Analgesic And Anti-Inflammatory Properties Of The Recipe From *Zanthoxylum Leprieurii* Bark, *Harungara Madagascariensis* Leaves And *Xylopia Aethiopica* Fruits In Wistar Rats.

ABSTRACT

The objective of this study was to evaluate the analgesic and anti-inflammatory potentials of extracts from a combination of organs of three plants. The analgesic and anti- inflammatory activities of the aqueous (DZHm) and hydroethanolic (EZHm) extracts (200, 400 and 800 mg/kg) were evaluated in rats as models of acetic acid-induced torsion and Carrageenan-induced hind paw edema, using Diclofenac and Ibuprofen as reference molecules.

Oral administration of ZHm extracts at doses of 200, 400 and 800 mg/kg produced anti-inflammatory and analgesic effects. These extracts significantly reduced edema and pain. These effects are time and dose dependent. In terms of analgesic effect, the dose of 800 mg/kg bw generated a high inhibition rate of 84.51% and 73.06% for DZHm and EZHm respectively, while the inhibition percentage of Ibuprofen was 78.3 ± 2.09 at the dose of 100 mg/kg bw. The anti-inflammatory effect was 60, 55% for DZHm, 56.08% for EZHm and 62.91% for Diclofenac at 10 mg/kg Pc. Diclofenac and Ibuprofen used for these two tests generated similar activities with ZHm extracts at the dose of 800 mg/kg Pc. The tested extracts (DZHm and EZHm) have analgesic and peripheral anti-inflammatory potentials justifying the use of this recipe in traditional environment.

***Keywords**: anti-inflammatory, analgesic properties, medicinal plants*

1. INTRODUCTION

Inflammation is a reactionary phenomenon implemented by the organism whenever the integrity of its morphological and biological constants are threatened [1]. It is therefore not synonymous with infection, but infection can be the cause of inflammation [2]. Inflammation has multiple origins among which are infections, physical and chemical agents [3]. Inflammation can be manifested by various symptoms such as edema (swelling or tumor), pain, fever often accompanied by anemia. Anemia is the main cause of hospitalization in sickle cell disease because it leads to painful vaso-occlusive crises. These painful attacks and anemia often dominate the symptomatology of the sickle cell patient under 5 years of age. Abdominal pain crises sometimes correspond to spleen infarctions [4]. However, sickle cell disease does not currently have sufficient curative treatment [5]. Thus to improve the quality of life or relieve patients, clinicians prescribe purely symptomatic treatment. Only bone marrow transplantation currently provides a curative solution, while gene therapy is in full development with the new Crisper-Cas9 technology [6]. However, these highly specialized therapies require appropriate infrastructures, qualified personnel with high financial means and a compatible donor, which generally explains their unavailability in Black Africa where the disease is more frequent. Natural substances could be a source of new types of drugs that could fight against these biological phenomena related to sickle cell disease. Several experimental studies have shown the anti-inflammatory, analgesic and antioxidant activities of *Zanthoxylum leprieurii*, *Harungara madagascariensis* and *Xylopia aethiopica in vitro* and *in vivo* [7, 8, 9]. In the context of the development of a phytomedicine for the management of sickle cell disease in Côte d'Ivoire and the sub-region, the present study was initiated to investigate the analgesic and anti-inflammatory activities of a recipe derived from different organs of *Zanthoxylum leprieurii*, *Harungara madagascariensis* and *Xylopia aethiopica*. Thus, in this study, it is important to

.

test the hypothesis that the different parts of these collected species would act synergistically to inhibit Carrageenan and acetic acid-induced edema and spasm in rats.

2. MATERIAL AND METHODS

2.1 Plant material

The plant material consists of fruits of *Xylopia aethiopica* (Dunal) A. Rich, barks of *Zanthoxylum leprieurii* (GUILL), and leaves of *Harungara madagascariensis* (LAM). These three plants were collected in January 2018 in the Indénié-Djuablin region of eastern Côte d'Ivoire.

Animal material

Nulliparous and non-pregnant Wistar rats with a weight between 104 and 130 g, from the animal house of the Ecole Normale Supérieure (ENS) in Abidjan (Ivory Coast) were used for the acute and subacute toxicity tests. All procedures and techniques used in this experiment were performed in accordance with the National Institute of Health guidelines for the care and use of laboratory animals [10].

2.2 Preparation of extracts

2.2.1 Preparation of the hydroethanol extract

The hydroethanol extract was prepared according to the method of Zirihi *[11]*. One hundred grams (100 g) of plant powder was dissolved in one liter of hydroalcoholic solvent comprising 70% ethanol and 30% distilled water. The mixture was then homogenized 10 times at a rate of 2 minutes per revolution using a Severin ® brand blender. The homogenate obtained was wrung out in a cloth square and then filtered three times on hydrophilic cotton and then on whatman paper (3 mm). The filtrate was evaporated at 45°C using a Venticell® type oven for 24 hours. The dry powder obtained was the 70% ethanolic extract coded EZHm.

2.2.1 Preparation of the aqueous extract by decoction

Following the method of Konkon [12], one hundred grams (100 g) of plant powder was boiled for 20 min in 2 L of distilled water. The decoctate cooled to room temperature (25°C) was filtered three times on absorbent cotton and once on Whatman 3 mm filter paper. The filtrate was then dried at 50°C using a Venticell® type oven. The powder obtained is the total aqueous extract coded DZHm.

