

REVIEW

The health benefits of rooibos tea in humans (*aspalathus linearis*)-a scoping review

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Abstract. Natural remedies in the treatment of health conditions are an appealing option for many individuals. Previous studies reported that fermented and unfermented rooibos tea have considerable anti-inflammatory and antioxidative properties. Most of this knowledge, however, originates from animal and cell culture studies. The aims of this review are to evaluate the existing, but limited, body of knowledge regarding rooibos tea interventions in humans and to identify the gaps in the literature. The PRISMA extension for Scoping Reviews (PRISMA-ScR) guidelines were followed in the collation of this scoping review. Among the databases searched were Google Scholar, PubMed, Cochrane Library, Scopus, and Web of Science. This review comprised 18 publications, with half (50%) of the studies being conducted in South Africa. There were 488 participants in all, ranging in age from six to 83 years, in the investigations. Rooibos tea was either fermented, unfermented, or black in 62% of the studies. Doses ranging from 200 to 1,200 ml were employed. In both healthy and at-risk individuals, rooibos has been shown to enhance lipid profiles, boost antioxidant status, and lower blood glucose levels. The existing findings suggest that rooibos consumption demonstrated to improve lipid profiles, boost antioxidant status, and lower blood glucose levels in both apparently healthy, and individual at-risk individuals or diagnosed of chronic conditions. Thus, it can be presumed that rooibos tea provides some health benefits, yet these findings are based on a limited number of human intervention studies and a small total sample size. Additionally, a variety of rooibos dosages and types

of tea in the experiments had inconsistent results that were probably impacted by the amount consumed. Future studies should include a dose-response study in humans, as well as large scaled clinical trials to evaluate the health effects of Rooibos.

Introduction

Many contemporary medicines have their roots in the long-standing practice of using plants for therapeutic purposes (1). According to the World Health Organization (WHO), approximately 80% of the world's population use herbal products for a variety of ailments. Herbs may be utilized for their purported anti-inflammatory, haemostatic, expectorant, antispasmodic, or immune-boosting properties (1,2). Numerous phytochemicals provide long-term health advantages for humans who consume them, and it can be used to treat a range of human afflictions (1). There has been increased attention on and consumption of herbal products in recent years due to claims that they are allegedly inexpensive and have few, if any, negative effects (1). Teas made from herbs, particularly Rooibos, have become increasingly popular worldwide (3).

Rooibos tea (produced from *Aspalathus linearis*), is free of caffeine and low in tannins (4), and has attracted a lot of research interest. It contains numerous minerals, especially flavonoids such as dihydrochalcones (aspalathin and nothofagin), phenylpropanoids, flavones, and flavonols (4,5), which may have a variety of positive health effects (6). Infusions made from Rooibos tea contain flavonoids and phenolic acids which have anti-oxidative activity (7) because they play an active role in preventing the formation of Reactive Oxygen Species (ROS) (8). Chronic illnesses including cardiovascular disease and diabetes mellitus can be prevented or treated by reducing highly oxidizing ROS and converting them into less harmful aroxyl radicals (9). In addition to the traditional method of consuming rooibos, a variety of rooibos tea drinks and dietary supplements in the form of capsules and tablets are easily accessible. As a result, it is warranted to promote Rooibos tea consumption and/or the consumption of its bioactive components.

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The bioavailability of Rooibos metabolites and their impact on non-communicable lifestyle diseases have been the subject of several studies (11-14). The dosages of various types of Rooibos tea that will have the maximum bioavailability and therapeutic effects in humans are a key subject that has generated a great deal of discussion. Six cups of tea (1,200 ml) each day, according to some researchers (13,14), may have positive effects. However, no human dose-response investigation has been carried out to date. Moreover, due to the scarcity of randomized controlled trials, it is uncertain if Rooibos consumers will experience hepatotoxicity from an overdose, or long-term consumption. Studies by Carrier *et al* (15) and Reddy *et al* (16) suggested that liver function may be negatively affected in regular Rooibos consumers.

With the majority of the confirmations coming from animal studies, the dosages that have been linked to advantageous effects in humans have been extrapolated from the various quantities utilized in animal studies. Given the growing interest in Rooibos's potential health benefits and the varied dosages that have been utilized, the main goal of this review is to evaluate the existing, albeit small, body of knowledge regarding Rooibos trials in humans and to offer suggestions for further research. Specifically, we aimed to:

- Identify all studies in humans and summarize the descriptive characteristics of the participants;
- Describe the various dosages (amount of Rooibos) that have been used in human studies;
- Describe the different types of Rooibos tea that have been used in human studies;
- Describe the various flavonoids in Rooibos that are detectable in urine and plasma;
- Describe the effect of the different dosages on health measures.

Materials and methods

Protocol and registration. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extension for Scoping Reviews (PRISMA-ScR) standards were used for this scoping review (19). To make sure that no scoping review had been registered or was being done to research a related topic, a search was conducted through the International Prospective Register of Systematic Reviews (PROSPERO).

Eligibility criteria. The following criteria was set to assess the eligibility of an article to be included in this scoping review: (a) the article must involve humans; (b) studies must evaluate the effectiveness of *Aspalathus linearis* in treating a chronic health condition, either alone or in combination with other medications; (c) articles must specify the dosage used; (d) articles must specify the type of Rooibos tea used (i.e., fermented (red), unfermented (green), capsule, or extract); and (e) articles must specify the population used in the study, and (f) the full article must be available and written in English or Afrikaans. Studies were included if the participants were healthy individuals, patients with any chronic condition, and persons who were at an increased risk of developing a chronic condition. In the case of interventional studies, the experimental group must have consumed Rooibos tea, while

the comparative group did not consume Rooibos tea or drank a different type of tea.

Data sources and search strategies. Databases including Google Scholar, PUDMED, Cochrane Library, Scopus, and Web of Science were utilized to search for articles. Databases spanning from the very first publications available until December 2022 were searched, to identify all studies conducted in humans. Where information was missing in an article, the authors were contacted personally and asked to provide the detail that was missing.

