Introduction

The protective role of tea (*Camellia sinensis*) against mutagenesis and carcinogenesis has been well established in the literature (1–5). Similar information regarding the protective role of the South African herbal teas, *Aspalathus linearis* (rooibos tea) and *Cyclopia* species (honeybush tea), is being established (6). These herbal teas, prepared from the indigenous Cape “fynbos” plants are consumed both locally and abroad with global consumption of rooibos composing more than 50% of its annual production of 7500 tons (http://www.emg.org.za/documents/rooibos.pdf), while 90% of honeybush annual production of 150 tons is exported (http://www.wesgro.org.za/-uploads/ssnatural_products_0800.pdf). The honeybush industry is only in a developmental stage and consists mainly of processed *Cyclopia intermedia* harvested from natural plant populations. Apart from these traditional processed herbal teas where production includes an oxidation step “fermentation” (7, 8), the manufacture of unprocessed (“green”) rooibos and honeybush teas has recently commenced. During this process, oxidation of the polyphenols is minimized to obtain a product with enhanced antioxidant and antimutagenic activity (9, 10).

Several health-promoting properties have been associated with the consumption of these herbal teas, but evidence is mostly anecdotal (11). Limited research has been conducted regarding the biological effects of these teas. Extracts of rooibos tea exhibited antimutagenic activity in Chinese hamster ovary (CHO) cells and in male ICR mice (12). Recent studies in the Salmonella mutagenicity assay showed that aqueous extracts of unprocessed (“unfermented”) and processed (“fermented”) rooibos and honeybush teas prevent mutagenesis induced by 2-acetylaminofluorene (2-AAF) and aflatoxin B1 (AFB1) (6). Several mechanisms have been proposed for the protective effect against mutagenesis in vitro. These include the reduction in the formation of the ultimate genotoxic intermediate by interfering with the cytochrome P450-mediated metabolic activation of mutagens and/or the direct interaction of nucleophilic tea components with the genotoxic intermediates, thereby preventing mutagenesis. Aqueous extracts of rooibos and honeybush teas have been shown to possess antioxidant activities in vitro (13, 14).
Unlike green or black teas, rooibos and honeybush teas contain no caffeine (11, 15) and have low tannin content (11, 16). The herbal teas also contain phenolic compounds that differ from each other and from green and black teas. Rooibos flavonoids predominantly consist of dihydrochalcones, flavonols, and flavones. Its flavonoid composition is unique in that it contains aspalathin, which to date has only been isolated from rooibos (17), and nothofagin, another rare β-hydroxydihydrochalcone (18). On the other hand, honeybush tea contains mainly xanthones, flavonones, and coumestans, with the xanthone, mangiferin, and the flavanone hesperidin constituting the major phenolic compounds (19–21). Processing results in a significant decrease in aspalathin and nothofagin in rooibos and mangiferin, isomangiferin, and hesperidin in honeybush (10, 18). At present, very little is known about the oxidized products present in the processed teas. In contrast, green tea mainly contains flavanols, while black tea contains their oxidized products, theaflavins and thearubigins (22, 23).

Although the herbal teas exhibit antimutagenic properties (6, 12) no information is available regarding the possible protective effects against carcinogen metabolism in vivo. It is known that the balance between the phase I (carcinogen activating) and the phase II (carcinogen detoxifying) enzymes is critical in the subsequent production of putative carcinogenic or mutagenic metabolites that are ultimately available to interact with the cell. The level and/or activity of these enzymes have been suggested to play an important role in the susceptibility of an individual for developing cancer (24). Several compounds that selectively enhance the activity of the phase II enzymes have been identified to impede the production of putative genotoxic metabolites and the formation of preneoplastic lesions and tumors (25–28). An increase in phase II detoxifying enzymes in rat liver treated by oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione] has been shown (29, 30) and postulated to be essential for effective chemoprotection against AFB1-induced hepatocarcinogenesis in rats (25). In a clinical trial conducted in residents of Qidong, a high incidence liver cancer area in China, oltipraz was shown to significantly reduce serum albumin DNA adducts of AFB1 (31).

The present study investigates the modulation of drug metabolizing enzymes in the liver of rats by rooibos and honeybush teas as compared to green and black teas. The antioxidant status as reflected by the redox state of glutathione and the oxygen radical absorbance capacity (ORAC) in liver of rats exposed to the various tea preparations were also determined.