2.3 Phytochemical screening

The phytochemical screening was performed according to the method used by Nemlin and Brunel [13]. The phytochemicals sought and the reagents used are presented in Table 1

Table 1**:** Reagents and tests for characterization of chemical groups

Groupes chimiques	Réactifs	Réactions caractéristiques
Alcaloïdes	Dragendorff Bouchardât	Précipité ou coloration orangée Précipité brun-rougeâtre
Polyphénols	Chlorure ferrique	Coloration bleue noirâtre
Flavonoïdes	Cyanidine	Précipitation rose-orangée
Stérols et les polyterpènes	Liebermann	Anneau vert
Tanins	Stiasny	précipité en gros flocons
Substances quinoniques	Bornstraëgen	Coloration rouge ou violet
Saponines	Agitation	Mousse persistante d'une hauteur de 10 cm

2.4 Determination of analgesic activity

.

The method used is that described by Koster [14] and modified by Collier [15]. It consists of testing the inhibitory action of extracts on pain induced in rats by intraperitoneal injection of a dilute solution of acetic acid. The pain is manifested by a stretching movement of the hind legs and twisting of the dorso-abdominal muscles (spasms). Induction of the twists was performed by intraperitoneal injection of rats with a 0.6% acetic acid solution at a rate of 10 ml/kg body weight (BW).
Eight batches of 5 rats were formed.
lot 1 or control lot (TM) received distilled water.
Lot 2 received Ibuprofen (10 mg/kg/pc).
Lots 3, 4 and 5 received ZHm decoctate (DZHm) at 200, 400 and 800 mg/kg/pc.
batches 6, 7 and 8 received ZHm hydroethanol extract (ZHmHE) at doses of 200, 400 and 800 mg/kg/pc.
One hour after the injection of the different solutions, 0.6% acetic acid at the dose of 10 mL/kg was injected into the rats [16]. Five minutes (5 min) after the injection of acetic acid solution, the number of abdominal twists was observed for 20 min. The analgesic activity which is the percentage reduction in the means of abdominal twists was calculated according to the equation:

$$\mathbf{PIC}(\%) = \frac{M0 - M1}{M0}$$

PIC : Pourcentage d'inhibition des cotorsions,
PIC: Percentage of inhibition of cotorsions,
M0 : Average of the torsions of the control batch,
M1 : Average of the twists of the treated batch.

2.5 Evaluation of anti-inflammatory activity

Test for inhibition of oedema of the rat paw with Carrageenan

The test for anti-inflammatory activity was performed according to the method of Winter *[17]*. Injection of carrageenan under the plantar fascia of the mouse or rat hind leg results in edema (clinical sign of inflammation) of the metatarsal region. The intensity of this edema, which reaches its maximum development in 5 hours, is assessed by the increase in the volume of the paw (compared to the initial volume). Preventive oral administration of an anti-inflammatory product significantly reduces the development of edema**.**
Rats weighing between 120 g and 150 g were fasted for 24 h. They were divided into 9 batches of 6 rats each. The diameter at time T0 of the right hind leg of each animal was determined using a digital caliper [18].
Lots 1 and 2 (normal and edematous rats) received distilled water. Rats in lot 3 (reference) received Diclofenac Sodium (5 mg/kg bw). The other six batches received decoctate and hydroethnolic extract at 200, 400 and 800 mg/kg bw, respectively.
One hour after gavage administration of the different solutions, 0.1 mL of the 1% carrageenan solution, was injected dermally into the plantar fascia of the right hind paw of each rat in batches 2, 3, 4, 5, 6,7, 8, and 9. The course of right edema was determined at 1h, 2h, 3h, 4h, 5h, and 6h [19].
The paw volume of all animals was measured with a caliper at 1h, 2h, 3h, 4h, 5h and 6h. The extent of edema was assessed by determining the average percent increase (% AUG) in rat paw volume according to the formula:

$$\%AUG = \frac{V1 - V0}{V0} \times 100$$

V1 : Volume de la patte au temps t
V0 : Volume initial de la patte

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

$$\%INH = \frac{\%\mathbf{AUG_{TM}} - \%\mathbf{AUG_{TT}}}{\%\mathbf{AUG_{TM}}} \times 100$$

$\%AUG_{TM}$: pourcentage augmentation des témoins négatifs
$\%AUG_{Tt}$: pourcentage d'augmentation des Traités

.

2.6 Statistical analysis

Statistical analysis of the data and graphical representation was performed using Graph Pad Prism 9.0 software (San Diego, California, USA). Values were given as means followed by the standard error on the mean (M ± SEM). The difference between two values was given by student's t-test which was completed by Dunnet and Turkey tests as post tests. The significance level was set at $P < 0.05$ for the expression of the results.

3. RESULTS

3.1 Phytochemical study

The qualitative phytochemical analysis performed on DZHm and EZHm revealed the presence of different groups of secondary metabolites. These results reveal the presence of polyphenols, tannins, flavonoids, alkaloids, steroids, triterpenes and quinone substances in DZHm and EZHm; in contrast, saponosides were absent in EZHm (Table 2).

Table 2: Results of the phytochemical study of the extracts of the ZHm recipe

Decocted (DZHm)							Hydroethanol extract (EZHm)						
S/T	**POL**	**FLA**	**TAN**	**SQ**	**ALC**	**SA**	**S/T**	**POL**	**FLA**	**TAN**	**SQ**	**ALC**	**SA**
+	+	+	+	+	+	+	+	+	+	+	+	+	-

(+) : Presence (-) : Absence

ALC: Alkaloids; TAN: Tannin, FLA: Flavonoid; POL: Polyphenols; S/T: Sterol / Triterpenes; SQ: Quinones; SAP : Saponosides.

