



Rooibos herbal tea reduces allergic basophil activation in adult atopic patients

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To the Editor

Allergic diseases are common and increasing in developed and low middle-income countries. Key *in vitro* surrogates for hypersensitivity include basophil activation – both via IgE-dependent and independent pathways – and mast cell degranulation with the release of a series of mediators (histamine, tryptase and leukotrienes) which can be easily quantified (Pedretti and Peter, 2017). Numerous herbal and alternative remedies are touted as having anti-allergic properties, yet few have robust human data to support use. In contrast, there remains a need for novel anti-allergic molecules and plant-based indigenous knowledge systems which may offer potential candidates. Thus, there is a need for *in vitro* human assay systems that could demonstrate proof-of-principle for candidate anti-allergic substances.

Aspalathus linearis, commonly known as rooibos, is a famous indigenous shrub of the Cederberg region in the Western Cape Province of South Africa. The leaves are used to make two types of herbal teas. The traditional red rooibos where leaves and stems are picked, bruised and fermented, and an unfermented green rooibos where leaves are quickly dried after picking to prevent oxidation. The unfermented rooibos brew contains higher levels of antioxidants. Rooibos herbal tea is rich in flavonoids and polyphenols which have beneficial antioxidant properties (Magcwebeba et al., 2016). Rooibos preparations are widely used as herbal remedy for alleviating numerous symptoms including allergic. Popular opinion is that rooibos has anti-allergic properties, but there is no human *in vitro* data examining if rooibos extracts can reduce IgE- or non-IgE mediated basophil and mast cell activation and degranulation;

and what the immunomodulatory mechanisms may be. There is no data on the dose, if any, of rooibos necessary to have a clinically meaningful impact on any allergic disease. Numerous studies have reported on the diverse physiological and pharmacological properties of rooibos including: hepato-protective, anti-tumour, and anti-viral (Canda et al., 2014; Marnewick et al., 2005; Rahmasaria et al., 2017; Sissing et al., 2011). Immunomodulatory proof-of-principle work has been conducted only with animal cells or model systems (Ichiyama et al., 2007; Kunishiro et al., 2001). Morishita et al. demonstrated that rooibos herbal tea could suppress antigen-stimulated degranulation in rat basophilic leukaemia cells (Morishita et al., 2017). A single *in vivo* study conducted in 1982, using a non-standardised or characterised rooibos extract, examined the impact of topical application and oral ingestion of rooibos on skin-prick test measurements in only 7 atopic patients; results were inconclusive (Hesseling and Joubert, 1982). We hypothesised that the addition of well-characterised fermented and unfermented rooibos extracts to a modified allergen-stimulated basophil activation assay, using *ex vivo* samples from atopic patients, would offer a rapid human *in vitro* method to assess the potential effect of this South African endemic herb on allergic inflammation.

A total of 9 subjects were enrolled in this study and all completed and signed informed consent forms for providing samples and for data processing as approved by the Human Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town (026/2017). Table 1 shows the characteristics of 9 atopic adults with confirmed IgE-sensitisation to two common aeroallergens included in the study. Whole blood collected from subjects was *in vitro* stimulated with anti-FcεRIα-

Abbreviations: anti-FcεRIα-Ab, antibody against the α chain of the high-affinity IgE receptor; fMLP, N-Formylmethionyl-leucyl-phenylalanine; HLA-DR, Human Leukocyte Antigen-DR isotype; IgE, immunoglobulin E; sIgE, specific IgE; SNOT-22, Sino-nasal Outcome Test; HPLC-DAD, High-Performance Liquid Chromatography with Diode-Array Detection; PBS, Phosphate-buffered Saline

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Table 1
Characteristics of participants (n = 9).