The primary search of the databases included all studies conducted in humans and consuming Rooibos tea. The search terms included 'Aspalathus linearis', 'rooibos,' 'human trials,' 'human studies,' 'adults,' and 'children.' EndNote version 10 (Clarivate Analytics, Philadelphia, USA) was used to manage the reference list and to remove duplicates.

Study selection. Articles included in the review were independently screened in three different steps and by two authors (AD and LE). First, duplicates were manually deleted. Then, a first selection was made based on the titles and abstracts of the articles. When articles failed to match the predetermined inclusion criteria, they were methodically inspected and eliminated. Additionally omitted were reviews, editorials, congress abstracts, and validation studies. Author disagreements were settled by consensus with a third reviewer (ET).

Data extraction. The first reviewer independently extracted the data from the included publications, and the second reviewer verified it. An already created data collection form was used to extract the data. The reviewers took notes on each article's participant characteristics (age, study location, type of participants, sample size), methods (study design, intervention length, type of Rooibos tea), outcome variables reported and findings. Data were only retrieved from the study arms that satisfied the inclusion criteria when a study included two or more study arms and one of the intervention arms did not.

Study quality assessment. According to Higgins *et al* (20) and Sterne *et al* (21), one reviewer (AD) evaluated the quality of the included research, which was then confirmed by the second (LE) and third reviewers (ET). The scale created by Jadad *et al* (22) was used to evaluate the studies' methodology. Randomization, blinding, and the explanation of withdrawals and dropouts were among the study quality factors that were evaluated. To elicit yes-or-no responses, the items were presented as questions. Depending on how well the methods for creating the randomized sequence and/or the mechanism of double-blinding were explained, points were given for items 1 (randomization) and 2 (blinding). If the trial was randomized and/or double-blind, but no explanation of the methods used to create the sequence of randomization or double-blind conditions was given, one point was awarded in each instance. An extra point was awarded if the method for creating the randomization sequence and/or the method for blinding were disclosed. High-quality studies were those that received at least three out of a possible five points.

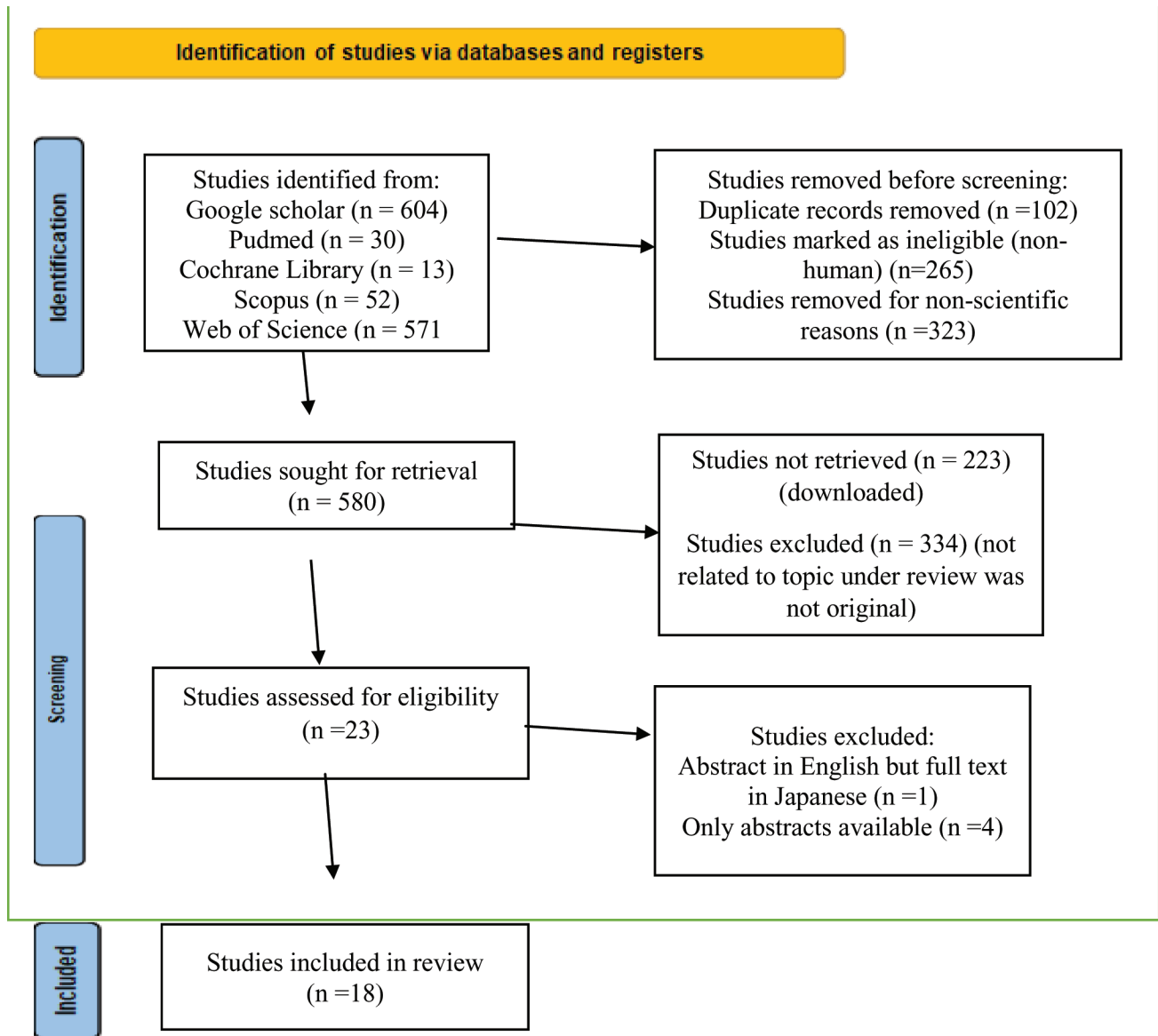


Figure 1. Flow chart of included studies.

