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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

J.L. Marnewick [a,*], W. Batenburg [a], P. Swart [b], E. Joubert [c], S. Swanevelder [d], W.C.A. Gelderblom [a]

[a] *PROMEC Unit, Medical Research Council, P.O. Box 19070, Tygerberg 7505, South Africa*
[b] *Department of Biochemistry, Stellenbosch University, Private Bag X1, Stellenbosch 7602, South Africa*
[c] *Agriculture Research Council Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa*
[d] *Biostatistic Unit, Medical Research Council, P.O. Box 19070, Tygerberg 7505, South Africa*

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Abstract

Male Fischer rats were given unprocessed (not oxidized) and processed (oxidized) rooibos and honeybush teas as well as green and black teas as a sole source of drinking fluid for 10 weeks, and sub cellular liver fractions were prepared. Cytosolic fractions of rats consuming the unprocessed herbal teas, green and black teas significantly ($P < 0.05$) protected against 2-acetylaminofluorene (2-AAF)-induced mutagenesis in the *Salmonella* mutagenicity test with strain TA 98, using Aroclor 1254-induced microsomes. A marginal or no protection was obtained with the processed herbal teas. The mutagenic response of aflatoxin B_1 (AFB_1) against *Salmonella* strain TA 100 was significantly ($P < 0.05$) inhibited by cytosolic fractions from rats treated with processed and unprocessed herbal teas, while no effect was obtained with the green and black teas. Microsomal fractions prepared from livers of rats treated with both the processed and unprocessed rooibos teas and the unprocessed honeybush tea, significantly ($P < 0.05$) reduced the activation of AFB_1 while no protection was observed against 2-AAF-induced mutagenesis. In contrast, microsomal fractions from rats treated with the green, black and unprocessed honeybush teas significantly ($P < 0.05$) enhanced the mutagenic response of 2-AAF. None of the tea treatments significantly affected the concentration of the microsomal liver cytochrome P450.

Keywords: Aflatoxin B_1; 2-Acetylaminofluorene; Mutagenesis; Ex vivo protection; Rooibos; Honeybush

1. Introduction

Food-borne mutagens and carcinogens are readily converted into reactive genotoxic intermediates by phase I metabolising enzymes. Following this metabolic step, the detoxification pathways convert the potential genotoxins to less reactive metabolites, which can be more readily excreted [1]. Studies in experimental animals revealed that many food-derived and/or synthetic components inhibit the carcinogenic response of food contaminants such as 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ),

* Corresponding author. Tel.: +27-21-938-0289; fax: +27-21-938-0260.
E-mail address: jeanine.marnewick@mrc.ac.za (J.L. Marnewick).

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a heterocyclic amine produced during the cooking of protein-rich food, benzo[*a*]pyrene (B[a]P), a polycyclic aromatic hydrocarbon formed upon heating food materials and aflatoxin B_1 (AFB_1), a mycotoxin produced by *Aspergillus flavus* [2,3]. Dietary constituents can selectively modulate the drug-metabolising enzymes thereby altering the metabolic fate of the carcinogen in the cell [4–6]. This could lead to either a decrease or in some cases even an increase in the level of the active carcinogenic metabolite(s) available to interact with DNA, which is known to be an important determinant in the development of cancer.

Epidemiological studies indicated that the frequent intake of certain food components plays an important role in reducing the risk for cancer development in humans [7–10]. With growing scientific evidence of the beneficial health properties of green and black teas [11–13] the health-promoting potential of two unique South African herbal teas, rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*) are of interest. Rooibos and honeybush teas have been used as herbal beverages and to a lesser extent as herbal 'medicines' in South Africa before the 1800s [14]. The popularity of these teas can be ascribed to the low tannin content [15,16] and the absence of caffeine [15,17]. The established pharmacological effects of rooibos tea and to a certain extent honeybush tea include anti-oxidative [18–20], anti-aging [21], anti-viral and anti-inflammatory [22] properties, and anti-mutagenic activity in test systems in vitro [23,24]. Most of the protective effects against mutagenesis of green and black teas were ascribed to the polyphenolic compounds, the catechins. Rooibos and honeybush teas contain a wide variety of phenolic compounds that not only differs from that of the green and black teas but also from one another. Rooibos tea predominantly contains dihydrochalcones, flavonols and flavones [25,26] and honeybush tea (*C. intermedia*) contains xanthones, flavanones, and coumestans [27,28]. In contrast, green tea mainly contains flavanols while black tea contains the oxidised polymeric compounds, the theaflavins and thearubigins [29,30].