**MATERIALS AND METHODS**

**Chemicals.** The 1-chloro-2,4-dinitrobenzene (CDNB) (97-00-7) and 3,4-dichloro-nitrobenzene (DCNB) (6306-39-4) were purchased from Merck. Reduced [70-18-8] and oxidized [27025-41-8] glutathione were purchased from Sigma Chemical Co. Phycoerytherin (PE) was purchased from Sigma Chemical Co. Ethanol (96%). UDP-glucuronic acid [63700-19-6], purchased from Roche. BDH laboratories were the suppliers of sodium chloride (AAPH) [2997-92-4] and Trolox [53188-07-1] were obtained from ProZyme, and 2,2'-azobis(2-aminopropane) dihydrochloride (AAPH) [2997-92-4] and Trolox [53188-07-1] were obtained from Aldrich Chemical Co.

**Enzyme Assays. Glutathione S-Transferase Assay.** The GST-α activity was measured according to the method of Habig et al. (35), using CDNB as substrate. The reaction mixture contained 0.1 M potassium phosphate buffer (pH 6.5), 30 mM GSH, and 20 mM CDNB. The reaction was initiated by the addition of cytosol (10 mg protein/mL), and the increase in absorbance at 340 nm due to the formation of CDNB–GSH conjugates was recorded for 3 min at 25 °C. The specific activity (pmol/min/mg protein) was calculated using a millimolar extinction coefficient of 9.6 for CDNB–GSH. The activity of GST-α was measured using 60 mM DCNB as substrate (35). The reaction mixture contained 0.1 M potassium phosphate buffer (pH 7.5), 86.1 mM GSH, and 60 mM DCNB as substrate cytosolic protein (0.3 mg/mL). Absorbance was measured at 344 nm for 3 min at 25 °C. The specific
activity was expressed as pmol/min/mg protein using a millimolar extinction coefficient of 8.5 for the DCNB–GSH conjugate.

**UDP-Glucuronosyltransferase Assay.** The activity of microsomal UDP-GT was determined spectrophotometrically using p-nitropheno1 and UDP-glucuronic acid as substrates (36). Liver microsomes (1 mg of protein/mL), activated with 0.25% (w/v) Triton X-100, were incubated with 0.1 M tris-HCl (pH 7.4), 50 mM MgCl₂, and 5 mM p-nitrophenol for 2 min at 37°C. The reaction was initiated by addition of UDP—glucuronic acid (30 mM) and was terminated after 10 min by the addition of 5% (w/v) trichloroacetic acid (TCA). After centrifugation (3000g), 2 M NaOH was added to the supernatant, and the absorbance was determined at 405 nm. The specific activity was expressed as nmol/min/mg protein using the millimolar extinction coefficient of 18.1.

**GSH Analysis.** Total glutathione (GSH and GSSG) was measured according to a modified method of Tietze (37). Liver cytosolic preparations were treated on ice with 6% (v/v) perchloric acid (PCA) containing freshly prepared 3 mM M2VP and 1 mM EDTA for GSSG and with ice-cold 15% (w/v) TCA for GSH determinations on ice. After centrifugation at 10000g for 10 min, 50 µL of the supernatant was added to glutathione reductase (1U) and 75 mM DTNB. The reaction was initiated by addition of 0.25 mM NADPH to a final volume of 200 µL. The change in absorbance was monitored at 410 nm for 5 min, and GSH and GSSG levels were calculated using pure GSH and GSSG as standards.

**Oxygen Radical Absorbance Capacity (ORAC).** The ORAC assay was based on the procedure described by Cao and Prior (38). Free radicals were produced by AAPH, and the oxidation of the fluorescent indicator protein B-PE was measured. Both reagents were prepared in 75 mM phosphate buffer (pH 7.0), and 50 µM Trolax was used as standards. The liver samples were homogenized in volumes of the phosphate buffer in a Thomas homogenizer (10 strokes) and centrifuged at 12 000g for 10 min at 4°C. The supernatant was deproteinized using 0.25 M PCA and centrifuged at 16 000g for 15 min. The resultant supernatants were then stored at −80°C prior to analysis. The reaction was performed in 96 well microtiter plates and consisted of 170 µL of B-PE (80 µg/mL) and 10 µL of diluted (1:1) sample incubated at 37°C for 15 min. The reaction was initiated by the addition of 20 µL of AAPH (240 mM), and the fluorescence (emission 590 nm, excitation 530 nm) was recorded every 5 min until reading had declined to less than 5% of initial reading. The ORAC values were calculated and expressed as µM Trolax equivalents/mg wet liver weight.

