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Original Article

Species-specific Spasmolytic Effect of *Aspalathus linearis* Aqueous Crude Extract

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ABSTRACT

Objectives: *Aspalathus linearis* (Burm. f.) R. Dahlgren, or rooibos as it is commonly known, is a local plant of South Africa. The plant is used traditionally in gastrointestinal (GI) conditions like diarrhoea, colic, and cramps. It is also used to help in respiratory disorders like asthma. This endeavour aimed to provide scientific justification for the traditional uses of this herb. **Methods:** An aqueous extract (Al.Cr) of the fermented variety of rooibos tea (dried leaves and stems) was prepared. This extract was tested for pharmacological activity on guinea-pig ileum and rabbit trachea tissue preparations. **Results:** Rooibos tea extract, in previous studies conducted in isolated rabbit jejunum tissues, had shown a GI relaxant effect predominantly mediated via a K^+ channel opening (KCO) mechanism. When Al.Cr extract was administered on K^+ 80 mM and 25 mM sustained contractions in guinea-pig ileum, it was unable to exhibit a blocking activity up to 10 mg/ml. This indicated that the extract does not possess any Ca^{2+} channel blocking (CCB) or K^+ channel opening (KCO) activity. The extract, from 1-5 mg/ml, was able to displace and suppress acetylcholine (ACh) concentration-response curves (CRC) in the ileal tissues. ACh mediated the GI stimulant effect from 0.01-3 µM. When investigated for a potential bronchodilator activity in rabbit trachea, Al.Cr, from 1-10 mg/ml, completely suppressed the carbachol (1 µM)-induced spasmogenicity. **Conclusion:** Findings from this study demonstrate a GI antispasmodic effect of Al.Cr in ileal tissues that came about through a non-CCB and non-KCO mechanism. Al.Cr also showed a bronchodilator effect in isolated rabbit trachea tissue preparations. These results point to the value of this herb in GI and respiratory disorders.
Key words: Rooibos, Guinea-pig ileum, Rabbit trachea, Gastrointestinal, Respiratory.

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INTRODUCTION

Aspalathus linearis (Burm. f.) R. Dahlgren (family Leguminosae - pea family) or rooibos (red bush) is a local plant of South Africa. The dried leaves and stems of rooibos are commonly used as an herbal tea in South Africa. Its popularity has grown globally and it is now consumed as a tea all over the world. Rooibos comes in both non-fermented and fermented varieties.[1] The non-fermented, in which the process of oxidation has been halted, is the green tea version while the fermented or oxidized variety is called red tea (fermentation leads to change of colour to red) or red bush tea. The green tea version is more expensive as other green teas. The red tea version is more popular and less expensive. They both taste different, although both are known for their aroma and flavour. The tea is low in caffeine and tannin content, which is an advantage compared to other teas as the low tannin content leads to less compromised iron bioavailability.[2]

Rooibos is known by the people of Africa to have several traditional medicinal uses, in particular its value in gastrointestinal (GI) disorders. It is used for diarrhoea, colic, nausea, vomiting, and for abdominal cramps.[3,4] It is also used traditionally in asthma.[5] There are several scientific studies that have been conducted on rooibos. The list of pharmacological activities is long and the plant is known for its antiglycemic and hypocholesterolemic;[6] antiproliferative;[7] cardiovascular protective;[8] neuroprotective;[9] antioxidant;[10,11] hepatoprotective;[12] renal protective;[13] and wound healing[14] properties. Many of these properties are attributed to the presence of a high level of total polyphenols in rooibos including, but not limited to, polyphenols, flavanols, flavones, flavanones, and dihydrochalcones like aspalathin and nothofagin.[15,16] The flavonoids in the plant are especially regarded for their cardioprotective and neuroprotective contributions.[17]

This study aimed to look further into the smooth muscle relaxant activity of an aqueous crude extract of a fermented type of rooibos tea. We have, in the past, reported a spasmolytic effect of rooibos tea extract.[18] In that study, we tested the rooibos tea extract on isolated rabbit jejunum tissues and found that the extract has a GI relaxant effect that was mediated predominantly via a K^+ channel opening mechanism (KCO). This was evident from the fact that the extract was more potent in relaxing K^+ (25 mM)-sustained contractions than K^+ (80 mM)-sustained spasmogenicity. Interestingly in the current study, we report a similar spasmolytic action of *Aspalathus linearis* in guinea-pig ileal tissue although it was not mediated through suppression of K^+-induced spasmogenicity. We also report a bronchodilator effect of the extract as determined in isolated rabbit tracheal tissues.

