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An investigation on the antimutagenic properties of South African herbal teas

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Abstract

The antimutagenic properties of South African herbal teas were investigated using the *Salmonella typhimurium* mutagenicity assay. Aqueous extracts of fermented and unfermented rooibos tea (*Aspalathus linearis*) and honeybush tea (*Cyclopia intermedia*) both possess antimutagenic activity against 2-acetylaminofluorene (2-AAF) and aflatoxin B_1 (AFB_1)-induced mutagenesis using tester strains TA98 and TA100 in the presence of metabolic activation. A far less inhibitory effect was noticed against the direct acting mutagens, methyl methanesulfonate (MMS), cumolhydroperoxide (CHP), and hydrogen peroxide (H_2O_2) using TA102, a strain designed to detect oxidative mutagens and carcinogens. Depending on the mutagen used, the unfermented tea exhibited the highest protective effect. A similar response regarding the protection against mutagenesis was obtained when utilising different variations of the double layer *Salmonella* assay. The double layer technique proved to be more effective to detect the protective effect of the different tea preparations against the direct acting mutagens. With respect to indirect mutagens, the highest protection was noticed when the carcinogen was metabolically activated in the presence of the tea extract as compared with when the tea extract was incubated in a separate layer with the bacteria. The current data suggest that two mechanisms seem to be involved in the antimutagenicity of the tea extracts towards carcinogens that require metabolic activation: (i) the tea components may interfere with cytochrome P450-mediated metabolism of these mutagens and (ii) the direct interaction between the tea constituents, presumably the polyphenolic compounds, with the promutagens and/or the active mutagenic metabolites. However, the mild and/or lack of protection and in some cases even enhancement of mutagenesis induced by direct acting or oxidative mutagens, provide new perspectives regarding the role of the polyphenolic compounds known to exhibit antioxidant properties, in the protection against mutagenesis in the *Salmonella* assay. The present study provides the first evidence on the antimutagenic activity of honeybush tea and further evidence on the antimutagenicity of rooibos tea.

Keywords: Rooibos tea; Honeybush tea; Antimutagenicity; *Salmonella* assay

1. Introduction

Epidemiological data suggest that more than 80% of cancers are attributable to lifestyle, of which at least one third is diet-related [1]. Diets containing substances that can modulate or prevent cancer may play a role in the general health of humans, especially if they are inexpensive and easily available. Tea is a widely consumed beverage throughout the world while the popularity of herbal health teas increased significantly during the past 20 years [2]. The anticancer properties of tea are well known, and the tumour inhibition

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potential of certain polyphenolic compounds from green and black tea has been well documented [3–7].

Both rooibos tea (*Aspalathus linearis*) and honeybush tea (*Cyclopia intermedia*), two South African herbal teas, have been shown to contain a complex mixture of polyphenolic compounds, of which aspalathin, a dihydrochalcone, is unique to rooibos tea while luteolin is one of the most important flavones in honeybush tea [8–10]. Processing of these teas include the following steps, fermentation of leaves and stems, followed by sun-drying and sieving and in the case of rooibos tea, steam pasteurisation of the dried product before packaging [11,12]. Rooibos tea is popular as a health beverage, as it contains no harmful stimulants and no caffeine with only trace amounts of tannins [13,14]. Extracts of rooibos tea have been found to decrease the number of chromosome aberrations when treating Chinese hamster ovary (CHO) cells with benzo[*a*]pyrene (B[*a*]P) in the presence of metabolic activation [15]. An in vivo study in male ICR mice indicated that the number of micronucleated reticulocytes (MNRET) induced by single dosage of mitomycin C (MMC) was significantly reduced by daily ingestion of rooibos tea. However, a single gavage treatment of rooibos tea 24 h before MMC injection did not reduce MNRET frequency [15]. Suppression of oncogenic transformation of mouse CH310T1/2 cells induced by X-rays in the presence of rooibos tea was also shown in a study by Komatshu et al. [16]. However, green tea extracts at an equitoxic concentration did not show any detectable suppression [16]. In a study done by Standley [17], it was reported that water soluble fractions of both fermented and unfermented rooibos tea possess antimutagenic activity against 2-acetylaminofluorene (2-AAF)-induced mutagenesis using strain TA98 in the *Salmonella* mutagenicity assay. Apart from these studies on rooibos tea very little is known about the antimutagenic and anticarcinogenic properties of the two South African herbal teas.

