International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2017.257.265

Research Article
Analgesic Efficacy, Quality and Safety of "Sarenta": An Herbal Preparation from Ivorian Traditional Medicine

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Abstract

Background: Sarenta a decoction from medicinal plants was prepared by a traditional health practitioner. However, there is no scientific data that can back up its reliability for consumers. The main objective of the study was to evaluate the know-how of Ivoirian's traditional health practitioners. **Objective:** The aim of this study was to carry out an ethnopharmacological survey about Sarenta, evaluate its analgesic activity, its quality through research of microbial contamination and eventually assess its acute toxicity. **Materials and Methods:** The ethnopharmacological survey was performed using an open questionnaire. The analgesic activity assessment through behavioral tests, abdominal constriction test in mice and formaldehyde-induced paw oedema test in rats. Thus, the microbial enumeration test based on the French pharmacopoeia and toxicity test 423 of Organization for Economic Cooperation and Development, helped to respectively evaluate microbial contamination and acute toxicity. Statistical test was performed using Wilcoxon test to compare groups. **Results:** The recommended dose set up by the traditional health practitioner was about 3 mg kg^{-1} b.wt. for adults. "Sarenta" at a dose of 5.10^{-8} mg kg^{-1} b.wt. (a preparation of 50 mg mL^{-1} was diluted to 10 millionth leading to 5.10^{-7} mg mL^{-1}, which was administered to rats at 1 mL/100 g b.wt.) inhibited abdominal constrictions in mice by 76% and licking of injected paw in rat by 70% at the inflammatory phase. Therefore, no fecal contamination germ, no yeast and mold were found beyond the French pharmacopoeia standards. Moreover, no deaf of rat was recorded at a dose of 5000 mg kg^{-1}. **Conclusion:** "Sarenta" exhibited analgesic and potential anti-inflammatory properties, a microbiological quality that complied with the French pharmacopoeia standards and contains no acute toxic substance. Thus, this first scientific study on herbal preparation could promote the know-how and professional practice of Ivoirian's traditional health practitioners.

Key words: Sarenta, analgesic, anti-inflammatory activity, decoction, medicinal plants, microbial quality, acute toxicity, safety of herbal remedies

Received: November 05, 2016 Accepted: December 19, 2016 Published: March 15, 2017

Citation: G. Kouakou-Siransy, K.E. Effo, G. Irié-Nguessan and E. Koua, 2017. Analgesic efficacy, quality and safety of "Sarenta": An herbal preparation from Ivorian traditional medicine. Int. J. Pharmacol., 13: 257-265.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

African traditional medicine is overflowing with varieties of remedies known for years immemorial by traditional health practitioners[1]. On account of sociocultural habits and the lack of access to modern medicines due to poor standard of living up to 80% of the African population relies on traditional medicine[2]. That's why since 1977, the World Health Organization (WHO) appealed to concerned member states to be committed to pay a special attention to the use of traditional health systems[3,4] by declaring that "traditional medicines that can ensure quality, safety and efficacy and contribute in achieving the goal of giving to everyone access to health care[5].

In Côte d'Ivoire, the Ministry of Health has integrated traditional medicine in the national health development plan by creating in 2001 the national programme for the promotion of traditional medicine (NPPTM). The NPPTM recorded more than 7,000 traditional health practitioners in 2007 with more than 2000 plants traditionally used by them to treat various diseases[6], this survey was crowned by the adoption of a recent legislation, law N°2015-536 of 20 July, 2015 laying down rules for the use and organization of traditional medicine. Besides rural traditional health practitioners giving to their customers varieties of medicinal plants and raw materials to be extemporaneously prepared, those from urban and suburban areas have traditionally developed from medicinal plants, aqueous preparations in form of finished products, packaged, labeled and ready for use. In several countries, traditional remedies are now well documented for their efficacy, safety and quality in the literature[7-14]. However, in Côte d'Ivoire, there is little scientific data on these new traditional health remedies that could guarantee their reliability for consumers.