Results

Search results and quality assessment. A flowchart of the search results is shown in Fig. 1. The search resulted in 1,270 articles. 580 articles were left after duplicates were eliminated and the titles and abstracts of the remaining articles were examined. Only 18 articles matched the eligibility requirements and were included in this scoping review. The quality of these 18 articles (10-14,23-35) was subsequently evaluated and ranged from poor to excellent. Most of the articles matched one or two of the three criteria for quality assessment and was classified as good quality. Six papers were deemed low quality (11,14,25,26,31,35), while two publications (23,28) were judged as high quality.

Sample size characteristics. The total number of participants in all investigations was 488, ranging in age from 6 to 83 years. The majority (193, 39.55%) of the participants were men, while 123 (25.20%), 94 (19.26%), and 56 (11.48%) were women, boys, and girls, respectively. The trials lasted from one day to three

months. Studies were conducted in South Africa (n=8), Italy (n=2), Germany (n=1), the United Kingdom (n=1), Argentina (n=1), New Zealand (n=1), Sweden (n=1), the United States (n=2), and Japan (n=1). Almost all studies featured participants who appeared to be in good health, except for Hesselting and Joubert (35), Marnewick *et al* (13,14), Rodgers *et al* (31), and Munnum *et al* (28), which included individuals with at-risk factors and those diagnosed with clinical conditions. While the at-risk individuals were defined as those with one or more risk factors for a chronic condition, the seemingly healthy populations comprised individuals who were said to have no diagnosis of any risk factor or chronic condition.

Bioavailability of the flavonoids found in human studies. Four studies (11,12,25,31) reported on the bioavailability of rooibos flavonoids in urine and plasma. The various flavonoids detected in both the urine and plasma samples are shown in Table III. Breiter *et al* (12) reported that the total concentration of flavonoids was $848 \pm 29 \mu\text{mol}$ in 500 ml with the major flavonoids being aspalathin ($636 \pm 20 \mu\text{mol}$), followed

by nothofagin ($79 \pm 3.1 \mu\text{mol}$). However, Stalmach *et al* (11), reported the total concentration in 500 ml of fermented and unfermented rooibos beverage to be 84 ± 2.9 and $159 \pm 6.5 \mu\text{mol}$ respectively. The concentration of the various flavonoids in urine was found to be 0.100 ± 0.001 (Aspalathin), 0.212 ± 0.002 (Orientin), 0.363 ± 0.007 (Isoorientin), 0.053 ± 0.001 (Isovitexin), 0.081 ± 0.001 (Vitexin), 0.501 ± 0.014 (Hyperoside), 0.015 ± 0.004 (Quercetin), 0.007 ± 0.001 (Luteolin), and 0.004 ± 0.000 (Chrysoeriol) (31). The recovery rates of flavonoids after the administration of rooibos tea were in the range of 0.2% (aspalathin) and 2.3% (vitexin). On average a total of 0.76 nmol of the quantified flavonoids were bioavailable during their maximum concentration in plasma, accounting for 0.26% of the total amount ingested ($758 \mu\text{mol}$) (12). However, Stalmach *et al* (11) did not detect any flavonoids in quantifiable amounts after consumption of either fermented or unfermented beverages.

Type of intervention (rooibos type), dosages used, and their outcomes. In general, almost all the studies used three types of rooibos: black, red (fermented), and green (unfermented), except for Chepulis *et al* (24) and Davies *et al* (27), which used rooibos extract and a standardized rooibos capsule. The dosages used throughout the studies are listed in Tables I and II and ranged from 200 to 1,200 ml of water (Tables I and II).

Health measures assessed. Several health parameters were measured across various studies included in this scoping review. Health parameters measured in both diagnosed and at-risk individuals, and apparently healthy populations included liver and kidney functions, iron status, inflammatory status (CRP), and physiological parameters (blood pressure, resting heart rate). Health measures assessed only among at-risk individuals and those diagnosed with chronic conditions included total serum immunoglobulin E (IgE), oxidative status, urinary and plasma biomarkers (urinary thiobarbituric acid reactive substances, Urinary N-acetyl- β -D-glucosaminidase and volume, and SS brushite), bone markers (type 1 intact amino-terminal propeptide), melatonin, and emotional and psychological domains. However, antioxidant status, total polyphenol intake, fasting and postprandial serum glucose, fasting insulin, angiotensin-converting enzyme, body mass, plasma osmolarity, blood oxygen saturation, acute mountain saturation, and peak torque extension and flexion were assessed in the apparently healthy population. Tables I and II provide a comprehensive description of the effect of rooibos consumption on the various health measures assessed in the included studies.

Findings from studies. One of the objectives of this scoping review was to report the effects of rooibos consumption on the various outcomes measured among at-risk and apparently healthy populations.

Effect of rooibos on health outcomes among at-risk population. After acute (short-term) consumption of 500 ml of black rooibos tea among asthmatic and hay fever individuals, Hesselting and Joubert (35) reported no changes in total serum immunoglobulin E (IgE) and the size of skin induration to 12 antigens. However, the skin reaction to four antigens (house dust, grass pollen, dog epithelia, and *Aspergillus fumigatus*)

was larger on the day of treatment with tea than on the control days ($P < 0.01$ to < 0.05).

Among individuals at risk of coronary heart disease (13), 6-weeks of rooibos tea consumption resulted in significant increases in Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), and creatinine function indicators by 35.9, 45.7, 21.3, and 24.4%, respectively, compared to the control period. Serum unconjugated bilirubin and total bilirubin levels decreased significantly ($P < 0.05$) by 34 and 24.08%, respectively, while a marked, but non-significant decrease (14.4%) was reported in serum glucose levels compared with the control period. Total cholesterol (TC) decreased by 8.6%, but this change was not statistically significant. However, significantly lower serum triglycerides (TG) (29.4%) and low-density lipoprotein (LDL-C) levels (15.2%) were reported ($P \leq 0.001$), while high-density lipoprotein (HDL-C) levels were significantly increased by 33.3% ($P \leq 0.001$).