Many studies focussed on the inhibition of mutagenesis by tea and tea polyphenols in vitro, but these modulating properties have not been fully elucidated in vivo [31]. Considering the potent anti-mutagenic activity of rooibos and honeybush teas in vitro [18,24] very little information is available whether similar protective mechanisms exist in vivo and what type of mechanism is involved. A study by Sasaki et al. [23] in hamster ovary cells and male ICR mice showed that extracts of processed rooibos tea decreased the number of chromosome aberrations and micronucleated reticulocytes, respectively, after exposure to benzo[*a*]pyrene (B[a]P) and mitomycin C (MMC). The present study investigated the ex vivo modulating effects of sub-cellular hepatic fractions of rats treated with processed and unprocessed herbal teas as compared with green and black teas against 2-acetylaminofluorene (2-AAF)- and AFB_1-induced mutagenesis in the *Salmonella* mutagenicity assay.

2. Materials and methods

2.1. Chemicals

2-AAF [53-96-3] and AFB_1 [1162-65-8], biotin [58-85-5], histidine [71-00-1], nicotinamide adenine dinucleotide phosphate (NADP) [1184-16-3], glucose-6-phosphate (G-6-P) [54010-71-8] and the enzyme, glucose-6-phosphate dehydrogenase (G-6-PDH) [9001-40-5] were purchased from Sigma (SA). Dimethyl sulfoxide (DMSO) [67-68-5] was obtained from BDH Laboratory Supplies (Poole, UK). Agar and Nutrient Broth No. 2 were purchased from the Difco Laboratories (Detroit, USA) and Oxoid (Hampshire, UK), respectively. All other solvents used were of analytical grade.

2.2. Tea preparations

Aqueous extracts of processed and unprocessed rooibos and honeybush and of green and black teas were prepared by the addition of freshly boiled tap water to the tea leaves and stems (2 g/100 ml) for processed and unprocessed rooibos, and black and green teas and 4 g/100 ml for processed and unprocessed honeybush tea. The herbal tea concentrations used are customary for tea making purposes [32,33] in South Africa. The mixture was allowed to stand for 30 min at room temperature, filtered (Whatman no. 4) and after cooling, dispensed into water bottles. Individual batches of processed and unprocessed rooibos and honeybush teas of the highest quality were supplied

by Dr. E. Joubert. The black tea (*Camellia sinensis* var *assamica*) was a blend of locally produced African tea and Sri Lankan teas (http://www.five-roses.com/about-us/default.htm) and was obtained from a commercial retail outlet in Cape Town, South Africa. The green tea (*C. sinensis* var *sinensis*), imported from China, was a gift from Vital Health Foods, Kuilsriver, South Africa.

2.3. Treatment of animals

Seventy male Fischer 344 rats, weighing 150–190 g, were obtained from the Primate Unit (Diabetic Research Group of the Medical Research Council of South Africa), randomly divided into seven treatment groups consisting of ten rats per group and housed individually in stainless steel wire-bottomed cages fitted with perspex houses in a closed environment (24–25 °C), with a 12 h light–dark cycle and 50% humidity. Rats had free access to the various aqueous tea extracts for 10 weeks as their sole source of drinking fluid while the control group received tap water. The rats were fed rat cubes (Epol Ltd., Johannesburg, South Africa) ad libitum and the fluid intake monitored on a daily basis while the tea was freshly prepared every second day. The general condition of the rats was monitored daily throughout the experiment while the body weights were determined on a weekly basis.

2.4. Preparation of microsomal and cytosolic liver fractions

The animals were sacrificed under pentobarbital anaesthesia and blood was collected from the abdominal aorta and subjected to clinical chemical analyses [34]. Livers were immediately excised, weighed and a sub-sample was homogenised in three volumes of ice-cold 0.15 M KCl, for 1 min using a Thomas homogeniser. The homogenates were filtered through double-layer cheesecloth, and homogenised in a glass tissue-grinder (10 strokes) using a loose plunger. The homogenates were centrifuged at 9000 × *g* for 10 min and the cytosolic and microsomal preparations collected after centrifugation of the resultant 'S-9' supernatant at 100,000 × *g* for 1 h. The microsomes were collected in 0.15 M KCl, homogenised in a glass tissue grinder using a loose plunger, centrifuged at 100,000 × *g* for 1 h, resuspended in 0.15 M KCl and stored with the cytosolic fractions at −80 °C. All procedures were performed under sterile conditions at 4 °C. Microsomal and cytosolic proteins were determined by the method of Bradford [35] using bovine serum albumin as protein standard.

