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Analgesic properties of aqueous leaf extract of *Haematostaphis barteri:* involvement of ATP-sensitive potassium channels, adrenergic, opioidergic, muscarinic, adenosinergic and serotoninergic pathways

DOI 10.1515/jbcpp-2015-0108
Received September 11, 2015; accepted April 21, 2016

Abstract

Background: Pain is the most common cause of patients seeking medical advice as a result of its association with different pathologies. This study evaluated the antinociceptive property of *Haematostaphis barteri* as well as the possible mechanism(s) associated with its antinociceptive property.

Methods: Mice were administered *H. barteri* (30–300 mg kg^{-1}; p.o.), followed by intraplantar injection of 10 μL of 5% formalin into the hind paws. The pain score was determined for 1 h in the formalin test. The possible nociceptive pathways involved in the antinociceptive action of *H. barteri* were determined by pre-treating mice with theophylline (5 mg kg^{-1}, a non-selective adenosine receptor antagonist), naloxone (2 mg kg^{-1}, a non-selective opioid receptor antagonist), glibenclamide (8 mg kg^{-1}; an ATP-sensitive K^+ channel inhibitor), and atropine (3 mg kg^{-1}; non-selective muscarinic antagonist).

Results: *H. barteri* (30–300 mg kg^{-1}) significantly and dose dependently precluded both first and second phases of nociception. Pre-treatment with naloxone had no effect on the analgesic activities of *H. barteri* in the first phase. Again, pre-treatment with atropine and glibenclamide did not significantly reverse the neurogenic antinociception of the extract in phase 1. However, theophylline reversed the analgesic effect of the extract in the first phase. In phase 2, theophylline had no effect on the analgesic activities of the extract. Naloxone, atropine, and glibenclamide significantly blocked the antinociception of *H. barteri* in the inflammatory phase of the formalin test.

Conclusions: *H. barteri* possesses antinociceptive property mediated via the opioidergic, adrenergic, muscarinic, ATP-sensitive K^+ channels, and adenosinergic nociceptive pathways.

Keywords: adenosine; antinociception; *Haematostaphis barteri*.

Introduction

Pain has been identified as the most common cause of patients seeking medical advice from health professionals due to its association with different pathologies. It also represents important medical and economic costs [1, 2]. Current analgesic therapies, despite their established efficacy in alleviating symptoms and providing pain relief, present with disconcerting side effects. For instance, NSAIDs produce gastrointestinal problems, renal damage, etc., while opioid analgesics cause respiratory depression, emesis, and tolerance and/or addiction [3–6]. This, among other reasons, has led to the use of herbs for the management of pain and other diseases. Plants have been employed as medicines for many centuries because they

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are cheap, easy to obtain, and often perceived to be safe [7]. Herbal medicines have a strong traditional or conceptual base, and the potential to be useful as drugs in terms of safety and effectiveness has led to the use of these plants for treating different diseases. Plants continue to serve as possible sources for new drugs and chemicals derived from various parts of plants [8].

Haematostaphis barteri, popularly known as blood plum, belongs to the Anacardiaceae family. The leaves contain phytochemicals and elemental constituents such as tannins, saponins and sodium, potassium, calcium, and magnesium. *H. barteri* is used traditionally for the treatment of malaria and inflammatory diseases such as hepatitis and swollen body parts. Due to its traditional use, the leaves were evaluated for their analgesic property [9].

Materials and methods

Plant collection and extraction

The fresh leaves of *H. barteri* were collected from the Wechiau Community Hippopotamus Sanctuary area in Ghana between the months of August and December 2013; authenticated by a botanist in the School of Biological Science, UCC; and air dried for 7 days. A voucher specimen (BIO/BMS/162) has been kept in the herbarium for reference. The dried leaves were pulverized with an electric mill. Two hundred grams of the powdered leaves was extracted with distilled water and maintained at 80 °C for 24 h. The filtrate was evaporated and lyophilized by freeze drying. The yield of the lyophilized freeze-dried aqueous extract of *H. Barteri* (AQ) was 64.44%.