Most of the research regarding the antimutagenicity and anticancer properties of tea, as well as chemoprevention and epidemiological studies in humans to date, has focused on green and black teas [18–24]. Most of these studies indicated that polyphenol preparations and/or water extracts from green and black tea dramatically decreased the mutagenicity of a variety of genotoxic carcinogens. As damage to DNA is likely to be a major cause of cancer and other chronic diseases [25,26], the protective effect of naturally occurring dietary constituents could effectively reduce the onset and/or progression of cancer.

The *Salmonella* mutagenicity assay has been extensively used to monitor the mutagenic potential of a variety of compounds that either occur naturally or are introduced artificially into the environment by various means [19,22,27,28]. In the present study, this test system was utilised to monitor the antimutagenic properties of aqueous extracts of fermented and unfermented rooibos and honeybush tea against a variety of direct-acting mutagens as well as two indirect mutagens that required metabolic activation.

2. Materials and methods

2.1. Chemicals and media

The mutagens, 2-AAF and aflatoxin B_1 (AFB_1) were purchased from Sigma Chemical Co. (SA) at the highest available purity. Cumolhydroperoxide (CHP) was purchased from Merck (Schuchardt, Germany), hydrogen peroxide (H_2O_2) from Saarchem (SA) and methyl methanesulfonate (MMS) from Aldrich Chemical Co. (SA). Stock solutions of the different carcinogens were freshly prepared on the day of the experiment in dimethyl sulfoxide (DMSO) purchased from BDH Laboratory Supplies (Poole, UK). All other solvents used were of analytical grade. Agar and Nutrient Broth No. 2 were purchased from the Difco Laboratories (Detroit, USA) and Oxoid (Hampshire, UK), respectively.

2.2. Antimutagenicity assay

2.2.1. Standard plate incorporation assay

Antimutagenicity of the tea extracts against different mutagens was assessed using the standard plate incorporation assay as described by Maron and Ames [29]. *Salmonella typhimurium* strains TA98, TA100, and TA102 were kindly provided by Dr. B.N. Ames (Berkely, CA). For the plate incorporation inhibition assay, 0.1 ml of each mutagen, 0.1 ml of the various tea extracts, 0.5 ml S9-mix and 0.1 ml of an overnight bacterial culture were carefully mixed with 2 ml of molten top agar, containing 0.05 mM biotin–histidine and dis-

persed onto minimal glucose agar plates. A liver S9 homogenate (0.72 nmol cytochrome P450/mg protein) was prepared by inducing male Fischer rats (200 g) with aroclor-1245 as described by Maron and Ames [29]. The S9 homogenate was incorporated into the S9-mixture at a level of 2 mg protein per ml. The mutagenicity of 2-AAF (5 μg per plate) and AFB_1 (10 ng per plate) were monitored against TA98 and TA100 in the presence of the S9 mixture, respectively. The direct acting mutagens were tested against strain TA102: MMS (10 μM per plate), CHP (400 μg per plate) and H_2O_2 (1.81 mg per plate) in the absence of the S9 mixture. A series of control plates containing only the tea extracts and the bacteria in the absence and presence of the S9 mixture were also included to screen the different tea preparations for mutagenic effects. Control plates containing only DMSO, which was used as the solvent vehicle, were also included to obtain the background or spontaneous revertant counts.

