SCIENTIFIC FEDERATION
ABODE FOR RESEARCHERS

Nsonde Ntandou GF, SF Pharma J, 2017, 1:1

SciFed Pharmaceutics Journal

Research Article Open Access

Central Mechanism of Analgesic Effect of The Aqueous Extracts of Schwenckia Americana and Lupeol Its Main Isolated Active Molecule

[*1,3]Nsonde Ntandou GF, [2]Makambila MC, [5]Banzouzi JT, [4]Ouamba JM, [1]Abena AA

*[*1]Laboratoire de Biochimie et Pharmacologie, Faculté des Sciences de la Santé, Université Marien NGOUABI, Brazzaville, B.P. 69, Congo*

[2]Institut National de Recherche en sciences de la Santé, Cité Scientifique (ex-ORSTOM), Route de l'Auberge de Gascogne, Makélékélé, Brazzaville-Congo

*[*3]Laboratoire de Physiologie et Physiopathologie Animales, Faculté des Sciences et Techniques, Université Marien NGOUABI, Brazzaville, BP 69,Congo*

[4]Unité de Chimie du Végétal et de la Vie, Faculté des Sciences, Université Marien Ngouabi, Brazzaville, B.P. 69, Congo

[5]Institut de Chimie des Substances Naturelles (ICSN-CNRS), 1 Avenue de la Terrasse-Bat 27, 91198 Gif-sur-Yvette Cedex, France

Abstract

Purpose

The aqueous extract of *Schwenckia Americana* is the most tolerated and widely used in traditional medicine. This work aims to isolate the main molecule responsible for the analgesic effect of *Schwenckia Americana* and elucidate the mechanism of this effect.

Materials and Methods

The extracts with DCM (SA1), ethanol (SA2) and water (SA2) are obtained after sequential extraction with increasing polarity. The SA4 extract used in traditional medicine is an aqueous decoction. HPLC coupled to mass, preparative HPLC and TLC allowed the fractionation of the SA1 extract and lupeol isolation. Experiments were performed in mice and rats. The tests of hot plate, abdominal cramps, formaldehyde and pressure of the paw were used. Evaluation of the analgesic effect of extracts and lupeol in the presence of naloxone 2 mg / kg elucidated the mechanism of this effect.

Results

SA3 and SA4 extracts at a dose of 250 mg / kg p.o. and lupeol at a dose of 50 mg / kg p.o. significantly; increase, the latency time on the hot plate test, the intensity and latency time on the paw pressure test and decrease the number of abdominal cramps caused by acetic acid, the duration of acute and tonic pain induced by formaldehyde. These effects are substantially identical respectively to paracetamol (100 mg /kg po) and morphine (2 mg / kg ip). In the presence of naloxone (2 mg / kg, ip) the analgesic effect of the aqueous extracts of *Schwenckia Americana* and lupeol disappears completely

Conclusion

Aqueous extracts of *Schwenckia Americana* and lupeol have analgesic effect which is mediated by central mechanism through opioid receptors. Alkaloids, terpenoids, flavonoids and tannins are believed to be responsible in part of this effect, a triterpen lupeol could be the main molecule responsible for this effect.

Keywords

Schwenckia Americana; Aqueous Extract; Phytochemstry; Isolation; Lupeol; Analgesic; Naloxone.

Introduction

S. Americana (Solanaceae) is located in Africa and tropical America. Its decoction is used as a drink against stomachache, onset of hernia, gonorrhea, heart ailments, edema, mouth infections, cough, intestinal worms, intercostal pain, swelling pain, rheumatism, osteoarthritis, gastric problems, asthma, respiratory insufficiency, and

***Corresponding author:** Nsonde Ntandou GF, Laboratoire de Biochimie et Pharmacologie, Faculté des Sciences de la Santé, Université Marien NGOUABI, Brazzaville, B.P. 69, Congo. E-mail: nsonde_ntandou@yahoo.fr

Received May 09, 2017; **Accepted** July 8, 2017; **Published** July 20, 2017

Citation: Nsonde Ntandou GF (2017) Central Mechanism of Analgesic Effect of The Aqueous Extracts of Schwenckia Americana and Lupeol Its Main Isolated Active Molecule. SF Pharma J1:1.

for its purgative properties in cases of poisoning. In steam baths it is used to treat feverish children. 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, sinusitis and conjunctivitis. The whole plant is crushed to make a paste that is used as effective fishing poison [1].