Drugs and chemicals

The drugs and chemicals used are Glibenclamide, Daonil®, purchased from Sanofi-Aventis, Guildford, UK; theophylline was purchased from BDH, Poole, England; Ondansetron was from Glaxo-SmithKline, Uxbridge, UK; atropine sulphate was purchased from E. Merck AG-Darmstadt, Germany; yohimbine was obtained from Walter Ritter GmbH+Co. KG, Germany; naloxone was from Troge Medical Gmbh, Hamburg, Germany; and morphine hydrochloride was from Phyto-Riker, Accra, Ghana.

Animals and husbandry

ICR mice (25–30 g) of both sexes were obtained from the animal facility of the Department of Biomedical and Forensic Sciences, University of Cape Coast, Ghana. The animals were housed in stainless steel cages with soft wood shavings as bedding, fed with normal commercial pellet diet, and maintained under laboratory. All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals. All protocols used were approved by the Departmental Ethics Committee.

Formalin test

The formalin test was carried out as described previously [10]. Each mouse was allowed to acclimatize to one of 20 formalin test chambers (Perspex chamber 15×15×15 cm) for 1 h. The mice were treated orally with 30, 100, and 300 mg kg^{-1} aqueous extract of *H. barteri* or 10 mL kg^{-1} saline. One hour later, pain was induced in the mice by intraplantar injecting 10 µL of 5% formalin into the hind paws. The animals were immediately returned individually into the testing chamber, and their nociceptive behaviors were captured (1 h) for analysis. A nociceptive score was determined for each 5-min time block by measuring the amount of time spent biting/licking of the injected paw. Tracking of the behavior was done using public domain software JWatcherTM, version 1.0. The average nociceptive score for each time block was calculated by multiplying the frequency and time spent in biting/licking. Data were expressed as the mean±SEM of scores between 0 and 10 min (first phase) and 20–60 min (second phase) after formalin injection.

Assessment of the mechanism of antinociception *of H. barteri* in the formalin test: involvement of adenosinergic, serotoninergic, opioidergic, ATP-sensitive K^+ channels, muscarinic, and adrenergic nociceptive pathways

The mechanism of analgesic action of *H. barteri* was investigated using various antagonists in the formalin test. The receptor pathways investigated were the adenosinergic, opioidergic, adrenergic, serotoninergic, ATP-sensitive K^+ channels, and muscarinic nociceptive pathways. The doses of antagonist, agonist, and other drugs were selected on the basis of previous literature data and in pilot experiments in the laboratory.

To investigate the roles played by these nociceptive pathways, seven groups of mice were pre-treated orally with theophylline (5 mg kg^{-1}, a non-selective adenosine receptor antagonist), naloxone (2 mg kg^{-1}, a non-selective opioid receptor antagonist), glibenclamide (8 mg kg^{-1}, an ATP-sensitive K^+ channel inhibitor), atropine (3 mg kg^{-1}, non-selective muscarinic antagonist), and yohimbine (3 mg kg^{-1}, an α_2 receptor antagonist) 60 min before the oral administration of 300 mg kg^{-1} AQ. One hour post-AQ treatments, pain was induced with 10 µL of 5% formalin in all the groups and nociceptive score was measured for 1 h.

Statistical analysis

GraphPad Prism for Windows version 5.0 (GraphPad Software, San Diego, CA, USA) was used for all data and statistical analyses. A p-value <0.05 was considered statistically significant. In all nociceptive tests, a sample size of five animals (n=5) was used. Total nociceptive score for each treatment was calculated in arbitrary unit as the area under the curve (AUC). Differences in AUCs were

analyzed using one-way ANOVA followed by Tukey's post hoc test. The various antagonists were compared to the vehicle group using t-test.

Results

Intraplantar injection of 10 µL of 5% formalin into the right hind paw produced the classical biphasic nociceptive response. The biphasic pain-like behavior was exhibited by licking and biting. Oral administration of AQ (30–300 mg kg^{-1}) precluded both first and second phases of nociception and dose dependently with the highest dose producing significant ($p<0.05$) antinociception in the neurogenic phase and inflammatory pain phase. Similarly, morphine used as the positive control drug at dose also reduced formalin-induced nociception in first and second phases significantly (Figure 1).

Pre-treatment with naloxone (2 mg kg^{-1}) had no effect on the analgesic activities of AQ in the first phase. Again, pre-treatment with atropine (5 mg kg^{-1}, p.o.), glibenclamide (8 mg kg^{-1}, p.o.), and yohimbine (3 mg kg^{-1}, p.o.) also did not significantly reverse the neurogenic antinociception of the extract in phase 1 (Figure 2). However, theophylline (5 mg kg^{-1}) reversed the analgesic effect of the extract in the first phase (Figure 2).