MATERIALS AND METHODS

Animals

Maximum effort was undertaken to minimize any kind of suffering to the animals at each step of this project. Experiments were performed under the laboratory animal and care directives of European Community guidelines, EEC Directive 86/609/EEC. Isolated smooth muscles were acquired from local rabbits and guinea-pigs of either sex weighing around a kg and 0.5 kg respectively. Animals were housed in Aga Khan University's pathogen-free animal facility where the temperature was kept between 23-25°C. The animals were provided a diet composed of (g/kg): wheat grain 380, roughage 380, syrup 12, table salt 5.8, Nutri-Vet L 2.5, food additive $K_2S_2O_5$ 1.2, grease 38, sea food 170, dairy powder 150. The animals were given water as needed while the food was withdrawn a day before the experiment.

Reagents and chemicals

The following standard pharmacological agonists and antagonists were purchased from Sigma Chemical Co. USA: acetylcholine chloride (ACh),

carbachol chloride (CCh), potassium chloride. Tyrode's and Krebs physiologic salt solutions were used to immerse the isolated tissues. Their ingredients were acquired from Sigma Chemical Co. and Merck, Germany. The composition of the salt solutions was as follows:

Tyrode's (mM) for guinea-pig ileum: 2.68 potassium chloride, 136.9 sodium chloride, 1.8 calcium chloride, 5.55 glucose, 11.9 sodium bicarbonate, 1.05 magnesium chloride, 0.42 sodium dihydrogen phosphate

Krebs (mM) for rabbit trachea: 4.7 potassium chloride, 118.2 sodium chloride, 2.5 calcium chloride, 11.7 glucose, 25.0 sodium bicarbonate, 1.2 magnesium sulphate, 1.3 potassium dihydrogen phosphate.

Preparation of extract

Fermented dry rooibos tea/red bush tea (Figure 1) was acquired from a market in Cape Town's township of Khayelitsha, Western Cape Province, South Africa. Most of the rooibos tea there originates from the mountainous Cederberg Wilderness Area (Figure 2) in the same Western Cape Province (geographical coordinates: Latitude 32°06'30.6"S, Longitude 18°57'19.1"E). It was sampled at the Herbarium of Aga Khan University with specimen # AL-LF-07-01-33. The extract of the tea was prepared in water (Figure 3). A total of about 150 g of the dry plant material was brewed for 10 min in a litre of distilled water. The material was allowed to sit aside to cool down for 20 min. The liquid was strained and then concentrated in a Rotavapor (Buchi Corporation, New Castle, DE, USA). A total of 31 g (yield 21% weight/weight) of a thick extract was obtained that was coded as Al.Cr. This was stored in the freezer until solubilized and diluted when needed.

Guinea-pig ileal tissues

The pharmacological actions of Al.Cr on this preparation were determined using a standard method.[19] Briefly, 2 cm long pieces of ileum were obtained from guinea-pigs. These were immersed in glass tissue baths with salt solution bubbled with mixture of oxygen and carbon dioxide (carbogen) at a physiological temperature. A tension of a gram was used on each tissue. Metric changes were noted with the help of Harvard equipment (oscillographs and force transducers). Tissues were left to normalize in the solution for 30 min before any drug was introduced. The preparations were left for half an hour to normalize and then they were stabilized with repeated doses of a standard agonist, ACh (1 μM). A 3 min gap was allowed between administered concentrations of standard or test agents. Guinea-pig ileum does not exhibit rhythmic contractions but only shows a flat quiescent baseline ideal for testing of potential spasmogenic agents. Spasmolytic activity can be tested with the help of an agonist. Contractions were induced with high 80 mM and low 25 mM K^+ and then the test extract was administered cumulatively to record for any relaxant activity. The antispasmodic effect was also quantified against ACh concentration-response curves (CRC). A control curve of ACh was obtained and later, the tissue was pretreated (for 30 min) with increasing concentrations of the test extract. The ACh CRCs were reconstructed, under the influence of test substance, to see if there is a change.