The S-9 homogenate used in the standard *Salmonella* mutagenicity assay was prepared from Aroclor 1254-induced rats as described previously [36], aliquoted in sterile vials and stored at −80 °C. For the preparation of Aroclor-induced microsomes the resultant S-9 homogenate was fractionated as described above.

2.5. Cytochrome P450 determination

The cytochrome P450 content of the S-9 homogenates and microsomal fractions was determined from the dithionite-reduced difference spectrum of carbon monoxide-saturated samples and expressed as nmol/mg protein using a millimolar extinction coefficient of 91 [37].

2.6. Salmonella mutagenicity/anti-mutagenicity assay

The *Salmonella* mutagenicity assay was conducted according to the method described by Maron and Ames [36] with minor modifications. Two known mutagens, 2-AAF and AFB_1, requiring metabolic activation, were used against tester strains TA 98 and TA 100, respectively. The tester strains were obtained from Dr. B.N. Ames (Berkeley, California, USA). Stock solutions of the different carcinogens were freshly prepared on the day of the experiment using dimethyl sulfoxide as solvent. The mutagenic response of 2-AAF and AFB_1 in the standard assay was conducted using S-9 fractions from Aroclor-1254 treated rats at a concentration of 2 mg protein/ml S-9 mixture and cytochrome P450 (CYP450) at 0.6 nmol/mg protein. Five replicate plates were included for each sample.

2.6.1. Cytosolic modulation assay

For the cytosolic modulation assay, the S-9 activation mixture consisted of phosphate buffer (0.1 M) pH 7.4, salt solution (0.03 M KCl; 8 mM $MgCl_2$), glucose-6-phosphate (5.3 mM), NADP (0.03 M) and

Aroclor-induced microsomes (1 mg protein/ml; 2.8 nmol/mg protein). The optimum cytosolic concentration to compare the protective properties of the different cytosolic preparations was established first. The various cytosolic preparations from the herbal tea- and water-treated rats (one rat per group) were tested at two protein concentrations (0.25 and 1 mg protein/ml S-9 mixture) against strain TA 98, with 2-AAF as a mutagen. Subsequent experiments were conducted using the different cytosolic preparations at a protein level of 0.25 mg/ml S-9 mixture, with 2-AAF (5 μg/plate) and AFB_1 (15 ng/plate) as mutagens. The total protein concentration (1.25 mg/ml) of the S-9 mixture used in the comparative study was below that proposed for mutagenicity testing (1.6 mg protein/ml S-9 mixture) of potentially mutagenic compounds when utilising the plate-incorporation assay [36]. This would have excluded the possible occurrence of certain artefacts induced by the different cytotosolic fractions such as bacterial cytotoxicity and/or the inhibition of mutagenesis due to the high level of protein added.

2.6.2. Microsomal activation assay

The mutagen-activating potential of microsomes isolated from tea-treated rats was determined using the standard plate-incorporation assay [36]. The activation mixture consisted of 0.1 M phosphate buffer (pH 7.4), salt solution (0.03 M KCl; 8 mM $MgCl_2$), glucose-6-phosphate (5.3 mM), NADP (0.04 M) and glucose-6-phosphate dehydrogenase (equivalent to 2 U/ml). The microsomes of the different tea-treated rats were incorporated at a level of 1 mg protein/ml in the activation mixture, while the mutagen concentrations used were 100 μg/plate for 2-AAF and 50 ng/plate for AFB_1.

Control plates, containing only DMSO (used as solvent vehicle) were also included to obtain spontaneous revertant counts. All plates were incubated at 37 °C for 48 h and the histidine revertants were counted using a Quebec Colony Counter (America Optical Corp., Buffalo, New York). All experiments were repeated twice and five replicates for each sample were included.

2.7. Statistical analysis

Analysis of variance (ANOVA) was performed using the statistical analysis system (SAS) programme. Data showing equal variances were analysed using the parametric Tukey *T*-test. When data showed unequal variances (Kruskal–Wallis test) the non-parametric Tukey-type test was used.

