International Journal of Pharmaceutics & Pharmacology

Available Online: http://ijpp.edwiserinternational.com

Evaluation of the Anti-inflammatory Activity and Ulcerogenic Risk of "Sarenta", an Ivorian Herbal Preparation

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Article info

Received 30 November 2018
Revised 20 December 2018
Published 28 December 2018

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Abstract

"Sarenta" is an Ivorian herbal preparation used for various purposes such its anti-inflammatory activity. On account of the ulcerogenic side effects of conventional anti-inflammatory drugs and in order to promote African traditional medicine, this study aimed to assess the anti-inflammatory activity and ulcerogenic risk of Sarenta.

The anti-inflammatory activity was evaluated by measuring the decrease in carrageenan- induced paw oedema in rat in acute model by "Sarenta", then in sub-acute model by the decrease of the formation of granuloma induced by cotton pellets implanted subcutaneously in rats. Ulcerogenic risk was assessed by administering "Sarenta" alone to rats that were anesthetized 4 hours later. The ulcerogenic risk on gastric mucosa was evaluated and codified using a magnifying glass.

"Sarenta" at 10 mg/kg and 50 mg/kg induced a decrease in carrageenan-induced paw oedema in rat with an activity on the acute inflammation above 60% for the first 3 hours, above 70% at the 4th hour and beyond 80% at the 5th hour. "Sarenta" at the same doses did not affect rats's gastric mucosa, showing a mucous membrane without erosions or petechiae. However, in a sub-acute inflammation model, "Sarenta" did not exhibit an anti-inflammatory activity 100 mg/kg and 200 mg/kg, a non-significant decrease in granuloma formation was recorded at a dose of 200 mg/kg.

In an acute inflammation model, "Sarenta" showed an anti-inflammatory activity with no ulcerogenic risk in animal. This herbal preparation represents an alternative for conventional non-steroidal anti-inflammatory drugs, as far as their ulcerogenic risks. However, in a sub-acute inflammation model, it did not show anti-inflammatory activity. Further investigations should be carried out that could lead to its introduction into anti-inflammatory therapeutic arsenal.

Keywords: Anti-inflammatory; Ulcerogenic risk; Sarenta

Introduction

In the face of socio-economic and accessibility problems with conventional medicines, more than 80% of the African population resort to traditional medicine for treatment [1]. Traditional medicine is indeed full of a wide range of remedies used by traditional medicine practitioners for centuries [2]. Moreover, since 1977, WHO is paying a particular attention to traditional medicine for it contributes in achieving the goal of providing primary health care for all [3,4].

In Côte d'Ivoire, the Ministry of Health has integrated traditional medicine in its national health development plan by creating the National Programme for Promoting Traditional Medicine in 2001 with the aim of improving national health coverage.

The purpose of this integration was to achieve tight collaboration between both conventional and traditional medicine practitioners. However, there is little scientific data on traditional medicine practitioners' remedies efficacy and safety. Among this, one can quote "Sarenta" an herbal preparation owned by Mr. Adou Tano Albert, a traditional medicine practitioner well-known by the Ivorian Ministry of Health.

"Sarenta" is an Ivorian herbal preparation used for various purposes such its anti-inflammatory activity. On account of the ulcerogenic side effects of conventional anti-inflammatory drugs and in order to promote African traditional medicine, this study was designed to assess the anti-inflammatory activity and ulcerogenic risk of "Sarenta".

Materials and Methods

The remedy "Sarenta"

"Sarenta" is a brownish aqueous suspension with a characteristic odour and a bitter taste. It was made from root barks and woody vines of various plants. The preparation was performed in a traditional way, packaged and sold in plastic bottles (Figure 1). This aqueous suspension was dried in an oven at 60°C for 36 hours to obtain dry residue which was used to prepare a range of concentrations in this study.

Solvents and Chemicals

For the carrageenan induced paw oedema test:

- Normal saline
- Indometacin (Indocid® 100 mg Hac pharma capsule)
- 1% Carrageenan

For the cotton pellet test:

- Normal saline
- Diclofenac (TEVA)
- Ether (GIFRER)

Study of the anti-ulcerogenic effect:

- Normal saline
- Indometacin (Indocid® 100 mg Hac pharma capsule)
- Ether (GIFRER)

Figure 1: Bottle of "Sarenta".

Animal Material

Adult Wistar rats of both sexes weighing between 120 and 250 g from the animal husbandry of the Department of Pharmacology, Faculty of Pharmaceutical and Biological Sciences of the University of Félix Houphouët-Boigny (Abidjan, Côte d'Ivoire) were used. They were kept in standard temperature conditions (26 ± 1 °C), 12 hours of light/dark cycle, fed, with free access to water ad libitum.