	Medium	Interquartile range
Age (years)	34.0	13.0
Male gender n/N (%)	33.3	
Allergic rhinitis severity (SNOT22 score)	20.0	6.0
Smoking n/N (%)	11.1	
Weekly alcohol intake (glasses)	2.0	2.5
Daily rooibos herbal tea intake (cups)	0.0	1.0
Daily coffee intake (cups)	1.0	1.8
House dust mite <i>D.pteronyssinus</i> sIgE level (Ku/L)	27.5	26.9
House dust mite <i>D.farinae</i> sIgE level (Ku/L)	14.4	25.0
Grass mix sIgE level (Ku/L)	5.2	16.5

Table 2
HPLC-DAD characterisation of rooibos extracts.

g compound/100 g extract	Fermented rooibos extract	Unfermented rooibos extract
S-Erio-6-glc	0.367	nd
R-Erio-6-glc	0.381	nd
S-Erio-8-glc	0.158	nd
R-Erio-8-glc	0.181	nd
PPAG	0.468	0.615
Aspalathin	0.472	11.77
Nothofagin	0.049	0.987
Isoorientin	0.755	1.147
Orientin	0.967	1.084
Quercetin-3-robinoside	0.864	0.829
Vitexin	0.213	0.212
Hyperoside	0.131	0.205
Rutin	0.226	0.341
Isovitexin	0.127	0.152
Isoquercetin	0.188	0.366

Ab and fMLP (IgE and IgE-independent positive controls) or allergens (grass pollen mix and *D. pteronyssinus* and *D. farinae*) as per a standard basophil activation assay. We used standardised spray-dried extracts of fermented and unfermented rooibos, which extract composition was characterised by HPLC-DAD as described previously (Walters et al., 2017) (Table 2). We selected the optimal extract concentrations and mixing conditions by examining cytotoxicity and flow cytometry plots using differing diluents (PBS versus stimulation buffer) and concentrations. For unfermented rooibos extracts we started with 1mg/ml but observed a high cytotoxicity (n = 2). For fermented rooibos extracts we also started at 1mg/ml and observed the same high cytotoxicity (n = 2), at 0.5mg/ml we showed an 18% decrease in viability (n = 2, background condition) and at 0.2mg/ml a 41% decrease in viability (n = 1, background condition). Therefore, three optimised decreasing concentrations of unfermented (0.1, 0.03 or 0.01mg/ml) or fermented rooibos extracts (0.05, 0.017 or 0.005mg/ml) were then added during stimulation in the efficacy experiments. Basophil activation determined by positive CD63 basophils (CD123+/HLADR-) was assessed using flow cytometry, gating only on live cells (7-AAD exclusion).

We found that both rooibos extracts inhibited basophil activation (CD63 upregulation) in a dose-dependent non-allergen specific manner; both anti-FcεR1α-Ab and fMLP positive control stimulation was reduced (Fig. 1). The inhibitory effect was stronger using fermented versus unfermented extract (Fig. 1). The suppression of basophil activation was still present with reducing concentrations of antigen (up to 10-fold).

In summary, these data are the first to demonstrate a strong allergen-independent inhibitory effect of well-characterised rooibos extracts on *in vitro* basophil activation in atopic patients, with a consistent inhibitory effect on IgE- and non-IgE-mediated basophil activation. These results are consistent with the study of Morishita et al. showing

