Original Research Article

Anti-inflammatory activity of the aqueous extract of *Daniellia oliveri* (Fabaceae)

SORO Tianga Yaya*, MIAN Jean Claude, COULIBALY Sirabana, NENE-Bi Semi Anthelme, TRAORE Flavien

University Felix Houphouet-Boigny (Côte d'Ivoire)
*Corresponding author email: **tiangaso@yahoo.fr**

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Abstract

Daniellia oliveri is a common plant in Africa and widely used by the population. It is found in the wooded savannahs as well as in dry forests. The medicinal properties of *Daniellia oliveri* are attributed to its different parts. Thus the leaves are used to treat various diseases such as inflammation, fever and pain. The comparative study of the aqueous extract of leaves *Daniellia oliveri* and the indomethacin (INDOCID) on inflammation induced by carrageenan on the right hind paw of the rats showed anti-inflammatory properties of this extract. The phytochemical analysis of the aqueous extract of leaves *Daniellia oliveri* showed the presence of flavonoids which could cause anti-inflammatory properties of this extract. The toxicological study of aqueous extract of leaves of *Daniellia oliveri* allowed to determine an LD50 = 436.51 mg/kg body weight. According Classification of Diezi this plant is toxic. These results justify the traditional use of leaves of *Daniellia oliveri* as anti-inflammatory.

Key words

Daniellia oliveri, Indomethacin, Anti-inflammatory, Flavonoids, Polyphenols.

Introduction

Daniellia oliveri (Rolfe, Hutch, Dalz) (Fabaceae) is prevalent worldwide. According Classification of Diezi [1] this plant is toxic. In Africa, it is found in wooded savannahs but in the Sudano-Guinean zone, it is common in dry forests [2]. This is a tall tree with conical crown whose shape is generally tapered. It can be recognized from the right appearance of his trunk, light gray bark. The leaves are paripinnately. Various parts of this plant such as bark, leaves, and branches are medically important. The decoction of the leaves and the young leaves are respectively used to treat tuberculosis and headache.

This study was undertaken to assess in the rat, the anti-inflammatory activity of aqueous extract of the leaf of *Daniellia oliveri* compared to indomethacin.

Material and methods

Vegetal material

It consists of a decoction of young leaves of Daniellia oliveri (Fabaceae). The leaves have been harvested in the north of Ivory Coast precisely in the Korhogo region, and identified by the botanical laboratory (UFR Biosciences), University Felix Houphouet Boigny, from a herbarium of the National Centre of Floristic. Young harvested leaves were dried in the shade at room temperature between 25 and 28 ° C. They were then ground to a powder from which the aqueous extract was made.

Animal material

It consists of rats and mice used respectively for the study of anti-inflammatory activity and acute toxicity.

The rats used belong to the species *Rattus norvegicus*, Wistar strain, their weight is between 200 and 300 g and are aged about 3 months. These rats were bred at the animal house of the UFR Biosciences, University Felix Houphouet Boigny, where the average temperature is 28 ± 3 ° C with a relative humidity of 70% and Photoperiod of 12/24 hours. The animals receive food and water ad libitum.

The mice are from the species *Mus musculus*, Swiss strain; they have a weight of between 25 and 30 g, and are kept in the same conditions as rats.

All these animals were adapted to laboratory conditions of seven days before the start of the experiments.

Chemical product

The Indomethacin (INDOCID): FROSST IBERICA SA, Madrid (Spain).

Methodology

Preparation of the aqueous extract of *Daniellia oliveri*

The aqueous extract of *Daniellia oliveri* is obtained from 250 g of powder of young dried leaves we put into 2 liters of distilled water, the mixture is boiled for an hour of time. The decoction obtained is filtered on paper Wattman. The filtrate is then placed in an oven at 50 °C until the obtainment of the extract.

Method of studying the anti-inflammatory activity

The injection of carrageenan in the footpad of the right hind paw of rats causes an inflammatory reaction which can be reduced by the anti-inflammatory substances [3].

The vigil rats distributed in batches of 7, were fasted 16 hours prior to experimentation. For each rat, the circumference (Co) of the right hind paw is measured. Then, we administer, the various treatments by gavage at 1 ml per 100 g body weight. This injection is supplemented with distilled water to a total volume of 5 ml, which provides uniform hydration, in all rats and minimizes the response of individual variations.