3.2 Analgesic power of the extracts of the ZHm recipe

3.2.1 Analgesic effect of DZHm on cotorsions

Analgesic activity was assessed according to the acetic acid test (chemical stimulus). Figure 1 summarizes the response of rats to pain induced by 1% acetic acid. During the 20 min after injection of the 1% acetic acid solution, the number of abdominal cramps observed in control rats was 53.33 ± 2.40. For the other substances tested after acetic acid injection, the number of twists was 11.67 ± 1.66; 18.33 ± 1.66; 8.66 ± 3.18 and 8.33 ± 1.66 for Ibuprofen and DZHm at 200, 400 and 800 mg/kg/pc, respectively. Treatment with Ibuprofen significantly reduced the number of twists with a percentage inhibition of 78.3 ± 2.09. As for the rats treated with 200, 400 and 800 mg/kg/pc of DZHm, the percentage of inhibition was 56.89 ± 0.64; 83.28 ±6.69 and 84.51 ± 2.80 respectively. The analgesic action of DZHm at 400 and 800 mg/kg is similar to that of Ibuprofen at 100 mg/kg (Figure 2).

3.2.2 Analgesic effect of EZHm on cotorsions

The results of this study are summarized in Figures 1 and 2. The mean number of contortions in untreated control rats was 53, 33 ± 2.40 following intraperitoneal injection of acetic acid. In general, the number of contortions in rats treated with Ibuprofen and EZHm were decreased compared to that of control rats. In the presence of Ibuprofen (100 mg/kg), the number of stretches in rats was 11.67 ±1.66 during the 20 min of observation, which corresponds to a percentage of protection of 78.3% compared to control rats. For rats treated with 200, 400 and 800 mg/kg/pc of EZHm, the mean number of stretches was 23.33 ±1.66; 12.33 ±1.45 and 14.33 ±0.66 or a percentage of inhibition of 55.81%; 77.02%; 73.06% respectively.

.

The analgesic effect of DZHm and EZHm is comparable to that of Ibuprofen at 400 and 800 mg/kg/pc. The reduction in the number of contortions is dose-dependent for both extracts.

3.3 Anti-inflammatory potential of the extracts of the ZHm recipe

Effect of extracts on acute inflammation

The results obtained show that all rats that received Carrageenan developed paw edema. The volume of paw edema varied over time.

For animals that received NaCl (9%), edema increased to a peak characteristic of an inflammatory reaction at 4e hours with a maximum of 56.66 ± 4.33%. Then, edema decreased at 5e and 6e hours with a percentage of 45.87% without reaching the initial volume (normal state). A significant ($p<0.01$) reduction in edema by the plant extracts and in a dose-dependent manner was observed after 3 hours (Figure 3). The reference molecule also significantly reduced the edema induced by Carrageenan.

Oral administration of the aqueous extract (DZHm) at the doses of 200, 400 and 800 mg/kg bw significantly ($p<0.01$) reduced edema compared with that of untreated rats 3, 4, 5 and 6 hours after Carrageenan injection. The percentage inhibition of DZHm at 200, 400, and 800 mg/kg Pc at 4e hours was 31.76%, 52.45%, and 62.25%, respectively, whereas that of Diclofenac was 62.91%. At the end of the 6e hours of experiment, the results show that Diclofenac at the dose of 10 mg/kg had better anti-inflammatory activity, with a percentage of inhibition of 82.82% and those of the aqueous extract were 28.44%; 45.44% and 60.55% at the different doses tested (Figure 4). The reduction of edema by the aqueous extract of ZHm *at* the dose of 800 mg/kg was similar to that of Diclofenac at 4e hours of observation.

Hydroethanol extract (EZHm) at the doses of 200, 400 and 800 mg/kg Pc also significantly ($p<0.01$) reduced the edema of treated rats compared to untreated rats from 4e , 5e and 6e hours after Carrageenan injection. Compared with the percentage of inhibition of Diclofenac which was 62.91% at 4e hours and 82.82% at 6e hours, the percentages of inhibition of ZHm hydroethanol extract were 28.43%, 53.97 and 56.08% at 4e hours and then 28.26%, 50.45 and 53.27% at 6e hours for the doses of 200, 400 and 800 mg/kg Pc, respectively (Figure 4).

During the six-hour experiment, both aqueous and hydroethanol extracts of ZHm significantly inhibited paw edema in rats from 4e hours at the dose of 800 mg/kg, compared with untreated rats. The inhibitory power of ZHm extracts was more pronounced with the aqueous extract after 4e hours.

.

