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REVIEW ARTICLE

A Review of the Bioactivity of South African Herbal Teas: Rooibos (*Aspalathus linearis*) and Honeybush (*Cyclopia intermedia*)

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Rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*) are popular tisanes in their native South Africa and have a growing worldwide market. Both herbal teas are used traditionally for medicinal purposes and are rich in polyphenols with rooibos a rare source of the dietary dihydrochalcones, aspalathin and nothofagin. The principal polyphenols in honeybush include the xanthone mangiferin and the flavonones hesperitin and isokuranetin. Despite their divergent phytochemical and nutrient compositions, rooibos and honeybush share potent antioxidant and antimutagenic activities *in vitro*. Animal model studies indicate both herbal teas possess potent antioxidant, immune-modulating and chemopreventive actions. However, human studies of rooibos are limited and of honeybush are absent. No adverse effects of rooibos or honeybush consumption as tisanes have been reported. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: honeybush; rooibos; tisane; herbal tea; aspalathin; mangiferin.

INTRODUCTION

Derivatives of plant origin have long been known to possess biological activity (Robak and Gryglewski, 1996). Many traditional cultures remain mostly dependent on plants for their food and medicine, and often consider them both in the same context (Huffman, 2003). According to the World Health Organization, approximately 80% of the world's inhabitants currently rely on indigenous or traditional medicines for their primary health needs, and most of this therapy involves the use of plant extracts, often in aqueous solutions (Zhang, 2002). Of the plant-based foods used as medicines, none have received more attention as a group than herbal remedies (Dubick, 1996). The use of herbal preparations, typically prepared by steeping or heating the crude plant material, has prevailed for centuries and healthcare providers in Europe and Asia today often prescribe herbal teas. However, such practices are largely based on folklore and schools of traditional medicine rather than evidence-based research.

In many cases, the bioactivity of these plants appears to be derived from 'secondary metabolites', such as the polyphenols (Huffman, 2003). Polyphenols, the most numerous and widely distributed class of phytochemicals, include classes of chromones, coumarins, lignans, stilbenes, xanthones and the ubiquitous flavonoids (Hertog *et al.*, 1994; Kromhout *et al.*, 1996). Within the past decade, many polyphenols, particularly the flavonoids, have been found to possess relatively potent antioxidant, antiatherosclerotic, antiinflammatory, antimutagenic, antitumor and antiviral activities (Nijveldt *et al.*, 2001).

Observational studies have repeatedly shown that diets high in plant-based foods and beverages are associated with a lower risk of chronic diseases, such as cardiovascular disease and some forms of cancer (Hertog *et al.*, 1996; Hertog *et al.*, 1993; Hertog *et al.*, 1995; Hollman *et al.*, 1999; Hu, 2003; Riboli and Norat, 2003; Rimm *et al.*, 1996) and suggest this correlation may be attributable to the phytochemical constituents as well as to the macro- and/or micronutrient content of these foods. While further research is necessary to better understand and quantify the contributions of phytochemicals to health promotion and disease prevention, virtually all of the dietary guidelines created by regulatory agencies and healthcare organizations include recommendations for generous intakes of plant foods, including fruits, vegetables and whole grain cereals. Interestingly, recommendations for the consumption of plant-based beverages (except for fruit juices) such as tea (*Camellia sinensis*) and tisanes (herbal teas) are absent despite their being particularly rich sources of polyphenols.

While there is an extensive literature suggesting health benefits associated with drinking black, green and oolong tea (i.e. *Camellia sinensis*) (McKay and Blumberg, 2002), evidence-based information regarding the effects of most herbal teas is quite limited. We review here the available scientific literature related closely or directly to the bioactivity and potential health benefits of two 'emerging market' herbal teas from South Africa, rooibos and honeybush. Consumer demand for rooibos

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has been increasing since trade sanctions against South Africa were lifted following the demise of apartheid in the 1990s (Erickson, 2003). Like many other herbal preparations used in traditional cultures, the therapeutic uses and purported health benefits of rooibos and honeybush are based largely on folklore rather than on scientific substantiation. Information regarding the phytochemical content, *in vitro* experiments, animal models and human studies available in the recent scientific literature is presented. The relevant literature was identified principally from the CAB Abstracts, Cochrane Library and Medline databases. The literature search employed both common and Latin names for each herb. In general, articles available in a language other than English were considered if the abstract was in English and/or if the full text article pertained to a human study and was completed or referenced elsewhere within the past 20 years.

ROOIBOS (*ASPALATHUS LINEARIS*)

Nomenclature

Rooibos or *Aspalathus linearis* (also *A. contaminata*, *A. corymbosus*, *Borbonia pinifolia* or *Psoralea linaeris*) is a shrub-like leguminous bush native to the Cedarberg Mountains in the Western Cape region of South Africa where it is extensively cultivated within this area for its commercial use as an herbal tea or tisane. After harvesting, the needle-like leaves and stems can be either bruised and fermented prior to drying or dried immediately. The unfermented product remains green in color and is referred to as green rooibos. During fermentation, the color changes from green to red with oxidation of the constituent polyphenols, so the final product is often referred to as red tea or red bush tea. Other names include rooibos tea, rooibosch, rooitea or rooitee.

Phytochemical and nutrient content

Brewed rooibos tea (1 tsp/cup) contains 300 mg protein with the amounts of Cu, Fl and Mn present in this single serving providing 7.8%, 5.5–7.3% and 1.7–2.2% of the U.S. Daily Value (DV), respectively (Erickson, 2003) while Ca, Fe, K, Mg, Na, PO_4 and Zn (Erickson, 2003; Hesseling *et al.*, 1979; Morton, 1983) constitute <1% DV. Vitamin C is present at ≤15.7 mg/100 mg dried tea according to Morton (1983) and 121.8–154.9 μmol/L infused tea according to Hesseling *et al.* (1979), although a more recent analysis failed to confirm its presence in either green or red rooibos (Erickson, 2003).

According to Habu *et al.* (1985), 99 compounds are present in the volatile oil of rooibos tea. The major components include guaiacol (24.0%), 6-methyl-3,5-heptadien-2-one isomer (5.2%), damascenone (5.0%), geranylacetone (4.2%), β-phenylethyl alcohol (4.1%) and 6-methyl-5-hepten-2-one (4.0%). Compared with the volatiles present in green and black teas, the concentration of guaiacol in rooibos was much higher, while linalool was moderately lower and geraniol was absent. Kawakami *et al.* (1993) confirmed the presence of these components (except for damascenone) in a later study, although the quantities determined in their analysis varied from those reported by Habu *et al.* (1985).

Several phenolic compounds are present in the brewed teas of both green and red rooibos, but the total concentration of flavonoids in each can differ by more than 10-fold (Bramati *et al.*, 2003; Bramati *et al.*, 2002). In 2% (w/v) solutions of rooibos tea, Marnewick *et al.* (2000) found a significantly higher percentage of total polyphenols (41.2% vs 29.7%), flavonoids (28.1% vs 18.8%) and non-flavonoids (13.1% vs 10.9%) in green compared with red rooibos, respectively, but no difference in soluble solid matter. Similarly, Standley *et al.* (2001) found a significant difference in total polyphenols between green and red rooibos (41.0% vs 35.0%) but, in contrast, also a difference in solids (2.3% vs 1.6%). These differences are attributable to the enzymatic and chemical modifications that occur during fermentation and to the processing methods (sun drying vs controlled drying) (Joubert, 1996; Standley *et al.*, 2001). Aspalathin, a dihydrochalcone present in green rooibos is extensively oxidized to dihydro-iso-orientin during fermentation and Bramati *et al.* (2003) reported its concentration in a comparison of the respective infused teas dropped from 49.9 to 1.2 mg/g. The C-glycosyl flavones isoorientin (3.6 mg/g), orientin (2.3 mg/g), isovitexin (0.7 mg/g) and vitexin (0.5 mg/g) are also degraded but to a lesser extent (range of differences post-fermentation is 2.7–0.2 mg/g). Nothofagin, a dihydrochalcone structurally similar to aspalathin, is degraded as well (Joubert, 1996; Joubert and Ferreira, 1996). The other predominant flavonoids detected in both types of rooibos tea are rutin (1.3–1.7 mg/g), isoquercetin and hyperoside (0.3–0.4 mg/g), quercetin (0.04–0.11 mg/g), luteolin (0.02–0.03 mg/g) and chrysoeriol (0.01–0.02 mg/g). The presence of the phenolic acids caffeic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, vanillic acid and protocatechuic acid have been reported in red rooibos tea (Rabe *et al.*, 1994). Quantitative and qualitative differences in phenolic content between wild and cultivated populations of *A. linearis* (van Heerden *et al.*, 2003) have also been reported.