Figure 1: Figure showing the fermented (red) dry rooibos tea (*Aspalathus linearis*) plant material.

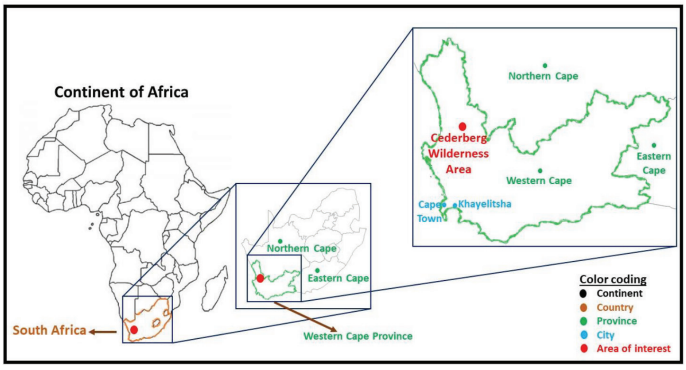

Figure 2: Map of the African continent (in black colour) showing the country of South Africa (in orange colour) from where the fermented (red) dry rooibos tea (*Aspalathus linearis*) plant material was acquired. It was bought from a market in Cape Town's (in light blue colour) township of Khayelitsha (in light blue colour), Western Cape Province (in green colour), South Africa. Most of the rooibos tea there originates from the mountainous Cederberg Wilderness Area (in red colour) (geographical coordinates: Latitude 32°06'30.6"S, Longitude 18°57'19.1"E) in the same Western Cape Province.

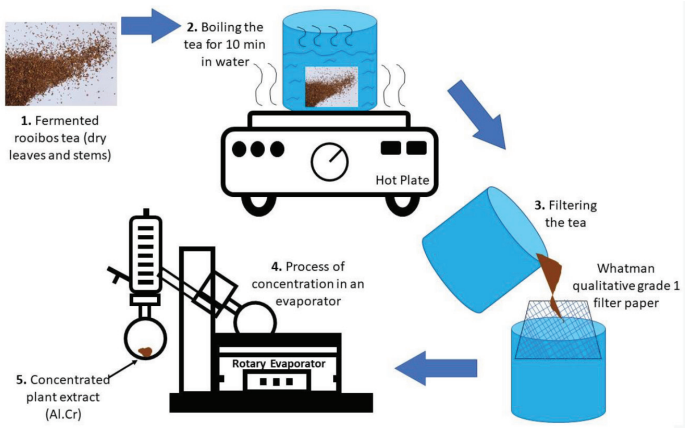

Figure 3: Figure showing the process of extraction for the fermented (red) dry rooibos tea (*Aspalathus linearis*) plant material.

Rabbit tracheal tissues

The pharmacological activity of Al.Cr on this tissue was determined using methods reported earlier.[20] Tissues were obtained and maintained in a

salt solution. Circular rings were formed out of the trachea. Each ring had an airway smooth muscle part and 2-3 cartilages. The ring was then sliced in such that the airway smooth muscle was in the middle, while the cartilages on the two sides. This made sure that once the tissues were hung in a glass muscle bath (with carbogen-aerated salt solution, physiologic temperature and preload of a gram), the thread was tied to the cartilage portions (above and below) while the airway smooth muscle was free to be observed for any changes in the middle. The preparation was left to normalize for an hour. A cholinergic agonist (CCh, 1 μM) was used to stimulate the preparation. Repeated concentrations of the agonist were administered until similar reposes were observed. A space of 45 min was allowed between bolus concentrations to the tissues. Once the tissues were ready to be used for experiments, the test extract was administered cumulatively up on the CCh spasmogenicity. This helped to quantify a potential bronchodilator/spasmolytic effect in trachea.

Statistical analysis

All results are presented as mean ± standard error of the mean (SEM). The letter 'n' shows the number of observations. A response was quantified using the median effective concentrations (EC_{50}) with 95% confidence intervals (CI). Concentration response patterns were compared for any statistical difference by the help of using one-way ANOVA followed by Bonferroni's test. A p value of < 0.05 was taken as significantly distinct (GraphPad, USA).

