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Study of pharmacological properties of the methanolic extract of *Dichrostachys cinerea* bark (L.) Wight et Arn (Leguminosae) in isolated myometrium from pregnant rats

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ABSTRACT

Ethnopharmacological relevance: The use of medicinal plants in Gabon contributes widely to the primary health care of the people of this area of Central Africa. This paper investigates the pharmacological properties of *Dichrostachys cinerea,* the plant barks are traditionally used by Gabonese and Ivorian populations to treat bronchial asthma, rheumatism, and other various diseases. Although *D. cinerea* barks have been reported to be used by population to facilitate childbirth, to the best of our knowledge no scientific evidence has been published.

Aim of study: In the present study, we investigated the pharmacological properties of *D. cinerea* methanolic extract, on isolated uterine smooth muscle and compared its effects to those of oxytocin, which is used by obstetricians to facilitate childbirth. We also explored the possible mechanism pathways of the in vitro uterine contraction induced by *D. cinerea.*

Materials and methods: The effects of different concentrations (3.2 μg/ml, 16 μg/ml, 80 μg/ml, 400 μg/ml, and 2 mg/ml) of the methanolic extract of *D. cinerea* on isolated strips of the uteri of pregnant rats were studied. These effects were compared to those of oxytocin (8.4×10^{-5} μg/ml, 8.4×10^{-4} μg/ml, 8.4×10^{-3} μg/ml, 8.4×10^{-2} μg/ml). The EC (50) and *E* (max) was determined graphically and statistically analysed using one-way ANOVA and Dunnett post hoc test.

Results: Cumulative concentrations of *D. cinerea* have caused rise in the contractile force of the uterine fragments that were isolated from the pregnant rats, as seen with oxytocin concentrations. We observed contractions amplitude of 30.41 mN (12%) at 80 μg/ml and amplitude of 39.68 mN (14.17%) at 400 μg/ml for *D. cinerea.* In parallel, oxytocin concentration of 8.4×10^{-3} μg/ml induced contractions of 45.82 mN with the highest concentration (8.4×10^{-2} μg/ml) that induced contractions of 55.82 mN.

Conclusions: Our results revealed that *D. cinerea* increased the contractile force and the frequency of muscle contractions. These findings support the use of *D. cinerea* to facilitate childbirth, as it has been used in traditional medicine.

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1. Introduction

Dichrostachys cinerea (L.) Wight et Arn. (Leguminosae) is a shrub or a small tree that grows up to eight feet in height with branches that end in spines (Walker and Sillans, 1995). This species is variable in appearance and covers a wide area that stretches from Africa to Australia. Similar to many vegetable species that are resistant to drought, *D. cinerea* exhibits most of the marine visual features. The species of the genus *Dichrostachys* are widespread from the Sudanese Sahel region to the Guinean areas. These plants grow in heavy soils and invade disturbed areas (e.g., fallow land and road embankments). The vernacular names in Gabon are mbara y'orové (Mpongwè) and ngamba (Punu) and in Ivory Coast, these names are N'gbagbémoto (Adioukrou) and Gboro (Malinké). The different parts of the plant are used by traditional healers to treat many diseases, such as rheumatism (Vergiat, 1970), infant cough, whooping cough in children (Vergiat 1969, Thomas, 1959), asthma (Ouattara, 2007), kidney disorders, gonorrhoea, abscesses,

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and measles (The Wealth of India, 1952, Dalziel, 1937). *D. cinerea* is used to treat snake bites (Schnell, 1949), to treat stomach aches, to protect pregnant women, to treat malaria, as an antibiotic, to treat fever in children, to treat pulmonary diseases, and as an analgesic. Moreover, *D. cinerea* is also used to facilitate childbirth (Sillans, 1951). To prevent abdominal spin after childbirth, the bark is crushed, boiled, filtered, and used as a vaginal enema; in addition, after processing into very fine chips, the bark is introduced into the vulvas of pregnant women to induce and facilitate childbirth (Vergiat, 1969, 1970). (Fowler and Lewis, 2013) stated the uses of *D. cinerea* which include the facilitation of delivery, the induction of abortion and the treatment of sterility. The different uses of this plant have been collected in Protabase (Fowler and Lewis, 2013). A pharmacological report on *D. cinerea* revealed that the methanolic extracts of this plant affect the contractility of tracheal smooth muscle isolated from guinea pigs (Aworet et al., 2011; Irie-N'guessan et al., 2011). This plant exhibits antispasmodic activity (Irie-N'guessan et al., 2010). Moreover, phenolic extracts of *D. cinerea* have also been shown to exhibit excellent anti-inflammatory activities (Aworet et al., 2014), and the methanolic extract has been shown to protect against anaphylactic shock induced by histamine in conscious guinea pigs (Aworet et al., 2014; Irie-N'guessan et al., 2011). This plant has also shown antibacterial and antiviral effects (Kambizi and Afolayan, 2001; Kudi, 1999). Several authors have proven that this species inhibit protein farnesyl-transferase activity (Jagadeeshwar et al., 2003; Achidi and Kinnoudo, 2008). Furthermore, chemical studies have revealed the presence of a new isomer of mesquitol (a primary active ingredient) that exhibits free-radical scavenging property and α-glucosidase inhibitory activities (Long et al., 2010). Phytochemical studies performed on *D. cinerea* extracts have revealed the presence of the following compounds: triterpenes, sterols, polyphenols, tannins, flavonoids, fatty acids, flavons and reducing sugar (Aworet et al., 2011, Joshi and Sharma, 1974; Koudou et al., 1994; Aiyeladgbe et al., 1996; Fotie et al., 2004) and cardiotonic heterosides (Tillement and Albengres, 1977). In the present study, we examined the pharmacological properties of *D. cinerea* methanolic extract on isolated uterine smooth muscle and compared its effects to those of oxytocin, which is used by obstetricians to facilitate childbirth.