2.2.2. *Double layer assay*

A modification of the double layer technique described by Glatt and Oesch [30] was also performed using two direct acting mutagens, MMS and CHP and one requiring metabolic activation (2-AAF). Two variations of the technique were used, one where the carcinogen and the tea preparation were included in the lower top agar layer, with the bacterial suspension in the upper top agar layer (referred to as α) and another where the carcinogen was included in the lower top agar layer with the tea preparation and bacterial suspension in the upper top agar layer (referred to as β). Two concentrations of the tea extracts (5 and 10% (w/v)) were used with MMS (β technique), while only a 10% (w/v) concentration was used with CHP and 2-AAF (α technique).

All plates were incubated at 37°C for 48 h. Thereafter, the histidine revertants were counted using a Quebec Colony Counter (American Optical Corp., Buffalo, New York). All the experiments were repeated once, and five replicates were included for each sample.

2.3. *Preparation of tea extracts*

Fermented and unfermented rooibos and honeybush tea were used. Aqueous extracts of the teas were prepared by the addition of freshly boiled water to the tea leaves and stems to a concentration of 2 g/100 ml for rooibos tea and 4 g/100 ml for honeybush tea. These concentrations are customarily used for tea making purposes [11,12]. The mixture was allowed to stand for 30 min at room temperature, filtered (Whatman No. 4), freeze-dried and stored in airtight containers at −20°C. Two different tea solutions (5 and 10% (w/v)) were prepared from the freeze-dried extracts in distilled water, centrifuged at 300 rpm and the supernatant sterilised through 0.45 and 0.22 μm filters. A methanol extract of unfermented rooibos tea was also prepared following excessive chloroform extraction. Thereafter, the solvent was evaporated at 48°C in vacuo. The protective effect of the methanol extract (5 and 10% (w/v) in DMSO) was also monitored against the different mutagens.

2.4. *Soluble solid and polyphenol determination*

The soluble solid content of each tea preparation (2 and 4% for rooibos and honeybush tea, respectively) was determined gravimetrically after drying 1 ml aliquots at 110°C for 12 h. Five determinations for each tea preparation were done. The scaled-down Folin–Ciocalteu assay was used to determine the total phenolic content [31] of the tea extracts (performed in triplicate), while non-flavonoids were determined after precipitation of flavonoids [32].

2.5. *Statistical analysis*

Analysis of variance (ANOVA) was performed using the statistical analysis system (SAS) programme. The Tukey *T*-test was used to determine whether the means of the control and positive control groups differed significantly. The Dunnett test was used to determine whether the means of the tea treatments differed significantly from the positive mutagenic control treatments. Bonferroni pairwise adjustment was used to compare the soluble solids, total polyphenols, flavonoids and non-flavonoids of the fermented and the unfermented tea preparations.

3. Results

3.1. *Analytical data*

The soluble solids, total polyphenol, flavonoid and non-flavonoid content of the different tea extracts used

Table 1
Soluble solid matter and total polyphenols in the different tea preparations[a]

Tea extracts	Total polyphenols (%)[b]	Flavonoids (%)[b]	Non-flavonoids (%)[b]	Solid matter (mg/ml)[c]
Unfermented rooibos (2% w/v)	41.15 ± 0.25a[d]	28.06 ± 0.25a[d]	13.10 ± 0.04a[d]	4.21 ± 0.44a[d]
Fermented rooibos (2% w/v)	29.74 ± 0.36A[d]	18.8 ± 0.35A[d]	10.94 ± 0.05A[d]	3.42 ± 0.22a[d]
Unfermented honeybush (4% w/v)	35.52 ± 0.03b	27.10 ± 0.02b	8.42 ± 0.05b	11.99 ± 1.09b
Fermented honeybush (4% w/v)	19.80 ± 0.26B	9.86 ± 0.18B	9.94 ± 0.08B	5.26 ± 0.37c

[a] Values are the mean of triplicate determinations. Statistical comparisons were made within a group. Means in columns followed by the same letter do not differ significantly. If the letters differ then $P < 0.01$. When the cases differ then $P < 0.001$.
[b] Soluble solid matter (g per 100 g).
[c] Aqueous tea extracts.
[d] Indicates significant differences between the two tea groups (i.e. unfermented rooibos vs. unfermented honeybush and fermented rooibos vs. fermented honeybush).

in this study are summarised in Table 1. Changes in the total polyphenol and flavanoid content of the two teas during processing are clearly noted, as they were significantly ($P < 0.001$) reduced during the fermentation process. The soluble solids were significantly higher in the honeybush tea extract, which is in agreement with the larger amount of the tea used in the preparation of the extract.