**Statistical Analysis.** Data were analyzed by two-way ANOVA using the generalized linear model procedure, and Tukey’s Studentized range test was used to determine whether the means differed statistically. When data showed unequal variances, the nonparametric Tukey-type test was used. Values were considered significant if \( P < 0.05 \). Bonferroni pairwise adjustment was used to compare GSH, GSSG, and UDP-GT values.

**RESULTS**

**Body Weight and Relative Liver Weight Gains.** No significant differences (\( P > 0.05 \)) in the daily tea intake between the various groups were noted. No differences were noted in the body weight gain and relative liver weight (percentage of bodyweights) as a result of the tea treatment (Table 1).

**Liver and Kidney Function Indicators and Serum Iron and Cholesterol Levels.** The effect of the different tea treatments on the liver function enzymes of the experimental animals is summarized in Table 1. No significant differences were noted in the activities of the liver function enzymes AST, ALT, and ALP. The level of total bilirubin, unconjugated bilirubin, and total protein was also not significantly different between the tea treated and control rats (results not shown).

**Soluble Solid, Total Phenolic, Flavonoid Content and Total Phenolic Intake.** The soluble solids were significantly higher (\( P < 0.001 \)) in the honeybush extracts than in the rooibos extracts, which is in agreement with the larger amount of tea used for the preparation of the honeybush extracts (Table 2). The soluble solids obtained from the processed tea extracts constituted approximately 50% of the unprocessed herbal tea extracts. The soluble solids of the black tea were significantly higher (\( P < 0.05 \)) when compared to the green, unprocessed rooibos and the processed herbal teas.

The total phenol content of the processed rooibos tea’s soluble solids did not differ significantly from its unprocessed counterpart, but processed honeybush tea had a significantly lower (\( P < 0.001 \)) phenol content when compared to the soluble solids of unprocessed honeybush tea. Green and black teas had a similar phenolic content (Table 2). The total phenolic content of unprocessed rooibos and green tea’s soluble solids was significantly higher than that of unprocessed honeybush tea. Black tea soluble solids exhibited phenolic levels very similar to those of processed rooibos and were significantly higher than those of the processed honeybush tea. The flavonoid content of the green and black teas was approximately 20 and 30% higher when compared with that of the processed and unprocessed herbal teas, respectively (Table 2). There were no significant differences in the phenolic intake of the rats that received the unprocessed rooibos, green, and black tea, while rats that consumed the unprocessed honeybush tea had the highest phenolic intake (\( P < 0.05 \)). Rats that received the processed herbal teas ingested the lowest amount of total phenols when compared with the unprocessed herbal teas and green and black teas. When considering the flavonoid intake, rats receiving the unprocessed honeybush tea, green, and black teas had a significantly (\( P < 0.001 \)) higher intake when compared to the rats consuming the unprocessed rooibos tea. The flavonoid intake of the rats receiving the processed herbal teas was significantly (\( P < 0.001 \)) less when compared with their processed counterparts.

**Cytosolic Glutathione S-Transferases.** Exposure of rats to various aqueous tea extracts did not significantly affect the activity of GST-µ (mu). However, there was a significant increase (\( P < 0.001 \)) in the activity of GST-α (alpha) in the
of the experimental animals was not significantly affected by unprocessed honeybush tea also marginally (P < 0.05). If letters in parentheses differ, then GST-

Table 2. Different Intake Parameters of Male Fischer 344 Rats Fed Various Tea Preparations over a Period of 10 Weeks