2. Materials and methods

2.1. Ethnobotanical information

The barks of *D. cinerea* were collected at Essassa (December 2009) near Libreville in Gabon during the rainy season. The plant material was authenticated by Raoul Niangadouma, a botanist of the Gabon National Herbarium (IPHEMETRA/CENAREST). A voucher specimen has been deposited (H.P.Bouroubou 387; M.S.M. Sosef 894, M.S.M 1097).

2.1.1. Chemicals and reagents

The following chemicals and reagents were used: petroleum ether (PubChem SID: 174530831), methanol (Sigma-Aldrich: 82762, Germany), oxytocin (PubChem CID: 439302), and EGTA (ethylene glycol tetra acetic acid; PubChem CID: 6207). Mac Ewen physiological solution was composed by: sodium chloride (PubChem CID: 5234); potassium chloride (PubChem CID: 4873); calcium chloride (PubChem CID: 24854): disodium hydrogen phosphate (PubChem CID: 24203); magnesium chloride (PubChem CID: 24584); and D-glucose (PubChem CID: 5793). The Mac Ewen physiological solution was composed of the following (in mM): NaCl: 130.05; KCl: 5.63; $CaCl_2$: 2.16; HPO_4Na_2: 0.91; HCO_3Na: 2.38; $MgCl_2$: 53; anhydrous glucose: 11.11; and distilled water: qsp

2.1.2. Preparation of the plant extract

D. cinerea barks were cut into small pieces and dried at room temperature for one month. Then, the barks were crushed into powder using a crusher (Retsch SK 100 Confort Geissen Germany). One hundred grams (100 g) of powdered *D. cinerea* barks was defatted with 500 ml petroleum ether for 24 hours at room temperature (27 ± 2 °C). The residue was dried and mixed with 500 ml of 96 ° methanol (Sigma-Aldrich, Germany) under magnetic stirring over 24 hours at room temperature. The mixture was filtered, and the filtrate was concentrated in a rotary evaporator to dryness at 45 °C. The obtained residue was subsequently maintained in dry, sterile vials.

2.1.3. Animals

Rats (*Rattus norgevicus)* weighing 225 ± 25 g were used for pharmacological studies. Animals were reared at room temperature (27 ± 2 °C) with a natural light–dark cycle with food and water available ad-libitum. Animals were treated according to the principles for care and use of laboratory animals for biomedical research approved by the ethical committee of the Pharmacopeia and Traditional Medicine Institute, Libreville.

2.1.4. Recording the contractile activities of the smooth muscle fragments

The recording apparatus comprised a 10 ml tank that contained the isolated organ in a 37 °C water bath. Biological preparation was immersed in the tank containing oxygenated, isolated Mac Ewen-type saline and glucose. The contractions were sensed with a HSE F30 372 type strain gauge (Hugo Sachs Instruments, Freiburg, Germany) and transmitted with a HUGO SACHS amplifier. Isometric force was measured and recorded using a Multipen Recorder Rikadenki polygraph (Hugo Sachs Instruments, Freiburg, Germany).

2.1.5. Isolation and collection of the uterine strips from the pregnant rats

At 15–20 days of gestation, rats were anaesthetised with ether and sacrificed by cervical dislocation. A midline laparotomy was performed on the abdominal part of the animal to isolate the myometrial muscle. The uterine body was opened, and the contents (embryo sacs) emptied. We performed dissections of fine strips across the width of the uterus (3–4 mm), and stored the strips in Mac Ewen-type saline solution. Nodes were made at both ends to connect the hooks of the strain gauge to the organ in the tanks (Yin et al., 2012).

2.1.6. Calibrating the pen

A one-gram weight was suspended from a hook and connected to the strain gauge for calibration. The movements of the pen recorder allowed us to determine the tension that was induced by the weight. A force on 1 g is 10 mN, which is equivalent to the stylus moving eight (8) cm.

2.1.7. Statistical analyses

All values are presented as means ± SEMs with n representing the number of different preparations. The differences in the pEC_{50} and *E*-max values for the compounds were evaluated with Dunnett's tests after an analysis of variance (ANOVA) had revealed that the samples represented different populations. Values of $p < 0.05$ were considered to indicate significant differences.

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