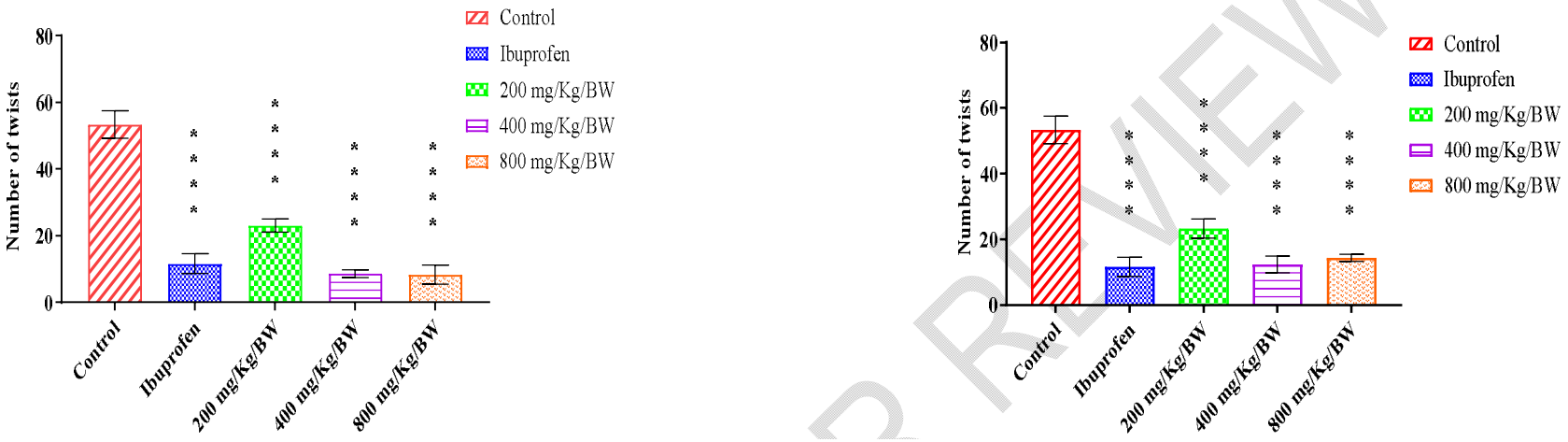

Figure 1: Number of twists in the presence of Ibuprofen and different doses of the ZHm recipe extracts

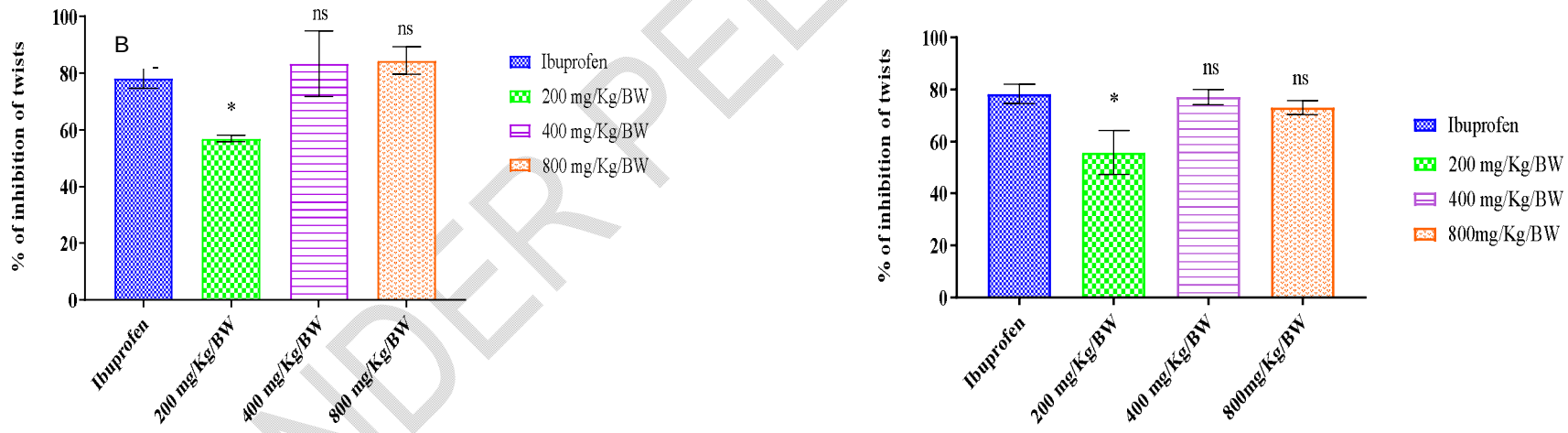

Figure 2: Pain inhibitory effect of rats treated with Ibuprofen and ZHm extracts after acetic acid injection

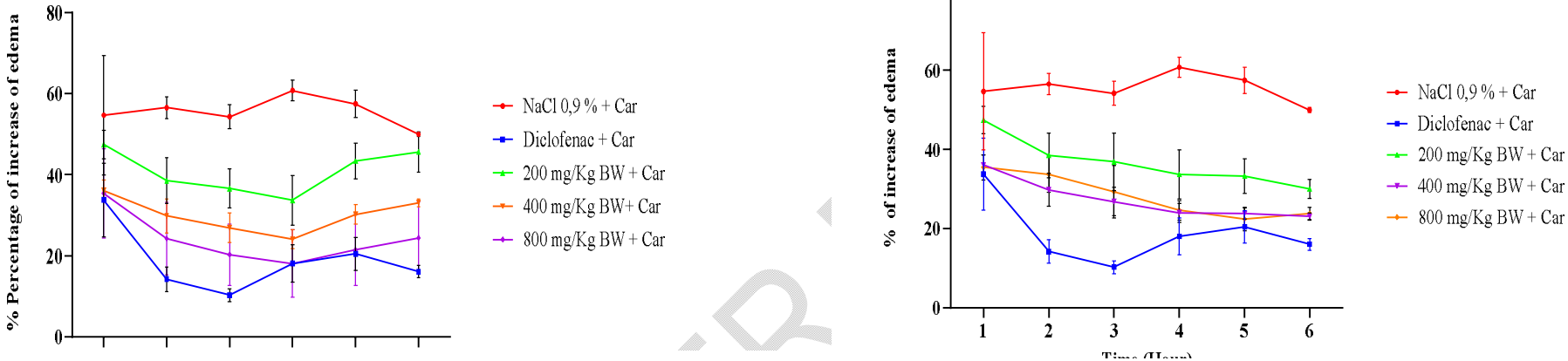

Figure 3: Variation in the percentage increase in edema of rats treated with ZHm recipe extracts and Diclofenac.