<table>
<thead>
<tr>
<th>treatment</th>
<th>soluble solids (mg/mL)</th>
<th>total phenolic content (mg gallic acid equivs/mg solute solids)</th>
<th>liquid intake/day/100 g BW (mL)</th>
<th>total phenolic intake (mg gallic acid equivs/day/100 g BW)</th>
<th>flavonoids (% total phenolic content)</th>
<th>total flavonoid intake (mg gallic acid equivs/day/100 g BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (water)</td>
<td>none</td>
<td>none</td>
<td>9.7 ± 1.45a</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Rg tea</td>
<td>2.59 ± 0.44a</td>
<td>0.32 ± 0.07a</td>
<td>7.90 ± 1.01a</td>
<td>6.40 ± 0.08a</td>
<td>63.13 ± 1.69a</td>
<td>2.94 ± 0.89a</td>
</tr>
<tr>
<td>Hp tea</td>
<td>5.96 ± 0.78b</td>
<td>0.15 ± 0.05b</td>
<td>8.10 ± 0.85a</td>
<td>7.29 ± 0.19a</td>
<td>63.09 ± 3.09a</td>
<td>3.73 ± 1.04a</td>
</tr>
<tr>
<td>Hg tea</td>
<td>11.78 ± 1.18c</td>
<td>0.23 ± 0.04ab</td>
<td>8.58 ± 0.92a</td>
<td>22.90 ± 0.36c</td>
<td>74.86 ± 1.39b</td>
<td>19.07 ± 2.37c</td>
</tr>
<tr>
<td>black tea</td>
<td>8.57 ± 0.66c</td>
<td>0.26 ± 0.03a</td>
<td>8.03 ± 0.52a</td>
<td>17.30 ± 0.11b</td>
<td>92.99 ± 2.35c</td>
<td>15.14 ± 2.09c</td>
</tr>
<tr>
<td>green tea</td>
<td>7.21 ± 0.28c</td>
<td>0.30 ± 0.04a</td>
<td>7.92 ± 0.35a</td>
<td>17.02 ± 0.06b</td>
<td>92.89 ± 0.7c</td>
<td>16.48 ± 1.91c</td>
</tr>
</tbody>
</table>

*Values in columns are means ± SD of 10 rats per group. Means followed by the same letter are not significantly different. If letters differ, then P < 0.01.

Table 3. Effect of Unprocessed and Processed Herbal and Green and Black Teas on the Activities of the Cytosolic Glutathione S-Transferases and the Microsomal UDP-Glucuronosyl Transferases in Livers of Rats Exposed to the Various Teas for 10 Weeks

<table>
<thead>
<tr>
<th>treatment</th>
<th>GST-μ (pmol/min/mg protein)</th>
<th>GST-α (alpha) (pmol/min/mg protein)</th>
<th>UDP-GT (pmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>2.41 ± 0.39a</td>
<td>2.39 ± 0.40a</td>
<td>31.55 ± 3.97a</td>
</tr>
<tr>
<td>rooibos processed tea</td>
<td>2.96 ± 0.77a</td>
<td>4.60 ± 1.10b</td>
<td>29.32 ± 3.09a</td>
</tr>
<tr>
<td>rooibos unprocessed tea</td>
<td>3.04 ± 0.64a</td>
<td>4.34 ± 1.03b</td>
<td>45.09 ± 4.48b</td>
</tr>
<tr>
<td>honeybush processed tea</td>
<td>2.50 ± 0.43a</td>
<td>3.87 ± 0.63b</td>
<td>37.7 ± 5.86ab</td>
</tr>
<tr>
<td>honeybush unprocessed tea</td>
<td>2.43 ± 0.74a</td>
<td>3.45 ± 1.13b</td>
<td>40.81 ± 6.56(b)</td>
</tr>
<tr>
<td>black tea</td>
<td>2.15 ± 0.47a</td>
<td>2.39 ± 0.66a</td>
<td>33.09 ± 3.67a</td>
</tr>
<tr>
<td>green tea</td>
<td>1.96 ± 0.38a</td>
<td>2.06 ± 0.46a</td>
<td>24.55 ± 2.57aa</td>
</tr>
</tbody>
</table>

*Values in columns are means ± SD of 10 rats per group. Means followed by the same letter are not significantly different (P > 0.05). If letters differ, then P < 0.05. Letters in parentheses indicate marginal effects (P < 0.1). Microsomal glucuronosyl transferases. A significant increase (P < 0.001) in the UDP-GT activity was evident following treatment of the animals with unprocessed rooibos tea, while unprocessed honeybush tea also marginally (P = 0.06) increased the activity of the enzyme. Animals receiving green, black, or processed herbal teas did not significantly alter the activity of the enzyme (Table 3).

