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# THE MODULATORY ROLE OF FERMENTED ROOIBOS ON OXIDATIVE STRESS ON SUBMAXIMAL EXERCISE PERFORMANCE IN ADULT MALES

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***ABSTRACT***

*Excessive production and accumulation of reactive oxygen and nitrogen species during exercise may cause oxidative stress and impact exercise performance. Rooibos with polyphenolic constituents may modulate the effects of oxidative stress during physical activities. This blinded randomised, cross-over placebo-controlled study included 30 adult males who consumed 375 ml of standardised fermented rooibos herbal extract or placebo beverage before completing a sub-maximal exercise, followed by 10 sprint bouts on a Wattbike. Rooibos appears to enhance the endogenous antioxidant defence system with increased levels of circulatory total and reduced glutathione in the participants and decreased levels in serum markers of muscle damage, creatinine kinase and aspartate aminotransferase. Mean submaximal trials (effort=75%–80% of maximum heart rate and/or 13 RPE Borg Scale) indicated after consuming rooibos, participants cycled for longer (291.83 s vs. 253.50 s: difference=14.05%), further (2790.30 m vs. 2434.93 m: difference=13.60%); produced 3.65% more power (W) and 2.42% more force (N). Mean cardiovascular responses during rooibos were modest when compared with the increased time and distance achieved, (134.55 bpm vs. 131.26 bpm: difference=2.40%) and relative oxygen uptake (27.32 ml/min/kg vs. 25.84 ml/min/kg: difference=5.53%). This suggests that rooibos may benefit metabolic responses during submaximal exercise, demonstrating its potential as an ergogenic aid.*

***Keywords:*** Antioxidant; Exercise; Oxidative stress; Performance; Polyphenols; Rooibos

## INTRODUCTION

The evidence about the health benefits of regular physical activity is well established (Piercy *et al*., 2018); however, under certain conditions, the increased oxygen consumption may lead to the continuous formation of reactive species, mostly reactive oxygen and nitrogen species (RONS) (Elejalde *et al*., 2021). During exercise, high oxygen consumption and utilisation results in the generation and accumulation of more RONS in skeletal muscles. This leads to exercise-induced oxidative stress and other ailments, such as fatigue, muscle damage, pain, and poor overall exercise performance, as well as prolonged recovery (Mastaloudis *et al*., 2001; Finaud *et al*., 2006; Powers & Jackson, 2008; Powers *et al*., 2020). Pingitore *et al*. (2015) reported that the relationship between exercise and oxidative stress is quite complex and it often depends on the mode, type, duration, and intensity of exercise. Regular, moderate exercise or training may also lead to increased oxidative stress; however, it appears to be more beneficial because the same stimulus is needed to strengthen the endogenous antioxidant defence system and other cellular molecular pathways, thereby improving the capacity of organisms and their ability to withstand greater stress. This exercise or training conditioning is referred to as exercise-induced hormesis (Peake *et al*., 2015). This means the impact of RONS depends on their concentration and may be harmful or beneficial because, at low and/or moderate concentrations, RONS are involved in physiological processes, e.g., cellular signalling, defence against infectious agents, upregulation of endogenous enzymatic antioxidant activity (Steinbacher & Eckl, 2015; Lesmana *et al*., 2022) and conditioning of the endogenous antioxidant system (D'Angelo & Rosa, 2020). However, at high concentrations, RONS may lead to the occurrence of oxidative stress and subsequent damage to important cellular macromolecules, such as lipids, proteins, and genetic material, resulting in alterations to their functions (Gupta *et al*., 2014; Elejalde *et al*., 2021).

Additionally, during exercise or physical activities, the body is exposed to other various forms of stress (thermal, metabolic, hypoxic and mechanical stress), which often trigger biochemical messengers, signalling pathways or other adaptive responses (Peake *et al*., 2015). Naturally, living beings are equipped with a complex network of an endogenous antioxidant defence system that can neutralise and/or stabilise these RONS (Poljsak *et al*., 2013; Elejalde *et al*., 2021). However, this system is not always 100% effective, especially in cases of excessive RONS production. Also, the natural effectiveness of the antioxidant defence system tends to decline with age, infection and/or when an individual's immune system is compromised (Yokota *et al*., 2001). Therefore, to mitigate the effects of RONS and other effects during exercise or physical activity, the intake of additional oral antioxidant supplements (exogenous antioxidants) to support the endogenous antioxidant defence represents a suitable noninvasive tool for preventing or reducing the deleterious effects of oxidative stress. However, excessive use of exogenous antioxidants may have detrimental effects on both the health and exercise performance of individuals (Teixeira *et al*., 2009; Theodorou *et al*., 2011; Morrison *et al*., 2015), indicating a need for balance. Hence, it is important to continuously support the endogenous antioxidant system and replace the antioxidant sources in the body, through the intake of naturally occurring antioxidant, so that when the body is subjected to a redox imbalance, it will be in a better position to defend itself (Elejalde *et al*., 2021). To date, there is no official protocol, approach or practice used to prevent or mitigate exercise-induced muscle fatigue (Wan *et al*., 2017). Nonetheless, many athletes opt to use certain non-specific treatments (synthetic drugs) or preventative approaches such as high/mega doses of single antioxidant

supplements. These approaches do have drawbacks such as high costs, may act as stimulant drugs, and can only be obtained through a medical prescription, and may result in serious side effects and low tolerance. Additionally, the safety and efficacy of these drugs have been questioned in some studies (Nieman *et al*., 2006; Wan *et al*., 2017). Therefore, the quest for novel or alternative interventions continues and more studies are investigating the use of dietary natural polyphenolics, as they contain components with differing structures and resultant differing mechanistic bioactivities, as possible nutraceutical supplements or ergogenic aids.

Rooibos, made from the plant *Aspalathus linearis*, is a popular indigenous South African herbal tea, harvested in the Cederberg mountainous region of South Africa, Western Cape Province. In the recent past, rooibos gained the attention of many researchers due to its rich and unique phytochemical and polyphenolic content and related health-promoting bioactivities, which are of potential health use to prevent, detoxify and attenuate exercise-induced oxidative stress effects, as well as improve exercise performance and recovery (Nagasawa & Kaori, 2017; Davies *et al*., 2019a). Although the antioxidant effects of rooibos herbal tea have been investigated and well documented (Marnewick *et al*., 2005; 2009; Persson *et al*., 2010; Marnewick *et al*., 2011; Chen *et al*., 2013; Canda *et al*., 2014; Smith & Swart, 2016; Dludla *et al*., 2017; 2020), its bioactivities in humans, concerning exercise-induced oxidative stress and exercise performance and recovery, remain debatable (Utter *et al*., 2010; Nagasawa & Kaori, 2017; Davies *et al*., 2019a).

## PURPOSE OF THE RESEARCH

This study employed a placebo-controlled dietary intervention model to assess the modulation of exercise-induced oxidative stress using a standardised fermented rooibos beverage and to elucidate further the effect of rooibos ingestion on submaximal exercise performance outcomes for the participants.

## MATERIALS AND METHODS

### Study design and sample size

The study design included a randomised, single-blinded, placebo-controlled cross-over intervention trial (RCIT), where healthy adult males (18–50 years old) consumed an acute dose of either fermented rooibos or placebo beverage (375 ml) together with a standardised snack, waiting 90 minutes (to ensure optimal bioavailability) before completing a modified submaximal exercise ramp test with 10 sets of sprints, on a Wattbike (Wattbike Ltd, UK) cycle ergometer. The study sample size was determined by the university statistician using t-test of a G-Power 3.1 software program with input: tail(s)=one; effect size dz=0.3; α error probability=0.05; power (1–β error probability)=0.8 and output: non-centrality parameter δ=2.5278449; critical t=1.6669145; df=70; total sample size=71; actual power=0.804. This sample size was for the full group thus requiring 36 per intervention, but 40 participants were recruited to provide for any participants who may not have completed the study or for non-compliance.