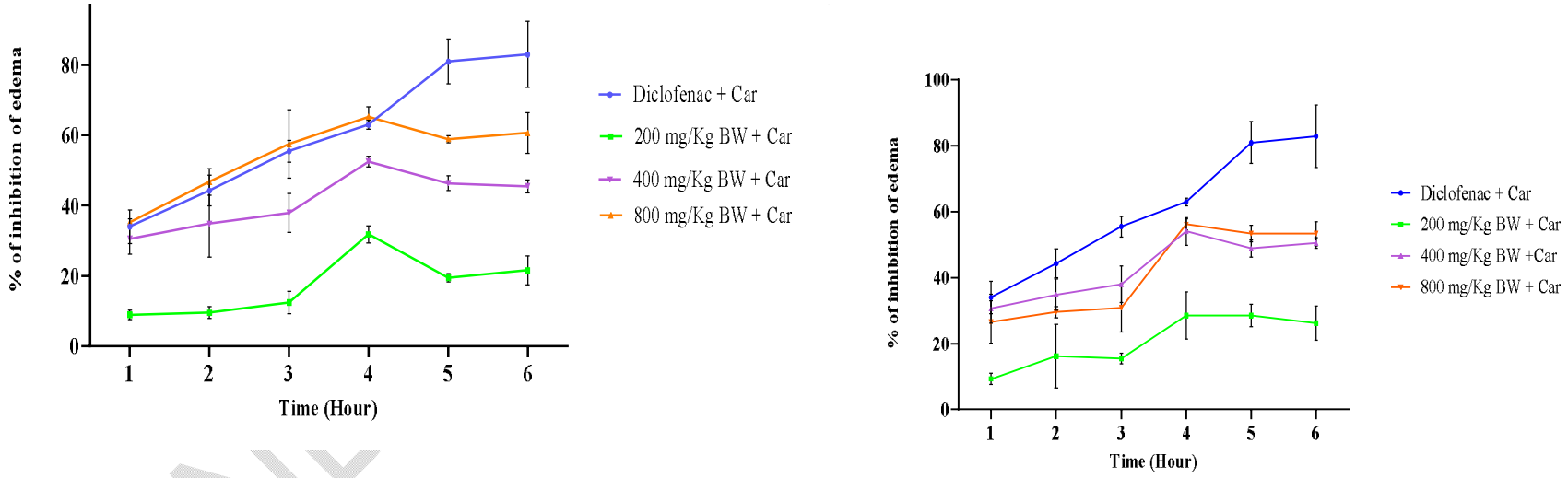

Figure 4: Variation in the percentage of edema inhibition of rats treated with ZHm recipe extracts and Diclofenac

Discussion

The aim of this study was to verify the analgesic and anti-inflammatory effect of the aqueous (DZHm) and hydro-ethanolic (EZHm) extracts of the combination of *Z. leprieurii*, *H. madagascariensis* and *X. aethiopica*.

Pain is one of the characteristics of inflammation. Many conditions cause pain and fever in humans. Some of these conditions often result in death. Although synthetic analgesics and anti-inflammatory drugs are available in pharmacies, most synthetic products are expensive and also have many adverse effects [20].

Thus, to mimic the painful attacks of sickle cell patients in rats, 1% acetic acid was injected intraperitoneally, a method used to study the peripheral analgesic effect of a substance. The pain elicited by injection of 1% acetic acid is due to the release of serotonin, histamine, bradykinin, and prostaglandins (PGE2, PGF2) [21]. Without analgesic, acetic acid administration generated an average of 53, 33 contortions in rats. A similar result was observed by Traoré *[22]*. These chemical mediators stimulate peripheral nociceptive neurons and induce increased vascular permeability. The result is a later, diffuse pain that manifests in rats as stretching of the hind legs and twisting of the dorso-abdominal musculature [23]. These seizures are the most disabling syndrome of the disease, moreover, they have an unpredictable and random nature [4]. Most often osteoarticular in nature in adults, they are associated with intense pain and variable frequency. Vaso-occlusive crises are the most frequent complications of sickle cell disease, so analgesics are the most commonly used medications by sickle cell patients for pain relief [4].

Administration of the extracts at different doses of 200, 400 and 800 mg/kg bw generated a reduction in the number of abdominal cramps caused by acetic acid. EZHm and DZHm at doses of 400 and 800 mg/kg bw, had an effect comparable to that of Ibuprofen. Indeed, the percentage of pain inhibition at the 400 mg/kg bw dose was 77.02%; 83.28% for EZHm and DZHm respectively and 73.06% and 84.51% at the 800 mg/kg bw dose while that of Ibuprofen was 78.3%. This activity of the extracts is dose-dependent. The analgesic effect of the extracts could be related to the inhibition of the release of pro-inflammatory mediators by the presence of alkaloids. Indeed, according to Allegra [24] ; Eom [25]; alkaloids are well known for their ability to inhibit pain perception. Furthermore, Koua [26], showed that the presence of flavonoids in *Crinum scillifolium* extracts would have inhibited acetic acid injection-induced spasms. The presence of these chemical compounds in ZHm extracts would therefore be responsible for its peripheral analgesic activity.

In an attempt to alleviate vaso-occlusive crises in sickle cell patients, inflammation was induced in rats. The Carrageenan-induced edema test has characteristics similar to those of acute inflammation, namely increased extravasation of cells and fluid into tissues. This test typically evaluates compounds for acute anti-inflammatory activity. This test is a classic model of inflammation and increased pain sensitivity, which has been widely used over the years to evaluate the anti-inflammatory properties of NSAIDs and selective COX-2 inhibitors [27]. The progression of edema after Carragenin administration is a biphasic event, dependent on age and weight. Histamine, serotonin, and bradykinin are the first mediators to be detected in the early phase [28] and a late prostaglandin-supported phase is mediated by bradykinin, leukotrienes, and polymorphonuclear cells [29]. The significant inhibition of inflammation by ZHm extracts after 2 hours of edema induction, suggests the ability of the extracts to act on both the initial phases of acute inflammation. Indeed, ZHm may have inhibited the release of vasoactive mediators that induce inflammation and, thus, protected the rats to a greater extent. ZHm significantly and dose-dependently inhibited edema formation by Carrageenan in rats. The inhibition of paw edema by ZHm was more pronounced in the presence of decoctate.

