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Modulation of Hepatic Drug Metabolizing Enzymes and Oxidative Status by Rooibos (*Aspalathus linearis*) and Honeybush (*Cyclopia intermedia*), Green and Black (*Camellia sinensis*) Teas in Rats

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Rooibos and honeybush teas significantly ($P < 0.05$) enhanced the activity of cytosolic glutathione S-transferase alpha. A significant ($P < 0.05$) to marginal ($P < 0.1$) increase in the activity of the microsomal UDP-glucuronosyl transferase was obtained with unprocessed rooibos and honeybush teas, respectively. Oxidized glutathione (GSSG) levels were significantly ($P < 0.05$) reduced in the liver of all tea treated rats while reduced glutathione (GSH) was markedly increased in the liver of the herbal tea treated rats. These changes resulted in a significant ($P < 0.05$) increase in the GSH/GSSG ratio by the unprocessed, processed rooibos and unprocessed honeybush teas. Green and black teas markedly to significantly decreased the oxygen radical absorbance capacity in liver homogenates, respectively. Modulation of phase II drug metabolizing enzymes and oxidative status in the liver may be important events in the protection against adverse effects related to mutagenesis and oxidative damage.

KEYWORDS: Rooibos (*Aspalathus lineariz*) tea; honeybush (*Cyclopia intermedia*) tea; glutathione S-transferases; UPD-glucuronosyl transferases; glutathione; ORAC; rat liver

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 B_1 (AFB_1) (*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*).

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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, flavanones, 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 AFB_1-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 AFB_1 (*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 Roche. BDH laboratories were the suppliers of sodium dithionite [7775-14-6], dimethyl sulfoxide (DMSO) [67-68-5] and ethanol (96%). UDP-glucuronic acid [63700-19-6], *p*-nitrophenol [100-02-7], glutathione reductase, perchloric acid [7601-90-3], 1-methyl-2-vinyl-pyridinium trifluoromethane sulfonate (M2VP), EDTA [60-00-4], and 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) [69-78-3] were purchased from Sigma Chemical Co. Phycoerytherin (PE) was purchased from ProZyme, and 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) [2997-92-4] and Trolox [53188-07-1] were obtained from Aldrich Chemical Co.

Plant Material. Individual batches of processed and unprocessed rooibos and honeybush (*C. intermedia*) teas of the highest quality were a gift from Dr E. Joubert, (ARC Infruitec-Nietvoorbij, South Africa). The black tea (*C. sinensis* var. *assamica*) is 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.

Preparation of Aqueous Tea Extracts. Aqueous extracts were prepared by the addition of freshly boiled tap water to the tea leaves and stems to a concentration of 2 g/100 mL for processed and unprocessed rooibos and black and green teas, while 4 g/100 mL was used for processed and unprocessed honeybush tea. These concentrations are customarily used for tea making purposes (*7*, *8*). The mixture was allowed to stand for 30 min at room temperature, filtered (Whatman no. 4), and after cooling, dispensed into water bottles.

Treatment of Animals. Male Fischer 344 rats, 150 g, were obtained from the Primate Unit of the Diabetic Research Group of the Medical Research Council of South Africa. They were individually housed 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. The rats were divided into seven groups consisting of 10 rats per group. The experimental groups each received the various aqueous tea extracts for 10 weeks as their sole source of drinking fluid, while the control group received tap water. Fresh tea was prepared every second day. The rats were fed ad libitum rat cubes (Epol Ltd, Johannesburg, South Africa), and the fluid intake monitored on a daily basis. Body weights were monitored on a weekly basis.

Total Phenolic, Flavonoid Content and Soluble Solid Determinations. The soluble solid content of each tea preparation was determined gravimetrically (sixteen repetitions) during the course of the study after drying 1-mL aliquots at 110 °C for 12 h. The scaled-down Folin−Ciocalteu method with gallic acid as standard was used to determine the total phenolic content (*32*) of the different tea extracts. The phenolic content of the extracts was determined before and after precipitation of the flavonoids to yield the nonflavonoid content, and by difference, the flavonoid content of the tea extracts. Precipitation of the flavonoids was done according to Kramling and Singleton (*33*).

Chemical Pathology. The animals were fasted (16 h) and sacrificed under pentobarbital anesthesia after 10 weeks, and blood was collected from the abdominal aorta. Serum samples were prepared for the determination of clinical biochemical parameters including creatinine, total cholesterol, total iron, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin (T.Bili), unconjugated bilirubin (D.Bili), and total protein. Serum analyses were conducted on a Technicon RA 1000 automated analyzer.