Marnewick *et al* (13) showed that total polyphenol significantly ($P \leq 0.05$) increased by 11.8%. However, the reduced glutathione (tGSH) increased (35.76%) significantly ($P \leq 0.001$), while the oxidized glutathione (GSSG) was significantly decreased by 30.53% ($P \leq 0.001$), and the ratio of GSH:GSSG increased by 87.47% ($P \leq 0.001$). Moreover, the conjugate dienes (CDs) and thiobarbituric acid reactive substances (TBARS) decreased by a significant 34.9 and 54%, respectively. Meanwhile, acute consumption of a single dose of 500 ml fermented rooibos tea (34) increased GSH levels from an initial baseline of $801 \mu\text{M}$ to reach a peak of $851 \mu\text{M}$ at 45 min (an increase of 6.24%), after which it returned to the baseline value ($P \geq 0.05$) among healthy individuals. The plasma GSSG levels also increased from an initial baseline value of $97 \mu\text{M}$ to reach a peak of $109 \mu\text{M}$ at 45 min (an increase of 12.37%) after which it decreased to below the baseline value at 90 to 180 min ($P \geq 0.05$) while the GSH:GSSG ratio decreased from a baseline value of 6.23 to 5.81 but increased to reach a peak of 7.98 at 90 min and then remained at this level up to 180 min ($P > 0.05$) (34).

Regarding other clinical parameters, chronic supplementation with rooibos tea three times per day for 3 months among older women diagnosed with osteopenia (28) did not have any significant ($P \leq 0.05$) effect on the bone markers procollagen type 1 intact amino-terminal propeptide (total-P1NP) or on P1NP (ng/ml) levels. For type I collagen C-telopeptide (CTX-I, ng/ml), CTX levels were reduced (-38.23 ± 22.63 ; -29.64%) in the individuals who were supplemented with rooibos. Although CRP was low at baseline, three months of rooibos consumption did not significantly ($P \leq 0.05$) affect it. Rooibos supplementation for three months statistically increased ($P = 0.06$) melatonin levels (ng/ml) by 22.97 ± 25.26 (54.21%) from the baseline (42.37 ± 19.40) but did not significantly affect blood pressure levels (systolic and diastolic) or the physical domain scores (impact because of physical problems) compared to the placebo. However, rooibos had a significant ($P = 0.09$) reduction in the emotional/psychological domain scores (impact because of emotional/psychological problems) by 2.25 ± 1.63 (5.98%) when compared to placebo (coriander group) (0.57 ± 1.1).

Among kidney stone formers (31), supplementation with rooibos tea for 30 days reduced urinary volume significantly by 17.83% ($P = 0.016$) but did not affect urinary TBARS

Table I. Apparently healthy individuals.

Author (s)	Number of participants	Age of participants	Study location	Comparative group(s)	Study design	Rooibos type	Reported dosage	Duration of intervention	Major findings	(Refs.)
Breet <i>et al</i>	150 children (94 boys; 56 girls)	6 -15 years	South Africa	Black tea (50 tea bags in 20 l of water) ^a	Randomized controlled trial	Fermented rooibos	50 tea bags in 20 l of water ^a	16 weeks	↑ Mean corpuscular volume (MCV), Serum transferrin (S-Tfn) and Total iron binding capacity (TIBC)	(22)
Chepulis <i>et al</i>	10 women	Not specified	New Zealand	1 green tea (1.0 g) tablet, 1.6 Alma berry (700 mg) capsule, 1 grape seed (500 mg) capsule, 6 propolis tincture (400 mg) capsule	Randomized, controlled trial	Rooibos tea extract	760 mg of rooibos tea extract (provided to participant in three size '0' gelatin capsules)	1 day	↓ Postprandial glucose	(23)
Davies <i>et al</i>	32 men	22.2 years	South Africa	3 standardized Placebo capsules	Randomized, crossover, study	Fermented rooibos capsule	Three standardized capsule (equivalent to 6 cups of fermented rooibos) daily	4 weeks	↔ Peak torque extension (Nm) ↑ Peak torque flexion (Nm) ↔ total work extension (Joules) and total work flexion	(26)
Davies <i>et al</i>	8 men	46.6±11.2 years	Argentina	No comparator group	Case study design	Rooibos extract	Three capsules of rooibos extract (equivalent six cups of fermented rooibos) daily	14 days	↓ Peripheral blood oxygen saturation and symptoms of acute mountain sickness ↑ Resting heart rate	(25)

Table I. Continued.

Author (s)	Number of participants	Age of participants	Study location	Comparative group(s)	Study design	Rooibos type	Reported dosage	Duration of intervention	Major findings	(Refs.)
Francisco	14 men and women	18-35 years	South Africa	Standardized fat meal (50.1 g) with commercial sucrose beverage (Control)	Randomized crossover design	Fermented rooibos tea with standardized fat (50.1 g) meal	2 g of rooibos tea leaves per 100 ml of water ^b	1 day	↓ Fasting serum glucose ↓ Serum total cholesterol (TC), High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) but ↑ Triglycerides (TG) ↓ High sensitive C-reactive proteins (hs-CRP) ↓ conjugated dienes (CDs) and Thiobarbituric acid-reactive substances (TBARS)	(28)
Hesseling <i>et al</i>	30 men	21-34 years	South Africa	Green tea (5 g per 200 ml of water with 40 ml milk and 20 g cane sugar) Boiled water (200 ml with 40 ml milk and 20 g cane sugar) groups	Randomized controlled trial	Rooibos tea leaves	5 g of rooibos tea leaves with 200 ml of water with 40 ml milk and 20 g cane sugar	1 day	↓ Ferritin, Transferrin and Iron absorption but ↑ Serum iron	(10)
Persson <i>et al</i>	20 men and women	20-31 years	Sweden	10 g of Green tea leaves in 400 ml of water and 10 g of black tea leaves in 400 ml of water	Randomized crossover design	Rooibos tea	10 g of Rooibos tea leaves in 400 ml of water	1 day	↓ Angiotensin-converting enzyme (ACE) activity ↔ Blood pressure (BP), Heart Rate (HR) and Nitric Oxide (NO)	(29)

Table I. Continued.