In order to provide consumers with a safe and reliable information and help traditional health practitioners to improve the quality of their remedies and respond to the political integration of traditional medicine in the national health system of Côte d'Ivoire, the Department of Pharmacology and Clinical Pharmacy of the Faculty of Pharmaceutical and Biological Sciences set out to evaluate the efficacy, safety and quality of traditional herbal remedies, such as "Sarenta". The owner of this herbal preparation is Mr. Adou Tano Albert, a traditional health practitioner registered in the Ivorian's ministry of health database. According to him, this herbal remedy is used to treat various ailments including pains and inflammations.

The present study aims to assess the analgesic efficacy, safety and microbial quality of "Sarenta" an herbal remedy sold in Côte d'Ivoire for over 20 years.

MATERIALS AND METHODS

Ethnopharmacological survey: The ethnopharmacological survey involved semi-structured individual interviews with Mr. Adou Tano Albert, owner of "Sarenta".

This interview was focused on the socio-professional, ethnobotany and ethnopharmacological aspects. Concerning the socio-professional aspect, the issue was about the mode of knowledge acquisition. As for the ethnobotany aspect and remedy preparation, attention was drawn on its composition, plants used, harvest location, harvesting season, harvesting time, storage, washing up, drying, remedy preparation, material or preparation of ustensils, preparation and preservation methods. The therapeutic aspect was on remedy administration, side effects and precautions for use, contra-indications and drug interactions.

Evaluation of analgesic activity

Materials

Preparation of dry residue from "Sarenta": "Sarenta" aqueous preparation (Fig. 1) was dried in an oven at 60°C for 36 h using porcelain dishes. The dry residue obtained was weighed, triturated and then resuspended in a volume of distilled water to prepare a range of concentration.

Chemical products

- **For the writhing test:**
- Distilled water
- Acetic acid 1%
- Doliprane 200 mg powder (Aventis Pharma)
- **For the formalin test:**
- Distilled water
- Formalin 1%
- Profenid® 100 mg tablets (Aventis Pharma)
- Aspirin 500 mg of Rhône® (RP-LAB)
- Trabar® 500 mg capsules (Mepha)
- Experimental animals

For the writhing test: Mice belonging to *Mus musculus* species of either sex, weighing between 17 and 30 g were used. Animals were fasted for 12 h prior to experimentation and were allowed free access to water.

For the formalin test: Wistar rats belonging to *Rattus norvegicus* species of either sex weighing between 150 and 250 g were used. Animals were fasted for 12 h prior to experimentation and were allowed free access to water.

Fig. 1(a-d): Principle stages of "Sarenta" preparation, (a) Harvesting or purchasing of plants on public markets, (b) Plants washing in an aluminium wash basin full of tap water, (c) Aqueous decoction on coal wood and (d) Bottle of 500 mL "Sarenta"

Preparation of administered doses: 5, 5.10^{-4} and 5.10^{-8} mg kg^{-1} b.wt.: A concentration of 50 mg mL^{-1} was prepared from dry residue of "Sarenta" in distilled water. This preparation was administered in the rat at a rate of 1 mL/100 g b.wt., corresponding to a dose of 5 mg kg^{-1} b.wt.

Then the preparation of 50 mg mL^{-1} was diluted to the thousandth to obtain a solution at 5.10^{-3} mg mL^{-1}, which administered to the rat at a rate of 1 mL/100 g b.wt., corresponds to a dose of 5.10^{-4} mg kg^{-1}.

To reach the dose of 5.10^{-8} mg kg^{-1} b.wt., the preparation of 50 mg mL^{-1} was diluted from one hundredth to one hundredth, 4 times consecutively (10 millionth) in order to obtain a preparation at 5.10^{-7} mg mL^{-1}. This preparation at 5.10^{-7} mg mL^{-1} was administered by gavage in the rat at a rate of 1 mL/100 g b.wt., which corresponds to a dose of 5.10^{-8} mg kg^{-1} b.wt.