The decoction of leaves is administered to pregnant women when the fetus develops too slowly. Breastfeeding women take it to prevent diarrhea in their baby. Ground leafy stems are applied to the skin against measles and chicken pox. A poultice made from leaves is applied to whitlow and athlete's foot [1, 3].The leaves infusion is taken to treat female infertility. The mastication of the roots is used to treat respiratory diseases in children. A decoction of roots is given to babies as a laxative and a purgative [3]. Roots and stems are used as chewing sticks to clean teeth. The aqueous extract is the most tolerated and widely used in traditional medicine [1, 3, 19]. In order to develop an improved traditional phytomedicine, this work aims to isolate the mean active pure compound of the analgesic effect of aqueous extract of *S.americana* and elucidate their mechanism of action.

Materials and Methods

1. Plant Material

The sample of *Schwenckia americana* was collected in 2007, in the Pool region. 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 chemical study. Botanical identification was done by professor Mountsamboté from national Herbarium. A specimen was deposited at the Natiotional herbarium under number MKC045.

2. Animal material

CDI male mice (Charles River France) weighing 18 to 20 g and male Sprague-Dawley rate strain (Charles River France) weighing 175 to 200 g were used. These animals were kept under standard conditions (21 ° C, 40-70% RH, 12h / 12h light / dark cycle). They had access to water and food ad libitum. The recommendations of the ethical rules published by the IASP (the "International Association for the Study of Pain") were observed [26]. Animals were divided in cages. The treatment was done in a randomized order. The experiments were made blind by the same experimenter.

3. Chemical Study

3.1. Preparation of extracts

Three extracts are prepared in solvents of increasing polarity in order to extract the maximum of compounds. 7 kg of plant powder are placed in an extractor to be cold macerated under mechanical agitation during 48 hours in dichloromethane (DCM). After filtration on filter paper, the remaining samples were extracted again twice. The three extracts thus produced have the same chemical profile on TLC. They are mixed and then concentrated at reduced pressure in a rotary evaporator to give the final extract SA1 (283 g with 4% yield). With the marc obtained at the first extraction, a second extraction is done in 95% ethanol using the same protocol to obtain SA2 extract (213 g, representing a 3.1% yield). The second marc from the ethanol extraction is again used for the aqueous extract preparation. This is a decoction prepared from 6 kg of Marc 2 in 3x60 liters. The mixture was boiled for 10 min and then filtered through cotton and then on filter paper. The extract is then centrifuged for 20 min at 3000 rev / min and then lyophilized for a dry extract (SA3, 700 g, with a 11.6% yield).

A traditional extract was also prepared, for this, a sample of 600 g of dry powder of plant material was boiled in 4 l of water during 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 50% yield). These solids (SA1, SA2, SA3 and SA4) were stored in a freezer - 4 ° C until their pharmacological evaluation.

3.2. Isolation of lupeol

100 g of the extract with dichloromethane from *Schwenckia americana* (SA1) were chromatographed on a silica column using dichloromethane / ethyl acetate / methanol (1: 1: 1) as migration system, which has enabled us 3 separate phases: a first, a second and a third. These three phases are mixtures contained respectively in dichloromethane, ethyl acetate and the mixture of ethyl acetate / methanol AcOEt/MeOH (8/2). Each phase was then subjected to flash chromatography on silica. The fractions obtained were analyzed by thin layer chromatography. At the end of that analysis, the fractions which presented the same chromatographic profile were mixed. This leads to the production of five (5) fractions SA1-A (4.4 g); SA1-B (7.2 g); SA1-C (4.17 g); SA1-D (1.0 g) SA1-E (4.1 g) in DCM phase; three

