

## Rooibos (*Aspalathus linearis*)

South Africa's most popular hot beverage is 'Red Bush Tea,' made from twigs and leaves of the Rooibos (*Aspalathus linearis*) shrub that grows in the mountains there.

Apart from adding taste to everything you cook, Rooibos actually makes your meals healthier too. Rooibos has a natural sweet taste - so you don't have to add sugar to your cooking. And because Rooibos contains lots of minerals and vitamins, it also lifts the nutrient level of the meal.

Rooibos is less astringent and has a lower tannin content than traditional teas, both black and green teas, made from leaves of *Camellia sinensis*, and because it inhibitory effects on iron is minimal when compared. <sup>6</sup> It is caffeine-free, but has a higher content of fluoride, which might help to protect against tooth decay.

Recent research has shown that this tea contains a complexity of flavonoids with anti-oxidant, <sup>1,3,7</sup> free radical scavenging, <sup>1,3,7</sup> and anti-mutagenic <sup>5</sup> ability that rivals that of green tea. Rooibos also processes anti-viral, <sup>2</sup> anti-allergenic, <sup>4</sup> radio-protective, <sup>7,8</sup> and immune regulating activity. <sup>1</sup>

Tea made with this herb is a South African bedtime favorite. South Africans also use it to improve appetite, calm the digestive tract and reduce nervous tension. Traditional medicinal uses of rooibos in South Africa include alleviation of infantile colic, allergies, asthma and dermatological problems. <sup>11</sup>

### A review of recent studies on Rooibos tea

#### Cancer Protective

New scientific studies provide evidence that rooibos red tea may protect against cancer, skin cancer, heart attack, and stroke. Rooibos, the antioxidant-packed South African herbal red "tea," has taken U.S. tea drinkers by storm with its combination of great taste, soothing effect, and multiple health benefits. Recent laboratory research on red tea's potent, unique antioxidant composition reveals that rooibos may help protect against free radical damage that can lead to varying types of cancer and heart problems. Studies show that rooibos (red) teas are antimutagenic--meaning they protect against induced DNA damage, and enhance the activity of certain important carcinogen-detoxifying enzymes in the liver. Rooibos teas may also offer new ways to fight the alarming increase in cases of skin cancer. Through in vivo studies, researchers find that rooibos tea extracts inhibit the promotion of cancerous tumors in mouse skin. <sup>11</sup>

"We concluded that rooibos extracts interfered with skin cancer in its promotion (later development) stage. This provides the first evidence of a protective effect for rooibos teas," states Jeanine L. Marnewick, MSc. at the Medical Research Council of South Africa. "The results obtained from the rooibos study look very promising. Mouse skin that was topically treated with the rooibos extracts before cancer promotion showed a 75% decrease in the development of skin papillomas." In addition, other studies revealed rooibos' protective effects against DNA damage when tested in vitro and in vivo. Although current skin cancer model tests have been conducted using live animals, Marnewick and her team hope the results will soon be extrapolated to humans.

In addition to being naturally caffeine-free, rooibos is also famed for helping relieve constipation, various types of inflammatory dermatitis, and milk allergies in babies. Rooibos also contains calcium, fluoride, magnesium, iron, zinc, copper and potassium.

Rooibos is currently a popular tea in Europe and Japan. Today, most major tea companies are selling rooibos tea in grocery, natural food stores and Tea/Coffee Houses throughout the USA and Canada. <sup>10</sup>

Effects of rooibos tea extract on antigen-specific antibody production and cytokine generation in vitro and in vivo.

Rooibos tea contains a large amount of flavonoids and acts as a potent antioxidant. In this study, we examined the effects of Rooibos tea extract on antigen-specific antibody production and cytokine generation in vitro and in vivo. The primary in vitro anti-ovalbumin (anti-OVA) or sheep red blood cell (SRBC) antibody production in murine splenocytes was markedly stimulated by the addition of the tea extract at concentrations of 1-100 microg/ml. On the other hand, a nonspecific antibody response elicited with lipopolysaccharide (LPS) in purified splenic B-cells was not modified by the extract. Rooibos tea extract caused an increase in the generation of interleukin 2 (IL-2) both in OVA- and anti-CD3-primed splenocytes at concentrations ranging from 10 microg/ml to 1000 microg/ml. In contrast, this tea extract suppressed the generation of interleukin 4 (IL-4) in OVA-primed splenocytes. Moreover, the reduction of

OVA-induced antibody production in serum of the cyclosporin A (CyA) -treated rats can be significantly restored and the IL-2 generation in murine splenocytes was stimulated, following oral administrations of Rooibos tea extract. Thus, our findings suggested *that Rooibos tea extract may facilitate the antigen-specific antibody production through selective augmentation of IL-2 generation both in vitro and in vivo. Collectively, Rooibos tea intake may be of value in prophylaxis of the diseases involving a severe defect in Th1 immune response such as cancer, allergy, AIDS, and other infections.*<sup>1</sup>

Anti-human immunodeficiency virus activity of oligosaccharides from rooibos tea (*Aspalathus linearis*) extracts in vitro.