Currently rooibos is the only known natural source of aspalathin and one of only two known sources of nothofagin (Joubert, 1996). Due to the lack of an authenticated standard for high performance liquid chromatography (HPLC) analysis, few studies have reported quantitative data on nothofagin (Joubert, 1996; Schulz *et al.*, 2003). Approximately 19 g/kg of dried, unprocessed rooibos consists of these dihydrochalcones (aspalathin and nothofagin) combined, while red rooibos contains only 7% of the amount present in the unprocessed plant material. The ratio of dihydrochalcones to total phenolics decreases during fermentation from 1.17 to 0.19.

In vitro studies

Antioxidant capacity. According to Schulz *et al.* (2003), the relative aspalathin content of green rooibos correlated well with the total antioxidant activity (TAA) of the dried plant material ($R^2 = 0.81$) when assessed with the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)

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(ABTS) radical cation-scavenging assay. The ratio of TAA to total phenolic content was 95.5 before fermentation and 60.5 after fermentation, with aspalathin contributing a very small amount of activity compared with the other phenolics present in fermented rooibos. The TAA of green rooibos was 2.8-fold higher than the fermented product (775.6 vs 274.5 μmol Trolox/g dry wt) (Schulz *et al.*, 2003), while a 2.0-fold difference was observed in brewed infusions (1 g/60 mL, 90 °C, 10 min) of the respective teas (0.8 vs 0.4 Trolox meq/g) (Bramati *et al.*, 2003). By comparison, the TAA of brewed green (1.8 Trolox meq/g) and black teas (1.7 Trolox meq/g) were more than double that found in rooibos with this assay.

In a study by Pazdzioch-Czochra and Widenska (2002), an assay based upon the oxidation of homovanillic acid was used to test the H_2O_2 scavenging activity of selected herbal and non-herbal teas including rooibos. The infusions were prepared with 1, 5 or 10 g dry material in 1 L boiling water and incubated at 95 °C for 10 min. The activity of rooibos tea, expressed as Trolox equivalents via a calibration curve (0.17 ± 0.01 mg/mL), was approximately half that found in green tea (0.31 ± 0.03 mg/mL) and black tea (0.32 ± 0.02 mg/mL). Rooibos' antioxidant capacity in this assay was also lower than other popular herbal teas including peppermint (0.27 ± 0.02 mg/mL) and hibiscus (0.20 ± 0.02 mg/mL).

The effects of fermentation, processing and preparation conditions on the antioxidant activity of rooibos have been examined with other assay methods as well. Standley *et al.* (2001) tested rooibos samples at five major stages of processing for their ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide ($O_2^{\cdot -}$) radicals. Compared with the unfermented sample, higher quantities of the extracts from later stages of processing were required to inhibit the DPPH and $O_2^{\cdot -}$ radicals, indicating a loss of antioxidant capacity with fermentation. Von Gadow *et al.* (1997a) used the DPPH and β-carotene bleaching methods to compare the antioxidant capacities of unfermented, semi-fermented, fermented rooibos teas with green (unfermented), oolong (semi-fermented) and black (fermented) teas derived from *Camellia sinensis*. All the teas were prepared by steeping 50 g dried leaves with 1 L boiling water for 30 min. a higher inhibition of the DPPH radical was observed in the unfermented versions of both types of tea, and the trend for both was unfermented > fermented > semi-fermented. The loss of antioxidant activity with fermentation was significant for green to black tea (90.8% vs 81.7%) but not for rooibos teas (86.6% vs 83.4%). Green tea was the most potent tea tested in this experiment and oolong tea the least with the antioxidant activities of the other teas not statistically different from one another. The results of the β-carotene bleaching method in this study correlated well with those obtained using the DPPH method.

Using the Rancimat method to measure the oxidation of lard over time, von Gadow *et al.* (1997b) found the antioxidant activity of rooibos tea was significantly higher if the brewing time was extended to 25–30 min. The concentration of total water-soluble solids in the brewed tea was significantly higher after 10 min (2.04 ± 0.03 vs 1.76 ± 0.11 g/100 mL at 5 min) and continued to rise until the experiment was ended at 30 min (2.42 ± 0.13 g/100 mL); no significant differences were observed with increased extraction time when the β-carotene bleaching method was employed. The antioxidant activity coefficient (AAC) peaked at 20 min, although the increase was not linear. Continuing to heat the tea extract for up to 30 min also resulted in significantly increased antioxidant activity with the Rancimat method, but not with β-carotene bleaching. Traditionally rooibos is brewed for longer periods than other herbal teas.

The DPPH radical and β-carotene bleaching methods were also used by von Gadow *et al.* (1997c) to compare the antioxidant activities of aspalathin and other individual phenolic components of rooibos with the antioxidant reference standards α-tocopherol, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Aspalathin inhibited the DPPH radical 91.4%, a higher potency than all of the reference standards (45.6–75.1%) and higher than all of the phenolic acids tested except for caffeic acid (93.7%) and the flavonoids quercetin (93.3%), (+) catechin (92.7%) and isoquercetin (92.0%). With the β-carotene bleaching assay, the AAC value of aspalathin (357.1) was lower than the reference standards (491.8–698.5), but higher than all of the phenolic acids and flavonoids except luteolin (522.7) and quercetin (455.3). Using the Rancimat method, the longest induction period was observed with (+) catechin (27.2 h), followed by quercetin (26.9 h), caffeic acid (18.9 h) and protocatechuic acid (15.5); aspalathin (2.6 h) and the reference standards (3.2–4.1 h) exhibited low activity with this assay.

Joubert *et al.* (2004) also evaluated flavonoids from green and red rooibos for their capacity to scavenge DPPH and $O_2^{\cdot -}$ radicals. In both assays, quercetin was the most potent radical scavenger. Aspalathin, orientin, luteolin and isoquercetin were slightly less active than quercetin towards DPPH, although aspalathin was as effective as quercetin towards $O_2^{\cdot -}$. Aspalathin was also more effective as a radical scavenger towards both DPPH and $O_2^{\cdot -}$ than BHT, BHA, α-tocopherol and Trolox. During fermentation, aspalathin is converted to orientin, which has a similar action towards DPPH, and to isoorientin, which was less effective. Both orientin and isoorientin were less effective scavengers of $O_2^{\cdot -}$ than aspalathin. The reduced ability of an aqueous extract of fermented vs unfermented rooibos to scavenge $O_2^{\cdot -}$ was also observed (IC_{50} = 78.2% and 69.2%, respectively). Inhibition of DPPH was also lower in the aqueous extract of fermented than unfermented rooibos (83.0% vs 87.3%, respectively) and in a crude polymeric fraction of rooibos (70.3% vs 87.7%, respectively); no difference in DPPH activity was observed between the ethyl acetate fractions of fermented and unfermented rooibos.

When assayed in a linoleic acid emulsion no differences in the inhibition of conjugated diene formation were observed between either the aqueous extracts of fermented versus unfermented rooibos (28.0% vs 28.6%, respectively) or in the crude polymeric fractions (23.5% vs 22.6%, respectively) (Joubert *et al.*, 2005). The inhibitory effect of the ethyl acetate soluble fraction of fermented rooibos was, however, significantly higher than that of unfermented rooibos (48.0% vs 11.0%, $p < 0.05$).

A small number of studies have reported the antioxidant activity of rooibos tea in cellular systems.

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Yoshikawa *et al.* (1990) observed that 20.1 μg/mL of an aqueous *A. linearis* extract inhibited the generation of $O_2^{\cdot -}$ induced by phorbol myristate acetate in human polymorphonuclear leukocytes (PMA-PMN) and measured by electron spin resonance spectrometry with 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as the spin trap agent. A 20.4 μg/mL of extract was equally effective in a comparative assay, the hypoxanthine-xanthine oxidase system (HX-XO). Quercetin, a flavonoid component of *A. linearis*, inhibited $O_2^{\cdot -}$ actions in the PMA-PMN system at a concentration of 46 μM, but a lower amount, 16 μM, was as effective in the HX-XO system. This difference is most likely the result of quercetin's inhibition of XO rather than a direct antioxidant action in the HX-OX system. In mouse leukemic cells, Ito *et al.* (1992) examined the activity of rooibos tea extract on the cytotoxicity of H_2O_2. Cells preincubated with rooibos extract (2.25 g leaves/200 mL water, boiled 20 min) exhibited a time- and dose-dependent increase in survival rate after exposure to 40 μM H_2O_2. Concomitant treatment with rooibos, however, afforded no protection against the cytotoxic effects of H_2O_2. Using a linoleic acid autoxidation system, Hitomi *et al.* (1999) compared the antioxidant activity of freshly brewed and freeze-dried rooibos tea extract on rabbit erythrocyte membrane, rat liver microsome and rat liver homogenate systems. In the rat liver homogenate, the freshly brewed tea exhibited strong activity, while in the erythrocyte and microsome systems, the freeze-dried extract had a strong, dose-dependent effect. Of the flavonoids purified from *A. linearis* and tested in the rat liver microsome system in this study, luteolin and quercetin showed the highest activity. Marnewick *et al.* (2005) examined the effects of ethanol/acetone soluble extracts of unprocessed and processed rooibos (0.01% in DMSO) against lipid peroxidation, in the presence of Fe^{2+} and absence of H_2O_2, using a rat liver microsomal preparation. In this assay, the formation of thiobarbituric acid reactive substances (TBARS), measured as malondialdehyde (MDA), was inhibited 91% with unprocessed rooibos compared with the control ($p < 0.001$). The inhibitory effect of green tea was 99%, which was not significantly different than the unprocessed rooibos. Processed, or fermented rooibos, was also found to be highly protective against lipid peroxidation (65%) in this experiment.