Author (s)	Number of participants	Age of participants	Study location	Comparative group(s)	Study design	Rooibos type	Reported dosage	Duration of intervention	Major findings	(Refs.)
Utter <i>et al</i>	23 men	19.6±0.3 years	USA	Regular bottled water, and carbohydrate (6% or 60 g l ⁻¹) beverage	Randomized, cross-over design	Fermented rooibos tea	Not specified	1 day	↓ Body mass ↓ Plasma Osmolarity (Posm)	(31)
Villano <i>et al</i>	15 individuals	Not specified	Italy	Water	Randomized crossover study design	Unfermented and fermented 'ready to drink' rooibos teas	1.5 g of rooibos tea extract powder per 1 l of water ^b	1 day	↑ Fasting blood glucose and Plasma total radical-trapping antioxidant potential (TRAP)	(32)
Wanjiku	8 men	20-35 years	South Africa	No comparative group	Pre-post intervention study	Fermented rooibos	2.5 g rooibos tea leaves in 180 ml boiling water ^b	1 day	↑ Ferric Reducing Antioxidant Potential (FRAP) and Oxygen Radical Absorbance Capacity (ORAC) ↓ The total polyphenols ↑ The reduced Glutathione (GSH) levels ↑ The plasma oxidised glutathione (GSSG) ↓ GSH:GSSG ratio	(33)

^aEach participant took 200 ml of either black tea or rooibos tea during first and second breaks, ^bParticipants consumed 500 ml of rooibos tea. FRAP, Ferric reducing antioxidant power; ORAC, Oxygen radical absorbance capacity; GSH, Reduced Glutathione; GSSG, Oxidised Glutathione; GSH:GSSG, Reduced-Oxidised Glutathione ratio; HDL, High Density Lipoproteins; LDL, Low Density Lipoproteins; TG, Triglycerides; BP, Blood Pressure; HR, Heart Rate and NO, Nitric oxide; TRAP, Total radical trapping antioxidant parameter.

Table II. Individuals with health conditions.

Author (s)	Number of participants	Age of participants	Study location	Comparative group(s)	Study design	Rooibos type	Dosages	Duration of intervention	Major findings	(Refs.)
Hesselling and Joubert	7 adults (Asthmatic or hay fever individuals)	Not specified	South Africa	No comparator group	Interventional cross-sectional study	Black rooibos tea	25 g of black rooibos tea per 1 l of water ^a	1 day	↔ Total serum immunoglobulin E (IgE) ↔ size of skin in-duration to 12 of the antigens ↑ The skin reaction to four antigens (house dust, grass pollen, dog epithelia) and <i>Aspergillus fumigatus</i>	(34)
Marnewick <i>et al</i>	40 men and women (Individuals with two risk factors of CHD)	30-60 years	South Africa	6 cups of water	Controlled clinical trial	Fermented rooibos tea	6 cups (one tea bag per 200 ml of freshly boiled water) daily	6 weeks	↑ aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and creatinine ↓ Serum unconjugated bilirubin and total bilirubin ↓ Serum TG and LDL-C levels ↑ HDL-C levels ↑ Total polyphenol, GSH and GSH:GSSG while their GSSG ↓ ↓ CD and TBARS	(13)

Table II. Continued.

Author (s)	Number of participants	Age of participants	Study location	Comparative group(s)	Study design	Rooibos type	Dosages	Duration of intervention	Major findings	(Refs.)
Mamnewick <i>et al</i>	40 men and women Individuals with two risk factors of CHD	30-60 years	South Africa	6 cups of water	Cross over study design	Fermented rooibos	6 cups (one tea bag per 200 ml of freshly boiled water) daily	6 weeks	↑ Total flavonoids ↔ serum iron, ferritin, transferrin, TIBC and % Fe saturation ↔ Hs-C-reactive protein and homocysteine	(14)
Munmun <i>et al</i>	35 women (with osteopenia)	36-83 years	USA	Placebo (coriander), tuisi or oolong groups	Randomized, Controlled trial	Rooibos tea	3 tea bags (1 g per tea bag) daily	3 months	↔ the bone markers procollagen type 1 intact amino-terminal propeptide (total-P1NP) and P1NP (ng/ml) levels ↔ CRP ↓ Melatonin levels ↔ Blood pressure ↓ the emotional/psychological domain scores when compared to placebo (coriander group)	(27)
Rodgers <i>et al</i>	28 men (8 kidney Stone formers and 20 apparently healthy individuals)	18-26 years (Healthy Control) and 30-60 years (Kidney stone formers)	South Africa and Japan	Control group 2 cups (2 tea bags per 125 ml of low mineral water per cup) of Japanese Green tea (JGT) (Control group 2)	Crossover study design	Freshpak Fermented Rooibos tea	Control group 2 cups (2 tea bags per 125 ml of low mineral water) Freshpak rooibos tea (Control group 1) Stone formers	30 days	Control groups: ↔ The mean urinary and plasma biomarkers (Urinary TBARS (μmol/g creatinine), Urinary N-acetyl-β-D-glycosaminidase (NAG) (U/g	(30)

Table II. Continued.

Author (s)	Number of participants	Age of participants	Study location	Comparative group(s)	Study design	Rooibos type	Dosages	Duration of intervention	Major findings	(Refs.)
				Stone formers; Japanese green tea (2 tea bags per 125 ml of low mineral water per cup) per day)			4 cups each of fresh Pak rooibos tea (4 tea bags of per 250 ml of low mineral water) per day		creatinine) and Plasma TBARS (nmol/ml)] for oxidative stress Stone formers: ↓ Urinary volume and SS brushite	

^a500 ml of black rooibos tea with sugar was taken by participants. AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; ALP, Alkaline Phosphatase; GSH, Reduced Glutathione; GSSG, Oxidised Glutathione; GSH:GSSG, Reduced-oxidised Glutathione ratio; TG, Triglycerides; LDL, C-Low density Lipoprotein-cholesterol; HDL-C, High Density Lipoprotein Cholesterol; CD, Conjugated Dienes; TBARS, Thiobarbituric acid reactive substances; PINP, type I intact amino-terminal propeptide; NAG, N-acetyl-β-D-glucosaminidase; CRP, C-reactive protein; TIBC, Total iron binding capacity; IgE, Immunoglobulin E.

($\mu\text{mol/g}$ creatinine), N-acetyl-β-D-glucosaminidase (NAG) (U/g creatinine), or plasma TBARS (nmol/ml).