(3) SA1-F (32.4 g); SA1-G (1.9 g); SA1-H (3.8 g) in AcOEt phase and also three (3) fractions SA1-I (0.3 g) SA1-J (12.8 g) SA1-K (4.0 g) in MeOH phase. For the continuation of this work, splitting focused on the SA1 fraction-A. To do so; 3, 9 grams of this fraction were dissolved in 5 ml of DCM, to which 5 ml of methanol were added, which led to a precipitation. After filtration, a solid residue (SA1-AS (1.4 g)) was obtained, while the filtrate (SA1-AL) gave a mass of 1.64 g after concentration.After this step, the purification process is only concerned the fraction SA1-AS. For this, the flash chromatography was further exploited. To this end, 954 mg of AS-SA1 fraction used helped to obtain six other fractions: SA1-AS1 (0.407 g), SA1-AS2 (0.041 g), SA1- AS3 (0.073 g), SA1 -AS4 (0.073 g), SA1-AS5 (0.129 g), SA1-SA6 (0.066 g). Three of these six (6) later fractions (SA1-AS1, SA1-SA5, SA1-SA6) were purified by preparative HPLC on a normal silica column. Thus from SA1-SA6, we obtained a pure product C1 (3 mg). And, SA1-AS5 gave two pure products SA1-AS5B1 (6.8 mg) and SA1-AS5B2 (1.7 mg). No pure product could be isolated from the fraction SA1-AS1. The SA1-AS5B1 representing the largest mass of pure product after comparative analysis has been identified as lupeol.

Comparative study of the analgesic effect of *Schwenckia americana* extracts and lupeol

The analgesic activity of these two aqueous extracts (SA3 and SA4) and lupeol was demonstrated by using four pain tests: the abdominal cramps test, the hot plate test, the formaldehyde test and the paw pressure test. The extracts dissolved in distilled water was evaluated at 250 mg / kg po, lupeol dissolved in 2% DMSO, at a dose of 50 mg / kg, p.o., paracetamol and morphine were used as reference products respectively at the doses 100 mg / kg po and 2 mg / kg, ip.; Naloxone was administered at 2 mg / kg intraperitoneally 45 min after the analgesic treatment. For each experiment the animals fasted during 24 hours before the test were divided into five (5) groups of five animals each (n = 5) corresponding to the different specific treatments. The pharmacological evaluation took place 1 hour after treatment.

4.1. Test of the hot plate

The test consists in causing a heat stimulus in the mouse by gently placing it on a hot plate (Ugo Basile DS-37) maintained at 56 ± 0.5 ° C [25]. With a chronometer, the time taken by the animal before reacting to thermal stimulation by the licking paws or jumping is noted. This time is selected as a parameter for assessing the antinociceptive effect. Analgesic substances increase the latency. Maximum exposure is limited to 2 min to avoid tissue damage.

4.2. Pressure paw test

The experiment was conducted in Wistar rats. This test is based on applying a mechanical stimulus that can induce pain. Increasing pressure is applied to the right hind paw until the pain threshold. This threshold leads to a voluntary withdrawal of the leg accompanied by a stereotyped flinch reaction. The maximum weight that can be applied is 750g [21]. The response to the mechanical pressure of the animal's paw was measured by using an algesimeter (Ugo Basile, Italy), as described by Randall and Sellitto [21]. It is expressed in current threshold supported by the animal before the withdrawal of the right hind leg pressed and latency.