Study of in vivo anti-inflammatory activity on carrageenan-induced paw oedema in the rat

The process was described by Winter et al. [5]. Animals were divided into 4 groups comprising six (6) rats in each group and treated by oral route as follows: Group 1 representing the positive control was treated with normal saline (10 ml/kg); groups 2 and 3 were treated

with "Sarenta" at doses of 10 mg/kg and 50 mg/kg respectively; group 4 representing the negative control was treated with indomethacin at a dose of 10 mg/kg.

One (1) hour after administration, the oedema was induced by a sub plantar injection of 50 µl freshly prepared 1% suspension of carrageenan. Paw volume was measured at 0h, 1h, 2h, 3h, 4h, and 5h after carrageenan injection using a digital micrometre and the percentage of inhibition of the oedema was determined. The inflammation (% I), was calculated according to the following formula [6]:

%I = (Tt-To)/To × 100

To: Initial paw volume of each animal

Tt: Paw volume at time t of each animal

In addition, the anti-inflammatory effect (% A) was calculated according to the formula below [6]:

%A = (1 - %Ie / %Ic) × 100

%Ic: Mean value of inflammation in the control group

%Ie: Mean value of inflammation experimental groups.

***In vivo* anti-inflammatory activity on cotton pellet-induced granuloma formation in rats**

The method was described by Swingle and Shideman [7]. 30 male rats were divided into 5 groups of 6 rats each. Two (2) sterilized cotton pellet granuloma models weighing 24 mg were implanted by sub-cutaneous route, one on each side of all rat abdomens after being anesthetized by ether through inhalation.

Subsequently, group 1 (positive control) was treated with normal saline. Groups 2, 3 and 4 were treated by "Sarenta" at doses of 67 mg/kg/day (recommended dose by the traditional medicine practitioner), 100 mg/kg/day and 200 mg/kg/day respectively. Group 5 was treated with diclofenac at a dose of 25 mg/kg/day. All animals were treated for 7 consecutive days. On day 8, each rat was anesthetized by ether inhalation and the implanted pellets were removed and dried at 60°C for 24 hours. Dry weights of the granuloma were recorded, and the percentage inhibition of the granuloma formation of tested compounds was calculated according to the following formula:

P = (WgrC - WgrT)/ WgrC × 100

WgrC: Represents the mean weight of the control granulomatous tissue

WgrT: Represents the mean weight of tested granulomatous tissue

Assessment of the ulcerogenic risk of "Sarenta"

The process was described by Alam et al. [8]. Thirty (30) rats were divided into 5 groups of 6 rats each and fasted for 48 hours. At the end of the 48 hours fasting, they were treated with oral route as follows: Group 1 (control) was treated with normal saline at 10 ml/kg. Groups 2 and 3 were treated with "Sarenta" at respective doses of 10 mg/kg and 50 mg/kg. Groups 4 and 5 were treated with indomethacin at doses of 10 mg/kg and 50 mg/kg respectively.

Four (4) hr later, rats were anesthetized through ether inhalation and the stomach of each rat was excised, cut open along the large curve and rinsed with saline water. It was then examined using a magnifying glass and the intensity of gastric lesions was evaluated using a modified scoring system described by Adami et al. [9]: 0, no lesions; 0.5, slight hyperemia or ≤ 5 petechiae; 1, ≤ 5 erosions ≤ 5mm in length; 1.5, ≤ 5 erosions ≤ 5mm in length and many petechiae; 2, 6-10 erosion ≤ 5 mm in length; 2,5, 1-5 erosions < 5 mm in length; 3, > 5-10 erosions > 5 mm in length; 3,5, > 10 erosions > 5 mm in length; 4, 1-3 erosions ≤ 5 mm in length and 0.5-1 mm in width; 4.5, 4-5 erosions ≤ 5 mm long and 0.5 to 1 mm wide; 5, 1-3 erosions > 5 mm long and 0.5 to 1 mm wide; 6, 4 or 5 grade 5 lesions; 7, ≥ 6 grade 5 lesions; 8, complete lesion of the mucosa with hemorrhage.

The mean ulcer score was calculated for each group. The protection rate was calculated using the following formula:

(Control Score - Test Score) / Control Score × 100

Statistical Analysis

The results were expressed as Mean ± standard deviation. The statistical analysis was performed by the SPSS v 18.0 software. The graphs were performed using the Graph Pad Prism 7.00 software. Means comparison was made using the Wilcoxon non-parametric test. A p value <0.05 was considered as statistically significant.