3.2. Antimutagenicity of tea extracts

The protective effect of the different tea extracts on the mutagenicity of the various chemical mutagens and carcinogens using the plate incorporation assay are summarised in Table 2. Data on the protective effect of the different tea extracts against the mutagenicity of the various compounds utilising the double layer technique are presented in Table 3. For data analyses the values of the two separate experiments were combined, as it did not differ statistically.

3.2.1. Mutagens requiring metabolic activation

1. 2-Acetylaminofluorene: Addition of fermented and unfermented rooibos and honeybush tea significantly ($P < 0.001$) reduced 2-AAF-induced mutagenesis. A clear dose response effect was noticed with the 10% (w/v) extract exhibiting the highest protective effect with both teas. A similar protective effect was obtained with the methanol extract of the unfermented rooibos tea at both concentrations, almost completely inhibiting 2-AAF-induced mutagenesis. At the 5% concentration level the fermented rooibos tea exhibited a higher protective effect than the unfermented counterpart. However, at the 10% concentration level there was no significant difference between the unfermented and fermented rooibos preparations. The unfermented honeybush tea preparation exhibited a significantly higher protective effect than the fermented tea at both concentration levels. When compared with fermented honeybush, the fermented rooibos tea exhibited a higher protective effect, whereas unfermented honeybush tea (5%) exhibited a higher protective activity than its rooibos counterpart. The total polyphenolic content related well to the protective effect exhibited by the different tea preparations as a higher total polyphenolic concentration is associated with a higher protective effect (Table 2).
2. Aflatoxin B_1: As described for 2-AAF the addition of both the 5 and 10% fermented and unfermented rooibos tea, significantly ($P < 0.001$) reduced the number of revertants induced by AFB_1 with the unfermented tea exhibiting a higher protective activity (Table 2). A similar protective effect (with no significant difference between fermented and unfermented) was obtained with honeybush tea at both concentration levels. No dose response effects were noticed with either rooibos or honeybush tea. In contrast to the effect on 2-AAF-induced mutagenesis, no difference could be detected between the protective effect of fermented and unfermented rooibos and honeybush tea. The methanol extract again showed a similar protective effect when compared with the unfermented rooibos tea preparations lacking any dose response effects. In the case of AFB_1-induced mutagenicity the higher total polyphenolic content did not relate with the higher protective effect.

Table 2
Protective effect of aqueous rooibos and honeybush tea extracts on mutagenicity of a variety of carcinogens used in the *Salmonella* assay[a]

Treatments	Total polyphenols (mg per plate)[b]	Revertants per plate				
Carcinogen		TA98	TA100		TA102	
		2-AAF (5 μg per plate)	AFB_1 (10 ng per plate)	CHP (400 μg per plate)	H_2O_2 (1.81 mg per plate)	MMS (10 μM per plate)
Control (−)		32 ± 5	120 ± 6	176 ± 18	317 ± 22	183 ± 16
Control (+)		513 ±14a	309 ± 22a	870 ± 144a	950 ± 122a	535 ± 34a
RBf (5%)	1.49	150 ± 29B	158 ± 21B	784 ± 66a	1028 ± 63a	592 ± 66a
RBf (10%)	2.97	88 ± 12C	145 ± 6B	614 ± 67b	712 ± 81b	577 ± 53a
RBu (5%)	2.06	272 ± 30D	128 ± 6CD	850 ± 100a	858 ± 146a	635 ± 62a
RBu (10%)	4.12	43 ± 6C	113 ± 11CD	702 ± 37a	697 ± 91B	676 ± 46B
Me (5%)	ND	40 ± 4C	115 ± 6C	702 ± 68a	805 ± 116a	442 ± 77b
Me (10%)	ND	30 ± 7C	109 ± 10C	706 ± 62a	650 ± 145b	522 ± 44a
HBf (5%)	0.99	318 ± 26D	157 ± 6B	926 ± 79a	968 ± 131a	602 ± 64a
HBf (10%)	1.98	182 ± 28E	131 ± 16BD	832 ± 68a	945 ± 59a	616± 43a
HBu (5%)	1.78	143 ± 16B	139 ± 13BD	854 ± 116a	1012 ± 33a	562 ± 34a
HBu (10%)	3.55	68 ± 12C	125 ± 14BD	674 ± 62b	805 ± 155a	606 ± 63a