4.3. Formaldehyde Test

The formaldehyde test described by Dubuisson and Dennis [5] can address the acute and tonic component of pain in animals. Pain was induced by injecting 10 µl of formalin (5% formaldehyde diluted in 0.9% NaCl buffered to pH 7) at the right hind paw. The spontaneous pain was quantified by measuring the licking time after intraplantar formalin injection at the leg [10]. The analgesic effect is characterized by diminution duration of licking. Licking behavior during this test is biphasic. The first phase (0-5 min) is the direct chemical stimulation of nociceptors by formalin, and the second (10-30min) is a response to inflammatory pain. The experiment was carried out in blind. The animals were placed in Plexiglas boxes (220 x165) 20 min before for acclimatation.

4.4. Antagonism of the analgesic effect

The antagonism of the analgesic effect of different products by naloxone was performed by evaluating the inhibition of the analgesic activity in the presence of a specific inhibitor of the morphine: naloxone. The evaluation of the disappearance of the analgesic effect was conducted by using the thermal test as described above. The mice (males) which reacts to pain within the 20 seconds that followed the moment when they were put on the hot plate were selected for the study.

5. Statistical Analysis

The results are expressed as mean ± standard error. The statistical significance of the analgesic activity was calculated by using an analysis of variance (ANOVA).

Significant differences were determined by using the test Duncan's multiple-range. P values <0.05 were considered significant.

Results

1. Thermal Test

SA3 and SA4 extracts at a dose of 250 mg / kg, and lupeol at a dose of 50 mg / kg orally increase significantly ($p < 0.01$) the latency time on the hot plate. However, no significant difference was observed between the different treatments (Figure 1).

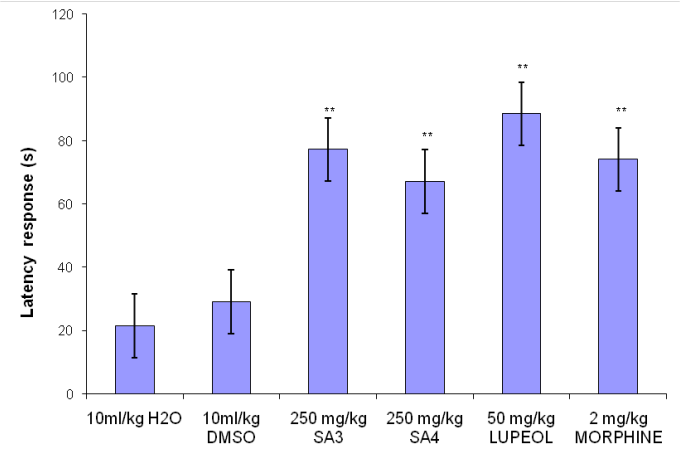

Figure 1: Analgesic effect of *Schwenckia americana* aqueous extracts (SA3 and SA4) and du lupeol: hot plate test. The values are expressed as mean ± SE, n = 5, **p < 0,01; compared with control .

2. Abdominal cramps test

The analgesic activity registered with lupeol at 50 mg / kg per os, is not significantly different with the two extracts (SA3 and SA4) at 250 mg / kg and with paracetamol 100 mg / kg per os, which is the reference drug (Figure 2).

3. Formaldehyde test

The SA3 and SA4 extracts at a dose of 250 mg / kg per os, lupeol at a dose of 50 mg / kg per os and morphine (2 mg / kg sc) confirmed their analgesic effect during both phases of the formalin test (Figure 3). But this effect is not statistically different between products.

4. paw Pressure test

Figures (4a and 4b) show the effects of both extracts at 250 mg / kg and lupeol at 50 mg / kg per os on paw pressure expressed on latency and intensity threshold.

5. Antagonism of analgesic effect

The analgesic effect of SA3 and SA4 extracts at 250 mg / kg per os, lupeol at 50 mg / kg peros and morphine 2 mg / kg sc. was completely inhibited by naloxone (Figure 5).