In the case of acute inflammation, the results obtained show that the aqueous extract of ZHm at doses of 400 and 800 mg/kg bw significantly inhibits the formation of rat paw edema like the reference drug, Diclofenac (10 mg/kg bw). It is a non-steroidal anti-inflammatory drug (NSAID) that exerts anti-inflammatory activity through inhibition of cyclooxygenase (COX1 and COX2) and suppresses the production of prostaglandin, histamine and serotonin. These substances are involved in increasing vasodilation and vascular permeability [30, 31]. The decrease in the volume of rat paw edema by ZHm extracts at 400 and 800 mg/kg bw could be explained by the presence of polyphenols in these extracts. These would have acted as substances with antagonistic action on the effects of histamine, serotonin, bradykinin and prostaglandin biosynthesis [26].

The extracts of ZHm having analgesic and anti-inflammatory effects could upstream attenuate the vaso occlusive crises in sickle cell patients. These different activities observed with ZHm suggest that there would have been an addition of molecules with anti-inflammatory and analgesic activity of the different plants from the ZHm recipe. Indeed, according to Johnson-Ajinwo and Nyodee [8] ; Njan *[32]*, *H. madagascariensis* has an anti-inflammatory, antipirytic and analgesic activity, moreover the work of Oso [33] ; Macebo *[7]* on *X. aethiopica* show that these extracts inhibit proinflammatory molecules and spasms caused by formalin and acetic acid. Furthermore; the work of Zondegoumba *[34]* and Evelyne [35] also showed that *Z. leprieurii* has anti-inflammatory activity *in vivo* and *in vitro*.

These observed effects were possible because of the therapeutic properties of the secondary metabolites identified during phytochemical sorting. Indeed according to the literature, alkaloids have antimalarial, antibacterial, analgesic and cardiotonic effects [36]. Flavonoids are known for their antioxidant, anticancer and anti-inflammatory activities [37]. As for terpenoids, they also possess antimicrobial, antiparasitic, antiviral, antispasmodic, anti-inflammatory, and immunomodulatory properties [38, 39]. As for saponins, they have antioxidant and anti-inflammatory properties and also participate in the delay of neural aging [40]. According to Gandonou [41] and Mpondo [42], tannin plants would be used as anti-inflammatory, anti-parasitic and analgesic.

Analysis of the phytochemical study results revealed the presence of alkaloids, catechic tannins, flavonoids, polyphenols, sterols, terpenes, and quinone substances in the aqueous and hydroethanolic extracts of the recipe (ZHm). The presence of these secondary metabolites in both ZHm extracts has been confirmed by different authors. Indeed, the phytochemical screening performed by Benson [9] ; Tine [43] and Gnamien [44] on *Zanthoxylum leprieurii* revealed the presence of tannins, flavonoids, terpenoids, alkaloids. The work of the following authors (Oso [33] ; Ehigiator and Adikwu [45] revealed the presence of tannins, flavonoids, leucoanthocyanins, terpenoids, saponosides, and steroids in the fruits of *Xylopia aethiopica*. These same compounds were found in extracts of *Harungara madagascariensis* [46,47]. Although these plant extracts in the recipe are rich in secondary metabolites and show promising biological activities, toxicological studies conducted by our team revealed that this recipe did not alter the biochemical and hematological parameters of test rats. These different results could therefore justify the use of this plant recipe in a traditional environment.

4. CONCLUSION

Oral administration of ZHm extracts at doses of 200, 400 and 800 mg/kg generated anti-inflammatory and analgesic effects. These extracts significantly reduced edema and pain. These effects are time and dose dependent. Diclofenac and Ibuprofen used in these two tests had similar activities with ZHm extracts at 400 and 800 mg/kg/Pc. These results give pharmacological credibility to the use of this herbal recipe as a natural remedy in the management of inflammatory pain.

REFERENCES

1. Cheriti A, Rahmani S and Belboukhari N. Evaluation of anti-inflammatory activity of aqueous extracts of Limoniastrum feei (Plumbaginacea) leaves. Algerian Journal of Arid Environment “AJAE”. 2016; 6(1): 80-86.

2. Varrassi G, Pergolizzi JV, Dowling P and Paladini A. Ibuprofen Safety at the Golden Anniversary: Are all NSAIDs the Same? A Narrative Review. Adv. Ther. 2020; 37:61–82.

3. Viladomiu M, Hontecillas R and Bassaganya-Riera J.. Modulation of inflammation and immunity by dietary conjugated linoleic acid. European journal of pharmacology. 2016; 785, 87-95.

4. Hierso R. 2015. Involvement of oxidative stress in the pathophysiology of sickle cell disease: vaso-occlusive crises, anti-band 3 antibody levels and red blood cell oxidation. Doctoral thesis University of the Antilles and Guyana, Faculty of Exact and Natural Sciences, Pointe-à-Pitre, France, 185p.

5. Wembonyama SO. Moringa oleifera, a boon in the management of major sickle cell syndrome? Journal of Medicine, Public Health and Policy Research. 2021; 1(1): 31-35.

6. Park SH and Bao G. CRISPR/Cas9 gene editing for curing sickle cell disease. Transfusion and Apheresis Science, 2021; 60(1).

7. Macedo T, Ribeiro V, Oliveira AP, Pereira D M, Fernandes F, Gomes NGM and Andrade PB. Anti-inflammatory properties of Xylopia aethiopica leaves: Interference with pro-inflammatory cytokines in THP-1-derived macrophages and flavonoid profiling. Journal of Ethnopharmacology. 2019.