Effect of rooibos consumption on health outcomes among apparently healthy individuals. Some of the results reported by Marnewick *et al* (13) were contrary to those found in healthy individuals, whereas others were similar. Francisco (29) showed that acute supplementation with 500 ml fermented rooibos herbal tea (2% w/v) significantly lowered serum glucose after 2 h (-22%) and 6 h (-18%) of consumption in the treatment group, but did not have an effect on serum insulin. However, compared with the control group, serum blood glucose tended to decrease with acute supplementation of 0.76 g rooibos by 35.5% ($P \leq 0.005$) and 32.9% ($P \leq 0.005$) using glucose and bread and ham tests, respectively (24). Moreover, serum total cholesterol levels were significantly lower in the treatment group at 2 h (15%; $P \leq 0.0001$), 4 h (10%; $P \leq 0.001$), and 6 h (6%; $P \leq 0.0001$) after consumption. Rooibos consumption also significantly ($P \leq 0.05$; $P \leq 0.0001$) reduced HDL-C levels at 2 h (4%), 4 h (2%), and 6 h (0.3%) and LDL-C levels at 2 h (18%), 4 h (11%), and 6 h (7%) (29). Triglyceride (TG) levels were also significantly lower ($P \leq 0.05$, $P \leq 0.001$) after supplementation with rooibos at 4 h (97%) and 6 h (78%). CRP levels were significantly ($P \leq 0.05$) lower (8%) at six hours post ingestion (29) among individuals supplemented with rooibos. The conjugated diene (CDs) levels in individuals supplemented with rooibos were significantly ($P \leq 0.001$; $P \leq 0.0001$) decreased at 2 h (14%) and 4 h (14%) after ingestion of rooibos, while TBARS was significantly ($P \leq 0.001$) lowered at 4 h (59%) after the intake of rooibos when compared with that of the control group (29).

When comparing the effects of both fermented and unfermented rooibos tea, Villano *et al* (33) reported that acute supplementation with fermented rooibos tea increased blood glucose by 32.0% ($P \leq 0.001$), whereas unfermented rooibos tea increased blood glucose by 21.6% ($P \leq 0.001$) compared with the baseline. However, neither tea affected the TG, total cholesterol, or uric acid levels. The consumption of fermented and unfermented rooibos tea increased by 4.8 and 1.7%, respectively, in plasma total radical-trapping antioxidant potential (TRAP) assay after 30 min, reaching a statistically significant increase after 1 h (6.6%; $P \leq 0.05$, fermented; 2.9%; $P \leq 0.01$, unfermented) when compared with the baseline and control groups ($P \leq 0.05$). However, the TRAP values associated with fermented rooibos tea began to decrease at 2 h (4.9%; $P \leq 0.05$ compared to the baseline) and returned to the baseline values after 5 h (+2.2%) (33). The TRAP values for those who consumed unfermented rooibos tea continued to increase at 2 h (+2.7%; $P \leq 0.05$) when compared with the control group before returning to the baseline values after 5 h (33). Wanjiku (34) also reported that Ferric reducing antioxidant power assay (FRAP) assay values increased to reach their peak (610 $\mu\text{mol/l}$) at 45 min (an increase of 6.10%) and then subsequently decreased to 584 $\mu\text{mol/l}$ after 180 min ($P \geq 0.05$) after a single dose of 500 ml of fermented rooibos in healthy individuals. ORAC values also increased slightly from a baseline value of 31.4 to 32.4 $\mu\text{mol TE/ml}$ (an increase of 3.18%) 45 min after the ingestion of the rooibos, remaining at this level up to 180 min ($P > 0.05$).

Table III. Bioavailability of rooibos flavonoids found in urine and plasma.

Author(s)	Sample size characteristics				Study methods			(Refs.)	
	Number of participants	Age of participants	Study location	Type of participants	Study design	Rooibos type	Study duration		
Breiter <i>et al</i>	12 men	Ages of 21 to 35 years	Germany	Apparently healthy men with body mass indices between 20.0 and 27.4 kg/m ²	Randomized cross over trial	10 g of unfermented rooibos tea leaves of 500 ml of water	5 weeks	Four different metabolites of aspalathin (sulphated, glucuronidase, methylated, and both glucuronidated and methylated, one metabolite of its aglycone (glucuronidated aglycone), one metabolite of nothofagin (glucuronidated) and one of its aglycone (glucuronidated aglycone) were detected Metabolites identified in plasma include aspalathin isoorientin, orientin, and vitexin	(12)
Courts and Williamson	6 (two men, and four women)	Ages of 22–28 years. They used neither routine nor spontaneous medication	UK	Apparently healthy individuals with a BMI range of 19–27 kg/m ²	Cross-sectional study design	14 g of green rooibos tea leaves per 1 l of water ^b	11 days	3-O-methylated aspalathin (O-MA) and 3-O-MA glucuronide excretion was detected but 4-O-MA was not detected. Urinary: Aspalathin metabolites was detected in the first sample (0–2 h) and remained in the urine of all subjects for 6 h after oral exposure to aspalathin	(24)
Stalmach <i>et al</i>	10 (five men and five women)	Not specified	Rome	Apparently healthy participants	Randomized controlled study design	500 ml of ‘ready-to-drink’ rooibos tea produced from unfermented	4 days	Rooibos teas: Eriodictyol-C-glucoside, luteolin-6-C-glucoside, luteolin-8-C-glucoside aspalathin, quercetin-O-	(11)

Table III. Continued.

Author(s)	Sample size characteristics			Study methods			Findings	(Refs.)
	Number of participants	Age of participants	Study Location	Type of participants	Study design	Rooibos type		
						leaves and fermented leaves ^a	rutinoides, apigenin-8-C-glucoside, apigenin-6-C-glucoside, quercetin-3-O-rutinoides, quercetin-3-O-galactoside, quercetin-3-O-glucoside, nothofagin, quercetin, luteolin Urine: Metabolites detected in urine samples after ingestion of both fermented and unfermented rooibos tea include aspalathin, nothofagin, eriodictyol-C-glucoside, luteolin-8-C-glucoside, luteolin-6-C-glucoside, apigenin-8-C-glucoside, apigenin-6-C-glucoside quercetin-3-O-galactoside, quercetin-3-O-glucoside, quercetin-3-O-rutinoides, quercetin-O-rutinoides isomer luteolin, quercetin Plasma: No flavonoids or their metabolites were detected	

^a Authors failed to report the exact quantity of rooibos tea leaves used in the preparation of the rooibos tea; ^b300 ml of rooibos tea was consumed by participants.