Chemopreventive potential. The proliferation and growth of chick embryo muscle cells was inhibited in a dose-dependent manner by a rooibos tea preparation (3.5 g dry leaves in 2 L boiling water, steeped 20 min) in study by Lamosova *et al.* (1997). Within 10 days of exposure to 2%, 10% or 100% of the tea extract, cell growth was inhibited 33.9%, 68.6% and 100%, respectively. After only 72 h, the 10% extract significantly lowered the DNA content of cultured primary cells to 66%, of fibroblasts to 85.1%, and of myoblasts to 37.0% compared with control cells containing 0% extract. The decrease in DNA content of the cells in the presence of rooibos tea extract roughly correlated with decreased DNA synthesis measured by [^{3}H] thymidine incorporation. Similar observations were made with regard to RNA content and *de novo* protein synthesis measured by incorporation of [^{3}H] leucine. The activity of ornithine decarboxylase (ODC), an enzyme involved in the signal transduction pathway for mitosis, was not affected by rooibos tea at lower concentrations, but significantly inhibited at concentrations of 100% in all cell types tested. The authors hypothesize that the growth inhibitory effect of rooibos was due to its radical scavenging activity, which prevented ODC from triggering mitosis in the presence of free radicals.

Komatsu *et al.* (1994) demonstrated the suppressive effect of rooibos tea on mouse embryo fibroblast cells subjected to x-ray induced oncogenic transformation. In this experiment, no growth inhibition (cellular toxicity) was observed after 2 weeks when cells were incubated in ≤2% tea extract prepared in the same manner as Lamosova *et al.* (1997) and, as in this latter report, cell survival decreased linearly at higher concentrations. One week after exposure to 3 Gy x-rays, the transformation frequency (transformants/surviving cells) of cells treated with 2% rooibos tea was no different than in cells treated with x-rays alone, but after 6 weeks of treatment, the transformation frequency was significantly lower by 50%. A significant effect was also seen with 10% rooibos (85% lower transformation frequency), but not at 0.5% (36% reduction). In the same study, 0.2% green tea (prepared as 1.8 g leaves in 100 mL boiling water for 15 min) had no effect on x-ray transformed cells after 6 weeks.

Sasaki *et al.* (1993) found rooibos tea significantly suppressed the number of chromosomal aberrations induced in Chinese hamster ovary (CHO) cells by benzo[a]pyrene (B(a)P) and mitomycin C (MMC). The tea extract used in this experiment was prepared by adding 1.5 L boiling water to 50 g dried leaves and kept boiling for 15 min, and then lyophilized. The clastogenicity of B(a)P was completely inhibited by simultaneous treatment with 1000 μg/mL rooibos extract, while all concentrations of rooibos tea extract (125–1000 μg/mL) significantly suppressed the induction of chromosomal aberrations in the presence and absence of S9 rat hepatic microsomal enzymes with MMC treatment. Post-treatment with rooibos tea extract also suppressed the induction of chromosomal aberrations by B(a)P and MMC with and without S9 mix, although the differences were significant only at the higher concentrations used in each experiment. Dose-related increases in the number of surviving cells were also observed at these higher concentrations. Green tea, examined under the same conditions, was not as robust in suppressing the effects of the mutagens at the lower concentrations used in this study.

Edenharder *et al.* (2002) tested various plant-derived beverages, including green, black and rooibos tea, for their protective effects against genotoxicity induced by 2-acetylaminofluorene (AAF) and 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP) in Chinese hamster lung fibroblasts genetically modified to express the cytochrome P450 dependent monooxygenase 1A2 (CYP1A2) and sulfotransferase 1C1 (SULT1C1) genes from rat using the Comet assay. Teas were prepared as 5 g infusions in 100 mL boiling water for 5 min or for rooibos, 10 min, and the concentrations of each solution required to inhibit genotoxicity by 50% (IC_{50}) were compared. The genotoxicity of AAF was significantly reduced by green, black and rooibos tea (IC_{50} 0.20%, 0.19%, 0.68% v/v, respectively); PhIp genotoxicity was reduced in a dose-dependent manner by green tea (IC_{50} = 0.20%), while black tea (1.25%)

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and rooibos tea (1.29%) were less active. Of the individual flavonoids tested with this system, 1 mM quercetin strongly inhibited the effects of PhIP (IC_{50} = 1.50 μM), although other flavonoids, 0.3 mM flavanone and 1 mM kaempferol, were more potent (IC_{50} = 0.42 μM, 1.30 μM, respectively). In another experiment designed to test the induction of the two rat genes independently, the genotoxic compounds BaP-7,8-OH and N-OH-PhIp were used to affect the CYP1A2 and SULT1C1 genes, respectively. Black tea and rooibos tea only modestly inhibited the genotoxicity induced by BaP-7,8-OH and had even less inhibitory action on N-OH-PhIP.

Using the *Salmonella typhimurium* mutagenicity assay, Marnewick *et al.* (2000) examined the antimutagenic properties of red and green rooibos tea and determined that they were both significantly effective against 2-acetylaminofluorene (AAF) and aflatoxin B1 (AFB1)-induced mutagenesis in tester strains TA98 and TA100 compared with controls. Teas were prepared with 2 g dried leaves in 100 mL boiling water and allowed to steep at room temperature for 30 min. The teas were then lyophilized and reconstituted at concentrations of 5% and 10% for use in this assay. Weaker inhibitory effects against the mutagens methyl methanesulfonate (MMS), cumoylhydroperoxide (CHP) and H_2O_2 in strain TA102 were observed. Both of the 10% rooibos solutions were effective against H_2O_2, while only the 10% red rooibos inhibited CHP, and the 10% green rooibos inhibited MMS mutagenesis. Standley *et al.* (2001) also reported the antimutagenic activity of both red and green rooibos tea against AAF using strain TA98 in the *Salmonella* mutagenicity assay. The effect of green rooibos tea against AAF-induced mutagenesis was significantly higher in both studies.

Immune responses. The effects of rooibos tea on the immune response were examined in a few studies conducted in Japan. Antigen-specific antibody production in murine splenocytes was markedly stimulated by 1–100 μg/mL rooibos tea extract, although in splenic B-cells a nonspecific antibody response elicited with lipopolysaccharide (LPS) was not according to Kunishiro *et al.* (2001). In primed splenocytes treated with rooibos tea extract, the production of interleukin (IL)-2 increased while IL-4 generation was suppressed. In studies by Nakano *et al.* (1997a; 1997b), acid polysaccharides extracted from the leaves of *A. linearis* suppressed the cytopathicity of HIV (HTLV-III) infected MT-4 cells, while polysaccharides from Japanese green tea leaves and a hot water extract of *A. linearis* did not. The polysaccharide, composed of reducing sugars (27%), neutral sugars (46%) and uronic acid (22%), almost completely inhibited the binding of HIV-1 to MT-4 cells as well.

Animal model studies

Immune responses. As suggested by the *in vitro* studies of Kunishiro *et al.* (2001) and Nakano *et al.* (1997a; 1997b) above, rooibos may have an immune modulating effect. Kunishiro *et al.* (2001) administered a rooibos tea extract p.o. to cyclosporin A-treated rats and found the production of antigen-specific antibodies in serum was restored and IL-2 generation in splenocytes was stimulated.

Gastrointestinal actions. Snyckers and Salemi (1974) tested fractionated rooibos extracts, containing quercetin and luteolin, for histamine antagonism in isolated guinea-pig ileum preparations. Extracts containing 5 μg/mL quercetin reduced acetylcholine-induced contractions by about 50%.