### Participant recruitment and screening

Potential participants were recruited by posting advertisements and leaflets at the study site, the laboratory, situated on the university campus, and distributing leaflets to various sports

clubs in the Cape Town area. Interested individuals were screened for study eligibility with inclusion criteria including physically active males with no current or past history of using anabolic steroids, not on any chronic illness medication (hypertension, hyperglycaemia, hypercholesterolemia or metabolic disorders), not using any ergogenic nutritional/antioxidant supplements, not consuming more than two alcohol beverages per day; no unusual dietary habits (e.g., vegetarian or vegan diets), and not having sustained any muscular or skeletal injury in the 2 months before the study. The participants were recruited because they represented a reasonable cross-section of habitually physically active young men from the general population with no apparent health issues. This convention aligns with the recommendations as advocated by Fox (1971) and Shookster (2020) for participant selection when utilising the calculation of 220 minus age heart rate as an indication of physiological strain during exercise. Additionally, screened fingerprick blood values were expected to be within the normal reference ranges for total cholesterol (3.5–5.0 mmol/L), fasting glucose (5. 6–7.0 mmol/L), haemoglobin (13.3–17.2 g/dL) and blood pressure (between 120/80 mmHg and 140/90 mmHg) as per normal reference ranges published by Ampath laboratory (South Africa) website https://www.ampath.co.za/pdfs/Desk-Reference-web.pdf. Exclusion criteria included females, as well as males who did not meet the aforementioned inclusion criteria. Those who met the inclusion criteria were recruited into the study. Because of differences in skeletal muscles mass and hormonal response to exercise between males and females (Accattato *et al*., 2017; Copetti *et al*., 2020) and in an attempt to have a homogeneous group, only males were recruited for this study.

**Randomisation and dietary intake**

Each study participant was assigned a unique study code that was used on all the blood sampling tubes, dietary records and health and fitness questionnaires that the participants completed, and as such none of the data collected contained any direct identifiers of the participants. Participants were randomly assigned into two groups, achieved by dividing them into two intervention groups using an online programme (randomizer.org). One group first received the rooibos intervention beverage and the other the placebo intervention beverage, while after a 7-day washout period the groups were reversed. Recruited participants were requested to keep dietary records for three non-consecutive days including two weekdays and one weekend day during the week before their exercise days. To assist them with this, each received a set of household measuring items (teaspoons, cups and a jar), dietary record booklets and a dietary restriction list (to ensure a minimal intake of flavonoid-rich foods and beverages). The participants were also asked not to change dietary habits and physical activity during the study period. However, participants were instructed to abstain from exercise for 48 h and avoid alcohol intake 24 h before their exercise days.

**Intervention beverages**

The intervention beverages (rooibos and placebo) were freshly prepared on the morning of exercise days. The placebo beverage was prepared by dissolving a store-bought powdered peach apricot-flavoured sachet (13.11 g) in 375 ml of still bottled water. In contrast, the rooibos beverage was prepared by dissolving the powdered fermented rooibos herbal tea extract (1.6 g equivalent to five cups of fermented rooibos) in 375 ml of peach apricot-flavoured still water. The cold water-soluble fermented rooibos extract was provided by Rooibos Ltd (Clanwilliam, South Africa).

**Study exercise test protocol and cross-over design**

After an overnight fast (8–12 h), participants reported at the Human Performance Laboratory of the university and were given a standardised light breakfast (162.8 g, two slices of white bread topped with margarine, pastrami and cheese) and consumed 375 ml of either the rooibos or placebo intervention beverage in an opaque bottle to ensure blinding of the study participant. This was done to ensure participants were blinded to their intervention and would not be subconsciously influenced to adapt their exercise performance. Ninety minutes after consuming the intervention liquid, participants commenced with the modified Wattbike submaximal ramp test protocol as detailed below:

- Five minutes warm-up, at 50–60 rpm on air resistance setting 3 on the Wattbike;
- Participant study code, age, mass (kg) and sex (male) were entered into the Wattbike computer and the maximum heart rate was predicted (HRmax=208–0.7×age);
- Pedal in a seated position for 1 minute at the starting power of 100 watts (W), at a cadence rate of between 70 and 100 rpm;
- Air resistance was increased and/or cadence adjusted as necessary every 1 minute to ensure a 15 W increase in power (W) output every 1 minute, to allow the body to adapt to the increasing workload and steady rate heart rate to be achieved (at that level);
- Power (W) output was increased by 15 W every minute until the participant reached the rate of perceived exertion (RPE) of somewhat hard-level 13 on the Borg scale RPE (approximately equivalent to submaximal exertion of 75%–80% maximum heart rate). Maximal heart rate was calculated using 220 minus age as described by Fox *et al*. (1971) and endorsed by Shookster *et al*. (2020), who in their review of age-predicted maximal heart rate equations indicate that the Fox equation is the best option for a general population;
- The test would be terminated if the rider/participant experienced any adverse symptoms during the exercise test prior to achieving their sub-maximum exertion of 75%–80% as per heart rate and/or prior to the participant rating the exertion level, as per the Borg scale, at 13 (somewhat hard);
- The test would also be terminated if the participant experienced an emergency (physical and/or mental) e.g. the participant felt unwell and/or became overly anxious;
- Once the participant reached level 13 on the Borg scale and/or a submaximal heart rate equivalent to 75-80%, he stopped cycling for 1 minute and was then asked to complete the second phase of the exercise test that included full-out 10-s sprints (with the aim of completing a total of 10 sprints or until the participant reached an RPE of maximal exertion-level 20 on the Borg scale), with each 10-s sprint separated by 15 s of passive recovery rest periods.

Cardiorespiratory data, notably relative oxygen uptake, was collected through a breath-by-breath analysis done on the CosMed Metabolic System (CosMed, The Metabolic Company, Italy). Participant heart rates were also monitored throughout the exercise regime by placing a heart rate signal (heart rate monitor: Polar, Finland) around the participant's chest with a beat per minute (bpm) recorded. This data (bpm) was transferred continually (via Bluetooth) to the Wattbike computer monitor for integration with other exercise data. Each participant attended two of these exercise sessions 7 days apart, serving as the washout period (Figure 1). The 7-day period was deemed sufficient to avoid any flavonoid carry-over effect from the first

intervention, as flavonoids are known to have a short elimination half-life (Scalbert & Williamson, 2000).

**Anthropometric and blood pressure measurement**

The anthropometrics, including stature, mass, and waist circumference, were recorded for all participants at the start of their first session. A digital weighing scale (Soehnle Professional, South Africa) was used to determine their mass (kg), while a free-standing stadiometer (Scales 2000, Rochdale Park, Durban, South Africa) was used to measure the stature (m). A 2-m non-stretchable measuring tape was used to measure the waist circumference, and the body mass index (BMI) was calculated manually using the standard formula (mass [kg]/height [$m^2$]). Resting blood pressure (systolic and diastolic) was measured on three occasions (baseline, before the first blood sample collected and then at 1 h and 24 h post-exercise) per session (Rossmax Medical®, Kraaifontein, South Africa).

