Antinociceptive and anti-inflammatory effects of *Dorstenia barteri* (Moraceae) leaf and twig extracts in mice

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Received 23 October 2003; received in revised form 19 May 2004; accepted 26 May 2004
Available online 14 August 2004

Abstract

The present study was undertaken to investigate the antinociceptive and anti-inflammatory activities of the leaf and twig extracts of *Dorstenia barteri* (Moraceae) in mice. Both the leaf and twig extracts of *Dorstenia barteri* at 50, 100 and 200 mg/kg showed significant ($P < 0.05$–0.01) antinociceptive activities in chemical-, mechanical- and thermal-induced pain test models. Intraperitoneal administration of the plant extracts at 50, 100 and 200 mg/kg significantly ($P < 0.05$–0.01) inhibited carrageenin-induced acute inflammation in oedema paw weight, pulmonary oedema and number of pleural leucocytes in a dose-dependent way. The twig extract was found to be more active than the leaf extract in all the experimental models used. The inhibitory effects of the plant extracts were comparable to those of the reference drugs acetylsalicyclic acid (ASA) and phenylbutazone (PBZ) at 100 mg/kg i.p. The significant reduction in acetic acid-induced abdominal contractions, the decrease in oedema paw weight as well as in the number of leucocytes in the pleural cavity exudates, and the significant increase in the reaction time and pain threshold of mice observed in this study suggest that *Dorstenia barteri* extracts possess both anti-inflammatory and antinociceptive activities. The present study, therefore, lend pharmacological support to the folkloric uses of *Dorstenia barteri* extracts in the treatment, control and/or management of arthritis, rheumatism, gout, headache and other forms of body pains in some parts of Africa.
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Keywords: *Dorstenia barteri*; Moraceae; Antinociceptive; Anti-inflammatory activities

1. Introduction

Species of the genus *Dorstenia* (Moraceae) are perennial, herbaceous plants that are widely distributed in tropical Africa, Middle-East, Central and South America (Abegaz et al., 1997; Dufall et al., 2003). In many parts of Africa, *Dorstenia* species are commonly used for the treatment and/or management of an array of human disorders, including: arthritis, rheumatism, gout, stomach disorders, cough, headache, skin diseases, and so forth (Bonquet, 1969; Abegaz et al., 1997; Dufall et al., 2003; Abegaz and Ngadjui, 1999). The leaves and twigs of *Dorstenia barteri* var. *subtriangularis* Bureau, are used in African folk medicine for the treatment of various human ailments. The crude drug is prepared by extraction of the plant material (10 g) in 500 ml of water. The decoction (10 ml) is orally taken three times a day. Recent investigations in our laboratories have shown that *Dorstenia barteri* leaf and twig extracts possess anti-trichomonas and antioxidant activities (unpublished data). Most of the folkloric uses of the genus *Dorstenia barteri* revolve around pain, inflammation and microbial infections. The present study was undertaken to investigate the antinociceptive and anti-inflammatory activities of the leaf and twig extracts of *Dorstenia barteri* in mice.

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doi:10.1016/j.jep.2004.05.022

2. Materials and methods

2.1. Plant material and preparation of the extracts

Plant samples were collected from Tombel in the South West Province of Cameroon in November 2001. Mr. Victor Nana, of the National Herbarium in Yaounde identified the plant. Voucher specimen (19534/SRF Cam) was deposited at the herbarium. The air-dried and powdered twigs of *Dorstenia barteri* var. *subtriangularis* (450 g) were extracted with CH_2Cl_2/MeOH (1:1) and MeOH for 24 and 2 h, respectively, at room temperature. The two extracts were combined. Removal of the solvents from the combined extracts was done under reduced pressure yielding 70 g of a dark green residue.

2.2. Animals

Swiss albino mice of either sex weighing 20–26 g were used. The animals were maintained under normal laboratory conditions of humidity, temperature (25 ± 1 °C) and light (12 h day:12 h night), and allowed free access to food and water ad libitum for at least 5 days, before the commencement of our experiments. The "principle of laboratory animal care" (NIH publication No. 85-23) guidelines and procedures were followed in this study (NIH publication revised 1985).

2.3. Drugs

The following reference drugs were used: [Disprin® (acetylsalicyclic acid – ASA)]-(Reckiti Benckiser), Carrageenin (Sigma) and Phenylbutazone (PBZ) (KGN Pharmaceuticals).