Results

Anti-inflammatory activity of "Sarenta" on carrageenan-induced paw oedema in rat

The oedema caused by carrageenan injection in rat paws gradually increased to its maximum 4 hours after injection. Indomethacin at a dose of 10 mg/kg and "Sarenta" at doses of 10 mg/kg and 50 mg/kg

significantly reduced carrageenan-induced inflammation during experiment (P <0.01). The inflammation percentage (%I) and the anti-inflammatory activity percentages (%A) were calculated and recorded in Table 1.

Table 1: Effect of "Sarenta" on the Percentage inhibition of carrageenan induced hind paw oedema in rat.

Group	Dose (mg/kg)	Time after injection of carrageenan									
		1 hours		2 hours		3 hours		4 hours		5 hours	
		(I%)	(A%)	(I%)	(A%)	(I%)	(A%)	(I%)	(A%)	(I%)	(A%)
Control		23.68 ± 6.68		24.1 ± 3.37		24.45 ± 4.98		24.95 ± 7.36		24.63 ± 6.62	
Indometacin	10	16.95 ± 125	28.4	15.04 ± 3.99	39.93	11.51 ± 3.81	52.91	8.37 ± 3.82	66.42	10.57 ± 3.04	55.84
Sarenta	10	4.24 ± 4.17**	82.08**	9.03 ± 3.59**	63.58**	8.25 ± 4.83*	66.25*	6.45 ± 4.52*	74.13*	4.88 ± 4.25**	80.16 **
Sarenta	50	8.3 ± 3.20**	64.93**	8.39 ± 3.67**	66.17**	8.66 ± 3.05*	64.55*	6.17 ± 2.44*	75.27*	3.03 ± 2.62**	87.66 **
Note: The values represent percentages of inflammation (I %) and percentages of anti-inflammatory activity (A %), 3=6 for each group. *Wilcoxon test: p<0.01 compared to indomethacin, **Wilcoxon test: p<0.001 compared to indomethacin.											

However, "Sarenta" seemed to have a higher anti-inflammatory activity than indomethacin (p <0.001). In the group of rats treated with "Sarenta" at a dose of 10 mg/kg, the anti-inflammatory activity appears to be greater the first hour after carrageenan injection than in the group of rats treated at a dose of 50 mg/kg (p <0.001). The anti-inflammatory activity was then equivalent to 2-hour, 3-hour and 4-hour. However, 5 hours after induction of oedema by carrageenan, a higher activity was observed at a dose of 50 mg/kg (p <0.001).

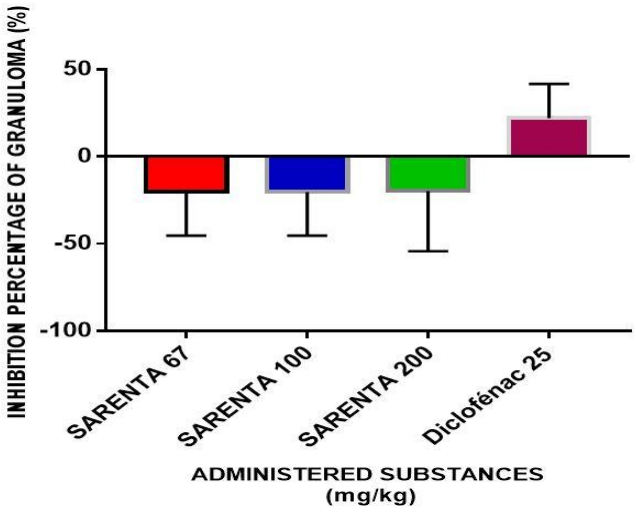

Figure 2: Inhibition percentage of granuloma formation by "Sarenta".

Anti-inflammatory activity on cotton pellet-induced granuloma formation in rats

In a subacute inflammation model, "Sarenta" at doses of 67 mg/kg/day, 100 mg/kg/day and 200 mg/kg/day did not exhibit an anti-inflammatory effect as shown in Figure 2. Inflammation continued during the 7 days of treatment while diclofenac at a dose of 25 mg/kg inhibited granuloma formation at about 22 %.

Table 2: Anti-ulcerogenic effect of "Sarenta",

Substance	Dose (mg/kg)	Score
Distilled water	-	0
Sarenta	10	0
Sarenta	50	0
Indometacin	10	0.83 ± 0.21*
Indometacin	50	1.96 ± 0.37**
Note: The values represent ulcer injury scores, n = 6 for each group, *Wilcoxon test: p<0.01 compared to negative control group, **Wilcoxon test: p<0.001 compared to negative control group.		