The active substances, acid polysaccharides, were extracted with 1% sodium hydroxide from the leaves of rooibos tea (*Aspalathus linearis*), Du Zhong Cha (*Eucommia ulmoides* Oliv.) and Japanese tea leaves (*Camellia sinensis* var. *sinensis*). The alkaline extracts of Rooibos tea and Du-Zhong tea leaves, but not Japanese tea leaves suppressed the HIV-induced cytopathicity using HIV (HTLV-III) infected MT-4 cells, having extremely low cytotoxicity: Its 50% effective concentration (EC50) was 12-67 micrograms/mL, while 50% cytotoxic concentration (CC50) was higher than 1.0 mg/mL. The active substances were purified with ethanol precipitation. The substances were composed of 27% of reducing sugar, 46% of neutral sugars and 22% of uronic acid. A LD50 of the alkaline extracts from rooibos tea was higher than 1.2 g/kg body weight. Acid degraded substances composed of disaccharides and trisaccharides, were also suppressed the HIV-induced cytopathicity. *From these results, it is probable that acid polysaccharides from rooibos tea were extremely safe, and that HIV infection may be suppressed by daily intake of the alkaline extracts of rooibos tea and Du-Zhong tea.*<sup>2</sup>

Quantitative characterization of flavonoid compounds in Rooibos tea (*Aspalathus linearis*) by LC-UV/DAD.

Rooibos tea originates from the leaves and stems of the indigenous South African plant *Aspalathus linearis*. It has gained much attention for clinical purposes in the case of nervous tension, allergies (dermatitis), and various indigestive problems. Recently, *antioxidative activity was also attributed to the tea on the basis of its flavonoid content.* Therefore, an HPLC method using a C(18) reversed phase column was developed for the assay of 10 flavonoids in aqueous and methanolic infusions. Main compounds determined were the dihydrochalcone aspalthin, rutin, and orientin, and their content was in the range of 1.0 to 1.3 mg/g. The identity of detected flavonoids was confirmed by comparing their retention times and UV and MS spectra with those of corresponding standards. In addition, the MS analysis showed evidence of the presence of other compounds such as nothofagin, dihydroisorientin, and dihydroorientin.<sup>3</sup>

### **Cellular protective effects of green tea, Po-lei tea and Rooibos tea in CHO cells and mice.**

The suppressing effects of crude extracts of three kinds of tea-green tea (GT) from Japan, Po-lei tea (PT) from China, and Rooibos tea (RT) from South Africa-on the induction of chromosome aberrations in cultured CHO cells and mice were studied. When CHO cells were exposed to each tea extract in the presence of rat liver microsomal enzymes (S9 mix) together with benzo[a]pyrene (B(a)P) or mitomycin C (MMC), a decrease in the frequency of chromosome aberrations was observed. PT and RT, but not GT, also suppressed the induction of chromosome aberrations by MMC in the absence of S9 mix. When cells were treated with tea extract after B(a)P or MMC treatment, RT suppressed the induction of chromosome aberrations in the presence and absence of S9 mix whereas GT and PT showed suppressing effects only in the presence of S9 mix. These data suggest that catechines, well-known antimutagens in tea samples, might account for the inhibitory effect in the case of GT and PT. Since RT contains few catechines, several unknown antimutagenic components could be responsible for its effect. The antimutagenic effects of tea extracts at concentration levels consumed by humans were examined in mice using micronucleus induction with B(a)P or MMC. When mice received oral gavage of 0.2% GT, 0.1% PT, and 0.1% RT at 1.0 ml/mouse 6 h before intraperitoneal injection of MMC, a decrease in the frequency of micronuclei was observed. The induction of micronuclei by B(a)P was suppressed by oral dosage of GT, PT and RT at 1.0 ml/mouse/day for 28 days. This was not due to a delay in the maturation of micronucleated reticulocytes. *In conclusion, intake of tea might suppress the mutagenic activity of certain potent mutagens in human beings.*<sup>5</sup>