3. Results

3.1. Tea intake profiles and effect on rat body weight parameters

The various tea treatments had no adverse effects on the body weight gain, relative liver weight, blood clinical chemical changes and daily fluid intake of the rats [34]. Tea intake profiles varied between 8 and 9 ml/100 g body weight per day with no significant difference between the various groups. The total flavonoid intake was significantly higher ($P < 0.001$) in the black, green and unprocessed honeybush tea-treated rats as compared with the rats consuming the processed and unprocessed rooibos and unprocessed honeybush teas (Table 1).

3.2. Cytosolic modulation assay

3.2.1. Pilot study

An increase in the mutagenic response of 2-AAF against strain TA 98 with Aroclor 1254-induced microsomes was noticed when the level of the cytosolic fraction from the control rats was increased from 0.25 to 1 mg protein/ml of the S-9 mixture (equivalent to 0.125 and 0.5 mg/plate) (Fig. 1). Significant ($P < 0.05$) protection was obtained with the different cytosolic fractions from the herbal tea-treated rats at concentrations of 0.25 mg/ml of the S-9 mixture. A similar inhibition pattern was obtained with the higher cytosolic content (1 mg/ml S-9) although a weaker protection was noticed with the processed and unprocessed honeybush teas. Based on these data a cytosolic level of 0.25 mg/ml of the S-9 mixture was used in the study for the different cytosolic fractions of the tea-treated rats, including those given the green and black teas. For comparative purposes, the subsequent studies with AFB_1 as a mutagen against strain TA 100, was also conducted at the same cytosolic protein concentration.

3.2.2. Tea-derived cytosolic liver fractions

The protective effects of the different cytosolic preparations obtained from liver homogenates

Table 1
Protective effects of liver cytosolic fractions isolated from rats consuming processed and unprocessed rooibos and honeybush teas, and green and black teas against mutagenicity of 2-AAF and AFB_1 in the *Salmonella* assay

Tea treatments	Total flavonoid intake (mg gallic acid equivalents per day/100 g BW)[a]	Revertants per plate	
		TA 98 2-AAF (5 μg/plate)	TA 100 AFB_1 (10 ng/plate)
Control (tap water)	–	220.3 ± 32.5 a	385.0 ± 42.0 a
Processed rooibos tea	2.94 ± 0.89 a	206.7 ± 36.0 a	304.9 ± 41.4 b
Unprocessed rooibos tea	11.97 ± 1.76 b	151.2 ± 30.1 b	303.3 ± 32.8 b
Processed honeybush tea	3.73 ± 1.04 a	191.1 ± 37.6 (b)	306.1 ± 43.9 b
Unprocessed honeybush tea	19.07 ± 2.37 c	161.3 ± 30.3 b	303.2 ± 45.1 b
Black tea	15.14 ± 2.09 c	116.2 ± 27.3 b	359.8 ± 34.8 a
Green tea	16.48 ± 1.91 c	111.6 ± 25.8 b	345.8 ± 50.0 a
Spontaneous revertants	–	30.3 ± 3.7	131.5 ± 11.9
Positive control[b]	–	487.7 ± 74.3	519.7 ± 31.2

Values in columns are means ± S.D. of 10 rats per group. Means followed by the same letter do not differ significantly. If letters differ then $P < 0.05$. Letter in parentheses indicates a marginal effect ($P < 0.1$). Cytosolic preparations from different tea-treated and water-treated rats were incorporated at 0.25 mg/ml activation mixture. Aroclor-activated microsomes (1 mg/ml) were added to the activation mixture.

[a] Data from Marnewick et al. [34].

[b] An S-9 preparation (2 mg protein/ml S-9 mix) from Aroclor-induced rats was used.

of tea- and water-treated rats against 2-AAF- and AFB_1-induced mutagenesis are summarised in Table 1. When 2-AAF was used as mutagen, only the cytosolic fractions of the rats consuming the unprocessed herbal teas and the green and black teas significantly ($P < 0.001$) decreased the mutagenicity as compared with the cytosolic fractions prepared from the water-treated control rats. The cytosolic fraction of the rats receiving the processed honeybush tea showed a marginally ($P = 0.053$) protective effect.