[a] Values are mean ± S.D. of five replications. Means in columns followed by different letters then $P < 0.05$, when the cases differ then $P < 0.001$. RBf, rooibos fermented tea; RBu, rooibos unfermented tea; HBf, honeybush fermented tea; HBu, honeybush unfermented tea; Me, methanol extract of rooibos unfermented tea (DMSO as solvent); ND, not determined.

[b] Calculated from the mean total polyphenol content of the respective tea preparations presented in Table 1.

3.2.2. Direct acting mutagens

1. Cumolhydroperoxide: Only fermented rooibos tea (10%) and unfermented honeybush tea (10%) preparations showed a significant ($P < 0.05$) decrease in the CHP-induced mutagenesis (Table 2). The methanol extract and the unfermented rooibos (10%) also slightly reduced the mutagenic response, but the reduction was not significant. Fermented honeybush tea (5%) tended to increase the number of revertant colonies in the presence of CHP, although not significantly. Only the unfermented honeybush tea preparation showed a higher protective effect associated with a dosage increase from 5 to 10%, which related well to the higher total polyphenolic content.
2. Hydrogen peroxide: Preparations of fermented (10%) and unfermented (10%) rooibos tea and the methanol extract (10%) showed significant decreases ($P < 0.05$ and $P < 0.001$) in the number of histidine revertants (Table 2). The total polyphenolic content of the rooibos tea preparations were in relation with the protective effect as the 5% rooibos tea preparation showed a far less inhibitory effect. No protective effect was noticed with fermented and unfermented honeybush tea although the 10% (w/v) unfermented honeybush tea also showed a slight (non-significant) protective effect.
3. Methyl methanesulfonate: No protective effect was noticed with the different tea extracts, while the methanol extract of unfermented rooibos tea (5%) significantly ($P < 0.05$) reduced MMS-induced mutagenesis. On the other hand, unfermented rooibos tea (10%) significantly ($P < 0.001$) enhanced the revertant counts.

3.2.3. The double layer technique (Table 3)

When utilising the double layer technique (β = MMS in lower layer of top agar, with the tea preparation and TA102 in the top layer) the methanol extract markedly and significantly reduced the number of MMS-induced revertants when incorporated at 5 ($P < 0.05$) and 10% ($P < 0.001$), respectively. In contrast to the plate incorporation assay, both the unfermented rooibos ($P < 0.001$) and honeybush ($P < 0.05$) tea (10%) significantly reduced the revertant counts (Table 3), respectively. No significant protective effect was noticed with the fermented counterparts. With respect to CHP no significant differences between the two variations of the double layer technique (α = CHP

Table 3
Effect of aqueous rooibos and honeybush tea extracts on mutagenicity of a variety of carcinogens in the double layer *Salmonella* assay[a]