**Blood sample collection and processing**

About 5 ml of blood was drawn from the participant's antecubital vein into a serum separate tube (SST), 3 ml into sodium fluoride (NaF) tube and 10 ml into ethylenediaminetetraacetic acid (EDTA) tubes. Blood samples were collected at five different time points (0 h – also referred to as baseline, 90 min after the ingestion of the study beverage and snack but before the exercise regime started, immediately after the exercise was completed (IAE), 1 h and 24 h post-exercise) during each session. Both 0 h and 24 h bloods were fasting samples. After collection, 100 µl of the EDTA-collected blood was aliquoted into a 2-ml Eppendorf tube for oxidised glutathione (GSSG) analysis. Another 50 µl of EDTA-collected blood was aliquoted into a 2 ml Eppendorf tube for reduced glutathione (GSH) analysis. The remaining blood in the EDTA tube was centrifuged at 2000× g, 4 °C for 10 min and thereafter aliquoted into different Eppendorf tubes. The NaF blood samples were centrifuged and aliquoted for glucose analysis, while the SST blood samples were first allowed to clot before being centrifuged to obtain serum aliquots. All sample aliquots were stored at –80 °C until the time of analysis.

**Blood biochemical analyses**

A method described by Singleton and Rossi (1965) was used to assess study beverages (fermented rooibos and placebo) and the total polyphenolic content (TPC) in the participants' plasma samples. Two assays, the ferric reducing antioxidant power (FRAP) assay and the trolox equivalent antioxidant capacity (TEAC) assay were used to determine the antioxidant capacity of the study beverages and the collected participant blood samples. These two assays were performed as described by Benzie and Strain (1996) and Re *et al*. (1999), respectively.

Assessment of participant blood redox status, including the total and reduced glutathione (tGSH and GSH) was carried out as described by Asensi *et al*. (1999). Lipid peroxidation was assessed by means of measuring oxidative damaged lipid products consisting of conjugated dienes (CDs), an earlier marker and thiobarbituric acid reactive substances (TBARS), a late marker. Study participants' plasma CDs and TBARS were measured as described by Glender and Recknagel (1984) and Draper *et al*. (1993), respectively. An automated chemistry analyser, Medica Easy Random Access (EasyRA, Massachusetts, USA) was used to analyse serum analytes including creatine kinase (CK), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as muscle damage biomarkers (Ferreira *et al*., 2020).

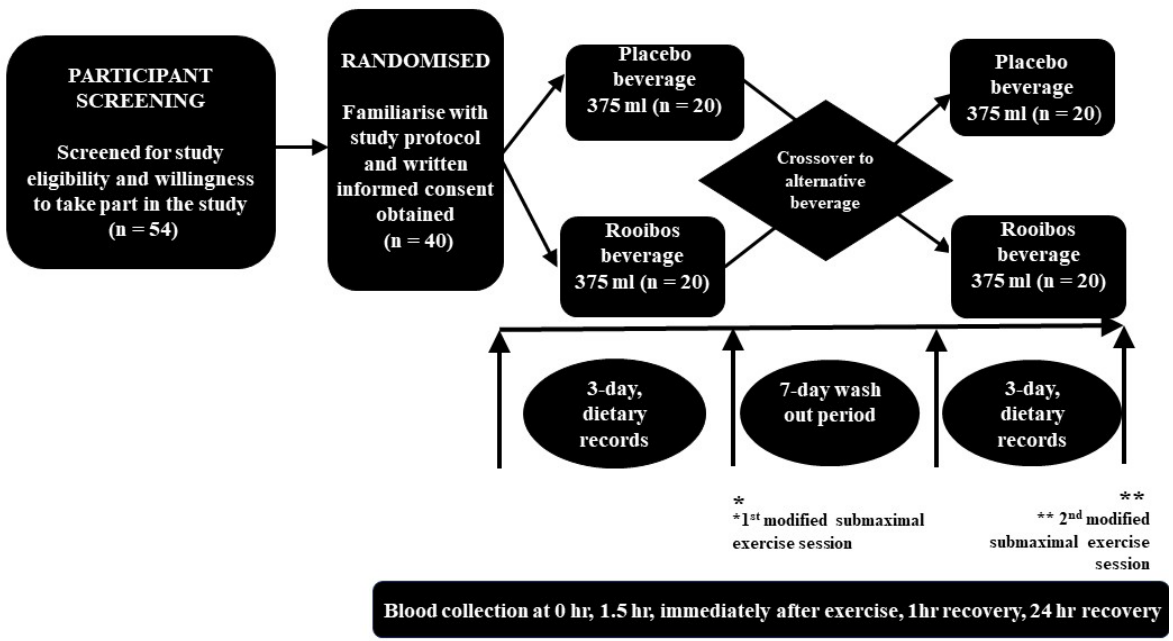


*Figure 1.* **SCHEMATIC DIAGRAM SHOWING THE STUDY TRIAL DESIGN AND PARTICIPANT FLOW**

### Submaximal exercise performance indicators

Submaximal exercise performance data included time (s) cycled to the withdrawal point for each participant along with the distance covered (m) and cycle performance parameters measured via the Wattbike computer monitor including power (W) and force (Newtons), which were downloaded via USB connection.

### Statistical data analysis

Data are presented as means and standard deviations (SDs). Statistical analyses for data and all assays were done in Excel 2016, Window 10 (Microsoft Office, USA) using a data analysis toolpack. The within-group (intragroup) analysis for both placebo and rooibos were performed using GraphPad Prism 5.00 software (San Diego, California, USA) as a repeated measure using analysis of variance (ANOVA), followed by post-hoc Bonferroni's multiple comparison test. Pearson correlation analyses were performed to analyse possible relationships between analysed plasma antioxidant capacity, muscle damage biomarkers and measured performance parameters. The results of all analyses were considered statistically significant at 5% ($p$<0.05).

### Ethical considerations

The Human Research Ethics Committee approved the study protocol (Project number REC 2018/H2) and followed guidelines as stated in the Declaration of Helsinki. Written informed consent was obtained from the study participants before the commencement of the study.

## RESULTS

### Characterisation of study participants

A total of 54 participants were screened for study eligibility. Forty physically active adult males (18–50 years old) adhered to the study criteria and were enrolled, randomly allocated to an intervention or control group, and participated in the cross-over trial. Twenty participants started with the placebo intervention beverage and the other 20 participants with the rooibos intervention beverage (Figure 1). Data of some participants were excluded during data and blood analyses as indicated in Figure 2.