RESULTS

Guinea-pig Ileum

The pharmacological activity of Al.Cr was first determined in guinea-pig ileum. As this tissue does not have a spontaneous behaviour, different standard agonists were used to test for a potential antispasmodic action. Al.Cr was administered on both strengths (80 and 25 mM) of K^+-induced spasmogenicity. The extract from 0.1-10 mg/ml, did not show any significant inhibitory effect on these sustained contractions (n=4, Figure 4).

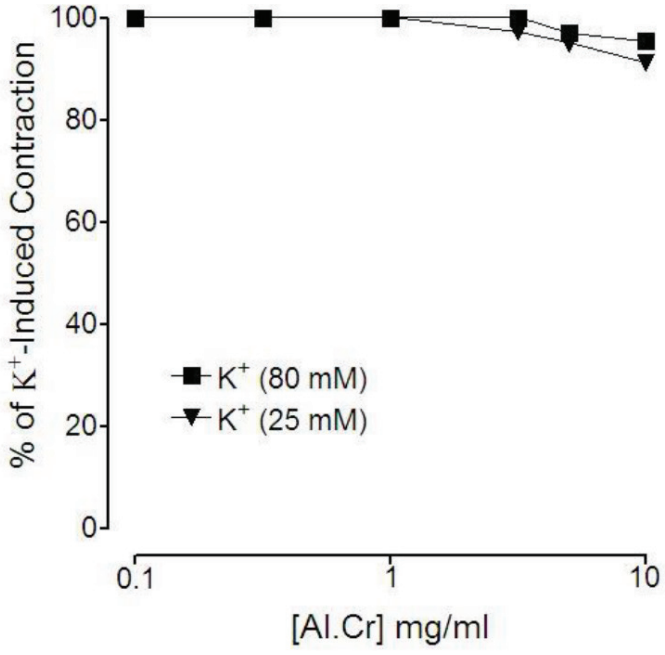

Figure 4: Graph presents the response of escalating concentrations of *Aspalathus linearis* aqueous extract (Al.Cr) upon K^+-induced contractions (80 and 25 mM) in ileal preparations of guinea-pig. The possible spasmolytic activity of extract is % of K^+-sustained contractions (results are mean ± SEM of 4 observations).

Later, ACh CRCs were constructed. ACh exhibited the ileal stimulant effect from 0.01-3 μM (n=4, Figure 5). These CRCs were first obtained without and then with escalating Al.Cr (1.0 to 5.0 mg/ml). The extract, from 1-5 mg/ml, not only shifted these curves to the right but also suppressed the ACh maximal effect (n=4, Figure 5). The ACh maximal effect of 100% was suppressed to 93.0 ± 2.3% by the Al. Cr concentration of 1 mg/ml, to 80.1 ± 7.7% by extract concentration of 3 mg/ml and finally to 63.8 ± 7.9% by extract concentration of 5 mg/ml. There was a significant difference between the controls ACh CRC and the CRCs constructed under influence of Al.Cr 3 mg/ml ($p < 0.05$) and Al.Cr 5 mg/ml ($p < 0.001$).

Rabbit trachea

Al.Cr was tested on CCh-induced contractions to determine if the extract has any bronchodilator effect. The extract, in increasing concentrations (1-10 mg/ml), relaxed the CCh-induced contractions (Figure 6). The EC_{50} for this effect was 4.8 mg/ml (2.5-9.3, 95% CI, n=4).

DISCUSSION

These experiments were performed to look into the effects of the rooibos tea aqueous extract on isolated guinea-pig ileum and rabbit trachea tissue preparations. Rooibos tea is traditionally used in Africa, and particularly in South Africa, for several GI and respiratory disorders.[4,5] We have in the past shown that an aqueous extract of rooibos elicits antispasmodic activity in isolated rabbit jejunum mediated predominantly via a KCO mode of action.[18] The current study aimed to see if the extract also has a similar antispasmodic effect in some other isolated tissues. For this purpose, we tested the extract on guinea-pig ileum. As this tissue does not have spontaneous spasmogenicity, the antispasmodic behaviour of a test substance can only be tested with the use of standard GI agonists/stimulants.[21] The extract was examined on two increasing strengths of K^+-induced contraction, 80 and 25 mM. The reason for using these two strengths is that inhibition of the 80 mM strength is indicative of blockade of Ca^{2+} channels (CCB) whereas blockade of 25 mM K^+ is for a