Ulcerogenic risk of "Sarenta"

Sarenta" at doses of 10 mg/kg and 50 mg/kg did not cause petechiae or gastric mucosa erosion in rats (Figure 3 and Table 2), while indomethacin at the same doses caused ulcerations with an increase in injury at a dose of 50 mg/kg (Figures 4 and 5, Table 2).

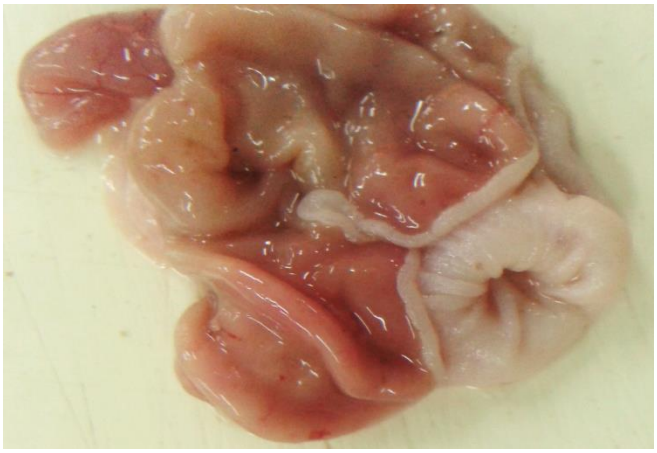

Figure 3: Photograph of rat gastric mucosa treated with "Sarenta" showing normal mucosa.

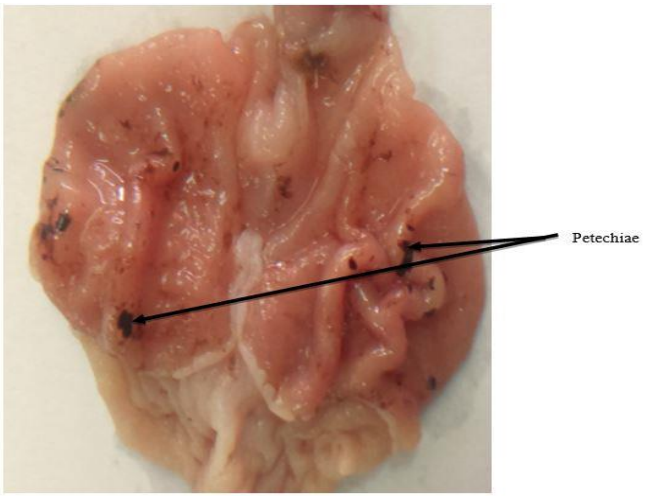

Figure 4: Photograph of rat gastric mucosa treated with indomethacin at a dose of 10 mg/kg showing petechiae.

Discussion

This study aimed to assess the anti-inflammatory activity and ulcerogenic risk of an herbal preparation "Sarenta", made of root barks and woody vines of various plants, traditionally used for more than twenty years in Côte d'Ivoire.

The study of the anti-inflammatory activity of "Sarenta" was motivated by the studies of Kouakou-Siransy et al. [10] which showed the analgesic activity, quality and safety use of this remedy. Indeed, Kouakou-Siransy et al. [10] demonstrated that "Sarenta" in addition to its analgesic activity, could have a potential anti-inflammatory activity by the formalin test, a non-specific test for studying anti-inflammatory activity [11]. The Carrageenan-induced paw oedema in rat test used in this study was a more specific test to study the anti-inflammatory activity. Carrageenan-induced inflammation is an acute and highly reproducible inflammatory model.

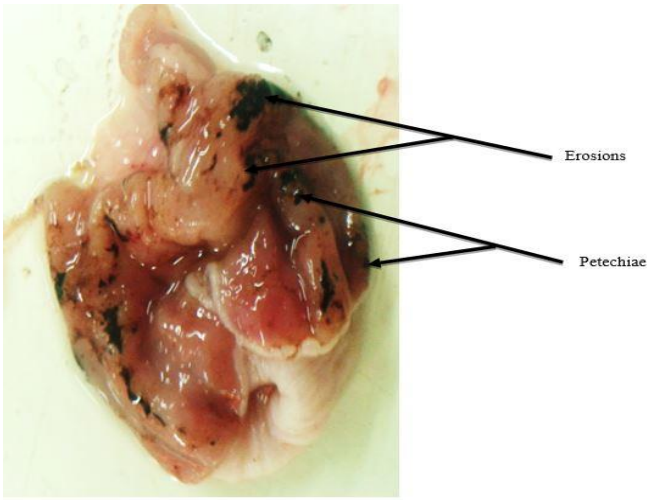

Figure 5: Photograph of rat's gastric mucosa treated with indomethacin at a dose of 50 mg/kg showing erosions and petechiae.