Methods

Abdominal constriction test: The method used was the one described by Koster *et al.*[15] and modified by Collier *et al.*[16]. Mice were divided into groups of 10 animals. Paracetamol and aspirin were administered to mice of the standard groups at doses of 200 and 50 mg kg^{-1} b.wt., respectively and each concentration from dry extract of "Sarenta" was respectively administered to three groups of mice at doses of (5, 5.10^{-4} and 5.10^{-8} mg kg^{-1} b.wt.). Dry extract of Sarenta, ketoprofen, aspirin and the acetic acid solution were diluted in normal saline prior to their administration to mice. Twenty minutes after the oral administration of drug, the acetic acid solution was intraperitoneally injected to mice. The pain was manifested by an outstretching of hind limbs and twisting of the dorsal-abdominal muscles in mice. The number of writhes was recorded 5 min after the injection of acetic acid for

a period of 10 min, then 35 min after injection of acetic acid for a period of 10 min. The percentage of pain inhibition was calculated by the following equation:

$$\text{Percentage of pain inhibition} = \frac{\text{No. of writhes performed in the control group} - \text{No. of writhes performed in treated group}}{\text{No. of writhes performed in the control group}}$$

Formalin irritation test: The method used was described by Dubuisson and Dennis[17] and modified by Tjolsen *et al.*[18]. Ketoprofen (Profenid®), aspirin (Aspirin of Rhône®) and tramadol (Trabar®) were administered to rats of the standard groups at doses of 100, 100 and 50 mg kg^{-1} b.wt., respectively and each concentration of dry extract of "Sarenta" was administered to two groups of rats at doses of (5 and 50 mg kg^{-1} b.wt.). Thirty minutes following the oral administration of drugs to each group of rats, 50 µL of formalin (1%) was injected into the plantar aponeurosis of the left hind paw of rats using a 30 gauge needle. Animals were kept under observation in a transparent plexiglas cage (20×20×30 cm). The cage was provided with mirrors on three sides and inclined up to 45° to the ground allowing a better observation of nociceptive behavior. Pain intensity was recorded by measuring the licking time of treated paw during the first phase (0-5 min) and the second phase (15-30 min). The percentage of pain inhibition was calculated by the following equation:

$$\text{Percentage of pain inhibition} = \frac{\text{Licking time of control group} - \text{Licking time of treated group}}{\text{Licking time of control group}} \times 100$$

Statistical analysis: Statistical analysis was performed using the non-parametric Wilcoxon test at 5% significant level. It involved comparing the mean of writhes performed and the mean of licking time at different doses of Sarenta to both control and standard groups.

Search for microbial contamination: This study was conducted by the National Public Health Laboratory (Côte d'Ivoire) according to the French pharmacopoeia 10th edition. This test helped to number mesophilic aerobic bacteria, yeasts and molds, Enterobacteriaceae, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella*.

Acute toxicity test: The acute toxicity test was performed using OECD 423 guidelines[19]. A group of 3 male rats with a known weight was administered "Sarenta" at a dose of 5000 mg kg^{-1} b.wt. (split into 3 doses for 24 h: 2000 mg kg^{-1} taken twice and 1000 mg kg^{-1} in a single intake, with 1 h interval for each intake). After administration of Sarenta, animals were observed each hour (1 h) for 4 h and then daily for 14 days. Furthermore, each animal was daily weighed for 14 days. For each rat the appearance or absence of acute toxicity signs such as apathy, agitation, breathing problems, excessive grooming, food refusal, refusal to drink, mouth bleeding, nasal bleeding, abdominal pain, coma, diarrhea, tremor, convulsions and death was recorded during the 14 days of experiment.

RESULTS

Ethnopharmacological survey: Mr. Adou Tano Albert is a traditional health practitioner since 1986. He attended primary and junior secondary school up to 4th form. He claimed to have positive experiences with "Sarenta" and treats up to 2,000 people per year. His remedy could soothe various pains such as painful menstrual periods, headaches, arthritis and stomach aches. Moreover, it could also treat various ailments such as chronic constipation, hemorrhoids, diabetes and anemia. His knowledge was acquired through divine revelation.