Chemoprevention. Sasaki *et al.* (1993) and Shimoi *et al.* (1996) examined the antimutagenic effects of rooibos in mice. Both studies employed the same preparation method: 50 g dried leaves in 1.5 L boiling water for 15 min. Sasaki *et al.* (1993) found the induction of micronucleated reticulocytes (MNRET) by MMC was significantly reduced after 24 and 48 h by 48% and 34%, respectively, in mice provided 1.0 mL of 0.1% rooibos tea extract p.o. 6 h prior to induction when compared with controls. Pretreatment with rooibos 24 h prior had no effect on the frequency of MNRET, nor did post-treatment for 3, 6, or 9 h after MMC induction. Pretreatment with 0.05–0.1% rooibos 1.0 mL daily for 28 days also significantly reduced MNRET frequencies 24–48 h after induction by either MMC or B(a)P. However, the 28 day pretreatment with rooibos tea had no apparent antimutagenic effects on 1.5 Gy γ-ray induced damage. A single gastric intubation of rooibos tea at 1.0 mL per mouse 2 h prior to γ-ray irradiation (1.5 Gy) did significantly reduce the frequency of MNRET in a different study (Shimoi *et al.*, 1996). A flavonoid-containing fraction, including luteolin and quercetin, isolated from rooibos tea also showed anticlastogenic activity in this experiment (n = 5) with a marked reduction in MNRET induction by γ-ray.

Marnewick *et al.* (2004) monitored the *ex vivo* anti-mutagenic activity of the cytosolic fractions of tea-treated rats. Liver cytosolic fractions from rats given green or red rooibos protected against aflatoxin B_1 (AFB_1)-induced mutagenicity in *Salmonella typhimurium* strain T100, while black and green teas had no significant effect in this assay. In contrast, green rooibos as well as black and green teas protected against AAF-induced mutagenicity in *S. typhimurium* TA98. Hepatic microsomal fractions of rooibos-treated rats also showed decreased AFB_1-induced mutagenic activity while green and black teas did not. None of the teas decreased microsomal activation of AAF. In fact, the number of histidine revertants was significantly increased with green and black teas (128–132%), while only a small and statistically non-significant increase with rooibos tea (50–52%), was observed. None of the teas affected the concentration of microsomal liver cytochrome P450.

Marnewick *et al.* (2005) also examined the effects of a topical application of methanol fractions of processed and unprocessed rooibos in a two-stage mouse skin carcinogenesis assay. Using a single application of 7,12-dimethylbenz[a]anthracene (DMBA) as the tumor initiator (200 nmol in 100 μL acetone), followed by 12-O-tetra-decanoylphorbol-13-acetate (TPA) as the cancer promoter (5 nmol in 100 μL acetone) 1 week later, the effect of the tea extracts was monitored following their application (100 μL) 30 min prior to TPA treatment. The treatments were repeated twice a week for a total of 20 weeks, and the final tumor yield and volume were determined upon termination. The mean number of tumors per mouse was significantly reduced by both unprocessed (1.1) and processed rooibos (0.7). There were 75% and 60% fewer tumor bearing mice in the

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processed and unprocessed rooibos groups, respectively (n = 10/group), compared with the positive control. Tumor size in both rooibos treated groups was also reduced. No tumors were detected, however, in the group treated with green tea extract.

Antioxidant capacity. Shimoi *et al.* (1996) examined the antioxidant activity of luteolin *in vivo*. Gastric intubation of 10 μmol/kg luteolin administered to mice 2 h prior to 6 Gy γ-ray irradiation significantly reduced lipid peroxidation, assessed via chemiluminescence, in bone marrow and spleen. In this model, luteolin also showed a non-significant trend toward protecting against the loss of endogenous ascorbic acid in bone marrow following irradiation.

Simon *et al.* (2000) investigated the antioxidant effect of rooibos tea on Japanese quail erythrocytes *ex vivo*. The fragility of erythrocytes to H_2O_2-induced hemolysis did not change after long-term consumption of rooibos tea; a similar lack of action was observed with hypotonicity-induced hemolysis. However, hemolysis was reduced after isolated cells were incubated with the tea *in vitro*; this effect was strongest after incubation with a boiled tea extract.

Inanami *et al.* (1995) examined the effect of long-term administration of rooibos tea on lipid peroxidation in rat brain. Lipid peroxides, assessed with the TBARS assay, were significantly higher in the frontal cortex, occipital cortex, hippocampus and cerebellum of 24 month old rats (n = 5) compared with animals at age 5 weeks. However, rats given rooibos tea *ad libitum* from age 3–24 months (n = 5) had no significant changes in TBARS. In addition, the signal intensities of the frontal cortex, hippocampus and cerebellum of rooibos-treated rats (n = 3), measured with magnetic resonance imagery (MRI), were similar to those observed in the 5 week old rats (n = 5), while those of the untreated 24 month old rats (n = 4) were significantly decreased.

Marnewick *et al.* (2003) tested the effects of rooibos and other teas on *in vivo* oxidative status and hepatic drug metabolizing enzymes in male Fischer 344 rats. For 10 weeks, rats were given either water (control), green or red rooibos, unfermented or fermented honeybush, or black or green tea as their sole source of drinking fluid (n = 10/group). Teas were prepared with 2 g/100 mL water except for honeybush (4 g/100 mL) and allowed to stand for 30 min. Unlike the green and black teas, neither green nor red rooibos tea had a significant effect on oxygen radical absorbance capacity (ORAC) values in the liver; however, rooibos did significantly increase the ratio of reduced to oxidized glutathione (GSH/GSSG), whereas black and green tea did not. Activities of the phase II enzymes cytosolic glutathione S-transferase alpha (GST-α) and microsomal UDP-glucuronosyl transferase (UDP-GT) were also enhanced by rooibos. Both types of rooibos increased GST-α by nearly 100%, while only the green rooibos increased UDP-GT by 50%; in this experiment, black and green tea had no effect on these drug metabolizing enzymes.

Ulicn *et al.* (2003) examined the hepatoprotective effects of rooibos tea in rats exposed to carbon tetrachloride (CCl_4), a potent pro-oxidant. Histological analyses revealed rooibos treatment resulted in a regression of CCl_4-induced hepatic steatosis and cirrhosis and reduced the production of hepatic malondialdehyde, triacyglycerols and cholesterol as well as plasma aminotransferase, alkaline phosphatase and bilirubin. Similar effects were obtained by treatment with the pro-GSH antioxidant, *N*-acetyl-L-cysteine.

Human studies and potential health/therapeutic applications

Rooibos tisanes have been employed as a folk remedy and indicated by some traditional medicines to treat asthma, colic, eczema, headache, nausea and mild depression. Rooibos has also been used as an anti-hypertensive, immune stimulant, laxative, sedative and spasmolytic agent as well as for the treatment of atherosclerosis and diabetes.

Iron bioavailability. Although no randomized clinical trials with rooibos tea have been conducted, Hesseling and Joubert (1982) and Hesseling *et al.* (1979) reported their observations from two small human studies. First, Hesseling *et al.* (1979) studied the effect of rooibos versus black tea and water on iron absorption in 30 healthy young men (21–34 years). No between group differences with regard to plasma measures of iron status (hemoglobin, ferritin, transferrin) were detected at baseline. All subjects were administered 1 μCi radiolabeled ^{59}Fe plus 16 mg elemental iron followed by either rooibos tea (n = 10), black tea (n = 10) or water (n = 10). The mean iron absorption, measured 2 weeks later with a whole body counter, was 7.25% with rooibos tea group, 1.70% with black tea and 9.34% with water. Thus, compared with black tea, rooibos did not have a significant effect on iron absorption and was not different than water in this regard.

Anti-allergenic actions. Hesseling and Joubert (1982) tested the potential anti-allergenic properties of rooibos tea in seven subjects diagnosed with either asthma or hay fever. The presence of allergic disease was confirmed with total serum IgE values and specific IgE for one or more of three antigens tested (cat and dog epithelium, grass pollen). A skin prick test was used to measure the degree of type 1 skin reaction during the intervention. Subjects refrained from using antihistaminic drugs for 1 week prior to the study, then skin prick tests of 16 commonly inhaled allergens were administered to subjects on both forearms 11–14 and 7 days prior to treatment (repeated controls) and then again following rooibos treatment. On the day of treatment, subjects refrained from all food and drink except for rooibos tea (25 g/L boiling water, 5 min), which was provided in three 500 mL doses given 3 h apart. During the two control tests, 14 of the 16 antigens were of similar size on both arms of each patient, while 2 antigens produced a significant positive reaction. In the skin prick test following ingestion of rooibos, 12 of the antigens remained unchanged from controls, while reactions to 4 antigens (house dust, grass pollen, dog epithelia and *Aspergillus fumigatus*) were significantly larger. The results of an experiment using a rooibos poultice (prepared by soaking 500 mL tea leaves in 500 mL cold water for 30 min) applied to one of the forearms of each subject for up to 15 min followed by skin prick tests gave similar results. Since neither ingestion nor topical application of rooibos exhibited any antihistaminic effect in this study, its therapeutic value

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as an anti-allergy treatment was not substantiated by these experiments.

Adverse reactions/toxicity

Using ten Fischer 344 rats Marnewick *et al.* (2003) examined a variety of indices for safety of green and red rooibos following a 10 week intervention with a 2 g/100 mL water preparation. Neither green nor red rooibos tea adversely affected body weight, liver weight, or liver and kidney parameters including serum aspartate transaminase, alanine transaminase, alkaline phosphatase, creatinine, total and unconjugated bilirubin, total protein, total cholesterol, or iron status.