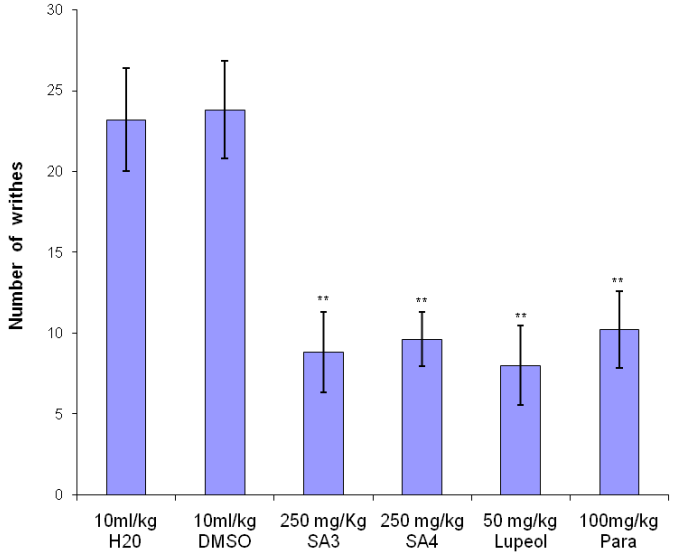

Figure 2: Analgesic effect of *Schwenckia americana* aqueous extracts (SA3 and SA4) and lupeol on the acetic acid induced abdominal writhes. The values are expressed as mean± SE, n = 5, **p < 0,01 compared with control.

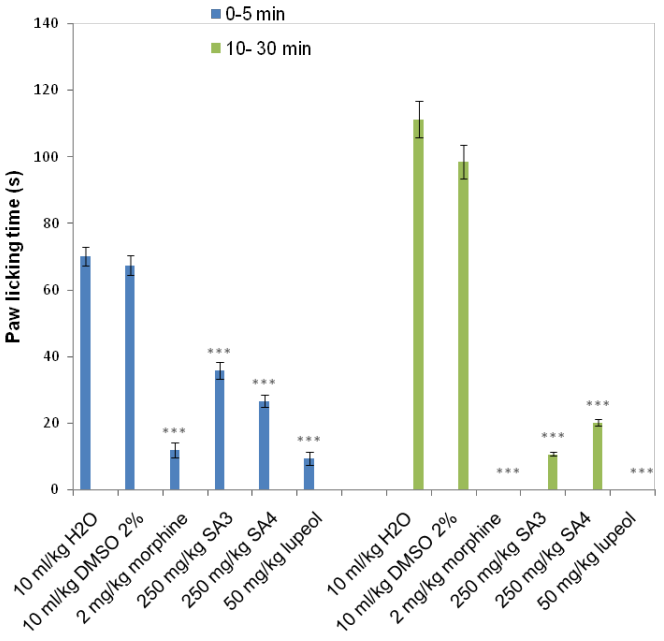

Figure 3: Analgesic effect of *Schwenckia americana* aqueous extracts (SA3 and SA4) and lupeol on pain behavior in the formaldehyde test. Values are expressed as mean ± SE; n= 5; ***p< 0,001, copared with control