2.4. Bioassays

2.4.1. Analgesic activity

Evaluation of the analgesic properties of the leaf and twig extracts of the plant was carried out by the chemical, mechanical and thermal noxious stimuli.

2.4.1.1. Acetic acid-induced writhing method. Control group of mice received distilled water (2 ml/kg i.p.) only. Mice in the test groups ($n = 6$ per group) received *Dorstenia barteri* extracts at 50, 100 and 200 mg/kg i.p. or acetylsalicyclic acid at 100 mg/kg i.p., respectively. ASA was used as the reference analgesic drug for comparison in this study. One hour following *Dorstenia barteri* extract-, ASA- or distilled water-administration, 0.1 ml of a 3% acetic acid solution was intraperitoneally injected to each of the test mice (Koster et al., 1959). The number of abdominal contractions that occurred within the next 20 min following acetic acid administration were counted and recorded. A significant reduction in the number of acetic acid-induced abdominal contractions of the extract-, ASA-, or distilled water-treated mice, compared to the contractions in the untreated control mice, was taken as an indication of analgesic activity.

2.4.1.2. Hot plate test method. The control group of mice ($n = 6$) received only distilled water (2 ml/kg i.p.). The control mean reaction time (in seconds) was determined and recorded. The test group mice (six mice per extract- or reference drug-dose) were treated with *Dorstenia barteri* extracts at 50, 100 and 200 mg/kg i.p. or ASA (100 mg/kg i.p.), respectively. One hour following the plant extract- or ASA-administration, the mice were separately placed in a glass beaker on a hot plate (Thermajust, Model 475,TechniLab Instruments, N.J., 07440) maintained at 50 ± 1 °C. For both the control and test animals, the reaction time (in seconds) was taken as the time when each of the mice jumped out of the beaker on the hot plate. The test mean reaction time (in seconds) was also determined for each plant extract dose and ASA.

2.4.1.3. Tail immersion test method. Control group of mice ($n = 6$) received only distilled water (2 ml/kg i.p.) and the mean reaction time (in seconds) was determined. Test groups of mice (six mice per extract- or reference drug-dose) were treated with *Dorstenia barteri* (DB, 50, 100 and 200 mg/kg i.p.) or ASA (100 mg/kg i.p.) respectively. One hour following the plant extract- or reference drug (ASA) administration, the tail (up to 5 cm) of each mice was immersed in hot water maintained at 50 ± 1 °C (in a 100 ml beaker). For both the control and test animals, the reaction time (in seconds) was taken as the time when the animals withdrew their tails completely from the hot water in the beaker (Parimaladevi et al., 2003). The test mean reaction time (in seconds) was calculated for each plant extract dose, ASA and distilled water control.

2.4.1.4. Analgesiometer method: pain threshold of mice. The middle tail of the mice used in this test were subjected to an increasing force (pressure) according to the method of Randall and Sellito (1957). One hour following the administration of the plant extracts (DB, 50, 100 and 200 mg/kg i.p.), the reference drug (ASA, 100 mg/kg i.p.) or distilled water (2 ml/kg i.p.) into the animals, the pain threshold was measured mechanically using an Ugo Basile Analgesiometer – Model 09380 (Milan, Italy). Squealing of the animals as a consequence of application of continuous pressure to their tails was taken as the reaction time of the animals. Thereafter, the pressure (force) stimulus was terminated, and the pain threshold was read off from the scale.

2.4.2. Anti-inflammatory activity

2.4.2.1. Acute inflammation. Carrageenin-induced paw oedema in mice was used as a model of acute inflammation. 0.1 ml of a 1% carrageenin solution was injected into the plantar surface of the right hind paws of the mice. Control group mice ($n = 6$) received distilled water (2 ml/kg i.p.) only, while animals in the test groups were treated either with *Dorstenia barteri* extracts (DB, 50, 100 and 200 mg/kg i.p.) or phenylbutazone, 100 mg/kg i.p.) 1 h before carrageenin injection. Two hours after carrageenin injection, the mice

were anaesthetized by dropping them in a jar containing cotton wool soaked with chloroform, and both the right and left hind limbs were cut identically at the ankle joint and weighed. The differences in weight gave the oedema developed in the right hind limbs (Subramoniam et al., 2001).