HONEYBUSH (*CYCLOPIA INTERMEDIA*)

Nomenclature

Honeybush or *Cyclopia intermedia* is a short, woody shrub grown in the mountain slopes of the Langkloof district between the Eastern and Western Cape regions of South Africa. Unlike other herbal teas, honeybush is not widely cultivated and most of the commercially available product is collected from natural plant populations. The leaves, stems and flowers of the plant are harvested for use in making an herbal tea infusion, which is variously called Heuningtee, Bergtee, Boertee, Bossiestee and Bush tea. Upon harvesting the plant material is cut to disrupt cellular integrity, fermented in either a curing heap or at elevated temperatures in a preheated baking oven, and then allowed to dry. During the fermentation process, the plant material changes color from green to dark brown as the phenolic compounds are oxidized.

Nutrient and phytochemical composition

There are no published reports regarding the macro- or micronutrient content of the *C. intermedia* plant or its infused tea. Some distributors list the mineral contents on their websites or on packages containing honeybush tea (du Toit *et al.*, 1998), however, the source of their analyses is not identified. The minerals most often listed include Ca, Cu, Fe, K, Mg, Mn, Na and Zn.

De Nysschen *et al.* (1996) identified the three major phenolic constituents in the leaves of *Cyclopia* species as mangiferin (a xanthone C-glycoside) and O-glycosides of the flavonones hesperitin and isokuranetin. According to Joubert *et al.* (2003), the relative quantities of these phenolics vary between the different *Cyclopia* species and within a geographical area. Over the course of the 3–4 month harvesting period, the mangiferin content of the plant decreases about 12%, but no significant changes in the content of the other major phenols have been observed. The quantities of mangiferin, isokuranetin and hesperidin (a rutinoside of hesperitin) present in unfermented *C. intermedia* were determined to be 1.7, 0.2 and 1.8 g/100 g dry wt, respectively. In the processed leaves and stems, Ferreira *et al.* (1998) also identified the xanthones mangiferin and isomangiferin, along with the inositol (+)-pinitol, luteolin, the hydroxycinnamic acid 4-coumaric acid, five isoflanones (formononetin, afrormosin, calycosin, pseudobaptigen and fujikinetin), four flavonones (hesperitin, hesperidin, naringenin, eriodictyol) and three coumestans (medicagol, flemichapparin and sophorocoumestan). In a later investigation, Kamara *et al.* (2003) identified additional flavonoids including the hydroxyphenylethanol tyrosol and a couple 4-O-glycosyl derivatives, five glycosylated flavonols (including monoglucosylated kaempferols), four flavanones, two isoflavones (including wistin) and two flavones.

After 72 h of fermentation, du Toit and Joubert (1998) found significant reductions of 26% in the concentration of total polyphenols compared with the amount present at 24 h (129.2 vs 95.6 g/kg soluble solids), of 32% in flavonoids over the same time period (92.0 vs 62.2 g/kg soluble solids) and of 60% in the tannin content (40.1 vs 16.0 g/kg soluble solids). Significantly reduced concentrations of these components were initially achieved between 24 and 36 h of fermentation. Marnewick *et al.* (2000) also reported significantly higher total polyphenols (35.3% vs 19.8%) and flavonoids (27.1% vs 9.9%) in a 4% aqueous extract of unfermented honeybush compared with an extract of the fermented product. The amount of non-flavonoids was, however, higher in the fermented than unfermented tea (9.94% vs 8.4%, respectively). Du Toit and Joubert (1999) reported that honeybush tea extracts prepared from fermented plant materials containing the flowers had significantly less total polyphenols, but more favorable organoleptic properties including a sweeter aroma, flavor and better quality overall.

In vitro studies

Antimutagenic activity. Marnewick *et al.* (2000) determined that aqueous extracts of both unfermented and fermented honeybush have antimutagenic activity. Extracts were prepared by pouring 100 mL boiling water over 4 g dried leaves and allowing it to stand at room temperature for 30 min before filtration and lyophilization. The freeze-dried tea was then reconstituted at 5% and 10% for use in the *Salmonella typhimurium* mutagenicity assay. In a dose-dependent manner, both unfermented and fermented honeybush teas significantly reduced mutagenesis induced by 2-acetylaminofluorene (AAF) in *S. typhimurium* strain TA98 compared with the control. The unfermented extracts had significantly fewer histidine revertants per plate (AAF, 5 μg/plate) compared with the fermented tea at concentrations of both 5% (318 vs 143, respectively) and 10% (182 vs 68, respectively). A similar protective effect with honeybush was observed after aflatoxin B_1 (AFB_1)-induced mutagenesis in *S. typhimurium* strain TA100 although, unlike AAF, the concentration and fermentation state differences reached with AFB_1 were not statistically significant. Only the unfermented honeybush preparation at 10% had a significant effect on cumolhydroperoxide mutagenesis, while none of the extracts affected mutagenesis induced by hydrogen peroxide and methyl methanesulfonate.

Antioxidant activity. Marnewick *et al.* (2005) determined the inhibitory effect of honeybush extracts on lipid peroxidation using a rat liver microsomal preparation

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and the TBARS assay. Ethanol/acetone soluble extracts of both unprocessed and processed honeybush teas effectively inhibited lipid peroxidation (63% and 13%, respectively) compared with the control ($p < 0.001$). However, neither honeybush extract was as effective as the other extracts tested. In this experiment, green tea exhibited the highest protective effect (99%), followed by unprocessed rooibos (91%) and processed rooibos (65%).

Hubbe and Joubert (2000) investigated the superoxide radical ($O_2^{\cdot-}$) scavenging activity of aqueous extracts from five different *Cyclopia* species (*C. sessiflora*, *C. genistoides*, *C. subternata*, *C. maculata*, *C. intermedia*) in a β-NADH/ phenazine methosulphate (PMS) system. Teas were prepared by steeping plant material in boiling water (20 g/500 mL) for 5 min, followed by filtration. The scavenging abilities of all the extracts were significantly lower after fermentation. Fermented *C. intermedia* and *C. maculata* had the lowest total polyphenolic contents and showed the weakest $O_2^{\cdot-}$ scavenging activity (IC_{50} 249.3 and 238.2 μg/mL, respectively). The phenolic components luteolin and eriodictyol showed the highest $O_2^{\cdot-}$ scavenging activity (IC_{50} 39.1–39.5 μg/mL) with the exception of the reference compounds quercetin (IC_{50} 16.8 μg/mL) and superoxide dismutase (SOD; IC_{50} 35.3 μg/mL). The effects of mangiferin and naringenin were more than 10-fold lower (IC_{50} 332.0–515.4 μg/mL) than luteolin, while hesperitin and hesperidin were even weaker (IC_{50} 880.9–1209.9 μg/mL). Formononetin and medicagol were not effective in quenching $O_2^{\cdot-}$.

The *in vitro* antioxidant effects of mangiferin alone have been reported in a few studies (Garcia *et al.*, 2002; Leiro *et al.*, 2003; Moreira *et al.*, 2001; Sato *et al.*, 1992; Shibnath *et al.*, 1996). In rat macrophages, Leiro *et al.* (2003) determined that concentrations of 1–100 μM mangiferin effectively scavenged the enzymatic production of $O_2^{\cdot-}$ by the hypoxanthine-xanthine oxidase (HX/XO) system. No inhibitory effects of mangiferin on XO were observed over this concentration range, so the scavenging effects of this phenol were not due to suppression of enzyme activity. Higher concentrations of mangiferin (10–100 μM) were required to inhibit $O_2^{\cdot-}$ generated by the NADH/PMS system. In both systems, 100 μM mangiferin was as effective at scavenging $O_2^{\cdot-}$ as 1 U/mL SOD. In a lipid peroxidation system consisting of ethylarachidonate and Fenton's reagent, Lee *et al.* (2003) showed that other phenolic compounds present in honeybush exhibited appreciable antioxidant activity. At 1.0 mM, hesperitin inhibited malondialdehyde formation by 83.7% while luteolin was even more effective with 93.8% inhibition at 0.5 mM. At 0.5 mM each, the antioxidant standards used in this experiment butylated hydroxytoluene (BHT) and α-tocopherol exhibited 96.4% and 87.1% inhibition, respectively. In a previous study by Garcia *et al.* (2002), 1–50 μg/mL mangiferin significantly reduced the phorbol myristate acetate-induced production of reactive oxygen species (ROS) by 88–97% in resident rat peritoneal macrophages and 68–92% in thioglycollate-elicited macrophages. The reduction obtained with 10–50 μg/mL mangiferin was similar to that obtained with 18 μg/mL (100 μM) of vitamin C. Reactive nitrogen species (RNS) were also suppressed by mangiferin. Thioglycollate-elicited macrophages stimulated *in vitro* for 48 h with lipopolysaccharide (LPS) and interferon (IFN) γ in the presence of 1–50 μg/mL mangiferin produced significantly less nitric oxide (NO) compared with cells stimulated in the absence of mangiferin (Leiro *et al.*, 2003). Leiro *et al.* (2003) concluded the mechanism of this action was a suppression of inducible nitric oxide synthase (iNOS) mRNA synthesis. At 100 μM mangiferin also significantly reduced mRNA levels of the cytokine TNF-α, an inducer of iNOS, and increased mRNA levels of the cytokine TGF-β, an inhibitor of iNOS; GAPDH mRNA, the control for gene expression in this experiment, was not affected by mangiferin.