8. Johnson – Ajinwo OR and Nyodee DG. Chemical characterization, phytochemical evaluation, analgesic and antipyretic activities of Harungana madgascariensis. Global Scientific Journals. 2021; 9 (4), 2457-2467.

9. Benson O, Jane N, Willy S, Mathias A and Robert B. Phytochemical screening, antimycobacterial activity and acute toxicity of crude extracts of selected medicinal plant species used locally in the treatment of tuberculosis in Uganda. Tropical Medicine and Health. 2022; 50:16.13p.

10. NAC (National Academy Council), 2011. Committee for the Update of the Guide for the Care and Use of Laboratory Animals Institute for Laboratory Animal Research. 8th Eighth Edition Washington D.C. The national academy press; 248p.

11. Zirihi G, Kra AKM and Guede-Guina F. Evaluation of the antifungal activity of Microglossa pyrifolia (Lamarck) O. Kantze (Asteraceae) "PYMI" on the in vitro growth of Candida albicans, Journal of Medicine and Pharmacy Africa. 2003; 17:11-18.

12. Konkon NG, Adjoungoua AL, Manda P, Simaga D, N'Guessan KE and Koné BD. Toxicological and phytochemical screening study of Mitragyna Inermis (willd.) O ktze (Rubiaceae), anti-diabetic plant. Journal of Medicinal Plants Research. 2008; 2(10):279-284.

13. Nemlin J and Brunel JF. Fascicle of Practical Work in Materia Medica (3rd year). National University of Ivory Coast. Pharmacological faculty. Department of Pharmacognosy. Phytology Laboratory, 1995; 47p.

14. Koster R, Anderson M and Debeer EJ. Acetic acid for analgesic screening. Federation proceedings. 1959; 18: 412-417.

15. Collier HOJ, Dinneen LC, Johnson CA and Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. British Journal of Pharmacology. 1968; 32(2): 295-310.

16. Siegmund EA, Cadmus RA and Lu G. Screening of analgesics including aspirin type compound based upon the antagonism of chemically induced writhing in mice. Journal of Pharmacology and Experimental Therapeutics. 1957; 119: 184–186.

17. Winter CA, Risley EA and Nuss GW. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine. 1962; 111: 544-547.

18. Bukhari. The central analgesic and anti-inflammatory activities of the methanolic extract of carthamus oxycantha; journal of physiologyand. 2013; 64(3): 369-375

19. Anupama AS, Kishor NR, Rahul DK and Kanchan SM. Evaluation of antiinflammatory and analgesic activities of Tamarindus indica seeds. International Journal Pharmacology Sciences Drug Research. 2012; 4(3): 213-217.

20. Ho KY, Gwee KA, Cheng YK, Yoon KH, Hee HT and Omar AR. Nonsteroidal anti-inflammatory drugs in chronic pain: Implications of new data for clinical practice. J.Pain Res. 2018; 11:1937–1948.

21. Wongrakpanich S, Wongrakpanich A, Melhado K and Rangaswami JA. Comprehensive Review of Non-Steroidal Anti-Inflammatory Drug Use in The Elderly. Aging Dis.2018; 9:143–150.

22. Traoré K, Haidara M, Denou A, Kanadjigui F, Sogoba MN, Diarra B, Maiga S and Sanogo R. Phytochemical screening and biological activities of four plants used in Mali in the management of malaria in children. European Scientific Journal. 2019; 15(6): 210-225.

23. Chatter R, Othman RB, Rabhi S, Kladi M, Tarhouni S and Kharrat R. In vivo and in vitro anti-inflammatory activity of neorogioltriol, a new diterpene extracted from the red algae Laurencia glandulifera. Marine Drugs. 2011; 9(7): 1293-1306.

24. Go gra M. Antioxidant and anti-inflammatory properties of plant extract. Antioxidants. 2019; 8(11), 549.

25. Eom T, Kim E and Kim J-S. In vitro antioxidant, anti-inflammatory and anticancer activities and anthraquinone content of root extract and fractions of Rumex crispus. Antioxidants. 2020; 9(8), 726.

26. Koua KBD, Irié-N'guessan AG, Effo KE, Kouakou SL, Ayoman TLD, Tetchi AF and Yapi HF. Evaluation of Anti-inflammatory Activity of Crinum scillifolium Extracts in Wistar Rats. International Journal of Biochemistry and Biophysics.2018; 6(4): 77-82.

27. Asante-Kwatia E, Jibira Y, Mensah AY, Osei-Sarfoh D. Macaranga barteri stem bark extract exerts anti-inflammatory and anti-hyperalgesia activity in murine models. Discovery Phytomedicine. 2019; 6(3): 130-137.

28. John KK, Fatou SOS, Georges A, N'guessan EZ, Koffi RK, Kouassi EB, Kple TKM, Jean-Jacques KK and Severin K. Anti-Inflammatory Activity and Phytochemical Studies of the Aqueous Extract of Distemonanthus Benthamianus Baill Bark . (Caesalpiniaceae: Leguminosae - Caesalpinioideae). European Scientific Journal. 2021; 17(7), 74.

29. Mandal S, Treuren WV, White RA, Eggesbo M, Knight R and Peddada SD. Analysis of composition of microbiomes: a novel method for studying microbial composition. Microbial Ecology in Health and Disease. 2015; 26(1): 1-7.

30. Mansour S. Evaluation of the anti-inflammatory effect of three medicinal plants: Artemisia absinthium L, Artemisia herba alba Asso and Hypericum scarboides. In-vivo study. Thesis in Biology, Algeria. 2015 ; 72p

31. Schultz F, Osuji OF, Wack B, Anywar G and Garbe L-A. Antiinflammatory Medicinal Plants from the Ugandan Greater Mpigi Region Act as Potent Inhibitors in the COX-2/PGH2 Pathway. Seedlings. 2021; 10, 351.