The aqueous extract, and indomethacin have concentrations of 60 mg/ml and 1 mg/ml. Dilutions are made with distilled water.

The oral administration of the product is carried out using a rigid probe of olivary end:

- Distilled water (Control);
- Vegetal extract;
- Indomethacin (Reference substance).

An hour after feeding, we injected to each rat, 0.05 ml of carrageenan solution 1% in the footpad of the right hind leg, then animals are returned to the cage. The development of edema is determined at times 1, 2, 3, 4, 5, and 6 hours after injection.

To assess the anti-inflammatory activity, the transmétatarsien diameter of the ankle and the

circumference of the leg at the metatarsal level (yaw) are determined using of an electronic display caliper branded COGEX SENSEMAT (France) [4].

Then, the percentage of inhibition of edema was calculated [5].

$$\text{Percentage of inhibition (\%)} = \frac{C - C1}{C} \times 100$$

C = Percentage (%) of average increase in the circumference of the edematous leg of the control group (group 1 in a given time).
C1 = Percentage (%) of average increase in the circumference of the edematous leg of the test group at the same time.

Phytochemical screening

The researches focused on identifying the main chemical groups such as sterols and polyterpenes, polyphenols, flavonoids, alkaloids, saponins, quinone substances and tannins by using the standard procedures reactions tubes as described by Trease and Evans [6] and Harborne [7].

The research of sterols and polyterpenes was made by the reaction of Liebermann. The characterization of compounds belonging to the group of polyphenols was made by the reaction with ferric chloride. The compounds belonging to the group of flavonoids have been identified by the reaction with cyanidin. Le research of the alkaloids was made using the general reagents of alkaloids characterization. Two reagents were used namely the Dragendorff reagent (reagent potassium iodobismuthate) and Bouchardat reagent (reagent iodo-iodide). The research of saponosides is based on the properties that have aqueous solutions containing saponins to foam after agitation. The free or combined quinone compounds were identified through the reaction Borntraeger. The compounds belonging to the group of tannins, have been shown by the reaction Stiasny. This study was conducted to determine the chemical constituents that may explain the effects of *Daniellia oliveri*.

Study of acute toxicity

Five groups of ten animals each are used for determining the 50% lethal dose (LD50). The increasing doses prepared from a stock solution of *Daniellia oliveri* of 60 mg/ml were administered by intraperitoneal route to the different groups of mice at a rate of one dose per group and 0.1 ml per 10 grams body weight. The doses of extracts are the following: 250, 400, 450, 600, 700 mg/kg BW. The treated mice are kept under observation for 24 hours, during which we note the mortality and all physical and behavioral changes.

The lethal dose 50% was determined according to the graphic method of Miller and Tainter [8]. This method is to note the percentages of dead mice in each batch and convert them into probits units. The doses corresponding to those percentages are determined as milligrams per kilogram of body weight. The curve expressing the mouse mortality (probit units) versus the logarithm of the dose (in milligrams per kilogram of body weight) is plotted. The linearization of this semi-logarithmic curve, permit to determine the LD50 which is the abscissa of the point corresponding to 50% mortality.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 4.0 software (San Diego, Mo, Ca, USA). The comparing of averages of the measurements between batches was made using the Student t test ($p < 0.05$).

Results

Anti-inflammatory activity

The **Table - 1** showed the effects of aqueous extract of *Daniellia oliveri* and indomethacin on evolution depending of the time, the circumference of the edema induced by carrageenan in the rat paw (n = 7).

After injection of carrageenan 1% into the right hind paw of the rat, the circumference of this paw passes from 14.56 ± 0.72 to 16.21 ± 0.78 mm after 1 hour and 19.89 ± 0.95 mm after 6 hours

by passing through a maximum which is equal to 20.23 ± 0.97 mm measured 4 hours after treatment. These values correspond to increases of 11.33 ± 0.54%; 36.6 ± 1.74% and 38.94 ± 1.86% for the maximum value (**Table - 2**).

Table - 1: Effet Indomethacin and the aqueous extract of *Daniellia oliveri* on the evolution depending of time of the circumference of the edema induced by carrageenan in the rat paw.