"Sarenta" is in the form of an aqueous suspension of brownish color, with a particular scent and a bitter taste. It is packaged and sold in plastic bottles (Fig. 1).

This herbal preparation (Fig. 1) encompasses medicinal plants such as *Ocimum gratissimum* Linn. (Lamiaceae), *Cassia occidentalis* Linn. (Fabaceae) and *Ageratum conyzoides* Linn. (Asteraceae) duly identified by the Ivorian National Floristic Center. Leaves, barks and roots were used. These plants were purchased on local market or harvested in the savannah and forests, in the towns of Abengourou, Bouaké and Abidjan.

Harvesting was done in all seasons but at specific times (early in the morning at 5 am and late in the afternoon to 5:30 pm to avoid the sun's effect, after 18 h the plant is "Resting". Plants were dried at the traditional health practitioner's household and then boiled in water for about 1 h. The decoction obtained was kept in barrels for a week and then packaged in plastic bottles bought in a local factory in order to be sold to customers. Preservation was done at room temperature.

"Sarenta" is an aqueous suspension orally administered at 99% and rectally administered at 1% using a rectal syringe with lukewarm water.

The prescribed dose varies from one teaspoon to a tablespoon and is taken twice a day or to a single administration every other day. That depends on the disease

being treated, its severity, the age, weight and very often of stool frequency. In cases of headaches or stomaches: 1 tablespoon in the morning and in the evening. In cases of hemorrhoids and chronic constipation, the dose is 4 tablespoons morning and evening. The next day according to stool frequency, it can be reduced to 2 tablespoons per day. For children, one teaspoon once a day.

The remedy is taken at any time of the day, even at night, because it could maintain sleep. Food could not influence medication. Because of its bitter taste "Sarenta" can be mixed up with food.

Few cases of side effects have been reported, if cases of diarrhea occur, it is required to stop treatment for about 4 days before resuming. "Sarenta" has no contra-indication and no harmful interaction.

Analgesic activity

Effect of "Sarenta" on abdominal constriction in mice: Different doses of "Sarenta" (5, 5.10^{-4} and 5.10^{-8} mg kg^{-1}) significantly inhibited writhing ($p<0.05$). Compared to paracetamol, the inhibition observed with the different doses of "Sarenta" was significantly higher ($p<0.05$). There was no significant difference between the inhibition percentage of the different doses of "Sarenta" and aspirin ($p>0.05$) (Fig. 2).

Effect of "Sarenta" on the licking time in rats: At the first stage of observation (0-5 min) "Sarenta" at a dose of 50 mg kg^{-1} showed a pain inhibition through a reduction of licking time while at a dose of 5 mg kg^{-1} it did not significantly reduced pain. Likewise ketoprofen (100 mg kg^{-1}) and aspirin (100 mg kg^{-1}) showed no pain inhibition (Fig. 3). At the second phase of observation (15-30 min) "Sarenta" at doses of 5 and 50 mg kg^{-1}, ketoprofen at a dose of 100 mg kg^{-1} and aspirin at a dose of 100 mg kg^{-1} significantly reduced licking time ($p<0.05$). Inhibition observed with both doses of significantly inhibited writhing ($p<0.05$). Compared to paracetamol, the inhibition observed with the different doses "Sarenta" was significantly greater than that of ketoprofen and aspirin ($p<0.05$). The inhibition percentage of both doses of "Sarenta" was not significantly different from each other.

Microbiological quality: Preparation sample for oral administration called' "Sarenta" (Table 1), in first batch brought by Mr. Adou did not meet the microbiological standards for oral preparation containing raw materials of natural origin according to the French pharmacopoeia 10th edition. After improving hygiene measures the preparation sample of the second batch for oral administration called "Sarenta" (Table 1), in 2nd batch brought by Mr. Adou complied with the microbiological standards for oral preparation containing raw materials of natural origin according to the French pharmacopeia 10th edition.