32. Njan AA, Iwalewa EO, Akinpelu LA, Ilesanmi OR, Daniyan OM, Fatuna OA, Fasina BA, Oyemitan IA and Olorundare OE. Analgesic effects of Harungana madagascariensis stem bark extract using four experimental models of nociception. Ife Journal of Science. 2015 ; 17 (3). 627-636.

33. Oso BJ, Oyewo BE and Oladiji AT. Phytochemical composition, antioxidant capabilities and Immunomodulatory In vitro indices of Xylopia aethiopica fruit extracts. Adv Pharm Journal. 2018; 3(1): 29-37.

34. Zondegoumba ENT, Dibahteu WL, de Araujo A, Vidari G, Liu Y, Luo S, Li S, Junior FJBM, Scotti L and Tullius M. Cytotoxic and Schistosomidal activities of extract, fractions and isolated compounds from Zanthoxylum leprieurii (Rutaceae) . International Journal of Sciences: Basic and Applied Research. 2019; 44: 209–222.

35. Evelyne AT, Guy BB, Fatimata N, Manon G, Esse LW, Allison L, Henri M, Zanahi FT, Michel F and Marie-Laure F. Seasonal Effect on the Chemical Composition, Insecticidal Properties and Other Biological Activities of Zanthoxylum leprieurii Guill. & Perr. Essential Oils. Foods. 2020; 9(5): 550p.

36. Randriamboavonjy JI, Loirand G, Vaillant N, Lauzier B, Derbré S, Michalet S, Pacaud P and Tesse A. Cardiac protective effects of Moringa oleifera seeds in spontaneous hypertensive rats. American Journal of Hypertension. 2016, 29(7): 873–81.

37. Togola I, Dembélé AA, Tounkara F, Diarra N, Konaré MA, Karembé M, Maiga SZ and Dembélé D. Evaluation of in vitro Antioxidant Activities of Ethanol Extracts of Datura innoxia Mill. Leaves and Seeds Harvested in Mali. Annual Research and Review in Biology. 2019; 33(2): 1–8.

38. Ji Y-J, Kim S, Kim J-J, Jang GY, Moon M and Kim HD. Crude Saponin from Platycodon grandiflorum Attenuates Aβ-Induced Neurotoxicity via Antioxidant, Anti-Inflammatory and Anti-Apoptotic Signaling Pathways. Antioxidants. 2021; 10, 1968.

39. Haake A, Nguyen K, Friedman L, Chakkamparambil B and Grossberg GT. An update on the utility and safety of cholinesterase inhibitors for the treatment of Alzheimer's disease. Opinion expert. Drug Safe. 2020; 19, 147–157.

40. Fabiolla R, Santos P, Heitor GA-F, Brenda SM, Saravanan S, Adriano ASA, Jackson RGSA, Parimelazhagan T and Lucindo JQJ. Anti-inflammatory and modulatory effects of steroidal saponins and sapogenins on cytokines: a review of preclinical research. Phytomedicine. 2022, 153842.

41. Gandonou DC, Ahissou H, Tokoudagba J-M and Dansou C. Ethnobotanical, phytochemical and toxicity analysis of a Beninese antihypertensive plant: Lippia multiflora. Int. J. Biol. Chem. Science. 2017; 11(4): 1816-1828.

42. Mpondo ME, Ngene JP, Som LM, Loe GE, Boumsong PCN, Jacques YJ and Dibong SD. Traditional knowledge and uses of medicinal plants in the Haut Nyong department. Journal of Applied Biosciences. 2017; 113: 11229-11245.

43. Tine Y, Diallo A, Ndoye I, Yang Y, Renucci F, Guèye RS, Baldé M, Diédhiou A, Fall D, Seck M, Desjobert J, Costa J, Wélé A and Paolini J. Flavonoid Compounds from Zanthoxylum leprieurii Guill . and Perr (Rutaceae) Extracts and their Antioxidant Activity against ABTS•+, Journal of Drug Delivery and Therapeutics. 2020; 10(2-s):120-124

44. Gnamien MA, Jacques AABA, Koffi JG, Adou KMK and Allic or JD. Antibacterial activity of aqueous and hydro-ethanolic extracts of Harungana madagascariensis, Zanthoxylum leprieurii and Xylopia aethiopica on the in vitro growth of Salmonella typhi and Escherichia coli. Pharmaceutical series. Med. Transl. Afr.2022; 21(2): 72-82.

45. Ehigiator BE and Adikwu E. Toxicity study of ethanolic stem bark extract of Xylopia aethiopica on fertility indices of male rats: An experimental study. International Journal of Reproductive BioMedicine. 2020; 18 (4): 265-274.

46. Medewase JO and Ezike AC. Preliminary investigation of the anti-inflammatory activity of H. madagas¬cariensis leaf extract Lam. Expoir (Hypericaceae). Full Length Research Paper. 2018 Item Number: 0D49695.

47. Eulogio J, Llorent-Martinez AR-R, Kouadio IS, Kouadio B, Maria LF-C, Carene P-A, Fawzi M, Hammad S and Gokhan Z. Exploring chemical profiles and bioactivities of Harungana madagascariensis Lam. ex Poiré.Leaves and stem bark extracts: a new source of procyanidins, Analytical Letters; 2020; 53:3, 399-412.

ABBREVIATIONS

DZHm: decocted extract of combination *Zanthoxylum leprieurii, Xylopia aethiopica, and Harungara madagascariensis*

EZHm: ethanolic extract of combination *Zanthoxylum leprieurii, Xylopia aethiopica, and Harungara madagascariensis*