When AFB_1 was used as mutagen, the cytosolic fractions from the rats treated with both processed and

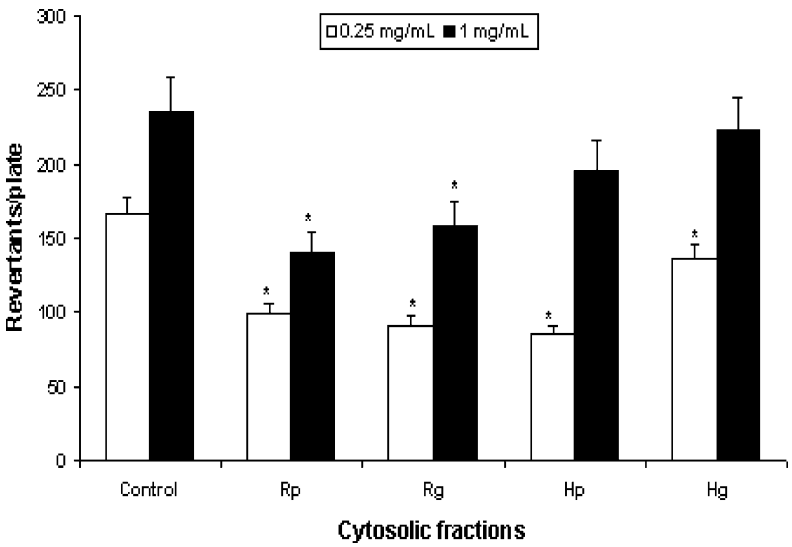

Fig. 1. Ex vivo protective effect of different cytosolic protein concentrations (0.25 and 1 mg/ml) on the mutagenicity induced by 2-AAF in *S. typhimurium* TA 98. Rp: processed rooibos, Rg: unprocessed rooibos, Hp: processed honeybush and Hg: unprocessed honeybush. Each value represents the mean and standard deviation ($n = 5$). $^*P < 0.05$ when different cytosolic concentrations were compared with the control. Microsomes (1 mg/ml activating mixture) of Aroclor-induced rats were used to induce metabolic activation of 2-AAF.

Table 2
Activation potential of hepatic microsomal fractions isolated from rats consuming the different teas on 2-AAF- and AFB_1-induced mutagenesis

Tea treatments	CYP450 (nmol/mg protein)	Revertants per plate	
		TA 98 2-AAF (100 μg/plate)	TA 100 AFB_1 (50 ng/plate)
Control (tap water)	0.35 ± 0.14 a	351.9 ± 80.0 a	1115.4 ± 167.1 a
Processed rooibos tea (2%)	0.31 ± 0.06 a	527.0 ± 197.5 a	878.4 ± 178.0 b
Unprocessed rooibos tea (2%)	0.38 ± 0.04 a	536.3 ± 114.0 a	907.9 ± 91.1 b
Processed honeybush tea (4%)	0.31 ± 0.06 a	492.1 ± 101.0 a	1102.7 ± 209.5 a
Unprocessed honeybush tea (4%)	0.45 ± 0.11 a	617.7 ± 75.9 b	941.2 ± 154.3 b
Black tea (2%)	0.41 ± 0.09 a	803.8 ± 132.0 c	1150.7 ± 244.3 a
Green tea (2%)	0.37 ± 0.03 a	818.0 ± 149.7 c	1375.2 ± 350.6 a

Values in columns are means ± S.D. of 10 rats per group. Means followed by the same letter do not differ significantly. If letters differ then $P < 0.05$. Microsomes from different tea-treated and control rats were incorporated at a level of 1 mg/ml activation mixture.

unprocessed rooibos and honeybush teas resulted in a significant ($P < 0.001$) protective effect as compared to the control. No significant protective effect was noted with the cytosolic fractions of the rats given the green and black teas at the specific concentration tested.

3.3. Microsomal activation assay

None of the microsomal preparations of the tea-treated rats caused a decrease in the mutagenicity of 2-AAF when compared to the control (Table 2). However, a significant ($P < 0.05$) increase in the number of histidine revertants was obtained with the liver microsomal fractions of the rats treated with the unprocessed honeybush, black and green teas. A marked but not significant increase in the number of histidine revertants was noticed with the microsomal fractions obtained from the rats treated with the processed honeybush tea and the processed and unprocessed rooibos teas.