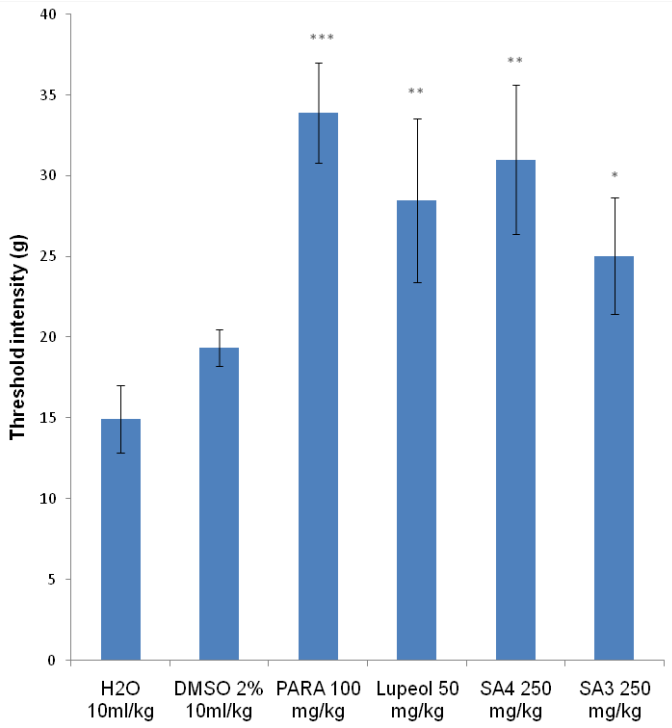

Figure 4a: Analgesic effect of *Schwenckia americana* aqueous extracts (SA3 and SA4) and lupeol in threshold intensity: paw pressure test. The values are expressed as geometric mean± SE; n= 5; *p < 0,05; **p <0,01; ***p < 0,001 compared with control.

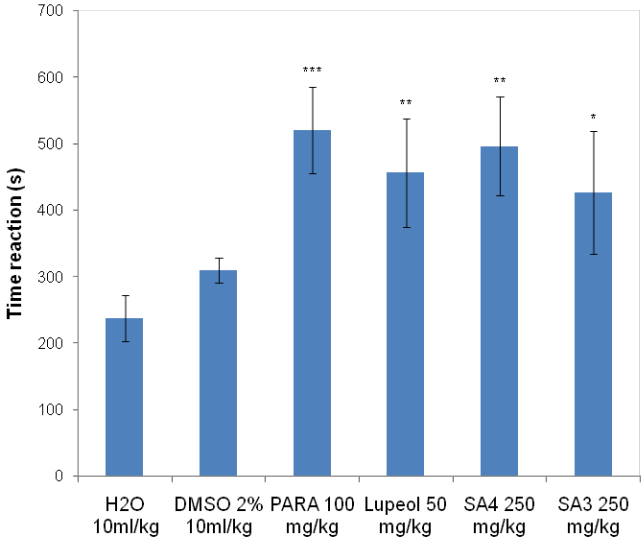

Figure 4b: Analgesic effect of *Schwenckia americana* (SA3 and SA4) and lupeol in time reaction response: paw pressure test. Results expressed as geometric mean± SE: n= 5; *p < 0,05; **p < 0,01; ***p < 0,001 compared with control.

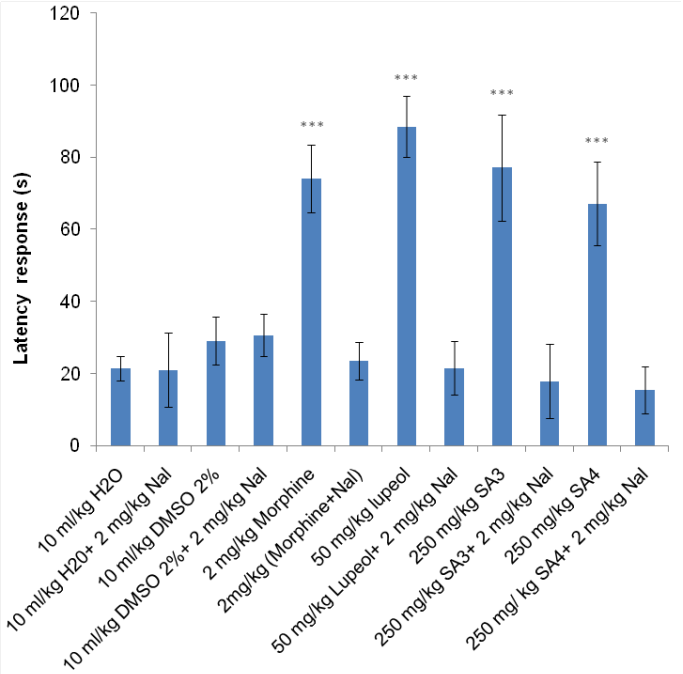

Figure 5: Inhibition of analgesic effect of *Schwenckia americana* aqueous extracts (SA3 and SA4) and lupeol in the presence of Naloxone (Nal). The values are expressed as mean ± SE; n=5; ***p < 0,001 compared with control.