### **The effect of rooibos tea on iron absorption**

A study was carried out to determine if rooibos tea (*Aspalathus linearis*) has a deleterious effect on iron absorption similar to that of ordinary tea (*Camellia sinensis*). Three groups of volunteers, each consisting of 10 healthy young men comparable with regard to iron status and body dimensions, were studied. After ingestion of 1 mCi <sup>59</sup>Fe and 16 mg of elemental iron, group A drank rooibos tea, group B ordinary tea

and group C boiled water. Iron absorption was measured 14 days later with a whole body counter. Mean iron absorption after ingestion of rooibos tea, ordinary tea, and water was 7,25%, 1,70% and 9,34% respectively. In contrast to ordinary tea (P less than 0,0001), rooibos tea did not affect iron absorption significantly. The ascorbic acid content of rooibos tea varies between 121,8 and 154,9  $\mu\text{mol/l}$ , depending on the method of preparation. Rooibos tea contains small amounts of iron, magnesium, phosphorus, sodium, chloride and potassium. These findings have practical nutritional and therapeutic implications.<sup>6</sup>

Protection by beverages, fruits, vegetables, herbs, and flavonoids against genotoxicity of 2-acetylaminofluorene and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in metabolically competent V79 cells.

Chinese hamster lung fibroblasts, genetically engineered for the expression of rat cytochrome P450 dependent monooxygenase 1A2 and rat sulfotransferase 1C1 (V79-rCYP1A2-rSULT1C1 cells), were utilized to check for possible protective effects of beverages of plant origin, fruits, vegetables, and spices against genotoxicity induced by 2-acetylaminofluorene (AAF) or 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). Antigenotoxic activities of juices from spinach and red beets against AAF could be monitored with similar effectivity by the HPRT-mutagenicity test (IC(50)=0.64%; 2.57%) and alkaline single cell gel electrophoresis (comet assay; IC(50)=0.12%; 0.89%) which detects DNA strand breaks and a basic sites. Applying the comet assay, genotoxicity of PhIP could, however, be demonstrated only in the presence of hydroxyurea and 1-[beta-D-arabinofuranosyl]cytosine, known inhibitors of DNA repair synthesis. As expected, AAF and PhIP were unable to induce any genotoxic effects in the parent V79 cells. Genotoxic activity of PhIP was strongly reduced in a dose-related manner by green tea and red wine, by blueberries, blackberries, red grapes, kiwi, watermelon, parsley, and spinach, while two brands of beer, coffee, black tea, rooibos tea, morellos, black-currants, plums, red beets, broccoli (raw and cooked), and chives were somewhat less active. One brand of beer was only moderately active while white wine, bananas, white grapes, and strawberries were inactive. Similarly, genotoxicity of AAF was strongly reduced by green, black, and rooibos tea, red wine, morellos, black-currants, kiwi, watermelon, and spinach while plums, red beets, and broccoli (raw) were less potent. Broccoli cooked exerted only moderate and white wine weak antigenotoxic activity. With respect to the possible mechanism(s) of inhibition of genotoxicity, benzo[a]pyrene-7,8-dihydrodiol (BaP-7,8-OH) and N-OH-PhIP were applied as substrates for the CYP1A family and for rSULT 1C1, respectively. Morellos, black-currants, and black tea strongly reduced the genotoxicity of BaP-7,8-OH, onions, rooibos tea, and red wine were less potent while red beets and spinach were inactive. On the other hand, red beets and spinach strongly inhibited the genotoxicity of N-OH-PhIP, rooibos tea was weakly active while all other items were inactive. These results are suggestive for enzyme inhibition as mechanism of protection by complex mixtures of plant origin. Taken together, our results demonstrate that protection by beverages, fruits, and vegetables against genotoxicity of heterocyclic aromatic amines may take place within metabolically competent mammalian cells as well as under the conditions of the Salmonella/reversion assay.<sup>9</sup>

#### **Radioprotective effects of antioxidative plant flavonoids in mice.**

Radioprotective effects of tea infusions and plant flavonoids were investigated by using the micronucleus test for anticlastogenic activity and the thiobarbituric acid assay for antioxidative activity. A single gastric intubation of rooibos tea (*Aspalathus linearis*) infusion at 1 ml per mouse 2 h prior to gamma-ray irradiation (1.5 Gy) reduced the frequency of micronucleated reticulocytes (MNRETs). After the fractionation of rooibos tea infusion, the flavonoid fraction was found to be most anticlastogenic and antioxidative. From this fraction, luteolin was isolated as an effective component. Then, anticlastogenic effects of 12 flavonoids containing luteolin and their antioxidative activities against lipid peroxidation by Fenton's reagent were examined. A good correlation ( $r=0.717$ ) was observed between both activities. Luteolin showed the most effective potency. A gastric intubation of luteolin (10 micromoles/kg) 2 h prior to gamma-ray irradiation (6 Gy) suppressed lipid peroxidation in mouse bone marrow and spleen and a trend of protective effect of luteolin against the decrease of endogenous ascorbic acid in mouse bone marrow after gamma-ray irradiation (3 Gy) was observed. *These results suggest that plant flavonoids, which show antioxidative potency in vitro, work as antioxidants in vivo and their radioprotective effects may be attributed to their scavenging potency towards free radicals such as hydroxyl radicals.* Therefore, the flavonoids contained in tea, vegetables and fruits seem to be important as antioxidants in the human diet.<sup>7</sup>