Glutathione levels. The concentration of GSH in the livers of the experimental animals was not significantly affected by any of the teas, although the South African herbal teas tended to increase the level (nonsignificantly) when compared with the control and black and green tea groups (Table 4). A significant decrease (P < 0.05) in GSSG levels was noted in the liver of rats exposed to the various tea preparations. Processed and unprocessed rooibos tea also caused a significantly lower (P < 0.05) GSSG level in the liver when compared to rats drinking the processed and unprocessed honeybush and green and black teas. Due to the changes in the GSH and GSSG levels, the GSH/GSSG ratio significantly increased (P < 0.05) in the liver of the rats drinking the unprocessed and processed rooibos and unprocessed honeybush teas, while processed honeybush tea marginally (P = 0.06) increased the ratio (Table 4).

Oxidative Radical Absorbance Capacity (ORAC). The ORAC values in the liver of the rats were significantly (P < 0.001) and marginally lowered by the green and black teas, respectively. No effect was noticed on the hepatic ORAC level in the rats treated with the herbal teas (Table 4).

DISCUSSION

Certain dietary constituents may influence the incidence of diseases (e.g., cancer) by modulating the enzyme systems responsible for the metabolic activation/detoxifying of chemical carcinogens in the cell (39). In this regard, the popularity of herbal teas has increased during the past twenty years (40) following advances made in our knowledge on the anticancer properties of green and black teas (3, 41, 42). Because the phenolic constituents of the two South African herbal teas differ from green and black teas, it is imperative to investigate the possible protective mechanisms of rooibos and honeybush teas against the adverse effects of xenobiotics in vivo.

Table 4. Effect of Unprocessed and Processed Herbal and Green and Black Teas on Reduced Glutathione (GSH), Oxidized Glutathione (GSSG), the Ratio GSH/GSSG and Oxidative Capacity (ORAC) in Livers of Rats Exposed to the Various Teas for 10 Weeks

<table>
<thead>
<tr>
<th>treatment</th>
<th>GSH (μM/mg protein)</th>
<th>GSSG (μM/mg protein)</th>
<th>GSH/GSSG ratio</th>
<th>ORAC (μM Trolox equivs/mg liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>9.35 ± 2.00a</td>
<td>1.23 ± 0.32a</td>
<td>7.98 ± 2.50a</td>
<td>10.49 ± 1.91a</td>
</tr>
<tr>
<td>rooibos processed tea</td>
<td>15.06 ± 5.38a</td>
<td>0.42 ± 0.13b</td>
<td>38.89 ± 12.19b</td>
<td>11.47 ± 1.11a</td>
</tr>
<tr>
<td>rooibos unprocessed tea</td>
<td>16.39 ± 5.33a</td>
<td>0.40 ± 0.13a</td>
<td>42.1 ± 6.66b</td>
<td>10.74 ± 1.98a</td>
</tr>
<tr>
<td>honeybush processed tea</td>
<td>13.6 ± 2.67a</td>
<td>0.70 ± 0.16c</td>
<td>20.99 ± 7.01b</td>
<td>11.28 ± 1.84a</td>
</tr>
<tr>
<td>honeybush unprocessed tea</td>
<td>18.56 ± 6.2a</td>
<td>0.51 ± 0.18c</td>
<td>43.73 ± 12.73b</td>
<td>10.10 ± 1.20a</td>
</tr>
<tr>
<td>black tea</td>
<td>10.67 ± 2.91a</td>
<td>0.87 ± 0.29c</td>
<td>12.9 ± 4.11a</td>
<td>7.99 ± 0.83b</td>
</tr>
<tr>
<td>green tea</td>
<td>9.2 ± 2.12a</td>
<td>0.76 ± 0.15c</td>
<td>13.3 ± 1.77a</td>
<td>8.72 ± 1.96ab</td>
</tr>
</tbody>
</table>

*Values in columns are means ± SD of 10 rats per group. Means followed by the same letter do not differ significantly (P > 0.05). If letters differ, then P < 0.05. Letters in parentheses indicate marginal effects (P < 0.1).
Consumption of aqueous extracts of rooibos and honeybush teas, as sole substitutes for drinking water, did not cause any adverse effects in the liver and kidney of the rats. The total serum iron levels were not altered, indicating that none of the teas, irrespective of the difference in phenolic composition, interfered with iron uptake in the present study. A study by Hesseling (43) showed that rooibos tea did not affect iron absorption in a human population; however, conflicting findings have been reported with respect to the effect of black and green teas on iron uptake in humans (44, 45).