MMS (10 μM per plate)		CHP (400 μg per plate)		2-AAF (5 μg per plate)	
Treatments	TA102 revertants/plate	Treatments (10%)	TA102 revertants/plate	Treatments (10%)	TA98 revertants/plate
Control (−)	170 ± 16	Control (−)	214 ± 21	Control (−)	28 ± 3
Control (+)	522 ± 39a	Control (+)	1065 ± 134a	Control (+)	450 ± 29a
RBf (5% β)	507 ± 58a	RBf-α	702 ± 83A	RBf-α	119 ± 18A[b]
RBf (10% β)	443 ± 30a	RBf-β	730 ± 174A	RBf-β	190 ± 32A
RBu (5% β)	453 ± 44a	RBu-α	960 ± 108a	RBu-α	40 ± 6A[b]
RBu (10% β)	395 ± 60A	RBu-β	752 ± 167a	RBu-β	67 ± 8A
Me (5% β)	442 ± 77a	Me-α	787 ± 77b	Me-α	ND
Me (10% β)	406 ± 17A	Me-β	725 ± 73b	Me-β	ND
HBf (5% β)	498 ± 45a	HBf-α	1295 ± 48a	HBf-α	32 ± 2A[b]
HBf (10% β)	489 ± 47a	HBf-β	1195 ± 149a	HBf-β	69 ± 12A
HBu (5% β)	502 ± 24a	HBu-α	940 ± 42a	HBu-α	32 ± 4A[b]
HBu (10% β)	423 ± 38b	HBu-β	813 ± 32b	HBu-β	79 ± 6A

[a] Values are the means of five determinations ± S.D.: α, carcinogen and/or S9 and tea extract in bottom layer, bacterial strain in top layer; β, carcinogen and/or S9 in bottom layer, bacterial strain and tea extract in top layer. Means in columns followed by different letters then $P < 0.05$, when case differ then $P < 0.001$. RBf, rooibos fermented tea; RBu, rooibos unfermented tea; HBf, honeybush fermented tea; HBu, honeybush unfermented tea; Me, methanol extract of unfermented rooibos tea (DMSO as solvent); MMS, methyl methanesulfonate; CHP, cumolhydroperoxide; 2-AAF, 2-acetylaminofluorene; ND, not determined.

[b] $P < 0.001$ (differences between α and β variations).

and tea in bottom layer and TA102 in top layer and β = CHP in bottom layer and tea and TA102 in top layer) were noticed. When compared with the standard plate incorporation assay the methanol extract ($P < 0.05$) and both the fermented rooibos preparations (5 and 10%) ($P < 0.001$) and the unfermented honeybush (10%) ($P < 0.05$) showed significant reductions in the revertant counts induced by CHP (Table 3).

In the case of the metabolically activated carcinogen, 2-AAF, both tea preparations significantly ($P < 0.001$) reduced the revertant colonies in both variations of the test. However, the α-method resulted in a significantly ($P < 0.001$) higher protection against mutagenesis than the β-method (Table 3).

3.3. Control tea treatment plates

No toxicity or mutagenic activity of the different tea extracts was detected in the presence of metabolic activation at the levels used in the *Salmonella* test (data not shown). The spontaneous revertant counts of the different strains in the presence and absence of DMSO were in the range of published values [29].

4. Discussion

Five different mutagens with diverse chemical structures and mode of action were used to determine the protective effect of two South African herbal teas against their mutagenicity. Aqueous extracts of unfermented rooibos and honeybush teas displayed a strong antimutagenic effect against both of the metabolically activated carcinogens, 2-AAF and AFB_1. In general the fermented tea preparations showed a high level of protection, but not as effective as their unfermented counterparts with the exception of 5% fermented rooibos tea, which exhibited a higher protective effect against 2-AAF-induced mutagenesis. In the case of 2-AAF, the fermented and unfermented rooibos (10%) tea preparations exhibited a higher inhibition potential than the corresponding honeybush tea preparations. When comparing the protection of the two herbal teas against AFB_1-induced mutagenicity, they showed a very similar protective effect despite the fact that no dose response effects were noticed, suggesting that a threshold was reached with the 5% tea preparation. As discussed above, the unfermented rooibos tea extract exhibited a higher protective effect than the fermented counterpart, whereas no such effect was noticed with the different honeybush tea preparations.