2.4.2.2. Carrageenin-induced pleurisy in mice. The method used in these experiments was modified from that described in detail by Vinagar et al. (1982) cited by Badilla et al. (2003). Five groups of six mice each, were treated with *Dorstenia barteri* extracts at 50, 100 and 200 mg/kg, phenylbutazone (100 mg/kg) and distilled water [control] (2 mg/kg i.p.), respectively. One hour after treatment, all the animals received an intrapleural injection of 0.25 ml carrageenin on the right side of the thorax. Two hours later, the mice were anaesthetized with chloroform, and the pleural cavity was washed with 1.0 ml of distilled water. The number of leucocytes in the pleural cavity was determined and recorded.

2.4.2.3. Pulmonar oedema. The lungs of the animals sacrificed in Section 2.4.2.2 were dissected free from the trachea and weighed. Significant changes in the test 'wet-lung weight' compared to the distilled water-treated controls, was considered to reflect pulmonary oedema (Staub, 1974). Pulmonary oedema was calculated from the fomula:

$$\text{Pulmonary oedema} = \left(\frac{\text{Lungs wet weight}}{\text{Body weight}}\right) \times \frac{10\,000}{1}$$

2.5. Statistical analysis

Data obtained for each set of experiments/tests were pooled and expressed as means ± S.E.M. Student's t-test was used to compare test- and control group-values. Differences in the test- versus control-values were considered to be statistically significant at $P \leq 0.05$.

3. Results

Dorstenia barteri leaf and twig extracts produced dose-related, significant ($P < 0.05$–0.01) antinociceptive and anti-inflammatory effects in mice. However, in the flick tail immersion test, only the twig extract of *Dorstenia barteri* (200 mg/kg i.p.) produced a significant increase ($P < 0.05$) in the reaction time of the mice. In the hot plate test method, the reaction time of the animals to thermal stimulus was significantly increased ($P < 0.01$) by both the leaf and twig extracts of *Dorstenia barteri*. Similarly, in the mechanically-induced pain test model (analgesiometer), there were significant increases ($P < 0.05$–0.01) in the pain threshold produced by *Dorstenia barteri* extracts DB, 50, 100 and 200 mg/kg i.p.) on the tails of the test mice (compared to the control group). In the acetic acid-induced writhing response, the abdominal contractions of the mice were reduced considerably by *Dorstenia barteri* leaf and twig extracts. The inhibitory effects of the plant extracts were dose-dependent and highly significant ($P < 0.01$, see Table 1).

The anti-inflammatory activities of the leaf and twig extracts of *Dorstenia barteri* on carrageenin-induced oedema in the right hind paw as well as in the pleural cavity of mice are shown in Table 2. In all the experimental models used, carrageenin-induced oedema in all the animals (compared with distilled water-treated control animals). However, in the test groups, the leaf extract reduced oedema paw weight. Percentage reduction was between 23.3% and 41.7%. At a dose of 200 mg/kg, the leaf extract of *Dorstenia barteri* produced a significant reduction ($P < 0.05$) in the oedema weight. The number of leucocytes obtained from the pleural cavity exudates, and the weight of the wet-lungs resulting in pulmonary oedema were also reduced by the leaf extract, but the difference was not very significant ($P > 0.05$). However, the anti-inflammatory activity of the twig extract of the plant was found to be more pronounced and highly significant compared to that of the leaf extract. Reduction in oedema formation weight was between 61.7% and 78.3%. Both the

Table 1
Antinoceptive effects of *Dorstenia barteri* leaf and twig extracts

Dose (mg/kg)	Tail immersion reaction time (s)	Hot plate reaction time (s)	Analgesiometer (pain threshold in g)	Acetic acid writhing contraction (No/20 min)
Control (distilled water 2 ml/kg i.p.)	2.3 ± 0.8	6.3 ± 0.6	104 ± 12	73.0 ± 4.8
Dorstenia barteri leaf extract (mg/kg i.p)				
50	2.6 ± 0.4	14.8 ± 1.7**	180 ± 38*	32.6 ± 9.4**
100	3.5 ± 1.2	13.9 ± 1.4**	182 ± 36*	38.4 ± 5.4**
200	3.8 ± 0.9	13.4 ± 1.0**	134 ± 26*	14.4 ± 8.3**
Dorstenia barteri twig extract (mg/kg i.p.)				
50	3.3 ± 1.7	16.9 ± 2.3**	224 ± 52*	30.4 ± 7.6**
100	4.5 ± 1.0	15.9 ± 1.7**	220 ± 63*	30.6 ± 9.8**
200	7.6 ± 2.4*	18.8 ± 2.0**	418 ± 40**	0.0 ± 0.0**
Disprin (mg/kg i.p.) (ASA,100)	3.6 ± 2.4	16.7 ± 1.1*	250 ± 38*	4.4 ± 3.2

Values given represent the means ± S.E.M. of six observations.
* $P < 0.05$
** $P < 0.01$ vs. control [Student's t-test].