Antioxidant activity has been demonstrated for rooibos (9) and honeybush tea extracts (46) in various in vitro systems (e.g., scavenging of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and superoxide anion radicals). These studies showed that honeybush tea exhibited a significantly lower antioxidant activity than rooibos tea. This could be attributed not only to the difference in the phenolic profile but also to the lower phenolic content of honeybush soluble solids. In the present study, the low levels were compensated for by the increased amount of tea used in preparation of these extracts. The latter did not negatively impact the consumption and/or the body weight gain and liver function of the rats. The phenolic intake of animals receiving green and black teas was similar or equal to the intake of animals receiving the unprocessed rooibos tea and 3–4 times higher when compared to the processed honeybush and processed rooibos tea. In a comparative study, it was demonstrated that aqueous extracts of green tea exhibit higher antioxidant potency than those of black tea, while unprocessed rooibos is comparable to black tea. Processed rooibos tea extracts exhibit the lowest antioxidant activity (47). When using the ORAC assay, the total antioxidant capacity of green and black tea compares favorably to other drinks prepared from fruit and vegetables (48). In the present study, however, green and black tea marginally (P < 0.1) to significantly (P < 0.05) reduced the hepatic ORAC level, respectively, while no effect was noticed with the herbal teas. It is not known whether a relationship exists between the reduced hepatic ORAC and the total phenolic intake of the rats fed the green and black teas under the present experimental conditions.

Reduced glutathione, a powerful intracellular antioxidant that plays an important role in stabilizing many enzymes (49), could also be considered as a good marker for the antioxidative capacity in tissue (50, 51). Several clinical conditions are associated with a decrease in the GSH level in the cell that may result in a lowered cellular redox potential (52). Van Acker et al. (51) showed that GSH has the ability to regenerate \( \alpha \)-tocopherol from its radical, maintaining the levels of \( \alpha \)-tocopherol at a level to protect the cell membranes against lipid peroxidation. In this regard, flavonoids mimic the antioxidant activity of \( \alpha \)-tocopherol, suggesting an interaction between flavonoids and GSH. Nanjo et al. (53) showed that the addition of green tea catechins to diets high in palm and perilla oil prevented a decrease in the plasma \( \alpha \)-tocopherol concentration in rats. Rooibos and honeybush teas significantly decreased the level of GSSG in the liver resulting in an increase of the GSH/GSSG ratio, presumably by stabilizing GSH. The level of GSH was markedly (not significantly) higher in the liver of the herbal-tea-treated rats. Green and black teas significantly (P < 0.05) reduced GSSG, while the GSH level was not affected. Changes in the GSH and GSSG levels resulted in a significant increase in the GSH/GSSG ratio in the liver of the rats fed the herbal teas, while it was markedly (not significantly) increased with the green and black teas. Sohn et al. (54) also showed that neither the black nor the green tea affected the liver concentrations of GSH in male F344 rats when treated with 2% aqueous extracts of green and black teas for 6 weeks. The GSH/GSSG ratios were not reported. Despite the differences in the type of flavonoids, the unprocessed herbal teas exhibited a similar effect on the GSH/GSSG ratio while there was an approximate 50% reduction in the case of the processed honeybush tea. The significant increase in the GSH/GSSG ratio in the liver of the herbal-tea-treated rats may be indicative of a reduced oxidative stress or an increased antioxidant capacity in the cell, thereby lowering the susceptibility to oxidative damage. The marked increase obtained with the green and black tea suggests that, in the present study, the phenolic components of the herbal teas were more effective to increase the antioxidant status in the liver.

Stabilization of GSH in the liver may also result in an increase in the endogenous detoxification capacity, as glutathione is known to, either directly or via the glutathione S-transferases, interact with reactive genotoxic metabolites, thereby reducing the likelihood of damage to cellular DNA (28, 55). With respect to metabolism, the carcinogenicity of compounds (e.g., the mycotoxin AFB\(_1\)) and 2-AAF) is modulated by the interaction with the phase I activating or the phase II detoxifying enzymes (27, 28, 56, 57). Studies by Siess et al. (28) in Wistar rats suggest that dietary flavonoids inhibit AFB\(_1\) carcinogenesis by decreasing the covalent binding of AFB\(_1\) to liver DNA as a result of the production of conjugated AFB\(_1\) metabolites via the induction of phase II enzymes GST-\(\alpha\) and UDP-GT. Rats treated with coumarin, a natural benzopyrone, were protected from developing hepatic AFB\(_1\)-induced preneoplastic lesions (27). The induction of GST-A5, a subunit of GST-\(\alpha\), was implicated as an important protective role. In the present study, the activity of GST-\(\alpha\) was increased in the livers of rats fed the processed and unprocessed rooibos and honeybush teas. The black and green teas showed no effect when compared to the control group, which is in accordance with studies conducted by Bu-Abbas et al. (58) and Sohn et al. (54) using male Wistar and Fischer rats, respectively. However, an increase in the activities of GST-\(\alpha\) and GST-\(\mu\) when using aqueous extracts of black tea in human Chang liver cells has been reported (1). Another study also showed a significant induction of GST by green tea leaves when fed to Wistar rats at various concentrations between 4 and 63 weeks (59).