When considering the protective potency of the teas against mutagens that require metabolic activation, cognisance has to be taken of the fact that the different tea preparations were tested on the same weight

basis. The total polyphenol content of the extracts are clearly more relevant for comparative purposes. The unfermented teas contain higher concentrations of polyphenolic compounds, which are significantly reduced during the fermentation process, due to oxidation [33]. In the case of 2-AAF, these oxidised compounds could either have a lower antimutagenic potential or have lost their antimutagenic potential, as the total polyphenol content of the various tea preparations corresponded well with protection against mutagenesis. No relation with the polyphenol content was noticed when comparing the protective effect of unfermented and fermented rooibos at the 5% level. When AFB_1 was used as the mutagen, unfermented rooibos tea exhibited a higher protective effect than the fermented tea, which again related well with the higher polyphenol content. However, both the unfermented and fermented honeybush tea exhibited a similar effect indicating that the oxidation of compounds during fermentation does not reduce the protective effect. Furthermore, fermented rooibos and honeybush tea exhibited similar protective effects whilst the unfermented rooibos tea exhibited a higher level of protection than honeybush tea. This data suggest that, when metabolic activation is required, differences exist in the degree of protection that not only depend on the type of tea and/or polyphenol content but also on the specific mutagen used.

The role of antioxidants has attracted much interest with respect to their protective role against free radical damage that may be the cause of many diseases including cancer [34]. The antioxidative effect of green tea is mainly due to the phenolic components, such as the flavonoids [35]. Some flavonoid and non-flavonoid phenolic compounds have been reported to also show alkylperoxyl radical scavenging activity thus reducing radical-mediated pathogenesis, e.g. carcinogenesis [36]. In the present study only a weak relation exists between the polyphenolic content of the herbal teas and the antimutagenic effect when using the oxidative mutagens, H_2O_2, and CHP and the direct acting mutagen MMS. Other investigators [18,37,38] have also noted this failure and/or weak protective effect against direct acting mutagens. Constable et al. [37] reported that the major polyphenols are not the only compounds responsible for the protective effects of the green and black teas, seen in the bacterial mutagenicity assay. As a reduction of the catechin content in fermented and instant teas did not correlate with the antimutagenic effects observed in the assay, other compounds formed during the processing conditions may be involved. This may well be the case for the SA herbal teas where the high oxidative properties of the fermented and unfermented teas did not correspond well with the low protective effect against the various oxidative mutagens used in the present study.

As discussed for the metabolically activated mutagens, differences exist with respect to the protective effect of the tea preparations and specific mutagen used. No protective effect was noticed against MMS, whereas with H_2O_2 fermented and unfermented rooibos tea (10%) and with CHP, fermented rooibos and unfermented honeybush (10%) tea showed a protective effect. Only a weak relationship exists with respect to the polyphenolic content of the teas and the protective effect against the direct-acting mutagens. Both the fermented and unfermented rooibos tea preparations (10% w/v) showed a similar inhibition when using H_2O_2 as direct-acting mutagen despite the fact that the total polyphenols was significantly lower in the fermented tea preparation. With CHP only the highest concentrations of the fermented rooibos and unfermented honeybush tea exhibited a protective effect. In some cases (CHP and H_2O_2), the addition of the tea preparations (except the methanol extract) resulted in a marked (non-significant) increase in the mutagen-induced response.

One possible explanation for this weak/lack of protection against the direct acting mutagens could be that the in vivo induction of antioxidative enzymes is required to protect against oxidative-like mutations. Another aspect that needs to be considered is the polarity of the mutagen used, compared with that of the protective principles in the aqueous tea preparations. The relatively polar constituents (including some polyphenols) of the aqueous extracts could be responsible for the low protective properties against direct acting mutagens. Due to the differences in polarity, molecules can be restricted to either hydrophilic or hydrophobic environments that will minimise their possible interaction. For example, the polar constituents of the aqueous extract will be associated with the polar constituents in the medium whilst the non-polar mutagens, MMS and CHP, are likely to be associated with the bacterial cellular matrixes. This became evident as a higher protective effect of the tea was obtained when

incubating the fermented rooibos tea and the methanol extract with TA102 (top layer) and the mutagen (CHP) in the bottom layer, which increased the possibility of the tea constituents to interact with the direct acting mutagen.