Treatment	Doses (mg/ kg p.c.)	Evolution of the circumference of the paw (mm)						
		Before treatment	1 h	2 h	3 h	4 h	5 h	6 h
Control (NaCl 9‰)		15.3 ± 0.82	17. ± 0.98	18.72 ± 0.83	19.2 ± 0.66	21.05 ± 0.95	20.92 ± 0.82	20.75 ± 0.85
Indomethacin	10	15.43 ± 0.78	16.55 ± 0.76*	16.61 ± 0.92*	16.72 ± 0.73*	17.98 ± 0.86*	17.74 ± 0.84*	17.71 ± 0.87*
Daniellia oliveri	400	15.37 ± 0.78	16.78 ± 0.94*	17.33 ± 0.95*	18.35 ± 1.2*	18.51 ± 0.97*	18.05 ± 0.93*	17.9 ± 0.93*

Values represent the mean (± SEM); n = 7 for each group. * p <0.05 versus control group. The data show the average circumference of the leg (mm)
The indomethacin at 10 mg / kg bw and the aqueous extract of Daniellia oliveri 400 mg / kg bw reduce the edema induced in the rat paw carrageenan.

Table - 2: Effect of indomethacin and the aqueous extract of *Daniellia oliveri* on increasing the circumference of the edema of the rat paw carrageenan-induced.

Treatment	Doses (mg/kg p.c)	Percentage increase in the circumference of the carrageenan-induced paw					
		1 h	2 h	3 h	4 h	5 h	6 h
Control (NaCl 9‰)		11.11 ± 0.74	22.35 ± 0.96	25.49 ± 1.08	37.58 ± 1.26	36.73 ± 1.55	35.62 ± 1.34
Indomethacin	10	7.25 ± 0.57*	7.64 ± 0.79*	8.55 ± 0.60*	16.52 ± 0.76*	14.97 ± 0.92*	14.77 ± 0.96*
Daniellia oliveri	400	9.17 ± 0.74*	12.75 ± 0.77*	19.38 ± 1.23*	20.42 ± 1.14*	17.43 ± 0.95*	16.46 ± 0.97*

Values represent the mean (± SEM); n = 7 for each group. * p <0.05 versus control group. The data show the mean percentage of the increasing of the circumference of the leg (mm)
Indomethacin at 10 mg / kg body weight reduced the edema induced in the rat paw carrageenan to a greater extent that the aqueous extract of *Daniellia oliveri* to 400 mg / kg body weight

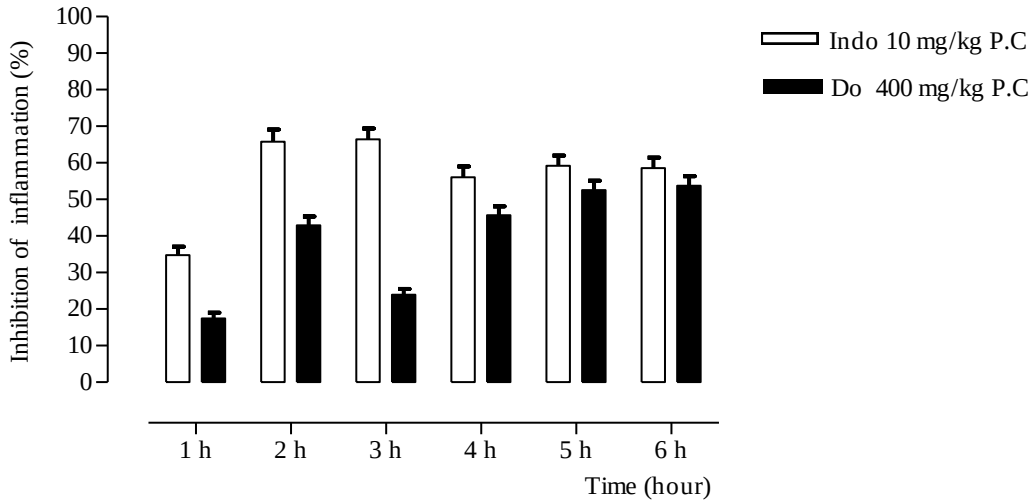
In the presence of indomethacin 10 mg/kg body weight and the aqueous extract of *Daniellia oliveri* 400 mg/kg body weight, we record the less important increases of the diameter of the paw, whose maximum is measured after 4 hours. These increases maxima are for indomethacin equal to 16.28 ± 0.86% and *Daniellia oliveri* equal to 20.26 ± 1.10%. These values are used to

plot the **Figure - 1** which showed inhibition of inflammation by indomethacin and the aqueous extract of *Daniellia oliveri* compared to the control.