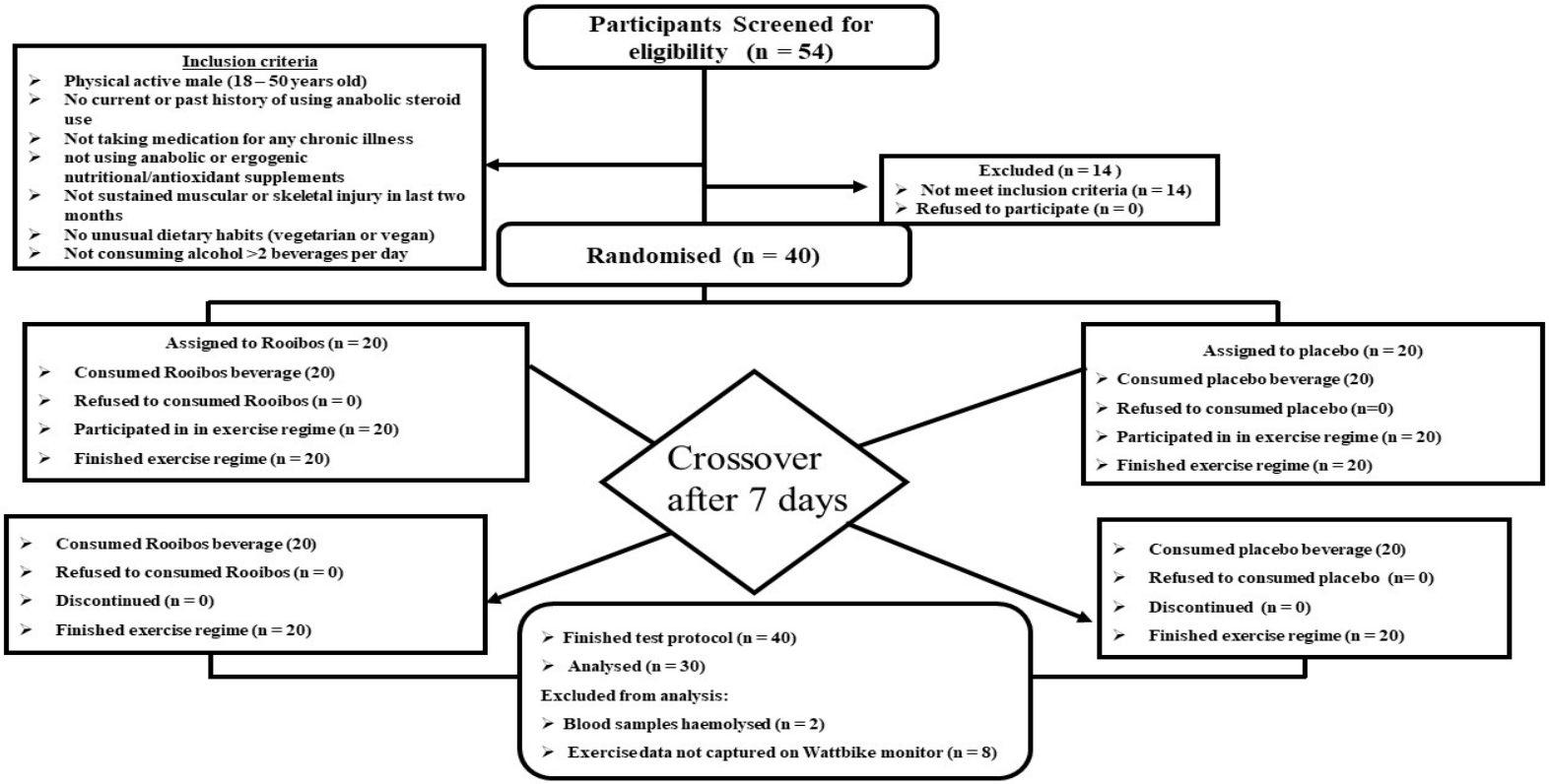


*Figure 2.* **CONSOLIDATED STANDARDS OF REPORTING TRIALS (CONSORT) FLOW DIAGRAM**

The final study cohort included 30 participants (*n*=30), and their anthropometrics and general health data are presented in Table 1.

*Table 1.* **ANTHROPOMETRIC AND GENERAL HEALTH CHARACTER-ISTICS OF STUDY PARTICIPANTS (*n*=30)**

| Variable | Mean (SD) |
|---|---|
| Age (years) | 26.23 (6.53) |
| Mass (kg) | 73.45 (14.31) |
| Stature (cm) | 1.73 (0.08) |
| Waist circumference (cm) | 102.00 (8.38) |
| BMI (kg/m) | 24.45 (4.24) |
| Resting SBP (mmHg) | 128.96 (14.39) |
| Resting DBP (mmHg) | 75.58 (8.44) |
| Hb (g/dl) | 16.25 (1.23) |
| Physical exercise frequency (days per week) | 3 |
| Physical exercise intensity | Moderate and vigorous |
| Cholesterol (mmol/l) | 4.77 (0.62) |
| Glucose (mml/l) | 4.25 (0.73) |

Values in columns are expressed as mean and standard deviation (SD).
BMI=body mass index DBP=diastolic blood pressure SBP=systolic blood pressure Hb=haemoglobin.

**Characterisation of the fermented rooibos and placebo study beverages**

The antioxidant content of the study beverages was quantified in terms of the total phenolic, flavanol and flavonol content using spectrophotometric analyses. As shown in Table 2, main rooibos phenolic compounds were identified by high performance liquid chromatography (HPLC) analysis and only the rooibos beverage contained phenolic constituents when compared with the placebo beverage. When considering the antioxidant capacity, the rooibos beverage showed a far superior antioxidant capacity (FRAP and TEAC) when comparing the two study beverages. The HPLC analysis authenticated the rooibos beverage by showing the presence of the unique rooibos dihydrochalcone, aspalathin, and confirmed that it was fermented rooibos, with the orientin and isoorientin quantities exceeding that of aspalathin (Table 2). The 1.6 g rooibos extract contributed 333.42 mg total polyphenols, which can be equated to about five cups of fermented rooibos.

*Table 2.* **QUANTITATIVE DESCRIPTION OF THE ROOIBOS AND PLACEBO STUDY BEVERAGES.**

| | Rooibos, mg/375 ml (SD) | Placebo, mg/375 ml (SD) |
|---|---|---|
| Flavonols (mg QE) | 93.7 (1.67) | 11.3 (5.03) |
| Flavanols (mg CE) | 37.6 (3.67) | 0 (1.60) |
| TPC (mg GAE) | 333.5 (1.72) | 0 (0.89) |
| FRAP (µmol AAE) | 1701 (4.55) | 52.6 (2.86) |
| TEAC (µmol TE) | 2118 (19.29) | ND (1.49) |
| Aspalathin | 8.37 (0.04) | ND |
| Orientin | 6.69 (0.03) | ND |
| Isoorientin | 15.50 (0.17) | ND |
| Isovitexin | 2.52 (0.05) | ND |
| Vitexin | 1.95 (0.03) | ND |
| Hyperroside | 7.26 (0.05) | ND |
| Quercetin | 2.37 (0.02) | ND |
| Luteolin | 0.57 (0.02) | ND |
| Chrysoeriol | 0.11 (0.00) | ND |

Values in columns are expressed as mean and standard deviation (SD), participants ($n$ = 30). GAE=gallic acid equivalents AAE=ascorbic acid equivalents TE=trolox equivalents TPC=total polyphenolic content FRAP=ferric reducing antioxidant power TEAC=trolox equivalent antioxidant capacity CE=catechin equivalent QE=quercetin equivalent ND=not detected.

**Analysed biochemical blood markers**

***Plasma total phenolic content and antioxidant capacity***

There were no significant ($p$=0.502 for TPC, $p$=0.628 for FRAP and $p$=0.994 for TEAC) intergroup differences in the antioxidant content and capacity measured when comparing the two intervention beverages (Table 3). When considering the intragroup differences, unlike placebo, fermented rooibos consumption significantly ($p$=0.03) increased participants' plasma TPC at 1.5 h and 1 h post-exercise when compared with baseline levels. The increased plasma TPC at 1.5 h corresponded with the significant ($p$= 0.015) increased plasma antioxiodant capacity (measured as FRAP) at 1.5 h when compared with baseline levels when rooibos was consumed. At 24 h post-exercise, plasma antioxidant capacity (FRAP) significantly ($p$=0.0001) decreased when compared with 1 h post-exercise when placebo was consumed, but such did not happen when rooibos was consumed. Rooibos consumption significantly ($p$=0.005) decreased intragroup plasma antioxidant capacity (TEAC) at 1 h post-exercise when compared with IAE levels, and then significantly ($p$=0.0021) increased at 24 h post-exercise when compared with one hour post-exercise.