Immunomodulating activity. Garcia *et al.* (2002) found that mangiferin affects phagocytic activity macrophages. The presence of this xanthone in assay medium at 0.1–100 μg/mL reduced phagocytosis by resident macrophages *in vitro* with an IC_{50} of 6.5 μg/mL. A similar reduction in phagocytosis was observed in thioglycollate-elicited macrophages from rats administered mangiferin i.p. at 50–250 mg/kg.

Chattopadhyay *et al.* (1987) assessed the effectiveness of mangiferin as a potential immunomodulator using murine lymphocytes. At doses of 5–40 μg/mL, mangiferin induced cellular proliferation in splenic cells and thymocytes of normal mice (4.4–6.8 mean stimulation index, SI), although at higher doses (50–100 μg/mL) proliferation was suppressed (1.7–2.7 SI). Splenocytes from tumor-bearing mice were significantly activated by 5 and 20 μg/mL mangiferin over a 12 day growth period (3.4–7.9 SI). While the responsiveness of the lymphocytes to mangiferin was lower at the end of the growth period, proliferation responses were significantly higher than that obtained with 5 μg/mL of the control mitogens phytohemagglutinin (1.5 SI) and concanavalin A (2.5 SI). Mangiferin was also shown to be effective in activating murine peritoneal macrophages *in vitro* by Guha *et al.* (1993). The mangiferin-treated macrophages showed increased lysosomal enzyme acid phosphatase activity and enhanced cytotoxicity and phagocytosis against ascitic fibrosarcoma (AFS) cells.

An antiviral activity of mangiferin has been reported. Zheng and Lu (1990) found mangiferin and isomangiferin inhibited type 1 herpes simplex virus (HSV–1) by 56.8% and reduced plaque rates by 69.5%. Zhu *et al.* (1993) found mangiferin effectively inhibited HSV-2 plaque formation in HeLa cells (EC_{50} 111.7 μg/mL) and reduced replicative yields at 33 μg/mL (EC_{90}) and 80 μg/mL (EC_{99}). Guha *et al.* (1996) reported that mangiferin was also able to antagonize the cytopathic effect of the human immunodeficiency virus (HIV) *in vitro*.

Lipolytic activity. Mangiferin isolated from the dried roots of the *Salacia reticulata* plant (0.11% yield) by Yoshikawa *et al.* (2002) exhibited lipolytic activity at a concentration of 100 mg/L in cultured rat epididymal fat-derived adipocytes. After incubation for 18 h, a significant reduction to 65% of the initial triglycerides present in the control cells remained after mangiferin treatment. Interestingly, mangiferin had no inhibitory effect on the lipid metabolizing enzymes lipoprotein lipase and pancreatic lipase.

Bone resorption. Li *et al.* (1998) found mangiferin showed a significant inhibitory effect on parathyroid hormone-stimulated bone resorption in an organ culture system using neonatal mouse parietal bones.

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Mangiferin also decreased the number of tartrate-resistant acid phosphatase-positive multinucleated cells during the formation of osteoclast-like cells.

Animal model studies

Antioxidant capacity. Marnewick *et al.* (2003) examined the *in vivo* effects of honeybush tea on antioxidant capacity as well as on hepatic drug metabolizing enzymes. They provided male Fischer rats (n = 10/group) either water or one of several different tea extracts, including a 2% solution of green tea, black tea, rooibos tea (unfermented or fermented) or 4% honeybush tea (unfermented or fermented), as the sole source of drinking fluid for a 10 week period. Tea extracts were prepared by adding boiling water to tea leaves and stems and allowing the solutions to stand for 30 min at room temperature prior to filtration. The hepatic antioxidative capacity, measured with the oxygen radical absorbance capacity (ORAC) assay, did not change in rats treated with either type of honeybush tea or rooibos tea. Unexpectedly, the green and black teas marginally lowered ORAC values compared with the control group. Levels of reduced glutathione (GSH) in the liver were not significantly affected by any of the teas, however, all of the teas examined reduced oxidized glutathione (GSSG) levels compared with the water control. GSSG concentrations in both the unfermented (0.51 ± 0.18 μM/mg protein) and fermented (0.70 ± 0.16 μM/mg) honeybush tea groups were comparable to levels found in the green (0.76 ± 0.15 μM/mg) and black (0.87 ± 0.29 μM/mg) tea groups, although levels in the unfermented and fermented rooibos tea groups were significantly lower (0.42 ± 0.13 and 0.40 ± 0.13 μM/mg, respectively). The GSH/GSSG ratios were significantly higher in the liver of rats drinking unfermented honeybush and marginally higher in the fermented honeybush group, while no changes were observed with green and black teas. The activity of cytosolic glutathione S-transferase alpha (GST-α) was only higher in rats given the honeybush teas or rooibos teas. Microsomal glucuronosyl transferase (UDP-GT) activity was modestly higher only following consumption of the unfermented honeybush and rooibos teas.

Sanchez *et al.* (2000) compared the effects of mangiferin (50 mg/kg/day) and a mangiferin-containing crude plant extract (*Mangifera indica*) (50–250 mg/kg/day) with the antioxidants vitamin C (100 mg/kg/day), vitamin E (100 mg/kg/day) and β-carotene (50 mg/kg/day) in male OF1 mice (n = 5/group). Each compound was administered p.o. for 7 days and on the following day all mice received 12-O-tetradecanoylphorbol-13-acetate (TPA) i.p. to induce oxidative stress. In blood samples taken prior to TPA injection, levels of superoxide dismutase (SOD) were significantly higher in the groups receiving mangiferin, *Mangifera indica* extract, β-carotene or a combination of vitamins C and E when compared with an untreated control group. After TPA treatment, SOD levels were higher in all groups, but statistical significance was achieved only with mangiferin and the *Mangifera indica* extract (increases of 21–24%). Glutathione peroxidase (GPX) levels following TPA treatment were lower (14%) only in the mangiferin group; the levels in all groups did not change prior to TPA. The TPA-induced production of ROS in peritoneal macrophages was lower in all groups when compared with the group given TPA only. Mangiferin decreased cytochrome c reduction by 44%, the same level achieved with the vitamin C and E combination, and inhibited the production of H_2O_2 by 40%, a greater change than was observed with either the single antioxidant vitamins alone or together (ranges: 6–13% for cytochrome c, 19–37% for H_2O_2). The effect of mangiferin on reducing the loss of TPA-induced hepatic sulfhydryl (TSH) levels (54%) was also greater than the antioxidant vitamins (15–43%). In addition, mangiferin protected against DNA fragmentation in the liver (35%) and brain (22%) compared with the TPA control and reduced lipid peroxidation in hepatic mitochondria (15%) and microsomes (13%), brain homogenates (39%) and serum (25%). Yoshikawa *et al.* (2002) demonstrated the scavenging activity of orally administered mangiferin on DPPH radicals (IC_{50} 5.9 μM) and its protective effects against carbon tetrachloride-induced elevations of serum aspartate and alanine aminotransferases as well as TBARS formation in male ddY mice at a dose of 100 mg/kg.

As several antioxidant polyphenols improve vascular responsiveness, Leiro *et al.* (2003) tested 1–100 μM mangiferin in pre-contracted and non-contracted rat aortic rings. However, these doses did not modify the resting tone or contractile responses elicited by 1 μM phorbol 12-myristate 13-acetate or phenylephedrine hydrochloride. These investigators concluded that mangiferin did not affect the balance between superoxide and nitric oxide production and was unable to modify endothelium-dependent vascular relaxation.

