



Ethnopharmacological communication

Anti-histamine effect of *Rubia tibetica*, used to treat anaphylaxis caused by tick bites in the Pamir Mountains, AfghanistanAnne S. Jeppesen^a, Uffe Kristiansen^b, Jens Soelberg^c, Anna K. Jäger^{a,c,*}^a Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Universitetsparken 2, 2100 Copenhagen, University of Copenhagen, Denmark^b Department of Pharmacology and Pharmacotherapy, Faculty of Pharmaceutical Sciences, Universitetsparken 2, 2100 Copenhagen, University of Copenhagen, Denmark^c NaturMedicinsk Museum, Faculty of Pharmaceutical Sciences, Universitetsparken 2, 2100 Copenhagen, University of Copenhagen, Denmark

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ABSTRACT

Ethnopharmacological relevance: The roots of *Rubia tibetica* are chewed as an antidote to anaphylaxis caused by bites of the tick *Ornithodoros lahorensis* by the Wakhi people in Afghanistan.**Aims of the study:** To test whether *Rubia tibetica* possess anti-histamine effect.**Materials and methods:** Water and ethanol extracts of roots of *Rubia tibetica* were tested for anti-histamine effect on the H₁-receptor in the guinea pig ileum assay. Fixed concentrations of plant extract with increasing concentrations of histamine were examined. Mepyramine was used as control.**Results and conclusion:** The ethanol extract of *Rubia tibetica* showed dose-dependent anti-histamine effect, whereas the water extract had little activity. The chewing of *Rubia tibetica* roots may alleviate the fatal swelling of the tongue during anaphylaxis.

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1. Introduction

It is well-known that bee-stings may cause anaphylactic shock in sensitive people, but other insects may cause allergic reactions as well (Estrada-Pena and Jongejan, 1999). The European pigeon tick (*Argas reflexus*) has caused anaphylactic reactions and sensitization which can persist for years (Kleine-Tebbe et al., 2006; Golden, 2007).

The Third Danish Pamir Expedition to the Wakhan Valley and Pamir Mountains of Afghanistan in the summer of 2010 recorded cases of severe anaphylactic reactions to bites of a tick. Both the tick *Ornithodoros lahorensis* and the antidote, the root of *Rubia tibetica*, were collected.

Anaphylaxis is triggered through release of histamine with the H₁ receptor as the prime receptor. The response includes smooth muscle contraction, the most notable is bronchial constriction (Hill et al., 1997). One of the most efficient assays for estimating the effect of histamine on H₁ receptors and the inhibiting potential of H₁ antagonists, is the guinea pig ileum assay (Hill et al., 1997). The

ileum contains no H₂ receptors and is thus free of interaction of the relaxant effects usually connected with these receptors (Bertaccini et al., 1979).

Extracts of *Crinum asiaticum* and *Andrographis paniculata* have previously shown anti-histamine effect in the guinea pig ileum assay (Samud et al., 1999; Karpakavalli et al., 2010). The research done on plants with antihistamine effect is rather limited, but these two studies support the concept that a plant may possess H₁ antagonistic principles.

The aim of this study was to provide scientific evidence for the traditional use of *Rubia tibetica* as an anti-anaphylactic remedy.

2. Material and methods

2.1. Ethnological data collection

Data on the use of a plant root against anaphylactic symptoms among Wakhi people was obtained as part of an ethnobotanical study in which 1.4% of the Afghan Wakhi population of approx. 17,000 people were interviewed. Nearly all community-based group interviews mentioned the use of a plant called *koh-e-rank* in Wakhi or *rank-e-kui* in Dari against the symptoms occurring after a bite by a tick called *köhna*. 16 independent Wakhi informants identified the plant and detailed its use through semi-structured interviews

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A specimen of the tick was collected in an old house under reconstruction in the village of Kharitsh in Upper Wakhan. It was morphologically identified and deposited at NaturMedicinsk Museum at the University of Copenhagen (Acc. no. 6506).

2.2. Plant extracts

Roots of *Rubia tibetica* Hook (Rubiaceae) were collected in the Wakhan corridor in the summer of 2010 and were identified by Soelberg. A voucher specimen was deposited at the Herbarium of University of Copenhagen (C) and Kabul University Herbarium (KUFS) (Soelberg 2010-160). The outer loose layer of the roots was removed and the woody part of the roots was ground in a hand-mill. 3.3 g root material was extracted twice with 50 ml of ethanol for 30 min on an ultrasound bath. The extract was evaporated to dryness resulting in 310 mg dry matter and redissolved in ethanol. 0.4 g root material was extracted twice with 10 ml water following the procedure above, yielding 58 mg, which was redissolved in water.

2.3. Antihistamine assay

A guinea pig (HsdPoc:DH, 400–449 g; Harlan, Holland) was anesthetized with CO₂ and killed by decapitation. The ileum was removed, and the intestine was gently rinsed with Tyrode's solution (Floyd, 1971). The ileum was then cut into 2–3 cm pieces and placed in a 10 ml organ bath with Tyrode's solution. The solution was heated to 37 °C and was bubbled through with 95% O₂ and 5% CO₂. The ileum pieces were connected to a transducer with 1 g tension. The transducer signal was recorded with the software LabChart 7. The data were processed in GraFit 5. The assay was carried out in duplicate.