Acute toxicity: No deaf of rat was recorded at a dose of 5000 mg kg^{-1} b.wt. Weight curve evolution (Fig. 4) showed weight gain in both control group (148.33 g on D1 to 173.85 g on D14 with a gain of 25.52 g) and group of rats treated with "Sarenta" at a dose of 5 g kg^{-1} (154 g on D1 to 162.4 on D14 with a gain of 8.4 g). Comparison of weight variations between control group and group of rats treated with "Sarenta" at a dose of 5 g kg^{-1} showed a significant

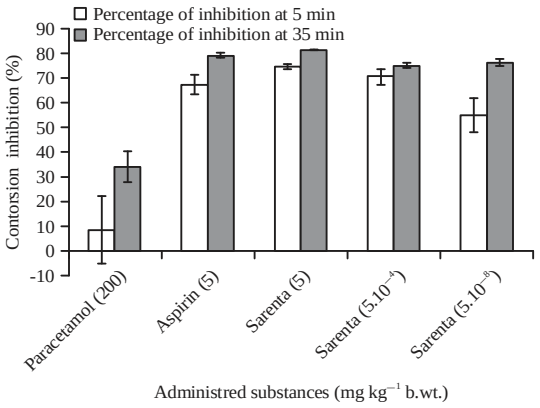

Fig. 2: Percentage of contorsions inhibition by "Sarenta" and standard substances, values are expressed as Mean±Standard Deviation (n = 6). All means showed a significant difference at $p<0.05$ when compared to paracetamol

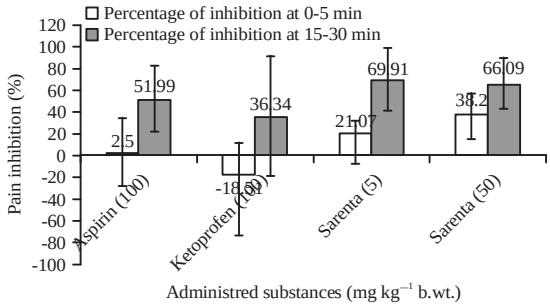

Fig. 3: Percentage of licking paw inhibition by "Sarenta" and standard substances, values are expressed as Mean±Standard Deviation (n = 7). All means showed a significant difference at $p<0.05$ when compared to control

Table 1: Results of "Sarenta" microbiological analysis

Microbiological analysis (according to French pharmacopoeia 10th edition)	Results 1st batch (CFU mL^{-1})	Results 2nd batch (CFU mL^{-1})	Norm French pharmacopoeia 10th edition (CFU mL^{-1})
Enumeration of mesophilic aerobic microorganisms in 1 mL (PCA agar at 30°C for 72 h)	2.10^5	10	$<10^4$
Enumeration of yeasts and moulds in 1 mL (sabouraud agar+chloramphenicol at 25°C for 5 days)	5.10^3	10	$<10^2$
Identification and enumeration of enterobacteriaceae in 1 mL (Agar VRBG at 37°C for 24 h)	90	10	$<10^2$
Identification of *Escherichia coli* in 1 mL (Fast media *E. coli* at 44°C for 48 h)	Absence	Absence	Absence
Identification of *Staphylococcus aureus* in 0.1 g (Baird Parker agar base 37°C for 48 h)	Absence	Absence	Absence
Identification of *Salmonella* in 10 mL BPW at 37°C for 3 h RV at 37°C for 24 h SS at 37°C for 24 h Adaptation of FN V 08-052	Absence	Absence	Absence