With respect to the mutagens that required metabolic activation the results obtained from the double layer assay provided insight into the possible mechanisms involved in the antimutagenic effects of the tea extracts. A higher degree of protection is obtained when 2-AAF, the S9 mixture and the different tea preparations are incubated in the bottom soft agar layer and TA98 in the top agar layer (α-method) than when the tea is incubated with the bacterium (β-method). In both cases, the activated metabolite has to diffuse from the bottom to the top agar layer to interact with the bacterium. To be mutagenic, 2-AAF, has to be metabolically activated by the cytochrome P450 enzyme system located in a hydrophobic pocket in the microsomes [39]. When the tea extracts are incubated with the bacterium the protective effect could be ascribed to a direct interaction of the activated mutagenic metabolite that diffuses from the bottom layer. However, when the tea is incubated with the enzyme preparation in the bottom layer, the inhibitory effect on 2-AAF mutagenesis can result from an interaction between the different components of the tea extracts and the enzyme system catalysing the metabolic activation of the various promutagens (inhibiting cytochrome P450-mediated activation of the carcinogen) and thus impeding the production of genotoxic intermediates. Tea polyphenols can serve as such electron acceptors directing the flow of electrons from NADPH away from cytochrome P450, the terminal component in the electron-transport chain [38]. The structure of flavanols (also referred to as catechins), the major polyphenol in green and black tea, provide strong nucleophilic centres which enables them to react with electrophilic carcinogens to form flavanol-carcinogen adducts that may result in the prevention of tumorigenesis [40,41]. This ability of aqueous green tea extracts to function as nucleophiles is supported by Bu-Abbas et al. [38]. Rooibos tea contains 4.4% tannins [13] consisting of flavanol chain extending units [42]. Unlike green and black tea, rooibos tea also contains other polyphenols like flavonols (*iso*-quercitrin), flavones (orientin, *iso*-orientin, luteolin) and dihydrochalcones (aspalathin, nothofafin) [33]. Honeybush tea has been shown to contain flavones (luteolin) and flavanones (hesperidin, hesperitin, naringenin, eriodictyol) [8]. The electron-rich aromatic B-ring system of the flavones can supply electrons that are required for the reduction of the active oxygen species [33]. These phenolic compounds presumably contribute towards the scavenging ability of the herbal teas. Presently, it is unknown whether all these different polyphenols may play a role in the antimutagenic activity of rooibos and honeybush tea. Apart from the interference with the metabolic activating system, the tea components could also directly interact with the genotoxic reactive intermediates that may result in the prevention of mutagenesis as discussed for the difference in protection obtained with β-method.

The antimutagenic effects of the different rooibos and honeybush tea preparations reported in this study could, in the case of 2-AAF that required metabolic activation, be related to the action of the different polyphenols. Studies are in progress to elucidate the mechanisms of antimutagenicity and to characterise the protective principles produced by these indigenous teas. It is known that different causative factors (e.g. carcinogens) are involved in the development of different cancers suggesting different mechanisms, which is organ specific. The present study indicated that the mechanism of protection obtained with the herbal teas differs not only when using direct-acting mutagens but also differs between mutagens that require metabolic activation. It can be argued that the herbal teas could protect against the activity of diverse mutagenic and possible carcinogenic compounds in vivo and their effectiveness, as potential chemopreventive drugs will depend on the mechanism of cancer development in a specific organ. In conclusion, it would appear that the herbal teas may not only be a good dietary source of natural antioxidants to counteract the damaging effects of free hydroxyl, superoxide and peroxyl radicals in vitro [31,43], but may also protect against mutagenesis.

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