The ileum pieces were allowed to stabilize for 30 min before adding of any agents. The response of each piece of ileum to histamine was determined by cumulative adding of histamine to the organ bath. The final histamine concentrations were: 1×10^{-8} , 3×10^{-8} , 1×10^{-7} , 3×10^{-7} , 1×10^{-6} and 3×10^{-6} M. This concentration scale was used throughout the experiments when adding histamine. Between adding of each series of histamine, the nutrient solution was washed out a couple of times to ensure no lingering agents before the next addition. Mepyramine was used as reference compound in a final concentration of 2×10^{-8} M (5.7 ng/ml), allowing a couple of minutes of contact time before adding histamine. The plant extracts were tested with a constant concentration of plant extract and increasing concentrations of histamine. The plant extracts were tested at 10, 30, 100, 300, 500 and 900 µg/ml as the final concentration in the organ bath.

The plant extracts were further tested for effects on the response of the ileum to acetylcholine in a final concentration of 1×10^{-5} M (1.46 µg/ml) and serotonin in a final concentration of 1×10^{-5} M to ensure that the response of the plant extract was due solely to inhibition of the H₁ receptor.

3. Results and discussion

Wakhi informants told of a tick, named *köhna* in Wakhi, which lives in old houses and occasionally bite people in their sleep. The tick was identified to be *Ornithodoros lahorensis* (Argasidae, sub-family Ornithodorinae) (Fig. 1). Bites were claimed to be potentially lethal, and several Wakhi informants recounted deaths in near or distant family. Symptoms are rashes on the chest and other parts of the body and swelling of the tongue which may lead to asphyxiation and death. Swelling of the tongue restricts speech, but the condition is so well-known that family members will react promptly. The correct action to be taken, as recounted by several Wakhi informants, is somewhat ritualized and take the form of three distinct actions:



Fig. 1. *Ornithodoros lahorensis* known as *köhna* in Wakhi.

(1) Catching the tick, throwing it on the fire place embers for the victim to inhale the smoke. (2) The victim should then vigorously chew the root of the plant known as *koh-e-rank*, or *rank-e-kui*, in Wakhi and Dari respectively. *Koh-e-rank* was identified as *Rubia tibetica*. Finger-sized pieces of root are kept in many households in Upper Wakhan, and many Wakhi carry a piece on them. (3) The victim should be immobilized and continuously over-poured with cold water.

The symptoms are consistent with some of the clinical manifestations of anaphylaxis (Oswalt and Kemp, 2007). Anaphylactic shock is mediated via the H₁ receptor, therefore the antidote, *Rubia tibetica*, was tested for antagonistic effect on the H₁ receptor.

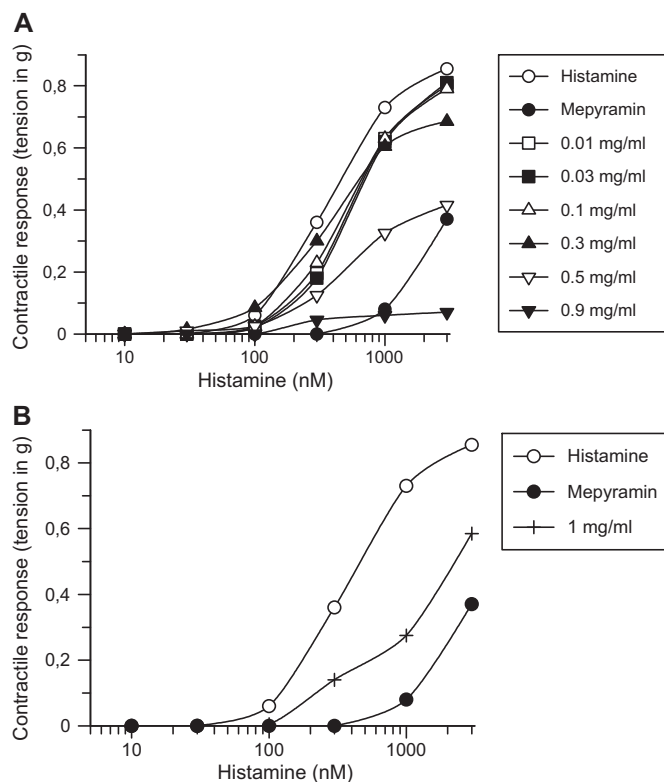


Fig. 2. Testing of (A) ethanol and (B) water extracts of *Rubia tibetica* in the guinea pig ileum assay. The plant extract was kept at a constant concentration and the histamine concentration was increased. Mepyramine was used as control at a concentration of 2×10^{-8} M (5.7 ng/ml).

The results from the anti-histamine testing of *Rubia tibetica* extracts showed that the ethanol extract was more active than the water extract (Fig. 2). The water extract showed only weak activity at 1 mg/ml. There was a significantly dose dependent effect of the ethanol extract ($p < 0.001$, Pearson product moment correlation), which at 900 $\mu\text{g/ml}$ had stronger anti-histamine effect than mepyramine at 5.7 ng/ml (Fig. 2). At the highest concentration, the ethanol extract had a wash-out period of up to 30 min (results not shown), indicating that the binding might be slowly reversible or non-reversible. The extract in itself did not affect the contractile state of the ileum. Tests with acetylcholine and serotonin indicated that the plant extract did not interact with these receptors.

It is difficult to estimate the dose of active plant constituents released during the chewing of the *Rubia tibetica* root, but it might exert a direct local anti-histamine effect on tongue and throat, preventing the fatal swelling of the tongue.

The results support the use of *Rubia tibetica* as a H_1 antagonist.

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