Contradictory results have also been reported for iron status. Acute supplementation with rooibos among adults (10) resulted in lower ferritin ($54.0 \pm 42.6 \mu\text{g/ml}$) levels compared to those in individuals supplemented with ordinary tea ($80.9 \pm 48.7 \mu\text{g/ml}$) and water ($68.1 \pm 57.9 \mu\text{g/ml}$). Nonetheless, these individuals had the highest serum iron levels ($22.7 \pm 7.6 \mu\text{mol/l}$) compared to those taking ordinary tea ($21.3 \pm 6.2 \mu\text{mol/l}$) and water ($21.7 \pm 4.2 \mu\text{mol/l}$) (10). However, Marnewick *et al* (14) reported no significant changes in iron status indicators (serum iron, ferritin, transferrin, total iron binding capacity (TIBC), and % Fe saturation), C-reactive Protein (CRP), and homocysteine levels among individuals at risk of coronary heart disease after six weeks of chronic supplementation. This was contrary to the results of Breet *et al* (23), who found increases in serum iron (S-Fe) ($1.378 \mu\text{mol/l}$; 10.69%), transferrin saturation (TS) (1.17%), and haemoglobin (Hb) (0.29; 2.34%). They also found a decrease in serum ferritin (S-Fer) ($0.20 \mu\text{g/l}$; 6.70%) among children after chronic supplementation, although it was statistically insignificant after chronic supplementation. Breet *et al* (23) reported a significant increase ($P \leq 0.001$) in the mean corpuscular volume (MCV) (1.66 fL; 2.20%), serum transferrin (S-Tfn) (0.18 g/l; 7.09%), and TIBC ($3.92 \mu\text{mol/l}$; 6.91%), as well as a significant decrease ($P \leq 0.0001$) in mean corpuscular Hb (MCH) (3.101 pg; 11.19%).

Acute supplementation with rooibos tea in young individuals (30) did not cause any significant changes in blood pressure or nitric oxide (NO) levels. However, Angiotensin-converting enzyme activity (ACE) was significantly reduced with the rooibos tea after 30 ($P \leq 0.01$) and 60 min ($P \leq 0.05$). A significant inhibition of ACE activity was also observed for genotype II 60 min after the intake of rooibos tea ($P \leq 0.05$). However, Davies *et al* (26) reported that the mean peripheral blood oxygen saturation decreased by 15% after three daily supplementations with three capsules of rooibos extract (after breakfast, lunch, and supper). However, resting heart rate increased by 19.12% and acute mountain sickness symptoms decreased by 37.5% compared to 80-90% of the data found in the literature (26).

When used as an ergogenic aid (27), peak torque extension (Nm) increased significantly by 7.85% in Bout 3 (31.6 ± 8.3) when compared with the placebo (29.3 ± 6.1) ($P = 0.21$). Peak torque flexion (Nm) also increased with rooibos intake by 10.80% in Bout 3 (39.0 ± 10.1) when compared with placebo (35.2 ± 7.6) ($P = 0.08$, effect size=0.42) and 10.33% in Bout 4 (36.3 ± 8.9) when compared with placebo (32.9 ± 7.2) ($P = 0.09$, effect size=0.42).

Discussion

The objective of this study was to identify all studies conducted on humans and summarize the descriptive characteristics of the participants. However, it specifically describes the different types of rooibos used and the flavonoids found in them. Moreover, various dosages and concentrations (of rooibos used) and their health outcomes were also evaluated.

First, three types of tea were used: green (unfermented), red (fermented), and black Rooibos teas. Aside from Hesseling and Joubert (35), almost all studies used either green or red Rooibos tea. Since black rooibos tea is no longer commercially produced, green and red rooibos currently dominate the market.

Through value addition, green rooibos tea has been produced in other forms, including extracts, cosmetic products, and animal foods (6). Red rooibos tea is produced by subjecting green leaves to natural fermentation. This gives red rooibos a distinct taste and aroma (3). The fermentation process results in the partial oxidation of the polyphenol content and subsequent colour change from natural green to reddish brown. Therefore, green rooibos contains approximately three times higher levels of total phenolic compounds than fermented rooibos (3,6). This was supported by Breiter *et al* (12), who reported that the free flavonoids luteolin and quercetin found in the green rooibos beverage were higher ($159 \pm 6.5 \mu\text{mol}$) than those in the red rooibos beverage ($84 \pm 2.9 \mu\text{mol}$). The high usage of fermented rooibos in previous studies could be attributed to the taste and aroma and the high solubility of rooibos tea in both cold and warm water.

Second, the review showed that the dosages used ranged from 200 ml (10) to 1,200 ml (13,14). High dosages were found to have a positive impact by improving various health parameters, against which rooibos was assessed during chronic supplementation. However, inconsistencies have been reported with acute supplementation. Low-dosage supplementation was also found to have no effect on chronic supplementation. Based on the data presented in this review, it appears that rooibos may have beneficial health effects in humans. These beneficial effects can be attributed to the various flavonoids found in rooibos. However, most of the flavonoids are absorbed and not bioavailable and quantified in both urine and plasma as reported by Stalmach *et al* (11), and Breiter *et al* (12). Interestingly, several flavonoids have been found to affect various chronic health conditions. A systematic review by Abdulai *et al* (36) reported that vitexin and isovitexin have an influence on several molecular drug points and pathophysiological and metabolic pathways involved in the development and progression of diabetes mellitus, and as such, have a positive effect on patients with diabetes mellitus. Therefore, it was not surprising that Francisco (29) and Chepulis *et al* (24) reported a reduction in glycaemia among their populations.