Phytochemical screening

Phytochemical tests performed on the aqueous extract of *Daniellia oliveri*, revealed the presence of sterols polyterpenes, polyphenols, flavonoids, alkaloids, quinone substances and catechic tannins. This extract is devoid of saponins and tannins gallic. The results of the phytochemical study of aqueous extract of *Daniellia oliveri* are shown in **Table - 3**.

Figure - 1: Inhibition of inflammation by indomethacin and the aqueous extract of *Daniellia oliveri* compared to the control.



Values represent the mean percentage ± SEM of inhibition of edema induced by indomethacin at a dose of 10 mg / kg bw and the aqueous extract of *Daniellia oliveri* at a dose of 400 mg / kg bw compared to the control rats (n = 7) for each group.

Table - 3: Chemical composition of the aqueous extract of stem bark of *Daniellia oliveri*.

	Sterols Polyterpenes	Polyphenols	Flavonoids	Alkaloids	Saponins	Quinone Substances	Tannins	
							Gallic	Catechic
Aqueous extract	+	+	+	+	–	+	–	+

+ = Presence of the compound.
– = Absence of the compound.

Acute toxicity

Intraperitoneal injection of *Daniellia oliveri* at doses between 250 and 700 mg/kg BW, caused in the first moments, an agitation of the mice. These mice move frequently. These displacements are followed of twisting the body. During the 30 minutes following the administration of the extract, the animals eat and drink little.

After this time, for non-lethal doses (250-350 mg / kg bw), the mice gradually, find again appetite. As against the lethal doses (400-700 mg / kg bw), cause jerky breathing in animals that show

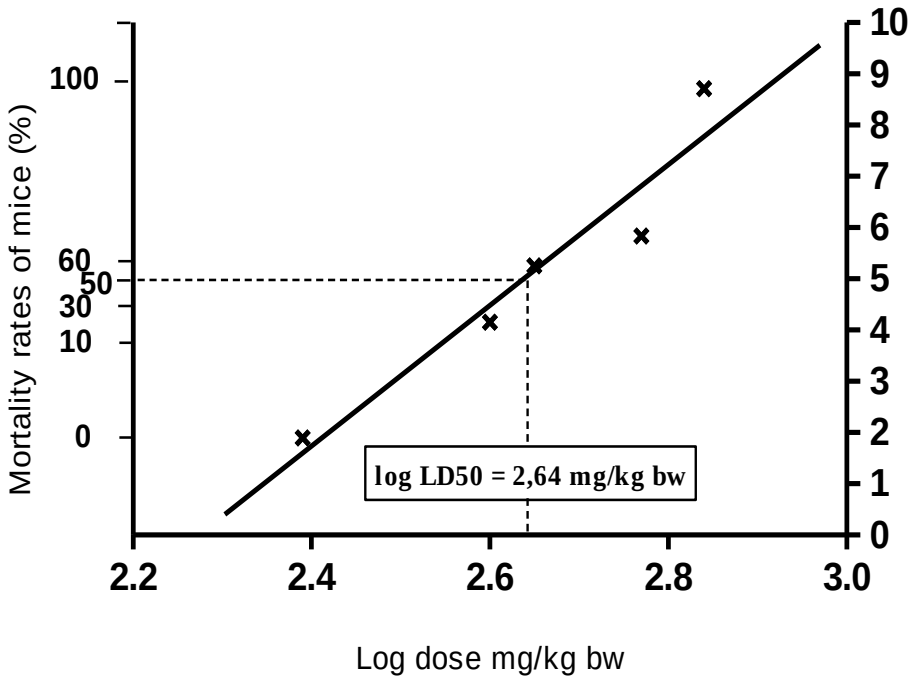
symptoms of fatigue. The first deaths were recorded 5 hours later.

The LD 50 graphically determined according to the method of Miller and Tainter [8], is 436.51 mg/kg body weight. At 700 mg / kg bw, the extract proved to be very toxic since all treated mice died (**Table - 4** and **Figure - 2**).