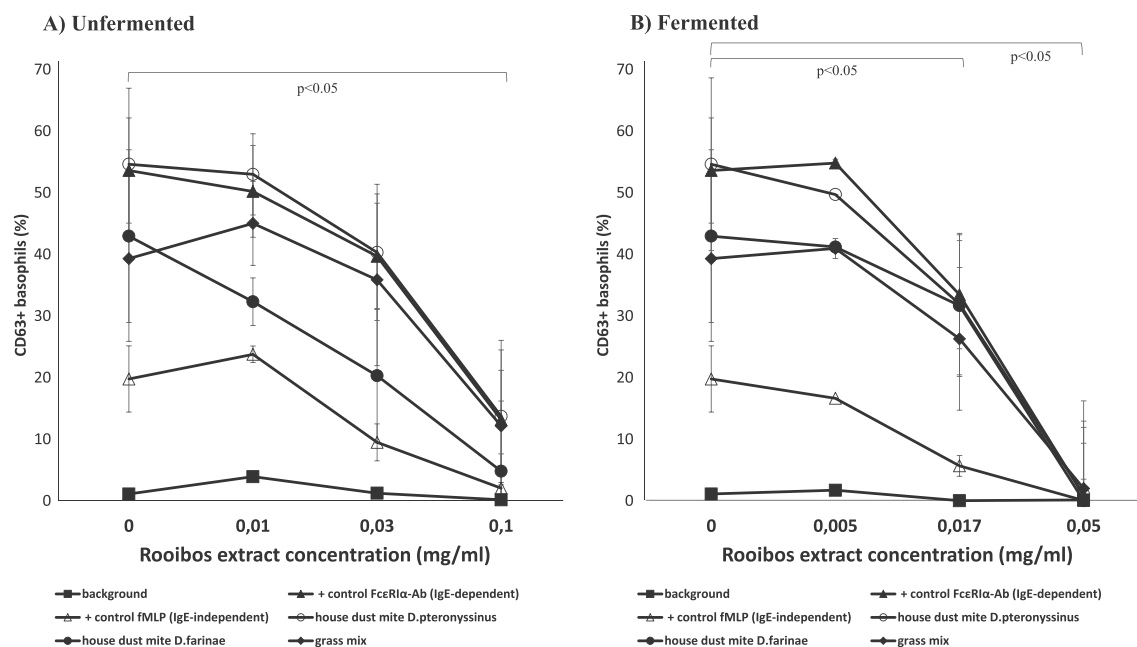


Fig. 1. Percentage of positive CD63 basophils representing activated basophils. Patients whole bloods were stimulated with stimulation buffer (background), IgE-dependent positive control (anti-FcεR1α-Ab), IgE-independent positive control (fMLP) or allergens (house dust mite *D.pteronyssinus*, house dust mite *D.farinae* or grass pollen mix). Increasing concentrations of unfermented (0.01, 0.03 or 0.1mg/ml) (A) or fermented rooibos extracts (0.005, 0.017 or 0.05mg/ml) (B) were added during stimulation. Data representative of mean ± SEM, n = 5–9. P-values were as follow vs no treatment: background: p = 0.023 for unfermented rooibos extract 0.1mg/ml, p = 0.029 for fermented rooibos extract 0.05mg/ml, p = 0.016 for fermented rooibos extract 0.017mg/ml; positive control: p = 0.001 for unfermented rooibos extract 0.1mg/ml; p = 0.0003 for unfermented rooibos extract 0.05mg/ml; p = 0.003 for fermented rooibos extract 0.017mg/ml; fMLP: p = 0.004 for unfermented rooibos extract 0.1mg/ml; p = 0.047 for unfermented rooibos extract 0.03mg/ml; p = 0.006 for fermented rooibos extract 0.05mg/ml; p = 0.007 for fermented rooibos extract 0.017mg/ml; house dust mite *D.pteronyssinus*: p = 0.006 for unfermented rooibos extract 0.1mg/ml; p = 0.007 for fermented rooibos extract 0.05mg/ml; p = 0.045 for fermented rooibos extract 0.017mg/ml; house dust mite *D.farinae*: p = 0.030 for unfermented rooibos extract 0.1mg/ml; p = 0.038 for fermented rooibos extract 0.05mg/ml; grass mix: p = 0.015 for unfermented rooibos extract 0.1mg/ml; p = 0.037 for fermented rooibos extract 0.05mg/ml; p = 0.023 for fermented rooibos extract 0.017mg/ml.

that rooibos herbal tea suppressed antigen-stimulated degranulation in rat basophilic leukaemia (RBL-2H3) cells (Morishita et al., 2017). Previously identified rooibos components quercetin, luteolin and chrysoeriol (Beltran-Debon et al., 2011; Gilani et al., 2006) were identified as contributing degranulation inhibitors and appeared to commonly target Syk phosphorylation in the upstream of degranulation signalling. In addition, these experiments are proof-of-principle that the basophil activation test can potentially be modified and use to assess the *in vitro* anti-allergic effects of candidate indigenous herbal allergy remedies.

Future work will focus further on mast cells, the mechanisms and specific active constituent(s) responsible for these *in vitro* effects, also trying to understand the differences seen between fermented and unfermented extracts. This human data, together with available animal data, justifies progression to a clinical study using standardised extracts, high and low-dosing strategies, and robust clinical end-points to determine if drinkable quantities of rooibos herbal tea can have a clinically meaningful effect on allergic rhinitis.

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Declaration of competing interest

There was no conflict of interest.

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