Cardinal signs of inflammation appear immediately after injection under the aponeurosis plantar of rat, resulting from pro-inflammatory agent actions [12]. The development of oedema is a two-phase event: the early phase (0 to 2.5 hr) involves the release of inflammatory mediators such as histamine, serotonin and bradykinin; the late phase (3 to 6 hr), is associated with the release of prostaglandins [12,13].

In this study, "Sarenta" and indomethacin significantly inhibited paw oedema in rat during both phases of carrageenan-induced inflammation, indicating that "Sarenta", as well as indomethacin, block the release of histamine and serotonin during the initial phase. They also prevent the release of some inflammation mediators by blocking the action of prostaglandins during the late phase. However, indomethacin has toxic effects because it inhibits isoenzymes responsible for prostaglandin production, promoting haemorrhagic ulceration in rats by oxidative stress and a decrease in prostaglandin production [14]. Moreover, non-selective cyclooxygenase inhibitors may cause kidney damage and more selective cyclooxygenase-2 inhibitors increase the risk of cardiovascular disease [15,16]. There is therefore an urgent need for effective and well-tolerated anti-inflammatory drugs. "Sarenta" at doses of 10 mg/kg and 50 mg/kg, did not cause any gastric

damage and could be a good alternative in the treatment of acute inflammation.

This anti-inflammatory activity of "Sarenta" could be explained by the presence in this herbal preparation of plants such as Ocimum gratissimum, Cassia occidentalis and Ageratum conyzoides. Indeed, various studies have been carried out on the analgesic activity of these plants using the formalin test and have shown their potential to inhibit the inflammatory phase of pain [17-19]. In addition, "Sarenta" showed an anti-inflammatory activity at doses of 10 and 50 mg/kg, while each plant quoted above showed their anti-inflammatory activity at higher doses. These doses ranged from 150 to 300 mg/kg for Cassia occidentalis [17], 2000 mg/kg for Ageratum conyzoides [18] and from 200 to 800 mg/kg for Ocimum gratissimum [19]. The low dose of "Sarenta" could be explained by a synergy action between active compounds found in this herbal preparation.

However, in subacute inflammation models, "Sarenta" at doses of 67 mg/kg/day, 100 mg/kg/day and 200 mg/kg/day for 7 days did not seem to possess an anti-inflammatory activity. Indeed, in the formation of granulomas induced by cotton pellets, responses can be divided into three phases. The first phase, called transudative, from 0 to 3 hours after the implantation of cotton pellets, is defined as a leakage of liquid into the blood vessels caused by an increase in vascular permeability. The second phase, known as the exudative phase, from 3 to 72 hours after the implantation of cotton pellets, is defined as the influx of low molecular weight proteins from blood circulation around the granuloma, caused by intensive maintenance of vascular permeability change. The proliferative final phase, from day 3 to day 6, is defined as the production of granulomatous tissues caused by the continuous release of a pro-inflammatory mediator [7,20]. According to Swingle and Shideman [7], steroids could significantly inhibit the transudative and proliferative phases.

According to Swingle and Shideman [7], steroids could significantly inhibit the transudative and proliferative phases. NSAIDs such as indomethacin, aspirin and diclofenac may have moderate effects. It seems that "Sarenta" remedy does not have a steroid-like effect and therefore does not possess an anti-inflammatory activity in the subacute inflammation model. However, Tamarindus indica one of the plants used for the preparation of "Sarenta", showed an anti-inflammatory activity in a subacute inflammation model at doses of 300 and 600 mg/kg [21]. The active compounds of the other plants might have antagonized this effect or should higher doses of "Sarenta" be used to exhibit the anti-inflammatory effect in a subacute inflammation model? Further investigations need to be undertaken in this regard.

Conclusion

"Sarenta" showed an anti-inflammatory activity with no ulcerogenic risk in animal in an acute inflammation model. This herbal preparation represents an alternative for conventional non-steroidal anti-inflammatory drugs, in the face of their ulcerogenic risks. However, in a sub-acute inflammation model, it did not show anti-inflammatory activity. Further investigations should be carried out on "Sarenta" in order to use it as an anti-inflammatory drug.

Conflict of Interest

None Declared.

Funding

None Declared.

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This manuscript was peer-reviewed
Mode of Review: Single-blinded
Academic Editor: Dr. Syed Sarim Imam