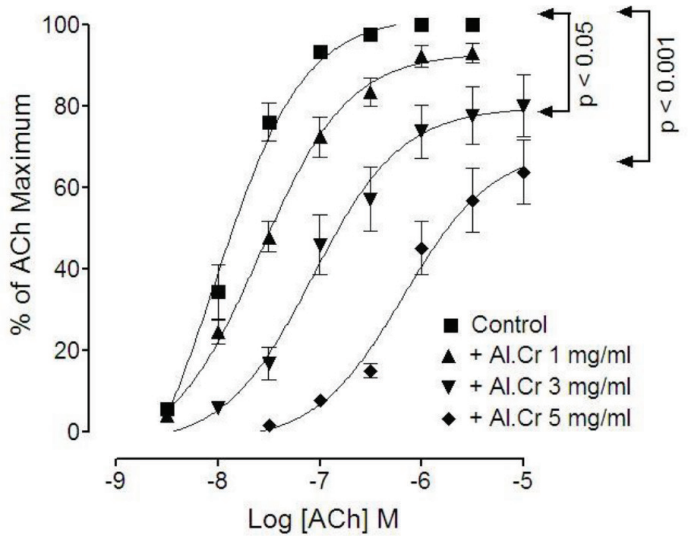

Figure 5: Figure showing acetylcholine (ACh) concentration-response curves acquired without (control) and with the influence of escalating doses of *Aspalathus linearis* extract (Al.Cr) in ileal tissues from guinea-pigs. Results are mean ± SEM with 4 observations. The control curve is statistically distinct from the Al.Cr pretreated curves of 3 mg/ml ($p < 0.05$) and 5 mg/ml ($p < 0.001$) (one-way ANOVA with Bonferroni's test).

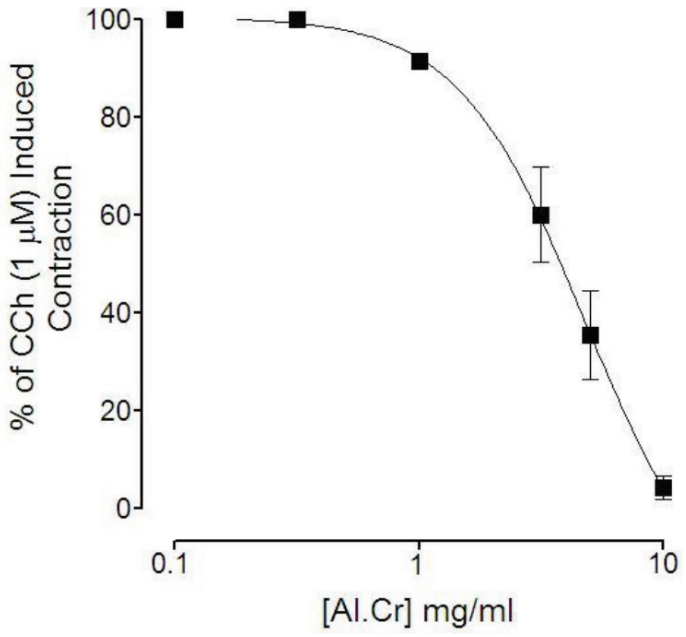

Figure 6: Curve showing spasmolytic effect of increasing concentrations of *Aspalathus linearis* aqueous crude extract (Al.Cr) when administered cumulatively on cholinergic agonist carbachol (CCh) 1 µM spasmogenicity in rabbit trachea smooth muscles. Bronchodilator activity is % CCh-sustained contractions (results are mean ± SEM of 4 observations).

mechanism involving the opening of potassium channels.[22,23] The crude extract did not exhibit any inhibitory effect neither on the 80 mM K^+ contraction, nor on the 25 mM K^+ contractions. This clearly showed that the extract is devoid of any CCB or KCO activity. This finding is quite in contrast to the general pharmacological results we normally get in our experiments with natural products. That is a similar kind of activity across the many tissues that we use to study the plant extracts on, although, there are exceptions. We have in the past reported such species-specific profiles and effects from some medicinal plants.[24-26]