Chemopreventive actions. Using the same experimental design described above, Marnewick *et al.* (2004) examined the *ex vivo* modulation of mutagenesis of honeybush tea. Using microsomal and cytosolic liver fractions isolated from tea-treated rats, mutagenicity induced with AAF and AFB_1 in the *Salmonella* assay was suppressed by honeybush tea. Using *S. typhimurium* strains TA100 and AFB_1, the number of histidine revertants per plate was significantly reduced with cytosolic fractions (0.25 mg/mL protein) from rats treated with unfermented and fermented honeybush (303.2 and 306.1, respectively) compared with rats provided water (385.0). The effect of unfermented honeybush was also significant using TA98 and AAF (161.3 vs 220.3 in controls), but the effect of fermented honeybush was marginal (191.1) and not statistically significant. No protective effects were observed when higher cytosolic protein concentrations (1 mg/mL) from honeybush-treated rats were used. The hepatic microsomal fractions from rats consuming unfermented, but not fermented, honeybush tea were able to protect against mutagenicities induced by AFB_1 in strain TA100, but increased the number of revertants obtained with AAF induction of TA100 (79%); in comparison, microsomal fractions from the green and black tea groups were more potent in increasing the mutagenicity of AAF (132% and 128%, respectively).

The topical application of both unprocessed and processed honeybush tea extracts was effective in reducing skin tumorigenesis in mice. Marnewick *et al.* (2005) applied DMBA and TPA to ICR mouse skin to respectively initiate and promote tumors. Tea extracts (green, rooibos or honeybush) were applied 30 min prior to

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the application of the promoting agent, and the process was repeated twice weekly for 20 weeks. Green tea extract completely inhibited the formation of mouse skin tumors. Unprocessed and processed honeybush and rooibos tea extracts significantly decreased tumor incidence ($p < 0.05$), reduced mean tumor volume and delayed the onset of tumor development. The unprocessed honeybush extract exhibited the highest decrease in the percentage of tumor bearing mice (90%), followed by the processed honeybush (84.2%), processed rooibos (75%) and unprocessed rooibos (60%) extracts.

In a study of rat colon carcinogenesis Yoshimi *et al.* (2001) tested the effects of dietary supplementation with 0.1% mangiferin. Aberrant crypt foci (ACF), indicative of preneoplastic lesions and tumorigenesis induced by the carcinogen azoxymethane (AOM) were both significantly reduced in the group treated with mangiferin. Compared with rats treated with AOM alone, ACF development in the AOM plus mangiferin-treated rats was reduced by 40%, the incidence and multiplicity of intestinal neoplasms were reduced by 47% and 42%, respectively, and cell proliferation in the colonic mucosa was reduced 65–85%.

Anti-diabetic actions. Muruganandan *et al.* (2002) investigated the protective role of mangiferin against streptozotocin (STZ)-induced insulin-dependent diabetes in rats. Compared with rats treated with saline, mangiferin administered i.p. at 10 or 20 mg/kg for 28 days post-STZ treatment significantly reduced glycosylated hemoglobin levels (15–21%), a biomarker of glucose control. In addition, the mangiferin treatment reduced serum creatine phosphokinase activity (54–56%) and malondialdehyde concentration in erythrocytes (39–43%), heart (27–37%) and kidney (25–34%). The treatment increased catalase activity in the kidney but, at the 20 mg/kg dose, catalase and SOD activities in heart were significantly reduced; in erythrocytes, these antioxidant enzymes were not affected by mangiferin.

Ichiki *et al.* (1998) reported that oral administration of mangiferin and its glucosides lowered blood glucose levels of KK-Ay mice, a strain used as a model of non-insulin dependent diabetes mellitus. Miura *et al.* (2001a; 2001b; 2001c; 2001d) also employed KK-Ay mice to study the effect of mangiferin on signs of diabetes. Mangiferin had no effect on blood glucose levels of non-diabetic mice but improved the hyperinsulinemia in KK-Ay diabetic mice, suggesting its affect on insulin sensitivity (Miura *et al.*, 2001b; 2001c). Mangiferin treatment (30 mg/kg) combined with exercise was also able to significantly reduce blood cholesterol and triglycerides in KK-Ay mice (Miura *et al.*, 2001d) as well as lower blood cholesterol levels in cholesterol fed mice (Miura *et al.*, 2001a).

Immune enhancement. Garcia *et al.* (2003a) examined the *in vivo* effects of mangiferin on humoral immune responses in mice induced by inoculation with spores from microsporidian parasites. The oral administration of mangiferin at 100 mg/kg/day for 28 days had no significant effect on the mean post-inoculation production of antibodies, but did significantly enhance the production of both IgG1 and IgG2B and raise the splenic index compared with mice inoculated with spores alone. Thus, while mangiferin does not affect the primary antibody response (principally IgM), it may stimulate the production of interleukins by helper T cells that in turn stimulate the conversion of Th-1 to Th-2 cells. Garcia *et al.* (2003b) also found that oral treatment with mangiferin at 50 mg/kg/day significantly reduced the number of *Trichinella spiralis* larvae encysted in the musculature of experimentally infected mice when administered either on the day of infection and throughout the parasite life cycle (~35 days) or for 20 days pre-infection to 11 days post-infection compared with untreated control mice. Mangiferin also reduced the levels of specific anti-*Trichinella* IgE in the serum of mice measured at 28 days and 35 days post-infection. When administered during the post-infection period only, however, mangiferin had no effect on the number of adult parasites implanted in the intestine. In a separate experiment, mast cell degranulation was completely inhibited in rats treated orally for 50 days with mangiferin at 50 mg/kg/day and then subjected to a passive cutaneous anaphylaxis test using serum IgE from mice previously infected with *T. spiralis* compared with untreated controls.

Human studies and potential health/therapeutic applications

Honeybush tisanes have been employed as a folk remedy and indicated by some traditional medicines to treat digestive problems, promote lactation and cure skin rashes. Honeybush has also been used as a laxative and as a sedative. However, no clinical trials or human studies examining the effects of *C. intermedia*, honeybush tea or mangiferin have been reported.

Adverse reactions/toxicity

No adverse effects or toxicities of *C. intermedia* have been reported, although the presence of microbial contaminants, particularly *Rhizomucor pusillus*, during the fermentation and drying of honeybush tea was noted by du Toit *et al.* (1999). Two thermophilic moulds, *Humicola grisea var thermoida* and *H. lanuginosa*, and five endospore-forming *Bacillus* species, *B. brevis*, *B. badius*, *B. stearothermophilus*, *B. subtilis* and *B. pumilus*, were isolated by these investigators. However, the elimination of these microbial contaminants can be achieved by processing the plant materials at a temperature >60 °C and with controlled curing conditions.

DISCUSSION

As with most plant materials, both rooibos and honeybush contain a wide variety of flavonoids and other phytochemicals (Table 1). Most of the individual flavonoids and other phytochemical constituents of rooibos and honeybush are found either exclusively in one or the other plant, i.e. flavan-3-ols, flavones, dihydrochalcones, proanthocyanadins and phenolic acids are predominantly found in rooibos while isoflavones, coumestans, inositols and xanthones predominate in honeybush. Eriodictyol and luteolin are the only individual components identified to date that are common

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Table 1. Phytochemicals present in rooibos and honeybush[a]

Class of phytochemical	Individual component	Rooibos	Honeybush
Flavan-3-ols	Catechin	✓	
Flavanones	Eriodictyol	✓	✓
	Hesperetin		✓
	Isokuranetin		✓
	Naringenin		✓
Flavones	Chrysoeriol	✓	
	Isoorientin	✓	
	Isovitexin	✓	
	Luteolin	✓	✓
	Orientin	✓	
	Vitexin	✓	
Flavonols	Kaempferol		✓
	Quercetin	✓	
Isoflavones	Afrormosin		✓
	Calycosin		✓
	Formononetin		✓
	Fujikinetin		✓
	Pseudobaptigen		✓
	Wistin		✓
Dihydrochalcones	Aspalathin	✓	
	Nothofagin	✓	
Proanthocyanadins		✓	
Phenolic acids	Caffeic acid	✓	
	Ferulic acid	✓	
	p-Coumaric acid	✓	
	p-Hydroxybenzoic acid	✓	
	Protocatechuic acid	✓	
	Syringic acid	✓	
	Tyrosol (and derivatives)		✓
	Vanillic acid	✓	
Coumestans	Flemichapparin		✓
	Medicagol		✓
	Sophoracoumestan		✓
Inositols	Pinitol		✓
Xanthones	Isomangiferin		✓
	Mangiferin		✓

[a] A checkmark indicates the presence of a particular compound identified in the specified herb. As no comprehensive and comparable analysis of the phytochemical profile of these herbs has been conducted, the absence of a checkmark does not necessarily imply the absence of that compound or other phytochemicals.

to both plants. Flavonoids are ubiquitously distributed among plants, and the profile of individual flavonoid compounds is known to vary markedly between different plant species and even between plants of similar species. As a result, different dietary consumption patterns of plant foods and beverages, including rooibos, honeybush and other tisanes, will provide distinctly different intakes of individual phytochemicals.