*Table 3.* **EFFECTS OF STUDY BEVERAGES ON PARTICIPANT (N=30) PLASMA POLYPHENOL CONTENT AND ANTI-OXIDANT CAPACITY**

| Different blood collection time points | Biomarker | | |
|---|---|---|---|
| | TPC (mg GAE) | TEAC (µmol TE) | FRAP (µmol AAE) |
| 0 h PL | 1523±17 | 7723±651 | 594±17 |
| 0 h Rb | 1512±19 | 8072±508 | 587±20 |
| 1.5 h PL | 1550±19 (1.77) | 7909±708 (2.41) | 629±20 (5.90) |
| 1.5 h Rb | 1542±17[a] (1.98) | 7790±576 (-3.49) | 635±20[e] (8.17) |
| IAE PL | 1622±19 (6.50) | 8060±511(4.36) | 650±18 (9.43) |
| IAE Rb | 1633±22 (8.00) | 8312±502 (2.97) | 659 ±19 (12.26) |
| 1 h PL | 1561±20 (2.50) | 7992±518 (3.48) | 826±23 (39.06) |
| 1 h Rb | 1562 ±19[b] (3.31) | 7635±474[c] (-5.42) | 839±33 (42.94) |
| 24 h PL | 1566±22 (2.83) | 8215±512 (6.37) | 639±22[f] (7.58) |
| 24 h Rb | 1545 ±22 (2.18 | 8107±502[d] (0.43) | 658±12(12.09) |

Values in columns are expressed as the mean ± standard error of the mean (SEM) at different time points during the study, and values in brackets indicate the percentage difference when compared with 0 h (baseline).
**No intergroup significant differences were noted**. Significant intragroup differences are as follows: [a]$p$=**0.03**, [b]$p$=**0.007** and [e]$p$=**0.015** compared with 0 h rooibos, [c]$p$=**0.005**) compared with IAE rooibos, [f]$p$<**0.0001** compared with 1 h placebo; [d]$p$=**0.0021** compared with 1 h rooibos. GAE=gallic acid equivalents AAE=ascorbic acid equivalents TE=trolox equivalents TPC=total polyphenolic content FRAP=ferric reducing antioxidant power TEAC=trolox equivalent antioxidant capacity IAE=immediately after exercise PL=placebo Rb=rooibos intergroup=comparing time point differences between the two study interventions intragroup=comparing time point differences within each intervention.

*Table 4.* **EFFECTS OF THE TWO INTERVENTION BEVERAGES ON PARTICIPANT (N = 30) LIPID OXIDATIVE DAMAGE BIOMARKERS**

| Different blood collection time points | Biomarker | |
|---|---|---|
| | CDs(nmol/ ml) | TBARS (nmol/ ml) |
| 0 h PL | 13.98 (0.41) | 10.37 (0.15) |
| 0 h Rb | 13.92 (0.47) | 10.40 (0.16) |
| 1.5 h PL | 13.65 (0.44) | 9.95 (0.19) |
| 1.5 h Rb | 13.92 (0.40) | 9.61 (0.18) |
| IAE PL | 14.68 (0.40)[a] | 10.49 (0.14) |
| IAE Rb | 14.41 (0.44) | 10.22 (0.17) |
| 1 h PL | 14.77 (0.55)[a] | 10.36 (0.20) |
| 1 h Rb | 14.42 (0.51) | 10.06 (0.16) |
| 24 h PL | 14.71 (0.37) | 10.64 (0.19) |
| 24 h Rb | 15.03 (0.33) | 10.41 (0.20) |

CDs=conjugated denies TBARS=thiobarbituric acid reactive substance PL=placebo Rb=rooibos. Values in columns are expressed as mean±standard error of the mean (SEM) at different time points during the study and values in brackets indicates % difference when compared with 0 h (baseline).
Significant intragroup differences are as follows: [a]$p$=**0.002** compared with 0 h placebo.

### *Blood glutathione redox status and lipid damage biomarkers*

Effects of acute consumption of the two intervention study beverages on participants' blood redox status biomarkers with significant ($p$<0.05) intergroup differences are shown in Figure 3 (A, B). The intergroup results showed a significant ($p$=0.021 and p=0.003) increase (14.43% and 17.49%) in blood total GSH (tGSH) levels and also a significant ($p$=0.017 and $p$=0.003) increase (15.39% and 17.88%) in blood GSH levels at 1.5 h and 24 h post-exercise, respectively, when rooibos beverage was consumed and compared with the placebo. No significant ($p$=0.635 and $p$=0.343 intergroup differences were noted in participants' blood GSSG and GSH/GSSG ratio levels. For the plasma lipid oxidative damage biomarkers (CDs and TBARS), no significant ($p$=0.481 and $p$=0.753) intergroup differences was noted. However, intragroup results (Table 4) showed a significant ($p$=0.002) increase in plasma CDs levels at IAE and 1 h post-exercise when compared with 1.5 h levels in the placebo group, while the rooibos beverage consumption did not result in these changes.

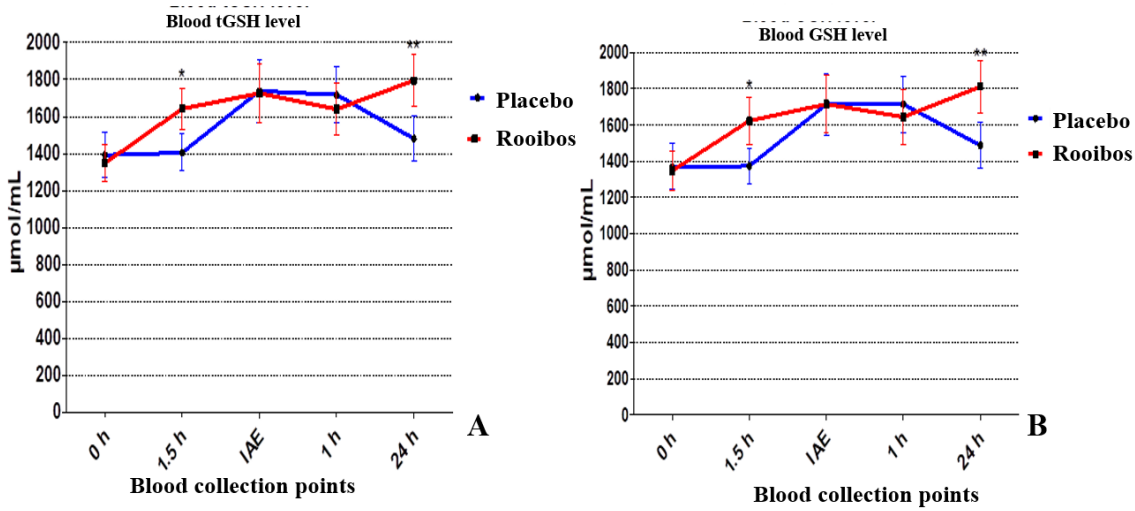


*Figure 3.* **EFFECT OF THE TWO STUDY BEVERAGES ON PARTICIPANT (N = 30) BLOOD GLUTATHIONE REDOX STATUS**

(A) Total glutathione and (B) reduced glutathione levels. Values are expressed as mean±standard error of the mean (SEM).

*, **$p$<0.05 when comparing the rooibos with the placebo intervention at the specific time points.

IAE= immediately after exercise GSH=reduced glutathione tGSH=total glutathione

### Muscle damage markers

The effects of the two study beverages on participants' serum muscle damage markers with significant ($p$<0.05) intergroup differences are shown in Figure 4 (A–C). Intergroup comparison results show that when rooibos was consumed, muscle damage indicator, serum CK level, was significantly ($p$=0.022) decreased by 25.38% at 1.5 h when compared with placebo level. Rooibos consumption also significantly ($p$<0.0001, $p$=0.002 and $p$=0.001) decreased serum aspartate aminotransferase (AST) by 17.26%, 12.88% and 13.33% at 1.5 h, IAE and 1 h post-exercise, respectively. At 1.5 h and IAE, when compared with placebo,

rooibos consumption significantly (p=0.007 and p=0.051) decreased serum alanine aminotransferase (ALT) levels by 19% and 15.93%, respectively. All these changes in muscles damage biomarkers still remained within the analytes' normal diagnostic reference ranges. When considering the intragroup results, significant ($p$<0.001) changes were observed in participant serum AST levels when both beverages were consumed, but no significant ($p$=0.340, $p$=0.337) changes in participant serum ALT levels were observed when either beverage (placebo or rooibos) was consumed. However, serum CK levels significantly ($p$=0.003) decreased at 1 h post-exercise compared with IAE serum levels when rooibos was consumed, but such changes did not occur when the placebo beverage was consumed.