In phase 2, theophylline (5 mg kg^{-1}) had no effect on the analgesic activities of the extract (Figure 2B). Naloxone (2 mg kg^{-1}), atropine (5 mg kg^{-1}), glibenclamide (8 mg kg^{-1}), and yohimbine (3 mg kg^{-1}) significantly blocked the antinociception of AQ in the inflammatory phase of the formalin test (Figure 2).

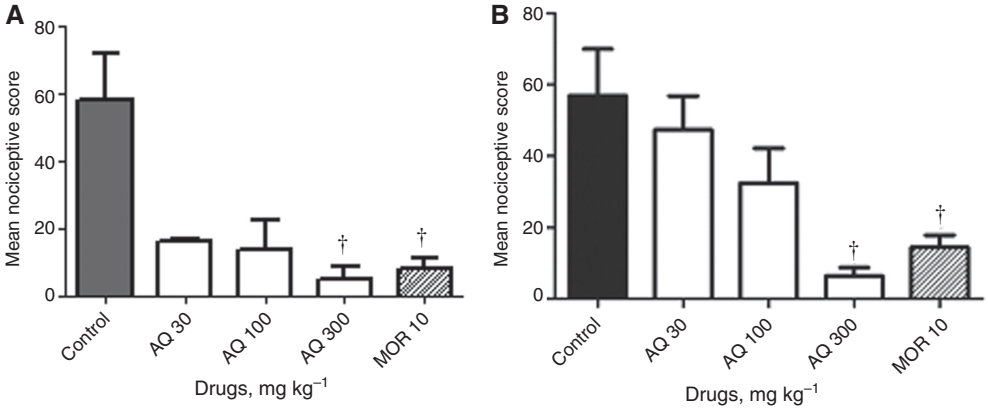

Figure 1: Effect of *Haematostaphis barteri* (AQ) (30–300 mg kg^{-1}, p.o.) and morphine (MOR) (10 mg kg^{-1}) on formalin-induced nociception in (A) phase 1 and (B) phase 2.
The graph represents the mean nociceptive score. Data are presented as mean±SEM, †$p<0.05$, one-way ANOVA followed by Tukey's post hoc test.

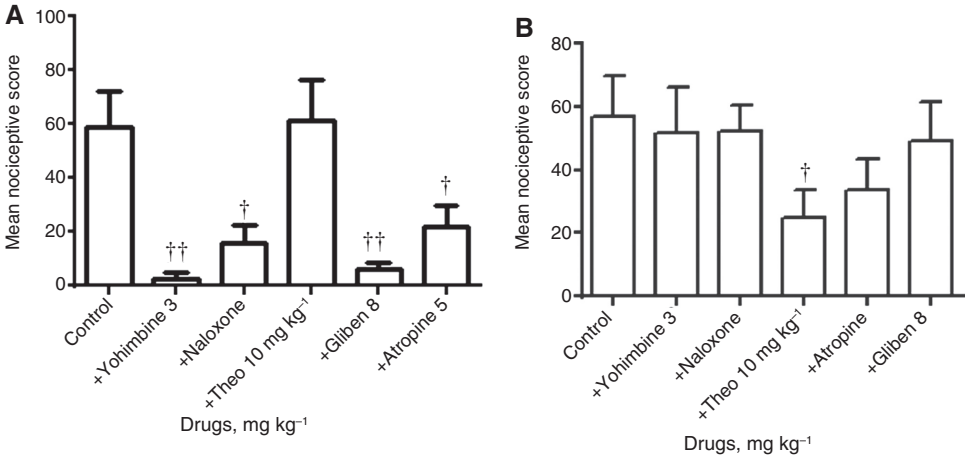

Figure 2: Effect of naloxone (2 mg kg^{-1}), theophylline (Theo) (5 mg kg^{-1}), glibenclamide (Gliben) (8 mg kg^{-1}), atropine (5 mg kg^{-1}), and yohimbine (3 mg kg^{-1}) on the antinociceptive effect of AQ (300 mg kg^{-1}) for (A) phase 1 and (B) phase 2 of formalin-induced pain.
Each column represents the mean±SEM. †$p\leq0.05$, ††$p\leq0.01$, compared to respective controls; one-way ANOVA followed by Tukey's post hoc test.