Table 2
Anti-inflammatory activities of *Dorstenia barteri* leaf and twig extracts

Dose (mg/kg)	Oedema formation size (g)	Inhibition (%)	Number of leucocytes	Pulmonary oedema
Control (distilled water 2 mg/kg i.p.)	0.008 ± 0.004	0	3.50 ± 0.9	64.2 ± 9.7
Carrageenin (with control)	0.06 ± 0.01	0	50.6 ± 19.6	109.2 ± 3.1
Dorstenia barteri leaf extract (mg/kg i.p.)				
50	0.046 ± 0.001	23.3	38.6 ± 2.3	94.4 ± 9.3
100	0.042 ± 0.005	30.0	30.6 ± 9.4	83.5 ± 2.1
200	0.035 ± 0.006	41.7	29.6 ± 12.9	83.9 ± 4.6
Dorstenia barteri twig extract (mg/kg i.p.)				
50	0.023 ± 0.003	61.7	29.4 ± 5.7	82.7 ± 3.5*
100	0.021 ± 0.006*	65.0	15.3 ± 1.6	81.8 ± 4.1*
200	0.013 ± 0.004**	78.3	4.5 ± 1.9*	49.8 ± 8.6*
Phenylbutazone (mg/kg i.p.) (PBZ, 100)	0.042 ± 0.001	30.1	3.92 ± 1.1*	80.5 ± 15.8

Values given represent the means ± S.E.M. of six observations.
* $P < 0.05$
** $P < 0.01$ vs.control [Student's *t*-test].

weight of the wet-lungs, and the number of leucocytes were highly reduced. At a dose of 100 mg/kg i.p., *Dorstenia barteri* twig extract was 2.2 times more potent than PBZ in reducing oedema formation in mice. The twig extract of *Dorstenia barteri* at 100 mg/kg was also found to be as potent as PBZ in reducing pulmonary oedema (see Table 2).

4. Discussion

In living animal tissues, inflammatory processes involve the release of several mediators, including: prostaglandins, histamine, chemo-attractants, cytokines, proteinase, and so on; as well as substances that regulate adhesion of molecules and the processes of cell migration, activation and degranulation (Hollander et al., 2003). Various forms and models of inflammatory reactions have been detected. For example inflammatory responses from the airways of asthmatic patients, bone and joints inflammation, microbial infection, anaphylaxis and allergic conditions, and so forth. Thus, the adoption of different analgesic and anti-inflammatory experimental models for the assessment of phytomedicines used in the traditional healthcare system for the management of pain, asthma, arthritis, rheumatism and so on, are considered desirable and justifiable.

The results of the present study indicate that the leaf and twig extracts of *Dorstenia barteri* possess antinociceptive activity in chemical-, mechanical- and thermal-induced pain test models. Furthermore, the plant extracts showed anti-inflammatory activities in carrageenin-induced hind limb paw acute inflammation, oedema paw weight, pulmonary oedema, and in the number of leucocytes from the exudates collected in the pleural cavity. From the present study, it is evident that in both the analgesic and anti-inflammatory test models used, the twig extract is more potent than the leaf extract. In most instances, however, the effects of the extracts were dose-dependent and significant; and were comparable with those of the reference drugs used for the analgesic (ASA) and anti-inflammatory (PBZ) activities respectively. The observed analgesic and anti-inflammatory effects of *Dorstenia barteri* extracts could be due to the presence of biologically active chemical compounds in the extracts.

In the tail immersion test method, the reference analgesic drug (ASA) and the leaf extract of *Dorstenia barteri* showed a low ($P > 0.05$) antinociceptive activity. However, relatively moderate to high doses of *Dorstenia barteri* twig extract (≥200 mg/kg i.p.) produced antinociceptive effect. It should be noted, however, that this test model of analgesic assessment is best reserved for evaluating compounds for centrally-acting antinociceptive activity. The results obtained in this experimental analgesic test model may suggest that *Dorstenia barteri* extracts as well as the reference analgesic drug used (ASA) do not produce their analgesic effects via central mechanisms. However, there are known non-steroidal anti-inflammatory drugs (NSAIDs) that alter motor performance of animals and still increase reaction time latency period in the hot plate test method without acting on the central nervous system (Parkhouse and Pleurvry, 1979). Acetic acid-induced abdominal contractions and the hot plate test techniques are other widely used methods for evaluating peripheral analgesic effects (Gene et al., 1998). Apart from the tail immersion test model, significant analgesic effects were obtained at all dose levels of *Dorstenia barteri* extracts employed, and for the reference analgesic drug ASA. This observation probably suggests that *Dorstenia barteri* extracts might be acting through peripheral mechanisms similar to those of the non-steroidal anti-inflammatory drugs (NSAIDs).