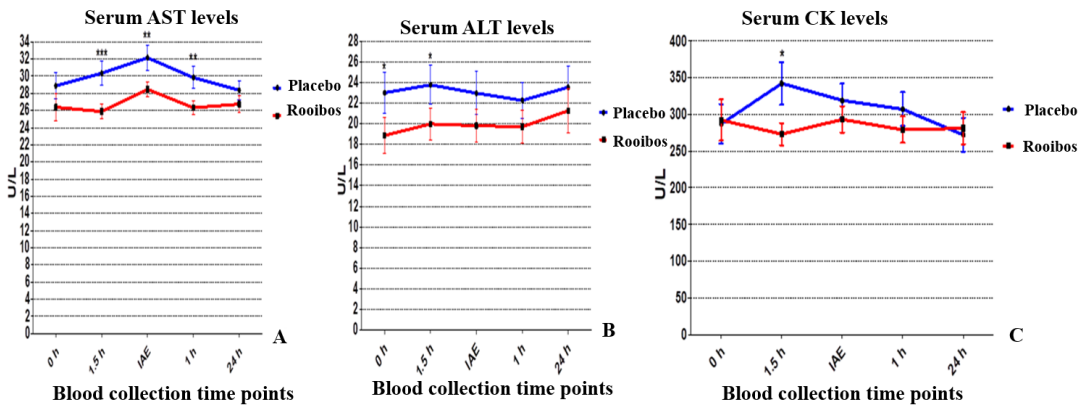


*Figure 4.* **EFFECTS OF STUDY BEVERAGES ON MUSCLE DAMAGE ANALYTES**

(A) Serum AST; (B) serum ALT; and (C) serum CK.
*, **$p$<0.05 when comparing rooibos with the placebo intervention at the various time points.
IAE=immediately after exercise ALT=alanine aminotransferase AST=aspartate aminotransferase CK=creatinine kinase

## Submaximal exercise performance

The effect of the intervention of study beverages on participants' exercise performance during the submaximal (75%–80%) ramp test on a Wattbike (Figure 5A–D) shows that during the submaximal phase of the test the participants on average exercised for 14.96% longer (254 vs. 292 s) after consuming the rooibos beverage and covered a 13.60% greater distance (2435 vs. 2790 m) compared with the placebo session. Participants also produced 2.42% more force (N) and 3.65% more power (W) during the rooibos session compared with the placebo session.

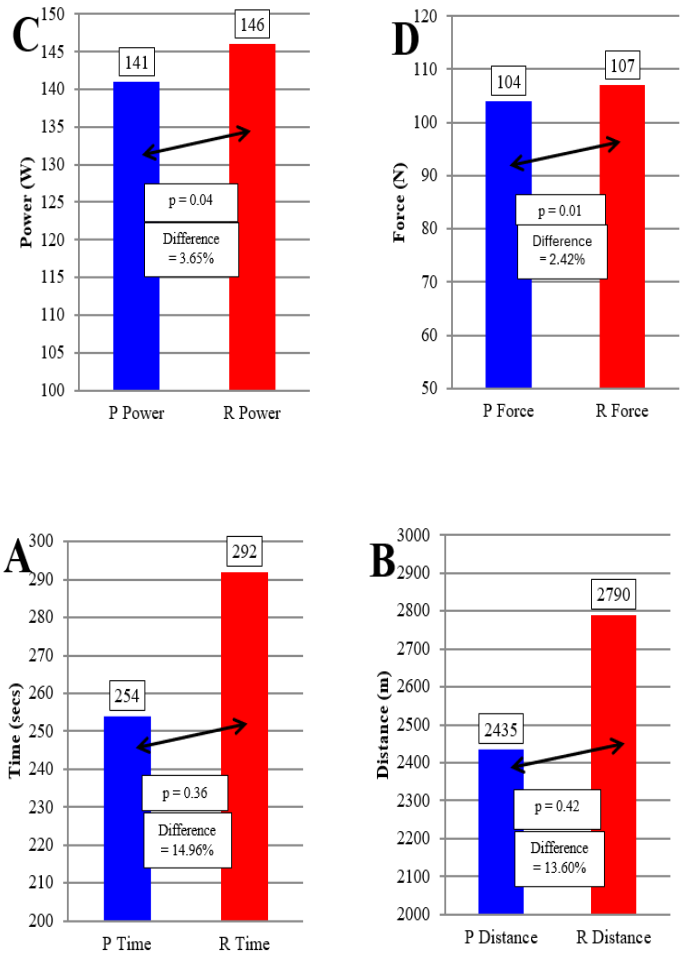


*Figure 5.* **PARTICIPANTS' (*N*=30) EXERCISE PERFORMANCE PARAMETERS DURING THE SUBMAXIMAL RAMP TEST**

(A) Time to withdrawal (s); (B) distance covered (m); (C) power (W); and (D) force (N).
Level of significance for a **one-way** ANOVA is indicated by *p* value (significance: $p<0.05$) when comparing the placebo with the rooibos interventions.
P=placebo R=rooibos.

### Submaximal cardiovascular response to submaximal exercise

The effect of intervention study beverages on participants' cardiovascular response during the submaximal (75%–80%) ramp test on the Wattbike (Figure 6A and B) show a modest elevation in both heart rate (2.47%) and oxygen uptake (5.53%) during the rooibos trial when referenced against the substantive increase in mean exercise time to withdrawal (14.96%) and distance completed (13.6%) during the rooibos trial.

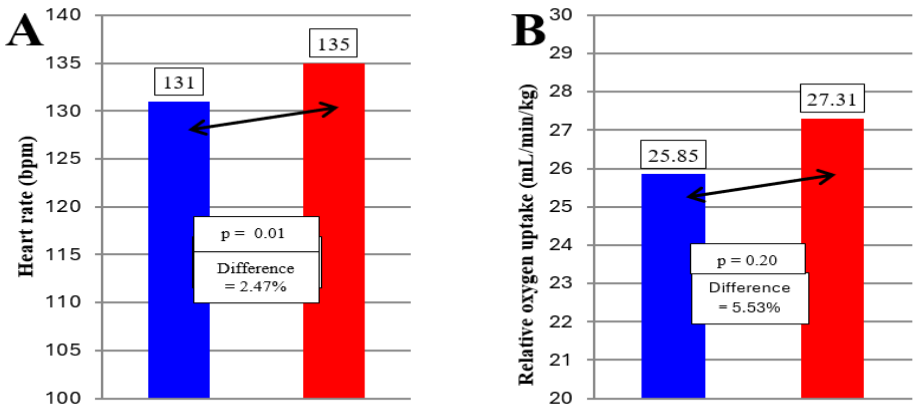


*Figure 6.* **PARTICIPANTS' (*N*=30) CARDIOVASCULAR RESPONSE DURING THE SUBMAXIMAL EXERCISE TEST**

(A) Heart rate; (B) oxygen uptake. Units for heart rate = beats per minute and relative oxygen uptake ml/min/kg. *p<0.05 when comparing the rooibos with the placebo interventions.
$O_2$=oxygen P=placebo R=rooibos.