Aspalathin and nothofagin are particularly interesting. Stalmach *et al* (11) and Breiter *et al* (12) reported that they made up more than 90% of the metabolites found in rooibos. This was similar to the findings of Kazuno *et al* (37) after determining and quantifying glycosyl flavonoids using liquid chromatography-triple quadrupole mass spectrometry. Orientin and isoorientin are the flavone analogues of aspalathin. The 8-C and 6-C- β -D-glucopyranoside derivatives of luteolin and rutin are the major monomeric flavonoids found in rooibos (38). Because aspalathin targets several key enzymes involved in fatty acid synthesis and oxidation, leading to enhanced glucose and fat metabolism (39,40), it is not surprising that rooibos intake improves glucose concentrations in the blood and improves overall glycaemic control.

Compared to Aspalathin, nothofagin was found in lower quantities (12,38). The flavone analogues of nothofagin found in rooibos include vitexin and isovitexin, which are 8-C- and 6-C- β -D-glucopyranoside derivatives of apigenin, respectively (38). Marques *et al* (41), reports that supplementation with nothofagin can lead to diuretic, natriuretic, and potassium-sparing effects. Interestingly, the diuretic and reno-protective properties associated with nothofagin have

been linked to its ability to improve antioxidative capability and enhance plasma NO bioavailability (41). However, in humans, the consumption of rooibos does not reduce high blood pressure (30). This could be attributed to the period during which supplementation took place. Four weeks could be too short for an effective change in blood pressure to have taken place. Another reason for the lack of improvement in blood pressure can be deduced from the population used. Rooibos could affect blood pressure in hypertensive individuals as was shown in hypertensive animal models but not in apparently healthy individuals.

Moreover, the supplementation period could also affect the effects of rooibos. Acute and chronic supplementation with 500 ml of both red and green rooibos tends to have beneficial effects on both healthy individuals and individuals diagnosed with a high risk of coronary heart disease. However, the magnitude of the effects tended to differ between acute and chronic supplementation. Serum glucose, triglycerides, LDL-C, total cholesterol, conjugated dienes, and TBARS levels were all reduced with both acute and chronic supplementation. Reduced serum glucose levels prevent diseases and reduce the risk of coronary heart disease, stroke, kidney diseases, and vision and nerve problems. There were enhancements in HDL-C levels, oxidation status as oxidized glutathione, and the ratio of reduced and oxidized glutathione. A similar trend was observed for both acute and chronic supplementation. HDL-C and GSH/GSSG decreased with acute supplementation of 500 ml but increased with 1,200 ml of fermented rooibos per day for six weeks while oxidized glutathione increased with acute supplementation but decreased with chronic supplementation. These results suggest that both acute and chronic supplementation with fermented rooibos could be effective in preventing and treating people at a high risk of coronary disease.

Rooibos supplementation did not have any effect on iron status among adults who were at risk of coronary heart disease but tended to affect iron absorption among healthy individuals. High levels of iron have been indicated as a potent risk factor for coronary heart disease, especially acute myocardial infarction (42). Hesselning *et al* (10) reported low ferritin and high serum iron levels in individuals who were supplemented with rooibos compared with individuals who were supplemented with other teas. However, in children, rooibos had some effect on iron status indicators. It tends to increase serum iron, haemoglobin, transferrin saturation, MCV, transferrin, and total binding capacity, while decreasing serum ferritin and MCH. Therefore, the results indicate that rooibos may not be a good supplement for children, as it may result in iron deficiency. Low ferritin levels and high serum iron reported among adults could also indicate the effect of rooibos tea on iron absorption, leading to iron deficiency. According to Fan (43), polyphenols found in food can affect the absorption of both heme and non-heme iron across cells. Fan (43) indicated a dose-dependent inhibitory effect of polyphenols on heme iron absorption. This explains the unexpected influence of tea consumption on iron status, irrespective of whether an individual consumes a normal diet (43). However, this negative effect on iron absorption could be helpful and beneficial for individuals

who are at high risk of iron overload, as tea consumption decreases iron accumulation in thalassemia and hereditary hemochromatosis (43).

Finally, unlike green tea, which has an effect on kidney stone formation (44) and blood pressure (45,46), rooibos supplementation did not affect blood pressure, nitric oxide levels, or kidney stone formation but had an effect on ACE in humans. This was contradictory to the results reported by Webster *et al* (47), who assessed the effects of first-line FDC drug treatment on lipid levels, myocardial ischemic tolerance, oxidative stress markers, and vascular endothelial function in male Wistar rats. Moreover, 30 days of supplementation with rooibos did not affect urinary TBARS, urinary N-acetyl- β -d-glucosaminidase (NAG), or plasma TBARS in kidney stone formers. Even though plasma TBARS was affected by acute (29) and chronic (13) supplementation, plasma TBARS was not affected among kidney stone formers.

Conclusions

From the studies reviewed and conducted among humans, it can be concluded that of all the rooibos products available, mostly red and green rooibos, are consumed and that green rooibos contain more flavonoids than red rooibos. However, the dosages used in these human studies tend to yield contradictory results. For example, rooibos consumption of six cups per day (1,200 ml) improved TBARS, reduced blood glucose concentration, and improved insulin sensitivity among healthy individuals and those at risk of coronary heart disease; however, chronic consumption of four cups (500 ml) per day failed to have an impact on blood pressure and TBARS among kidney stone formers. Moreover, the number of human studies and sample sizes used make it very difficult to either confirm or refute the notion that rooibos has a significant effect on humans. Based on the different types of rooibos used and the contradictory results reported in these few human studies, a dose-response study that investigates the dosages that offer the greatest benefits to humans in individuals with both healthy and chronic conditions is warranted. Researchers should also investigate the effect of rooibos consumption in large-scale clinical studies with larger sample sizes.

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Authors' contributions

All authors had full access to the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. DA conceived and drafted the study. DA and LE were responsible for literature retrieval, data collection, and analysis. ET and BOE contributed to the revision of the manuscript. All authors contributed to the planning, conduct, interpretation of the data, reporting, drafting and finalization of the manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Conflict of interest

The authors declare no potential conflict of interest.

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