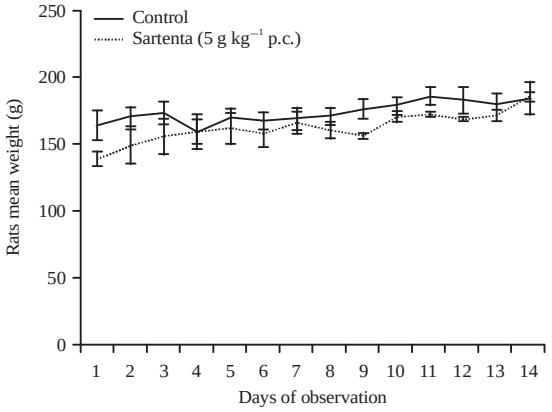

Fig. 4: Evolution of animals mean weight at a dose of 5000 mg kg^{-1}

difference ($p<0.05$). No manifestation of apathy, agitation, breathing problems, excessive grooming, food refusal, refusal to drink, buccal bleeding, nasal bleeding, abdominal pain, coma, tremors and convulsions were observed. A case of loose stools was noticed on D2 in a rat treated with "Sarenta".

DISCUSSION

Our study was aimed at investigating on the existence and analgesic efficacy of a herbal preparation called "Sarenta" on one hand and ascertained its microbial quality and safety on the other hand. That was the first study carried out according to scientific methodologies on this remedy empirically used in traditional medicine for twenty years in Côte Ivoire. Like many herbal remedies found on public markets and places in Abidjan and basically in Côte d'Ivoire, "Sarenta" an aqueous decoction is traditionally made from leaves, bark and roots of various medicinal plants identified in Côte d'Ivoire. Indeed, we witnessed the preparation of Sarenta at the owner's household to be sure of the absence of pharmaceutical products or other chemicals in its composition. Combination of various plants in the same preparation was also observed by several researchers[20-22]. However, this may vary depending on both plant species availability and traditional health practitioner financial means. Attributing a wide range of properties to his remedy and with the expertise acquired for many years Mr. Adou Tano treats various ailments involving or not inflammation and pain and argues that this remedy could recover patient's sick organs.

Investigation on the analgesic activity of Sarenta was to assess its inhibitory effect on acute pain. Animal models are commonly used to evaluate plant extract analgesic activity[23]. The writhing test or abdominal constriction test is a screening test. This test is not specific to pain, because anticonvulsant substances respond to it as well, but is characterized by its high sensitivity. The results showed a significant inhibition of writhes by "Sarenta", greater than that obtained with paracetamol. In addition, at a dose of 5.10^{-8} mg kg^{-1}, "Sarenta" has similar inhibitory effects as aspirin administered at a dose of 5 mg kg^{-1}.

By analogy with the effect of the acetic acid, "Sarenta" could possess inhibitory effects on the release of mediators involved in pain. Therefore, the acetic acid is involved in peripheral mechanisms of pain, inducing the release of many chemical mediators such as histamine, prostaglandins PGE2, PGEα, serotonin, bradykinin in high proportions in rodents peritoneal exudates[24,25]. This analgesic effect of "Sarenta" helps to justify the interest of African population for herbal remedies like *Ocimum gratissimum*, *Cassia occidentalis* and *Ageratum conyzoides* being part of its composition. Those plants were subjected to numerous scientific studies regarding their many therapeutic virtues in traditional medicine. Their analgesic activity, in the same study model was demonstrated by several researchers[26-29]. Combination of these plants in the same preparation could help creating a synergy justifying the inhibitory effect of pain by "Sarenta". Synergy effects of plant extracts are increasingly described by literature[20-22].

The formalin test, more specific to pain than the abdominal constriction test was also used to evaluate the analgesic effect of "Sarenta". This test characterizes both phases of pain. The first phase of this test is related to a direct chemical stimulation of nociceptors while the second depends on peripheral inflammation[18]. The second phase or inflammatory phase is mediated by the release of PGE2 prostaglandins, nitric oxide, tachykinin, histamine, sympathomimetic amines, tumor necrosis factor and interleukins[30-33]. Anti-inflammatory analgesics inhibit more substantially the second phase[34].