Discussion

In order to develop an improved traditional phytomedicine, this work aims to isolate the mean active pure compound of the analgesic effect of aqueous extract of *S.americana* and elucidate their mechanism of action, in order to validate the use of *Schwenckia americana* in the treatment of pain in traditional medicine. The results show that under our experimental conditions, all samples prepared as the lupeol isolated from the AS1 extract of this plant are active against the pain. This species is already known for its analgesic and anti-inflammatory effects [11]. A lupeol is a triterpene extracted and isolated from many plants [16, 20]. Its analgesic and antiinflammatory properties have already been demonstrated [2, 15, 17]. Anterior investigation of analgesic activity of this species has concerned organic extracts [19]. In this study we have evaluated its aqueous extract which is used in traditional medicine in Congo. Concerning pharmacological methods used in this study, *Schwenckia Americana* was already investigated by using acetic acid induced abdominal cramps test and hot plate test. The study is the first to used paw-pressure test and formaldehyde on analgesic assessment of

aqueous extract of *Schwenckia Americana* in comparison with lupeol. The acetic acid abdominal cramps test, hot plate test, paw-pressure test and formaldehyde test are well known and proved very effective in the evaluation of the analgesic properties of medicinal plants [7, 9, 16, 23].

Cramps caused by acetic acid linked to the awareness of local peritoneal prostaglandin receptors PGE2et PGF2a [2, 12, 14]. The hot plate test and the paw pressure test are well accepted for evaluating analgesic effect of medicine with central mechanism of action.

By contrast, the formaldehyde test is used to study either peripheral or central analgesic activity or both. By using abdominal writing, hot plate and paw pressure tests; the finding results showed similar analgesic effect between *Schwenckia americana* aqueous extracts (250 mg/kg) with lupeol (50 mg/kg). But, with the formalin test we found most analgesic effect with lupeol.The analgesic activity of extracts is due to the presence of terpenoïds chemical compounds, mainly by lupeol [22, 25]. The lupeol isolated and evaluated here is a triterpenlupan that is found in the large group of plant. To confirm the hypothesis of a central mechanism of action, we evaluated the analgesic effect in presence of naloxone well known as a specific inhibitor of morphine, it is an antagonist of μ receptors located in the central nervous system [13, 18]. The results confirm that morphine, the two extracts of *Schwenckia americana* and lupeol would act through receptors located in the central nervous system. This result is in accordance with those of Lucetti et al. [15]. This therefore shows that *Schwenckia americana* has good analgesic activity as was also demonstrated by the team Jimoh et al. [8, 11]. These results could justify the traditional use of this plant in the treatment of pain [1, 3].

References

1. Adjanohoum EJ, Ahyi MRA, Ake Assi L (1988) Contribution aux études ethnobotaniques et floristiques en République du Congo ACCT Paris 605.

2. Ahmad SF, Pandey A, Kour K, et al. (2010) Down regulation of pro-inflammatory cytokines by lupeol measured using cytometric bead array immunoassay Phytother Res 24: 9-13.

3. Aké-Assi L, Guinko S, Aya-Lazare A (1991) Plantes utilisées dans la médecine traditionnelle en Afrique de l Ouest Edition Roche Basel Switzerland 151.

4. Boissier JR, Simon P La (1962) Reaction d exploration chez la souris Therapie 17: 1225-1232.

5. Boissier JR, Simon P (1964) Dissociation de deux composantes dans le comportement d investigation de la souris Arch Inter Pharmacodyn 147: 372-387.

6. Dubuisson D, Dennis SG (1977) The formalin test a quantitative study of the analgesic effects of morphine meperidine and brain stem stimulation in rats and cats Pain 4: 161-174.

7. Hajare SW, Chandra S, Tandan S K, et al. (2000) Analgesic and Antipyretic Activities of *Dalbergia sissoo* Leaves Indian Journal of Pharmacology 32: 357-360.