#### **Inhibitory effects of Rooibos tea, *Aspalathus linearis*, on X-ray-induced**

Oncogenic transformation of mouse C3H10T1/2 cells induced by X-rays was suppressed in the presence of extract of Rooibos tea, *Aspalathus linearis*. Transformation was reduced with increased concentration of

the extract, so that at an extract concentration of 10%, transformation incidence was similar to the spontaneous level. Suppression was also dependent on treatment time with the extract and was maximal when present during the entire incubation period. In contrast, green tea extract at an equitoxic concentration showed no detectable effect on transformation incidence.<sup>8</sup>

**References:**

1. Kunishiro K, Tai A, Yamamoto I., Effects of rooibos tea extract on antigen-specific antibody production and cytokine generation in vitro and in vivo. *Biosci Biotechnol Biochem.* 2001 Oct;65(10):2137-45. Department of Immunochemistry, Faculty of Pharmaceutical Sciences, Okayama University, Japan.
2. Nakano M, Nakashima H, Itoh Y. Anti-human immunodeficiency virus activity of oligosaccharides from rooibos tea (*Aspalathus linearis*) extracts in vitro. *Leukemia.* 1997 Apr;11 Suppl 3:128-30. Institute for Medical Science of Aging, Aichi Medical University, Japan.
3. Bramati L, Minoggio M, Gardana C, Simonetti P, Mauri P, Pietta P. Quantitative characterization of flavonoid compounds in Rooibos tea (*Aspalathus linearis*) by LC-UV/DAD. *J Agric Food Chem.* 2002 Sep 25;50(20):5513-9. Istituto Tecnologie Biomediche, CNR, Via Fratelli Cervi 93, 20090 Segrate (Milan), Italy. [bramati@itba.mi.cnr.it](mailto:bramati@itba.mi.cnr.it)
4. Hesselting PB, Joubert JR. The effect of rooibos tea on the type I allergic reaction. *S Afr Med J.* 1982 Dec 25;62(27):1037-8.
5. Sasaki YF, Yamada H, Shimoi K, Kator K, Kinai N. The clastogen-suppressing effects of green tea, Po-lei tea and Rooibos tea in CHO cells and mice. *Mutat Res.* 1993 Apr;286(2):221-32. Laboratory of Food Hygiene, School of Food and Nutritional Sciences, University of Shizuoka, Japan.
6. Hesselting PB, Klopper JF, van Heerden PD. The effect of rooibos tea on iron absorption [Article in Afrikaans] *S Afr Med J.* 1979 Apr 14;55(16):631-2.
7. Shimoi K, Masuda S, Shen B, Furugori M, Kinai N., Radioprotective effects of antioxidative plant flavonoids in mice. *Mutat Res.* 1996 Feb 19;350(1):153-61. Laboratory of Food Hygiene, School of Food and Nutritional Sciences, University of Shizuoka, Japan.
8. Komatsu K, Kator K, Mitsuda Y, Mine M, Okumura Y. Inhibitory effects of Rooibos tea, *Aspalathus linearis*, on X-ray-induced C3H10T1/2 cell transformation. *Cancer Lett.* 1994 Feb 28;77(1):33-8. Department of Radiation Biophysics, Nagasaki University School of Medicine, Japan.
9. Edenharder R, Sager JW, Glatt H, Muckel E, Platt KL. Protection by beverages, fruits, vegetables, herbs, and flavonoids against genotoxicity of 2-acetylaminofluorene and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in metabolically competent V79 cells. Department of Hygiene and Environmental Medicine, University of Mainz, *Obere Zahlbacher Str. 67, D-55131 Mainz, Germany.*
10. Marnewick JL, Batenburg W, Swart P, Joubert E, Swanevelder S, Gelderblom WC. Ex vivo modulation of chemical-induced mutagenesis by subcellular liver fractions of rats treated with rooibos (*Aspalathus linearis*) tea, honeybush (*Cyclopia intermedia*) tea, as well as green and black (*Camellia sinensis*) teas. *Mutat Res.* 2004 Mar 14;558(1-2):145-54.
11. Joubert E, Gelderblom WC, Louw A, de Beer D. South African herbal teas: *Aspalathus linearis*, *Cyclopia* spp. and *Athrixia phylicoides*-A review. *J Ethnopharmacol.* 2008 Jun 2