**Correlation between muscle damage, plasma antioxidant capacity and performance variables**

Pearson correlation analysis assessed possible relationships between different measured variables. Comparisons of muscle damage markers with antioxidant markers showed the following significant relationships; serum CK levels and TEAC was negatively correlated at 1 h post-exercise for the rooibos group ($r$=-0.380, $p$=0.039). Both rooibos and placebo interventions showed negative correlation between CK and tGSH and GSH at IAE ($r$=-0.454, $p$=0.012; $r$=-0.364, $p$=0.048). In addition, the FRAP and AST levels were negatively correlated for the rooibos group at the 1 h and 24 h post-exercise time points (r=-0.425, p=0.019; $r$=-0.400, $p$=0.029. When considering the relationship between exercise performance and participant antioxidant parameters, significant ($p$<0.05) correlation analyses were also observed and are presented in Table 5.

*Table 5.* **PEARSON COEFFICIENT CORRELATIONS BETWEEN HEART RATE AND THREE PERFORMANCE VARIABLES, DISTANCE COMPLETED, FORCE AND POWER DURING SUBMAXIMAL EXERCISE**

| | Placebo trial | | |
|---|---|---|---|
| **Heart rate correlation** | Distance | Force | Power |
| | 0.602** | 0.886** | 0.925** |
| | **Rooibos trial** | | |
| **Heart rate correlation** | Distance | Force | Power |
| | 0.610** | 0.886** | 0.925** |

* $p<0.05$ and **$p<0.01$ indicate significant heart rate correlation

In general terms, rooibos consumption appears to have modulated participants' cardiorespiratory response in relation to submaximal exercise (Figure 6A and B). Following rooibos ingestion, the heart rate increased only 2.47% which is less when compared with the increased time to withdrawal of 14.96% and distance completed of 13.60%, while relative oxygen uptake (ml/min/kg) increased 5.53%, perhaps demonstrating that rooibos has a beneficial effect on metabolic responses during submaximal exercise by possibly improving and/or optimising the efficiency of the oxidative phosphorylation system.

## DISCUSSION

In this study, we hypothesised that an acute intake of a fermented rooibos beverage with its high and unique antioxidant capacity and content (Table 2) would modulate exercise-induced oxidative stress and associated damage and may subsequently enhance submaximal exercise performance outcomes, when compared with the placebo.

The study recruited 40 healthy young adult males, and all participants completed the 3-week study trial successfully. None of the participants reported any adverse effects from the exercise regime or beverages consumed. The ingestion of 375 ml was to ensure participants ingest a sufficient volume of liquids to remain well-hydrated during the exercise. Ingestion at 90 min before the submaximal Wattbike exercise regime, was to ensure enough time was allowed for the absorption of the phytochemical polyphenols as reported by Mao-Jung *et al*. (2002), which entailed participants ingesting/drinking a single dose of green tea (394 mg polyphenols) that was accompanied by the highest plasma catechin content 1.5 h after the intake. In the current study, a similar result was obtained when the participants consumed the rooibos beverage with a significant increase in both antioxidant content (TPC) and antioxidant capacity (measured as FRAP) after 1.5 h of intake. Both intervention groups showed an increased antioxidant capacity immediately after the exercise was completed, after 1 h and 24 h recovery, but rooibos showed a notable increased activity at these time points over the placebo beverage, and this can be ascribed to the significantly enhanced antioxidant capacity after rooibos was consumed. Contributing to the endogenous antioxidant defence system is the increased level of glutathione and total glutathione in the study participants, which stayed elevated after the 24 h rest period when the placebo beverage decreased to levels similar to that of time 0 h or baseline. This protective and modulatory potential of rooibos through the increase of glutathione has also been

identified in previous human and experimental animal studies (Marnewick *et al*., 2011; Ajuwon *et al*., 2014). Furthermore, the strong significant trend or improvement in blood redox status (tGSH and GSH) observed in this study at 1.5 h and 24 h post-exercise when rooibos was consumed might also be an indication of rooibos' ability to upregulate the synthesis of GSH, as suggested by Moskaug *et al*. (2005). This increased glutathione level together with the increased antioxidant capacity, confirms rooibos to support the endogenous antioxidant defence system. This mechanism, using plant-derived antioxidants to increase the concentration of endogenous substances, such as glutathione, to take part in redox reactions in the body has been proposed by others (Franco *et al*., 2019).

The exercise regime in the current study is seen as strenuous with enhanced oxidative stress and different to the type of exercise that was involved with the hormesis theory. The type and intensity of exercise, as well as its duration, frequency and training status of the individual, influence whether it causes an increase in the formation of pro-oxidants and thus induced oxidative stress and damage or increase in antioxidants, supporting the defence system (Pingitore *et al*., 2015; Thirupathi *et al*., 2021). ROS causing the oxidative stress has been shown to have a biphasic effect that is either pathological or physiological and the cutoff point between these effects has not been clearly defined (Lesmana *et al*., 2022). In the current study it is proposed that the exercise regime is strenuous enough to have caused an increased ROS and accompanying increased physiological damage (measured as CK, AST, ALT and lipid peroxidation markers), with the intervention of rooibos mitigating the induced oxidative stress via enhancing the body's antioxidant defence system. Similar findings were also reported by da Silva *et al*. (2018), whereby green tea extract (polyphenolic-rich) intake lowered and minimised participants' serum CK activity after an exercise-induced delayed onset of muscle soreness. In the current study, compared with the placebo, consumption of the fermented rooibos beverage did show a promising trend (intragroup results) in the reduction of the analysed lipids biomarker (CDs), with plasma CDs levels reduced at 1.5 h and 1 h post-exercise after rooibos consumption. This observation is not surprising because unlike the TBARS, which represent the end phase of lipid peroxidation, CDs represent the early phases of lipid peroxidation damage, hence, their reduction by rooibos consumption in this study, unlike placebo, further indicates rooibos potential protective effect against oxidative stress lipid damage.

Additionally, both AST and ALT are mostly associated with liver function; however, when muscle damage occurs, these enzymes are released from muscles into the bloodstream as well, hence proving to also be a good indicator of tissue/muscle damage (Ferreira *et al*., 2020). Unlike the liver, skeletal muscles have more AST and ALT due to a larger tissue mass, thus, in case of muscular disorders or injury, AST and ALT levels may be elevated. In our study, both AST and ALT were slightly increased IAE regardless of the beverage consumed. This could be an indication that there was muscular injury/damage, which also further affirmed the effectiveness of our exercise regime model used to induce oxidative stress and muscle damage. A study by Vecchio and colleagues (2017) reported a positive correlation between increased lipid peroxidation markers with CK and AST and suggested that biomarkers of muscle damage during exercise are indicative of oxidative stress occurrence. In the current study, the consumption of rooibos caused a significant reduction in serum AST at 1.5 h, IAE and 1 h post-exercise, and serum ALT levels at 1.5 h and IAE. These findings, if viewed from a muscular damage point of view, suggest that rooibos intake was able to mitigate muscular damage in the study participants unlike the placebo, rendering the fermented rooibos beverage to be considered as an ergogenic aid for sport athletes or individuals. A study by Ferreira and

colleagues (2020) also concluded that a hydro electrolyte beverage (whey permeate with the phenolic extract of jabuticaba peel) attenuated markers of muscular and oxidative stress damage in trained individuals indicated by a significant reduction in AST, ALT and CK serum level. When considering the serum CK level during rooibos at 1 h post-exercise had a significant negative correlation ($r$=-0.380, $p$=0.039) with plasma antioxidant capacity (TEAC) at 1 h post-exercise during rooibos. This may be due to increased plasma antioxidant capacity (TEAC) levels at 1 h during the rooibos session, which may have resulted in improved plasma antioxidant capacity and decreased RONS accumulation, contributing to lower muscle damage and vice versa. Serum CK levels are considered to be the most common reliable muscle damage biomarkers. During the rooibos session at IAE, serum CK levels increased, resulting in a significant negative correlation with blood tGSH at IAE, which suggest that more RONS were produced. More tGSH molecules were oxidised, reducing the antioxidant capacity and leading to oxidative stress with associated muscle damage. Also, the fact that this negative correlation occurred during both placebo and rooibos intervention is to be expected but appeared to have a stronger correlation in the placebo than in the rooibos intervention ($r$=-0.364 compared with $r$=- 0.454). These results suggest that rooibos intervention reduced muscle damage occurring during the exercise intervention, compared with the placebo group (Ferreira *et al*., 2020). It may be reasonable to argue that this slight sparing in muscle damage through the rooibos intervention not seen during the placebo intervention may be part of the reason participants were more efficient in cycling while on the rooibos intervention and may then explain the improved power and distance.