8. Hodouto KK (1990) Etude chimique des plantes à flavonoïdes du Togo Bulletin de Médecine Traditionnelle et Pharmacopée 4 : 31-48.

9. Hossain MM, Ali MS, Saha A, et al. (2006) Antinociceptive activity of whole plant extracts of *Paederia foetida* Dhaka University Journal of Pharmaceutical Science 5: 67-39.

10. Hunskaar S, Fasmer OB, Hole K (1985) Formalin test in mice a useful technique for evaluating mild analgesics J Neu rosci Methods 14: 69-76.

11. Jimoh AO, Chika A, Umar MT, et al. (2011) Analgesic effects and anti-inflammatory properties of the crude methanolic extract of *Schwenckia americana* Linn (Solanaceae) Journal of Ethnopharmacology 137: 543-546.

12. Koster R, Anderson M, De Beer J (1959) Acetic acid for analgesic screening Federal Proccedings 18: 412-417.

13. Kieffer B, Matthes H, Maldonado R (1997) Mécanisme d action de la morphine. Med Sci 13: 232- 235.

14. Leme JG, Hamamura L, Leite MP, et al. (1973) Pharmacological analysis of the acute inflammatory process induced in the rats paw by local injection of carrageenan and by heating British Journal of pharmacology 48: 88-96.

15. Lucetti DL, Lucetti ECP, Bandeira MAM, et al. (2010) Anti-inflammatory effects and possible mechanism of action of lupeol acetate isolated from *Himatanthus drasticus* (Mart) Plumel Journal of Inflammation 7: 1-11.

16. Muhammad I, Naveed M, Barkatullah HK, et al. (2012) Antinociceptive and anticonvulsant activities of essential oils of *Zanthoxylum armatum* Phytopharmacology 3: 191-198.

17. NsondeNtandou GF, Banzouzi JT, Mbatchi B, et al. (2010) Analgesic and anti-inflammatory effects of *Cassia siamea* Lam Stem bark extracts J of Ethnopharmacol 127: 108-111.

18. Nsonde ntandou GF, Bassoueka DJ, Banzouzi JT, et al. (2016) *Cassia siamea* lam extracts analgesic mechanism of action and pharmacodynamic interaction with paracetamol (acetaminophen) European J of Res in Medical Sci 4: 1-13.

19. Olumayokum A, Olajide S, Olubusayo A, et al. (2000) Studies on the anti-inflammatory antyperitic and analgesic properties of *Alstonia boonei* Stem bark Journal of Ethnopharmacology 71: 179-186.

20. Ongoka PR, Banzouzi JT, Poupat C, et al. (2008) Steroids isolated from *Millettia versicolor* Baker (Fabaceae) African Journal of Biotechnology 7: 1727-1730.

21. Randall LO, Selitto JJA (1957) Method for measurement for analgesic activity on inflamed tissue Arch Int Pharmacodyn Ther 111: 409-419.

22. Rousset P (2008) Guide technique pour une utilisation énergétique des huiles végétales Brasilia Cirad 288.

23. Ronaldo AR, Mariana LV, Sara MT, et al. (2000) Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by Zymosan and acetic acid in mice European Journal of pharmacology 387: 111-118.

24. Takeda T, Narukawa Y, Hada N (1999) Studies on the constituents of *Leonotis nepetaefolia* Chem Pharm Bull 47: 284-286.

25. Woolfe G, Mac Donald AD (1944) Evaluation of the analgesic action of pethidine-hydrochloride J Pharmacol Exp Ther 80: 300-307.

26. Zimmermann M (1983) Ethical guidelines for investigations of experimental pain in conscious animals Pain 16: 109–110.

Citation: Nsonde Ntandou GF (2017) Central Mechanism of Analgesic Effect of The Aqueous Extracts of Schwenckia Americana and Lupeol Its Main Isolated Active Molecule. SF Pharma J1:1.