When AFB_1 was used as mutagen, the microsomal preparations of the rats treated with processed and unprocessed rooibos and unprocessed honeybush teas showed a significant ($P < 0.05$) decrease in the number of histidine revertants. No effect was obtained with the microsomal fractions of the rats treated with the green or black teas, or with the processed honeybush teas.

The concentration of microsomal cytochrome P450 (nmol/mg protein) of the different microsomal preparations did not differ significantly as a result of the different tea treatments (Table 2). However, the levels tended to be higher (not significantly) in the microsomes prepared from rats that had received the unprocessed honeybush tea and the black tea.

3.4. Spontaneous revertant counts

The spontaneous revertant counts of strains TA 98 and TA 100 were in the range (30–50 and 120–200, respectively) of published values [36].

4. Discussion

Several mechanisms for the modulating role of black and green tea extracts or purified phenolic compounds on the in vitro mutagenic response against various metabolically activated mutagens have been suggested. These include the inhibition of CYP450-dependent bio-activation of the pro-mutagens and/or a prevention of DNA damage by the interaction of tea components with the ultimate carcinogens [12,38–42]. With respect to rooibos and honeybush teas, similar in vitro protective mechanisms against 2-AAF- and AFB_1-induced mutagenesis in the *Salmonella* mutagenicity assay were suggested previously [24]. In that study, the anti-mutagenic activity of the aqueous herbal tea preparations were directly monitored at levels of 5 and 10% (w/v) without any toxic effect to the *Salmonella* bacteria used. The present study was conducted to establish whether these protective mechanisms would also prevail in vivo by monitoring the ex vivo anti-mutagenic activity of the cytosolic fractions of tea-treated rats. Rats exposed to the various

tea preparations (2 and 4% in their drinking-water) exhibited no hepatotoxic effects when monitoring the serum levels of enzymes indicative of liver function [34]. No cytotoxic effects were observed when incorporating the different cytosolic fractions (0.25 mg protein/ml S-9 activation mixture) in the presence of Aroclor-induced microsomes (1 mg protein/ml S-9 activation mixture). In this regard, no reduction in the spontaneous mutation rate or any cytotoxic effects to the tester strains were monitored when using the Aroclor-induced S-9 containing a higher protein content (2 mg protein/ml S-9 mixture).

When considering the metabolic fate of AFB_1 and 2-AAF, chemopreventive agents have been characterised that selectively reduce their carcinogenic effects. With respect to AFB_1, studies focused on the fate of the reactive mutagenic metabolite, the AFB_1-8,9-epoxide, by either inhibiting its CYP450-dependent activation and/or enhancing its detoxification via conjugation with glutathione (GSH) catalysed by the cytosolic glutathione-*S*-transferases (GSTs) [43–45]. With respect to the aromatic amines (2-AF, 2-AAF), two different mechanisms exist in the production of the active genotoxic metabolites, one involving the rat liver microsomes and the other both the microsomal and cytosolic cellular compartments [46,47]. In both cases the initial step involves N-hydroxylation with the subsequent addition of either an acetyl group by the microsomal N,O-acetyltransferases or a sulfate via the sulfotransferases in the cytosol [48]. Modulation of the formation of active mutagenic metabolite(s) derived from these aromatic amines could therefore occur at different levels involving both the cytosolic and microsomal compartments [1].

In the present study, liver cytosolic fractions from rats that had received processed and unprocessed rooibos and honeybush tea protected against AFB_1-induced mutagenicity ex vivo, while green and black tea showed no cytosolic protection. Unprocessed herbal teas, and green and black teas showed a significant and processed honeybush tea a marginal ($P < 0.053$) cytosolic protective effect against 2-AAF-induced mutagenesis, while processed rooibos tea showed no protection. It is not known whether a protective effect could have been obtained at higher cytosolic levels (>0.25 mg protein/ml S-9 fraction). However, in the case of 2-AAF a lower protective effect was observed at the higher cytosolic concentration (1 mg/ml) in the pilot study. In this regard the presence of the cytosolic sulfotransferases would have increased the mutagenic response of 2-AAF and, as discussed above, could have decreased the protective effect of the cytosol.

