on the painful behavior induced by the chemical stimulation. The test of abdominal cramps with acetic acid and the paw pressure test have proven to be very effective in evaluating the analgesic properties of medicinal plants (Sampath et al., 2012, Muhammad et al. 2012). The contractions caused by acetic acid are related to the sensitization of the local peritoneal receptors of prostaglandins PGE2 and PGF2α (Koster et al. 1959). The paw pressure test is well recognized for the evaluation of analgesic activity having a central mechanism of action. This latter test appears to be more specific, insofar as it makes it possible to characterize truly analgesic behavior (Woolfe and MacDonald 1944). The effect on acetic acid test is expressed in a decrease of the number of abdominal cramps compared to the control. However, it does not make it possible to make a categorical decision on the anti-nociceptive effect of a product with a central mechanism of action only with a peripheral mechanism of action. This important activity of the *Schwenckia americana* plant prompted us to continue the evaluation of its activity by the method of paw pressure in order to confirm its analgesic potential. In addition, the paw pressure test is performed on a different animal species. The paw pressure test therefore confirmed the existence of a significant dose-dependent analgesic activity of the *Schwenckia americana* extracts. This analgesic activity is therefore due to the presence of chemical compounds belonging to different chemical groups: alkaloids, flavonoids, terpenoids, saponins and tannins (Nsonde Ntandou et al.,2017). Some of these analgesic compounds would have a peripheral and other central mode of action or both. Some substances may increase or decrease the spontaneous mobility of mice according to whether they are excited or sedative. Tests of the photoelectric actimeter and rotating rod or Rota-Rod are often used to assess the spontaneous motility of mice (Boissier and Simond, 1964). The actimeter allows to do an assessment of motility. The rotating stem allows to study equilibrium reflexes and the coordination of the movements of the animals. *Schwenckia americana* does not interfere with the observed analgesic effect. This shows that *Schwenckia americana* possesses an analgesic activity as was also demonstrated by the team of Jimoh (2011) and Banzouzi (2010). These results may

justify the traditional use of this plant in the treatment of pain (Adjanohoun et al., 1989). Carrageenan is a mucopolysaccharide used for its phlogistic properties. This model is typically associated with the activation of the cyclooxygenase pathway and is sensitive to glucocorticoids by antagonism of prostaglandin synthesis. The development of carrageenan-induced edema corresponds to events in the acute phase of inflammation. These events are mediated by histamine, bradykinin and prostaglandins produced by cyclooxygenase. The results show an anti-inflammatory effect of the *Schwenckia americana* extracts as is often observed with some modern analgesic drugs. This effect is very remarkable with the extracts SA1 and SA2, which are significant after 1h and 1h30. In our experimental conditions, the anti-inflammatory activity obtained with SA1 is greater than that of aspirin. The bibliographic analysis shows that the SA1 extract is rich in alkaloids and flavonoids whereas the SA2 extract contains terpeniodes and saponins, and SA3 and SA4 extracts are rich in tannins (Banzouzi et al., 2010). In addition to these secondary metabolites, there are also primary metabolites: lactones / esters and proteins / amino acids (Makambila et al., 2010). The toxicity of the extract SA1 may be due to the presence of its alkaloids and flavonoids as had already been observed by Bruneton (1999). Antiinflammatory and analgesic activies are due to the presence of secondary metabolites. It is well known that these plants contain lupeol as active molecules, really interesting for fighting pain and inflammation (Luceti et al.,2012; Nsonde Ntandou et al., 2010;2017).

VI. Involvement in research and practice

Without scientific verification and validation, the users of this plant are exposed to the risks of poor primary health care and intoxication, which can lead to other diseases. This is a serious public health problem. About 80% of the populations of developing countries are at great risk. They use traditional medicine or plant for their treatment. The traditional form of use deserves to be improved to increase efficiency, improve safety and the pharmaceutical forms of use and preservation. Moreover this plant can be source of new active principles against pain and inflammation and lead to the development of a new class of drug.

VII. CONCLUSION

In this study, we confirmed the analgesic effect of this plant; demonstrates an anti-inflammatory effect and establishes that the extracts of *Schwenckia americana* do not alter the reflex of equilibration and coordination of movement in the animal. We have also shown that extracts of *Schwenckia americana* do not present acute toxicity in the form of traditional use.

VIII. Future Research

In perspective, we will evaluate the cytotoxicity, subacute toxicity in rats and mice of active extracts, isolate or concentrate the active ingredients; To prepare drug formulations, to carry out the clinical trials in phase I and then in phase two (II) and three (III) with *Schwenckia americana* and *Cassia siamea* (another very active plant) and also to look for the effects of synergies with the medicines already known.

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