Importantly, this study assessed whether rooibos consumption improves submaximal exercise performance. Results show that consumption of fermented rooibos beverage influenced participants' exercise performances differently when compared with placebo. When rooibos was consumed, participant exercise duration was improved (14.9% difference), accompanied by improved sustained exercise endurance, as participants covered a greater distance (13.6% difference). Particpants' mean performance during the submaximal trial also resulted in more power (3.65%) and force work (2.42%) when compared with the placebo. Generally speaking, one would have expected a concomitant increase during the rooibos trial in heart rate and oxygen uptake when compared with the increase in endurance time (14.9%) and distance (13.6%). However, mean heart rate and oxygen uptake increased only moderately (2.47%) and (5.53%) respectively. The increased endurance performance (time and distance achieved) following ingestion of rooibos beverage may be indicative of its ability to optimise metabolic and cardiovascular processes that support aerobic metabolism. The delayed voluntary withdrawal following rooibos ingestion from the submaximal exercise test regime could also suggest potential antifatigue effects (delay in fatigue onset) as a result of rooibos consumption and/or the ability to sustain a marginally higher heart rate and aerobic uptake that facilitated a substantive increase in exercise time and also overall distance achieved when compared with the placebo trial. These results corroborate previous human and animal studies (Watanabe *et al*., 2014; Davies *et al*., 2019b) that reported on the potential antifatigue and ergogenic potential of rooibos beverage/products. It should be noted that the heart rate measure reported on in the study was a measure of average heart rate across the exercise period. This would mean that participants who cycle longer at higher heart rates would have some of the real-time heart rate increases buffered by the calculation of the average. It may well be that if measured differently, heart rate changes for both groups may reflect differently. As such the results of the current study should be seen to reflect on the hemodynamic changes over a period of exercise and recovery, rather than short term or time point-based comparisons.

When we looked at the correlation between exercise performance parameters and participants antioxidant blood status, a significant negative correlation was observed between oxygen uptake and blood tGSH at 1 h post-exercise during placebo, while at 24 h post-exercise during rooibos session a significant negative correlation was also observed between oxygen uptake and blood tGSH and GSH. However, although this result was indicative of more blood tGSH and GSH oxidation during both placebo and rooibos session, the results further indicate that rooibos ingestion might have provided some buffering to oxidation, as rooibos ingestion the oxidation of glutathione molecules were delayed. This was evidenced by the increased oxidation only becoming apparent at 24 h post-exercise for rooibos groups, unlike during placebo where it occurred at 1 h post-exercise. The delay in glutathione oxidation during rooibos could be suggestive of good antioxidant status that stabilise and neutralise produced RONS post-exercise, which subsequently may have assisted with participant recovery.

Therefore, based on the present submaximal exercise results, it can be speculated that rooibos beverage/product consumption may have beneficial effects for athletes and other individuals involved in sport and fitness activities, by delaying fatigue onset, improving exercise performance and/or enhancing cardiorespiratory function, especially at lower exercise intensities. However, inappropriate or excessive use of nutritional or antioxidant supplements may become detrimental or blunt the positive responses to exercise. Some studies cautioned against antioxidant use by reporting lung cancer, jaundice, arthralgia, diarrhoea and gastric upset (Wooltorton, 2003), delay in wound healing and muscle strength after exhaustive exercise (Theodorou *et al*., 2011), as well as failure to protect against LPO, muscle damage and inflammation (Teixeira *et al*., 2009). Also, some studies (Gomez-Cabrera *et al*., 2005; Wadley *et al*., 2013; Morrison *et al*., 2015) suggested that the use of antioxidant supplements may hamper or prevent the signalling of important adaptation processes such as muscle mitochondrial biogenesis, insulin sensitivity and hypertrophy. Therefore, a balance is needed because the controversy regarding antioxidant supplementation on exercise performance or adaption is still under debate in the scientific community. Merry and Ristow (2016) suggests that the type of antioxidant utilised (general vs. targeted), duration of treatment (pretreatment vs. treatment only during training) and the volume of training determine the antioxidant supplementation effect on exercise performance, training and the associated adaptations.

**Study limitations and recommendations**

Although the proposed number of participants (stemming from the power calculation) were enrolled, some participant data had to be excluded (according to exclusion criteria) from the final number, which decreased sample size and may have contributed to increased variability and loss of significance in some results. The recruited participants appeared to been at different levels of physical fitness, which may have served as a confounder to the study results, given that the production of waste products and free radicals may differ in individuals of varying levels of fitness (Furrer *et al*., 2023) Further investigation looking into the impacts of interindividual differences such as fitness levels are needed as this may have played a role or influenced the results. Also, studies with a bigger sample size are required to comprehensively understand the effective dosage, bioavailability, metabolic pathways and other cellular mechanisms involved that possibly underscore positive exercise/performance effects observed when rooibos beverages/products are consumed. Furthermore, the effects of rooibos in different exercise/physical activity modalities/regimes also need to be further investigated.

Additional research is needed to better understand the biological changes experienced during strenuous exercise such as inflammation, oxidative stress and related muscle fatigue, while additional future studies ought to consider individual genetic variation, and the need to better the understanding of exercise genotype–phenotype relationship as these may influence the changes and extent of exercise outcomes. Another limitation of this study was the inability to collect post-exercise cardiorespiratory data. This was due to the participants having to dismount from the Wattbike and disengage with its computer and the metabolic analyser immediately after exercise testing and move to the designated health regulation compliant blood sampling/collection room.

## CONCLUSION

Consumption of the fermented rooibos beverage enhanced the endogenous antioxidant defence system of the body, decreased circulating levels of muscle damage markers and improved submaximal endurance performance, notably in terms of the mean withdrawal point. It appears that rooibos supplementation supported and enabled participants to cycle longer and, consequently, further than the placebo trial. The accompanied moderate increases in heart rate and oxygen intake infer that fermented rooibos may benefit the efficiency of aerobic metabolism during submaximal exercise.

Therefore, it is tentatively suggested that the use of rooibos herbal teas and/or nutraceutical supplementation may be beneficial in reducing exercise-induced oxidative stress damage effects and thus improve endurance performance at submaximal (<80%) exercise levels. In terms of the foregoing comments one must balance this against the varied fitness level of participants, which might have influenced the extent of the intervention rooibos beverages' effects on measured physical performance outputs. Future studies looking at short term cardiovascular and respiratory changes during and after exercise may be important to reveal the underlying mechanisms of rooibos-induced benefit.

### Conflict of interest statement

The authors declare no conflicts of interest.

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