Despite the differences in their respective phytochemical profiles, *in vitro* and animal studies have found that extracts from both rooibos and honeybush have antioxidant properties, chemopreventive potential and immune modulating effects. One component found in honeybush alone, mangiferin, was also shown to have a protective effect in diabetic mice, perhaps unrelated to its antioxidant activity. *In vivo* experiments examining the potential health effects of rooibos and honeybush were conducted almost exclusively in animal models (Tables 2 and 3, respectively). No published reports describing controlled clinical trials employing either rooibos or honeybush tisanes or their extracts were reported in English language peer-reviewed journals. Only two reported studies of rooibos were conducted in human subjects (Table 4), and no reports on the effects of honeybush in humans were discovered.

Overall herbal tea consumption has been increasing at an annual rate of 15–20% per year, fueled primarily by aging 'baby boomers' and other health conscious consumers seeking out products that will help them live longer, feel better and stay healthier (Sage Group, 2004). According to a 2003 Gallup study (Sage Group, 2004) nearly half of all adults reported making at least some effort to consume food and beverages high in antioxidants, including different types of teas and tisanes. While the marketing for some teas and tisanes do not appear fully substantiated, information being proffered to consumers should be based upon sound, research-based evidence to justify health claims (Balentine *et al.*, 1999). As 'emerging' herbal teas, rooibos and honeybush have not been well studied in humans, so claims of benefit are not truly warranted

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Table 2. Summary of animal studies examining the effects of rooibos[a]

Reference	Animal model	Dose	Duration	Outcome
Sasaki *et al.*, 1993	Mice	1.0 mL of 0.1% tea solution	6 h prior to MMC induction 24 h prior to MMC induction 3,6 or 9 h post-MMC induction	Reduced induction of MNRET No effect on MNRET frequency No effect on MNRET
	Mice	1.0 mL/day of 0.05–0.10% tea solution	28 days, plus 24–48 h MMC or B(a)P induction 28 days, plus 1.5 Gy γ-ray irradiation	Reduced MNRET frequency No antimutagenic effect on γ-ray-induced damage with any tea tested
Inanami *et al.*, 1995	Rats	*Ad libitum*	3–24 months	Prevented age-related accumulation of lipid peroxides (TBARS) in brain
Shimoi *et al.*, 1996	Mice	1.0 mL	2 h prior to 1.5 Gy γ-ray irradiation	Decreased frequency of MNRET
	Mice	10 μmol/kg luteolin (fractionated from rooibos tea)	2 h prior to 6 Gy γ-ray irradiation	Reduced lipid peroxidation in bone marrow and spleen; protected against loss of endogenous ascorbic acid (NS)
Simon *et al.*, 2000	Japanese quail[c]	Tea supplemented food	Long term	No change in susceptibility to peroxide or hypotonia-induced erythrocyte hemolysis
Kunishiro *et al.*, 2001	Rats	10–1000 μg/mL tea extract	Post cyclosporin-A treatment	Restored production of antigen-specific antibodies in serum; stimulated IL-2 generation in splenocytes
Marnewick *et al.*, 2003	Rats	Sole source of drinking fluid (2 g/100 mL)	10 weeks	No change in ORAC; increased ratio of GSH/GSSG; enhanced phase II enzyme activities
Ulicn *et al.*, 2003	Rats	Average daily consumption of 32 mL/animal	7 days prior to exposure to CCl_4	Had anti-fibrotic effect in experimental model of hepatic cirrhosis
Marnewick *et al.*, 2004	Rats[c]	Sole source of drinking fluid	10 weeks	Hepatic fractions from treated animals had reduced AFB1-induced mutagenic activity
Marnewick *et al.*, 2005	Mice[b]	100 μL extract applied to skin	1 week after DMBA, 30 min prior to TPA; 20 weeks total	Reduced number and volume of skin tumors

[a] p.o. route of administration for all studies except for [b] which was topical and [c] *Exvivo*.
MMC, mitomycin C; MNRET, micronucleated reticulocytes; B(a)P, benzo[a]pyrene; AFB_1, aflatoxin B_1; TBARS, thiobarbituric acid reactive substances; ORAC, oxygen radical absorbance capacity; GSH/GSSG, reduced/oxidized glutathione; CCl_4, carbon tetrachloride; NS, non-significant; DMBA, 7,12-dimethylbenz[a]anthracine; TPA, 12-O-tetra-decanoylphorbol-13-acetate.

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Table 3. Summary of animal studies examining the effects of honeybush and its major phenolic component, mangiferin[a]

Reference	Animal model	Dose	Duration	Outcome
Ichiki *et al.*, 1998	Mice	30–90 mg/kg mangiferin isolated from *Anemarrhena asphodeloides*	7 h	Lowered blood glucose levels in diabetic (NIDDM) mice
Sanchez *et al.*, 2000	Mice	50 mg/kg/day mangiferin	7 days followed by one i.p. dose of TPA	Significantly higher SOD activity compared with controls; lower GPX activity, cytochrome c reduction; protected against DNA fragmentation and reduced lipid peroxidation
Yoshimi *et al.*, 2001	Rat	0.1% mangiferin in basal diet	4 weeks	Reduced markers of colon carcinogenesis
Miura *et al.*, 2001a; 2001b	Mice	90 mg/kg/day of water extract from *A. asphodeloides* root containing mangiferin	3 weeks	Improved hyperinsulinemia in diabetic (NIDDM) mice
Miura *et al.*, 2001c	Mice	30 mg/kg/day mangiferin plus exercise	2 weeks	Significantly reduced blood cholesterol and triglycerides in diabetic (NIDDM) mice
Miura *et al.*, 2001d	Mice	30 mg/kg/day mangiferin-containing extract from *A. asphodeloides*	3 weeks	Lowered blood cholesterol levels in cholesterol-fed mice
Yoshikawa *et al.*, 2002	Mice	100 mg/kg mangiferin	1 h prior to CCl_4 treatment (4 h)	Protected against CCl_4 induced enzyme activities, reduced TBARS formation
Muruganandan *et al.*, 2002	Rats[b]	10 or 20 mg/kg/day	28 days	Reduced glycosylated Hb levels, reduced serum CPK, MDA activities in STZ-induced IDDM rat
Marnewick *et al.*, 2003	Rats	4% solution as sole drinking fluid	10 weeks	No change in ORAC; marginally higher GSH/GSSG, increased GST-α activity/hepatic drug metabolizing enzyme activities
Garcia *et al.*, 2003a	Mice	100 mg/kg/day mangiferin	28 days	Improved humoral responses in mice inoculated with parasites
Garcia *et al.*, 2003b	Rats	50 mg/kg/day	50 days	Inhibited mast cell degranulation
Marnewick *et al.*, 2004	Rats[d]	Sole drinking fluid	10 weeks	Suppressed AAF, AFB_1 mutagenicity in *Salmonella* assay
Marnewick *et al.*, 2005	Mice[c]	100 μL extract applied to skin	1 week after DMBA, 30 min prior to TPA; 20 weeks total	Reduced number and volume of skin tumors

[a] p.o. route of administration for all studies except for [b] which was i.p, and [c] which was topical.
[d] *Ex vivo.*
AFB_1, aflatoxin B_1; TBARS, thiobarbituric acid reactive substances; ORAC, oxygen radical absorbance capacity; GSH/GSSG, reduced/oxidized glutathione; CCl_4, carbon tetrachloride; GST-α, glutathione S-transferase alpha; AAF, 2-acetylaminofluorene; NS, non-significant; TPA, 12-O-tetradecanoylphorbol-13-acetate; STZ, streptozotocin; IDDM, insulin dependent diabetes mellitus; NIDDM, non-insulin dependent diabetes mellitus; CPK, creatine phosphokinase; MDA, malondialdehyde; DMBA, 7,12-dimethylbenz[a]anthracine; TPA, 12-O-tetra-decanoylphorbol-13-acetate.

Table 4. Summary of human studies examining the effects of orally ingested rooibos

Reference	Subjects	Dose	Duration	Outcome
Hesseling *et al.*, 1979	30 healthy young men (21–34 years)	Rooibos tea, black tea or water following one time ingestion of radiolabeled Fe	2 week follow up	Compared with black tea, rooibos had no significant effect on Fe absorption, and was no different than water
Hesseling and Joubert, 1982	7 subjects diagnosed with hay fever or asthma	25 g rooibos/L boiling water, provided in three 500 mL doses given 3 h apart on same day skin prick tests of antigens	1 day	No antihistaminic effects were observed compared with control tests given without rooibos treatment

despite their long history of use in some traditional medicines. According to the 2004 'Tea is Hot' report (Sage Group, 2004), the labeling and promotion claims for some brands of rooibos and honeybush teas are questionable, with claims of antioxidant superiority over green tea and the ability to treat selected health disorders. Nonetheless, the apparent bioactivity of rooibos and honeybush tisanes and their constituents evident from *in vitro* and *in vivo* studies suggests further investigations in this area are warranted. Such research, when placed in the context of dietary guidelines, suggests as well the consumption of tisanes as a ready way to increase the intake of a variety of potentially health-promoting phytochemicals

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