Administration of *Dorstenia barteri* extracts over a period of 3 h showed a dose-dependent, anti-inflammatory activity in the three models of acute inflammation used. *Dorstenia barteri* twig extract (DB, ≥ 100 mg/kg i.p.) was more active than phenylbutazone at 100 mg/kg. Under the same experimental conditions, *Dorstenia barteri* leaf extract produced dose-related, but low significant anti-inflammatory effects in all the models. The inhibitory effects of *Dorstenia barteri* extracts on the number of leucocytes, pulmonary oedema and

acute oedema induced by carrageenin in the right hind limb paws of mice may be due, at least in part, to enzyme inhibition, chemical mediators released during inflammation and/or reduction of white blood cell (WBC) movement into the site of injury (Pathak et al., 1991) Thus, the use of an array of anti-inflammatory experimental models in this study lends further weight to the possible wide spectrum of activity of the extracts in the management of inflammatory conditions such as arthritis, rheumatism, asthma, bronchitis, acute inflammation, type-IV hypersensitivity reaction in anaphylaxis, and allergy (Insel, 1990) which are due to the release of inflammatory mediators and migration of WBC.

In one of our studies (unpublished data), we found that the twig extract of *Dorstenia barteri* was more active than its leaf extract in terms of its anti-trichomonal and antioxidant properties. Various chemical constituents of *Dorstenia barteri*, such as quercetin, quercitrin, bartericin A and B, stigmasterol and 6,8-diprenyleriodictyol, were shown to produce moderate to high antioxidant activities. These compounds could have contributed to the analgesic and anti-inflammatory properties of *Dorstenia barteri* extracts observed in the present study. In the same manner, *Dorstenia barteri* and other *Dorstenia* species (*Dorstenia elliptica, Dorstenia psilurus, Dorstenia ciliata, Dorstenia mannii, Dorstenia prorepens, Dorstenia zenkeri and Dorstenia poinsettifolia*) have been shown to contain various phytochemical constituents (secondary metabolites) of great importance. Phytochemical screening of the extracts showed the occurrence of flavonoids, terpenes and sterols. Flavonoids and polyphenolic compounds have been reported from many *Dorstenia* species (Tsopmo et al., 1998,1999; Dufall et al., 2003; Kansci et al., 2003; Abegaz et al., 2004; Ngameni et al., 2004). They occur as monoprenylated, diprenylated and geranylated derivatives including chalcones and other flavonoids. Other compounds identified in *Dorstenia* species are monoterpenoids, furanocoumarins and β-sitosterol. These compounds have been reported to exhibit antioxidant activity (Dufall et al., 2003; Kansci et al., 2003).

The common link between oxidants and inflammatory reactions, infection and other disorders has been well established (Mongelli et al., 1997; Wang et al., 1999). In chronic infection and inflammation, release of leucocytes and other phagocytic cells readily defend the organism from further injury. They do this by releasing free oxidant radicals, NO, O_2^-, H_2O_2, and OH^-, as powerful oxidant mixtures (Ames et al., 1993; Mongelli et al., 1997). Antioxidants appear to inhibit the actions of some of the oxidants generated in inflammation (Ames et al., 1993). No wonder, therefore, that the extracts of *Dorstenia barteri* exhibited both anti-trichomonal and antioxidant properties in our earlier study (unpublished data). The antioxidant properties of these chemical constituents of *Dorstenia barteri* extracts could be used to explain, at least in part, the anti-inflammatory activities obtained in the present study. Nevertheless, the antioxidant property found in our unpublished work may have contributed generally not only to the anti-inflammatory activities, but also to the antinociceptive activities observed with *Dorstenia barteri* extracts.

Acknowledgements

One of us (JAO) is grateful to the University of Durban-Westville, Durban, South Africa; and to South African National Research Foundation (NRF); for financial support to carry out a part of this study.

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