Results on the licking inhibition showed an activity of "Sarenta" at doses of 5 and 50 mg kg^{-1} b.wt., significantly more active at the inflammatory phase of pain than the anti-inflammatory drugs used as standards namely aspirin and ketoprofen with a respective administration dose of 100 mg kg^{-1}.

The observed inhibition was about the two phases of pain, but more important in the second phase corresponding to the inflammatory phase. Thus "Sarenta" could possess an analgesic activity coupled with an anti-inflammatory effect. This activity could be explained by the presence of *Ocimum gratissimum*, *Cassia occidentalis* and *Ageratum conyzoides*. Various researchers reported the analgesic effect of leave extracts of those three plants, through the formalin test showing a preferential inhibition of the inflammatory phase of pain[28,29,35]. While this study highlights the inhibitory effect of "Sarenta" on the inflammatory phase at a dose of 5 mg kg^{-1} b.wt., the above quoted authors reported an inhibitory effect at doses of 200-800 mg kg^{-1} for *Ocimum gratissimum*[35], 150-300 mg kg^{-1} for *Cassia occidentalis*[29] and 2000 mg kg^{-1} b.wt. for *Ageratum conyzoides*[28]. A part from some hypotheses on interaction of active principles from different plants in the same preparation could result in a synergetic effect, entailing the inhibitory effect of "Sarenta" at doses far below the inhibitory doses of each plants tested separately.

Lack of training and failure to apply the West African economic and monetary Union good manufacturing practices guidelines in its annex 3, relating to medicinal plants[36] could explain poor microbiological quality of "Sarenta" first preparation that was analyzed. Indeed, after improving hygiene measures advocated in that guidelines, the second preparation tested showed, absence of yeasts and molds and so a microbiological quality product complying with the European pharmacopoeia standards.

It is therefore important to emphasize on health control in traditional medicine centers, as a recent law, law No. 2015-536 of 20 July, 2015 recognizes its practice. It should be also better to assist traditional health practitioners by initiating them to good manufacturing practices in order to improve the quality of their remedy and to provide our population with reliable products, approved by drug regulatory authorities.

The acute toxicity study performed, allowed us to say according to the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) that "Sarenta" does not contain a deadly, toxic or hazardous product and is therefore considered as a non acute toxic product. Compared to standard analgesic drugs such as paracetamol and aspirin belonging to the 4th toxicity category according to GHS, that's to say labeled harmful if swallowed, "Sarenta" could be less toxic. Moreover, the presence of anthracene derivatives in medicinal plants used for Sarenta preparation could explain the case of loose stools observed. Weight evolution for 14 days showed a weight gain lower than the control group. Thus, "Sarenta" at high doses could exhibit an anorectic effect at a dose of 5 mg kg^{-1} which was far below the recommended dose by the traditional health practitioner.

CONCLUSION

Inclusion of traditional health practitioners in the national health system requires the evaluation of efficacy, quality and safety as recommended by regulatory authorities in general. "Sarenta" showed an excellent analgesic efficacy, potential anti-inflammatory properies and microbiological quality profile since basic hygiene rules was followed. "Sarenta" should not be listed among acute toxic products. A reasonable use of "Sarenta" should be considered.

Thus, this first scientific study on herbal preparation could promote the policy of integrating traditional medicine into modern health care systems. This could allow our populations and healthcare staff to have access to reliable good quality and standardized plant-based products. Furthermore, the proven efficacy, quality and safety of "Sarenta" should upgrade the know-how and professional practice of Ivoirian's traditional health practitioners.

ACKNOWLEDGMENTS

We are grateful to Dr. Kroa Ehoulé, Coordinator of the National Program for the Promotion of Traditional Medicine (Abidjan) who did his uttermost to make possible traditional health practitioners survey. Our appreciations to Mr. Assi Jean, Botanist (National Floristic Center, University of Felix Houphouet Boigny) and to Professor Loukou Yao Guillaume (microbiologist) respectively for their contribution in plants identification and microbiological control of "Sarenta".

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