Discussion

The leaves of *H. barteri* have demonstrated antinociceptive property in the formalin test, a model used widely to evaluate the effects of analgesic compounds in laboratory animals. Formalin injection produces a biphasic pain response in animals; phase 1 reflects acute pain resulting from the activation of primary afferent sensory neurons, while the second phase (inflammatory pain) is as a result of the combined effects of afferent input and central sensitization in the dorsal horn [11–13]. Results from the present work suggest the effectiveness of the plant against neurogenic and inflammatory pain types. In order to determine the possible mechanism of action of *H. barteri*, naloxone, theophylline, yohimbine, glibenclamide, and atropine were used to assess the opioidergic, adenosinergic, ATP-sensitive K^+ channels, and muscarinic receptors, respectively. These antagonists did not produce antinociception when they were administered alone. The antinociception mechanism of *H. barteri* involved the opioidergic, ATP-sensitive potassium channels, adrenergic, muscarinic, adenosinergic, and serotoninergic pathways. These nociceptive pathways have been reported for several analgesic candidates as well as some clinically used analgesics. The opioid receptors – μ, κ, and δ opioid receptors – are G-protein-coupled receptors (GPCRs) and are located in the periphery, the dorsal root ganglion, the spinal cord, and supraspinal regions where they modulate pain. Although almost all the pharmacological opioid receptor agonists used to treat pain activate the μ receptors, research is providing insight into the usefulness of the κ and δ receptors in pain management [14, 15]. It is not clear which sub-set of opioid receptor(s) is activated by *H. barteri*, but it is likely that the δ receptor played a major role in the antinociception of *H. barteri*. Naloxone reversed the antinociception of *H. barteri* in the inflammatory phase (phase 2) of the formalin test but not the neurogenic phase. It has been reported that for δ opioid agonists to be effective, a state of inflammation may be required. Inflammatory states induce δ opioid receptors to migrate to the surface of neuronal cells and thereby become accessible to δ opioid agonists [14]. The role of κ opioid receptors in the antinociception of *H. barteri* cannot be ruled out for reasons that κ opioid agonists that solely act peripherally have been developed to target κ opioid receptors located on visceral and somatic afferent nerves for relief of inflammatory, visceral, and neuropathic chronic pain.

Theophylline reversed the antinociception produced by *H. barteri* suggesting the possible involvement of the adenosinergic nociceptive pathway. In rodents, adenosine A_1 receptor activation at peripheral nerve terminals produces antinociception via down regulation of cyclic AMP, while adenosine A_2 receptor activation produces pain. Adenosine A_3 receptor activation produces pain secondary to the release of histamine and 5-hydroxytryptamine from mast cells. *H. barteri* may have produced antinociception by acting on A_1 receptors [10]. Furthermore, it has been reported that adenosine A receptor activation in the spinal cord produces antinociceptive property in acute nociceptive, inflammatory, and neuropathic pain tests by increasing K^+ conductance as well as presynaptic inhibition of sensory nerve terminals to impede the release of substance P and perhaps glutamate [16]. The significance of the extract on K^+ channels in the antinociception of the extract was further highlighted by the reversal of glibenclamide of the antinociception of *H. barteri*. Similarly, it has been proposed that a multi-receptor complex comprising A_1, μ-opioid, and α_2-adrenergic receptors is likely to be activated by some analgesic candidates [10, 17] and *H. barteri* is no exception. This phenomenon was confirmed by the reversal of the antinociceptive effect of *H. barteri* by theophylline, naloxone, and yohimbine. It was also not surprising that atropine reversed the analgesic effect of the extract. It has been demonstrated that the analgesic efficacy of some α_2-adrenergic agonists such as clonidine is dependent on the spinal release of acetylcholine. Indeed, the analgesic efficacy of clonidine is enhanced by the administration of neostigmine, an anticholinesterase [18]. It is likely that the analgesic effect of *H. barteri* may involve the release of acetylcholine, which may be dependent on the α-adrenergic antinociceptive pathway activation, although it has not been confirmed.

Conclusions

H. barteri inhibits nociception in mice by modulating the opioidergic, adrenergic, muscarinic, and ATP-sensitive K^+ channels and adenosinergic nociceptive pathways.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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