Later, to find out if the extract has any antispasmodic activity, it was investigated against ACh CRCs. The extract, in increasing concentrations (1-5 mg/ml), inhibited and suppressed the ACh CRCs in guinea-pig ileum. This indicated that the extract does have antispasmodic activity in guinea-pig although not of the KCO or CCB type. ACh is one of the main neurotransmitters in the GI tract responsible for GI stimulation and overall regulation of the GI tone.[27] A suppressive effect of the extract on ACh points to its utility to relax the GI tone. The inhibitory effect of Al.Cr against ACh CRCs shows that rooibos tea has medicinal potential in GI disorders like diarrhoea, colic, and stomach cramps.

As rooibos tea is also used traditionally to relax the airways in respiratory conditions like asthma,[5] its crude extract was elucidated for an activity in leporine tracheal tissues. The extract exhibited a concentration-dependent blockade of CCh-induced bronchoconstriction. CCh is a standard cholinergic agonist.[28] The extract concentration of 10 mg/ml, completely suppressed the induced spasmogenicity. Bronchodilator activity of rooibos has been reported in the literature although that was done by using guinea-pig tracheal smooth muscles and on K^+ induced contractions.[29] We, in the current study, are reporting the bronchodilator effect of rooibos in rabbit trachea and via inhibition of CCh. Acetylcholine has a major involvement in the modulation and regulation of the respiratory system. Cholinergic agonists are used clinically to diagnose airway hyperresponsive disorders like asthma[30] while cholinergic inhibitors are a medication of choice in the treatment of airway diseases.[31] The inhibitory effect of Al.Cr against CCh-induced contractions points to a possible benefit of this herb in airway hyperresponsive disorders.

CONCLUSION

Findings from this study shed light on the spasmolytic behaviour of this very popular herb used as a soothing tea in the African continent and beyond. The extract was unable to block K^+-induced contractions in guinea-pig ileum but was able to displace and suppress ACh CRCs indicating its ability to relax the intestinal tissues. The rooibos extract also exhibited an inhibitory potential on agonist-induced contractions in the rabbit trachea. All in all, the results provide scientific justification for the utility of *Aspalathus linearis* in hypertonic conditions of the GI and respiratory tracts.

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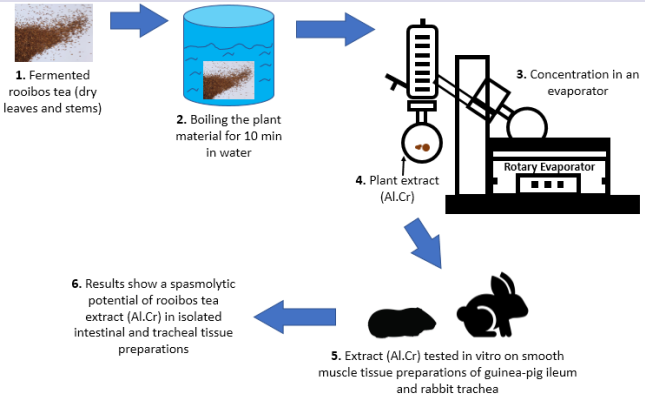
PICTORIAL ABSTRACT



SUMMARY

- *Aspalathus linearis* (Burm. f.) R. Dahlgren, or rooibos, is a local plant of South Africa. The plant is used traditionally in gastrointestinal (GI) and respiratory conditions like diarrhea and asthma.
- We report species-specific spasmolytic effect of an aqueous extract of the fermented variety of rooibos tea (dried leaves and stems) on guinea-pig ileum and rabbit trachea tissue preparations.
- In previous studies, rooibos extract showed GI relaxant effect in rabbit jejunum tissues via a K^+ channel opening (KCO) mechanism. When tested in guinea-pig ileum, the extract was unable to exhibit a KCO-like effect although it was able to displace and suppress acetylcholine concentration-response curves in ileal tissues.
- The extract also showed a bronchodilator activity in rabbit trachea exhibited as suppression of carbachol-induced contractions.
- These results demonstrate a pharmacological basis for the traditional use of rooibos in GI and respiratory disorders.

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