Similar discrepancies seem to exist with respect to the induction of UDP-GT by green and black teas. Under the present experimental conditions, no induction of UDP-GT activity was found in male Fischer rats treated with 2% tea in their drinking water for 10 weeks. The induction of UDP-GT activity in the microsomes has been suggested as a major mechanism of tea as a chemopreventive agent (60). Unprocessed rooibos significantly increased the activity of the microsomal UDP-GT in the liver, while unprocessed honeybush tea only showed a marginal significant (P < 0.1) increase. The increase in the glucuronidation capacity was suggested to facilitate the metabolism of chemical carcinogens into inactive, readily excretable products, thereby reducing their possible interaction with cellular DNA (58). The lack of induction of UDP-GT activity in processed rooibos and honeybush tea could be due to the change in phenolic composition during processing of these teas (9, 10, 21). The present study showed no difference in the induction of the activity of the enzyme by the green and black teas. In contrast, several studies indicated that aqueous extracts of black tea and to a lesser extent green tea induced the activity of UDP-GT using \( p \)-nitrophenol and 2-aminophenol as substrates for the enzyme (54, 56, 60, 61). However, when using 4-methyl-umbelliferone as a substrate none of the teas increased the
activity of the enzyme. Decaffeinated black tea also failed to enhance the activity of the enzyme.

It would appear that many variables exist that could determine the induction of the activity of phase II enzymes by green and black tea. These include the rat species used (58), the amount of tea used, duration of treatment and route of administration (59), and the substrate used in the enzyme assay (58, 61). Some of these parameters could have contributed to the apparent lack of the induction of enzyme activity by green and black tea reported in the present study.

A preliminary report by Marnewick et al. (62) indicated that the mutagenicity of AFB1 and 2-AAF decreased with the addition of liver cytosolic fractions from male Fischer rats that consumed the South African herbal teas for 10 weeks when compared with controls given water. The present study indicates that enhanced activities of the important phase II enzymes, GST-α and UDP-GT, as well as the increased oxidative status in the liver, may account for this protective effect in the mutagenicity assay. Apart from the induction of phase II enzymes by rooibos and honeybush teas, other mechanisms of antimutagenicity may play a role. Nucleophilic tea components may also directly interact with the genotoxic reactive intermediates of mutagens to reduce their mutagenic potential. However, this may vary with the type of mutagen and needs further investigation (6, 63). Because the phenolic constituents of the two South African herbal teas differ from those of green and black teas, the mechanisms involved in protection against DNA damage could also differ. The induction of phase II hepatic drug metabolising enzymes and the increased antioxidant status of the liver by these herbal teas may represent a promising tool for chemoprevention against cancer in humans as they are consumed on a regular basis locally and are becoming increasingly important as a neutraceutical product globally.

ABBREVIATIONS USED

CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 3,4-dichloronitrobenzene; GSH, reduced glutathione; GSSG, oxidized glutathione; UDP-GT, uridine 5′-diphospho-glucuronosyl-transferase; GST, glutathione S-transferase; M2VP, 1-methyl-2-vinylpyridinium trifluoromethane sulfonate; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; β-PE, β-phycocerythin; ORAC, oxygen radical absorbance capacity; DPPH, 1,1-diphenyl-2-picrylhydrazyl.

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LITERATURE CITED


(27) Kelly, V. P.; Ellis, E. M.; Manson, M. M.; Chanas, S. A.; Moftai, G. J.; McLeod, R.; Judah, D. J.; Neal, G. E.; Hayes, J. D. Chemoprevention of Aflatoxin B1 hepatocarcinogenesis by
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