Table - 4: Mouse mortality rates depending of the dose of the aqueous extract of *Daniellia oliveri.*

Groups	Dose of *Daniellia oliveri* injected to mouse in mg / kg bw	Number of dead mice	Percentage of mice mortality (%)	Percentage of mice mortality (unités probits)
1	250	0	0	1.90
2	400	2	20	4.15
3	450	6	60	5.25
4	600	8	80	5.84
5	700	10	100	8.71

Figure - 2: Toxicity curve of the aqueous extract of *Daniellia oliveri* in mice.



Discussion

The aqueous extract of the young leaves of *Daniellia oliveri* has significant effects on edema of the rat paw induced by carrageenan, which is considered by Leme, et al. [9] as one of the most effective phlogiston. The effects of the aqueous extract of *Daniellia oliveri* to 400mg/kg body weight being similar to those of indomethacin 10 mg/kg BW, this natural substance has anti-inflammatory properties.

However the anti-inflammatory properties of the natural substance are much lower than those of indomethacin especially during the three (3) first hours of the experiment which lasts six (6) hours.

According to Sharma, et al. [10] and Khan, et al. [11], the carrageenan inducing edema in the rat paw, is a model to show the effects of natural products in the acute inflammation.

This phlogiston injected into the rat's right hind leg causes a severe inflammatory response that is observed after thirty minutes [12, 13].

It is well known that the edema induced by carrageenan injection takes place in two phases involving different mediators: in the first stage (during the first two hours after injection of carrageenan), mediators such as histamine and serotonin are involved while in the second phase (3 to 5 hours after injection of carrageenan) kinins and prostaglandins are involved [14, 15].

Furthermore according Thomazzi, et al. [16], induction of edema by carrageenan injection is a phenomenon that takes place in two phases. The first phase (90 -180 min) inflammation is due to the release of histamine, serotonin and similar substances and the last stage (270-360 min) is due to the activation of substances releasing of kinins, prostaglandins, proteases and lysosome.

In our study, indomethacin, an inhibitor of cyclooxygenase and the aqueous extract of *Daniellia oliveri* significantly reduce, the inflammation induced by carrageenan during both phases. We can conclude that this natural substance not only contains anti-sérotoniques compounds and antihistamines but also inhibitors of prostaglandins, kinins, lysosomes and proteases.

Previous studies of plant extract showed that the flavonoids, have a pharmacological role in inflammation and allergies [17]. Flavonoids are indeed capable of inhibiting the oxidants released by leukocytes and other phagocytes in inflammatory zone which maintain inflammation [18, 19, 20, 21, 22, 23, 24, 25].

The phytochemical screening (**Table - 3**) showed that the aqueous extract of *Daniellia oliveri*contains among other chemical, the flavonoids. The presence of flavonoids in the aqueous extract of *Daniellia oliveri* was also demonstrated by Adaku and Okwesili [26]. The effect of the aqueous extract of *Daniellia oliveri* on reducing inflammation induced by carrageenan could be related to the presence of these flavonoids.

The LD50 of the aqueous extract of *Daniellia oliveri* is equal to 437.5 mg/kg bw. According the classification of Diezi [1], when:

- DL50 <5mg / kg: the substance is highly toxic;
- 5 Mg / kg <LD50 <500 mg / kg: the substance is toxic;
- 500 Mg / kg <LD50 <5000 mg / kg: the substance is of low toxicity;
- DL50> 5000 mg / kg: the substance is non toxic.

It is concluded that the aqueous extract of *Daniellia oliveri* is toxic in mice. The LD50 obtained in this study was lower than that obtained by Adaku and Okwesili [26] on another aqueous extract of *Daniellia oliveri* which have obtained an LD50 greater than 5000 mg/kg bw. *Daniellia oliveri* is also less toxic than the extract of *Ximenia americana* stem bark [27], whose LD50 is equal to 219 mg / kg body weight.

It should be noted that, the toxicity of the plant is related to many conditions, such as geographical distribution and the harvest season. It is also recognized that the metabolism and the pharmacological activities are very different depending on the animal models used [28, 29]. As a result, this plant should be used with caution in men.

Conclusion

Based on this study, our results show that the aqueous extract of *oliveri Daniellia* has a very significant anti-inflammatory effect compared to that of indomethacin which is a reference molecule. This action may be related to its chemical composition characterized by the presence of flavonoids which are antioxidants.

Accordingly, subsequent experiments using fractions rich in flavonoids are planned to confirm this hypothesis and understand the mechanism of action of the active principles of this plant.

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