Microsomal fractions obtained from rats treated with the herbal teas, except processed honeybush tea, also showed a decreased metabolic conversion of AFB_1 to its reactive mutagenic intermediate, while the processed honeybush tea and the green and black teas did not significantly alter the activation of AFB_1. In contrast to AFB_1 mutagenesis, none of the herbal, green and black teas decreased the microsomal activation of 2-AAF. Instead, 2-AAF mutagenesis was increased markedly to significantly when utilising microsomal preparations of the different teas.

The present study provides evidence that active components involved in the ex vivo modulation of 2-AAF and AFB_1 metabolism are absorbed from the gut to exhibit their protective effect. Therefore, oral administration of rooibos and honeybush teas is likely to reduce the liver microsomal activation of AFB_1 significantly, while cytosolic component(s) would further reduce the availability of the active mutagenic metabolite to interact with DNA. In contrast, only the cytosolic components are likely to protect against 2-AAF-induced effects and the balance between enhanced microsomal activation versus cytosolic protection could determine the biological outcome in vivo. Differences in the ex vivo modulation of mutagenesis induced by 2-AAF could be ascribed, as mentioned above, to differences in the metabolic pathways resulting in the formation of the active metabolite. Whether induction of the microsomal N,O-acetyltransferases could also contribute to enhanced activation of 2-AAF is not known at present.

The exact mechanisms involved in the ex vivo protection of the different tea preparations against AFB_1- and 2-AAF-induced mutagenesis are presently unknown. However, a recent study by Marnewick et al. [34] indicated that the herbal teas significantly enhance the activity of the microsomal UDP-glucuronosyl transferases, and the cytosolic glutathione-*S*-transferase alpha (GST-α), which could be important in explaining the protective effects of the different sub-cellular liver fractions. The herbal teas also stabilize the level of reduced glutathione (GSH) that may result in an increased anti-oxidant capacity in the cell. In the present study these parameters could

play an important role in the ex vivo protection of the cytosolic fractions against AFB_1 and 2-AAF-induced mutagenesis. The protective activity of green and black teas against 2-AAF appears not to proceed the modulation of GST-α or UDP-GT as they failed to induce the activity of these enzymes under the present conditions [34]. In this regard the stabilization of GSH by the green and black tea seems to be involved. It is not known whether the activity of the sulfotransferases is modulated by green and black teas, thereby reducing the cytosolic activation component of 2-AAF. However, other cytosolic components such as the tea phenols could also be involved in the direct scavenging of reactive AFB_1 and 2-AAF metabolites under the present experimental conditions.

Differences in the microsomal conversion of AFB_1 and 2-AAF could be related either to the induction of specific CYP450 isoforms involved in their metabolism or to direct metabolic competition between the flavonoids and the carcinogens. Various flavonoids are metabolised by certain CYP450 isoforms, e.g., CYP1A2, and they may competitively reduce the CYP1A2-mediated activation of AFB_1, thereby inhibiting its mutagenic activity [4]. Different flavonoids have also been shown to selectively induce the activity of enzymes of the CYP family [49] e.g., the flavanones induce CYP2B1/2 and the flavones CYP1A and 2B isozymes [50]. Differences in the induction pattern of these isozymes could therefore play an important role in the metabolic fate of a potential mutagen or carcinogen in vivo. Treatment of rats with green and black tea from 4 to 6 weeks significantly induced different isoforms of CYP450 including CYP1A1, 1A2, 2B, 4A1 [51–53]. In the present study the total daily flavonoid intake was shown to be significantly ($P < 0.05$) higher in rats fed the black, green and unprocessed honeybush teas, suggesting that the induction of the CYP450 isoforms could have enhanced 2-AAF metabolism yielding the active mutagenic metabolite. With respect to the protective effect of the herbal teas against AFB_1 metabolism it would appear that the phenolic constituents of the herbal teas selectively induce different isoforms of CYP450 that could direct the metabolism away from the formation of the active mutagenic metabolite.

Various phenolic tea constituents exhibit different protective mechanisms in vitro depending on the chemical nature and metabolic fate of the carcinogen [12]. Variation in the protective effects of the herbal, green and black teas might also be ascribed to the difference in phenolic constituents and their selective effects on the drug-metabolising enzymes. The present study confirms that the in vitro anti-mutagenic activity of the South African herbal teas against AFB_1- and 2-AAF-induced mutagenesis is likely to be active under in vivo conditions as well, although the mechanisms may differ. This is the first report on the ex vivo protective effects of rooibos and honeybush teas and it provides valuable information regarding their possible health-promoting properties.

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