Preparation of Microsomal and Cytosolic Liver Fractions. Upon sacrifice, the liver was excised immediately, weighed, frozen in liquid nitrogen, and stored at −80 °C until analyzed. For the preparation of the subcellular fractions, a sample was homogenized in 3 volumes of ice-cold 0.15 M KCl solution for 1 min, using a Thomas homogenizer. The homogenates were filtered through double layer cheesecloth and homogenized with a glass dounce (10 strokes) using a loose pestle. The homogenates were centrifuged at 9000*g* for 10 min, and the cytosolic and microsomal preparations were collected after centrifugation of the supernatant at 100000*g* for 1 h. The microsomes were resuspended in 0.15 M KCl using a glass dounce, centrifuged at 100000*g* for 1 h, resuspended in 0.15 M KCl, and stored with the cytosolic fractions at −80 °C. All procedures were performed at 4 °C. Microsomal and cytosolic proteins were determined by the method of Bradford (*34*) using BSA as standard protein.

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 30 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-μ was measured using 60 mM DCNB as substrate (*35*). The reaction mixture contained 0.1 M potassium phosphate buffer (pH7.5), 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

Table 1. Effect of Tea Treatments on the Body Weight Gain, Relative Liver Weight, and Selected Blood Clinical Chemical Parameters Related to Liver and Kidney Function[a]

treatment	body weight gain (g)	relative liver weight (g)	AST (U/L)	ALT (U/L)	ALP (U/L)	creatinine (μmol/L)	cholesterol (mmol/L)	total iron (μmol/L)
control (water)	109.3 ± 17.7a	7.26 ± 0.97a	333.6 ± 68.2a	179.4 ± 64.33a	134.8 ± 10.2a	82 ± 6.7a	1.31 ± 0.19a	15.10 ± 1.54a
rooibos processed tea	112.0 ± 15a	7.00 ± 0.80a	351.5 ± 55a	123.9 ± 24.5a	144.6 ± 22.4a	88.5 ± 6.5a	1.64 ± 0.19a	16.45 ± 2.65a
rooibos unprocessed tea	115.4 ± 18.8a	6.96 ± 1.2a	391.8 ± 66.9a	146.8 ± 47.7a	127.7 ± 36.7a	88 ± 6.2a	1.54 ± 0.23a	13.9 ± 1.6a
honeybush processed tea	117.8 ± 19.3a	6.72 ± 0.96a	320.6 ± 56.7a	145.4 ± 91.6a	133.1 ± 25.3a	86.1 ± 3.5a	1.55 ± 0.28a	13.8 ± 2.1a
honeybush unprocessed tea	106.1 ± 15.4a	7.53 ± 0.77a	311.7 ± 39.1a	132.1 ± 30.3a	144.5 ± 30.5a	85.7 ± 4.35a	1.58 ± 0.27a	16.75 ± 4.23a
black tea	127.8 ± 18.2a	6.53 ± 0.65a	356.2 ± 100.7a	182.3 ± 94.3a	132.6 ± 12.9a	76.1 ± 5.22a	1.46 ± 0.25a	14.48 ± 2.19a
green tea	121.3 ± 10.6a	7.04 ± 0.85a	386.7 ± 63.2a	143.1 ± 67.4a	133.7 ± 18.9a	82.5 ± 7.1a	1.53 ± 0.1a	14.26 ± 2.83a

[a] Values in columns are means ± STD of 10 rats per group. Means followed by the same letter are not significantly different.

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*-nitrophenol 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 $MgCl_2$, 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 (3000*g*), 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 10000*g* for 10 min, 50 μL of the supernatant was added to glutathione reductase (1U) and 75 μM 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 Trolox was used as standard. The liver samples were homogenized in 4 volumes of the phosphate buffer in a Thomas homogenizer (10 strokes) and centrifuged at 12 000*g* for 10 min at 4 °C. The supernatant was deproteinized using 0.25 M PCA and centrifuged at 16 000*g* 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 Trolox 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). Similarly, the levels of creatinine, a marker for kidney function, total cholesterol, and total plasma iron were not altered.

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

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

treatment[b]	soluble solids (mg/mL)	total phenolic content (mg gallic acid equivs/ mg soluble solids)	liquid intake/ day/ 100 g BW (mL)	total phenolic intake (mg gallic acid equivs/ day/100 g BW)[c]	flavonoids (% of total phenolic content)[d]	total flavonoid intake (mg gallic acid equivs/ day/ 100 g BW)
control (water)	none	none	9.7 ± 1.45a	none		none
Rp tea	2.59 ± 0.44a	0.32 ± 0.07a	7.90 ± 1.01a	6.40 ± 0.08a	63.13 ± 1.69a	2.94 ± 0.89a
Rg tea	5.36 ± 0.81b	0.36 ± 0.06a	8.29 ± 0.74a	16.12 ± 0.18b	76.02 ± 2.29b	11.97 ± 1.76b
Hp tea	5.96 ± 0.78b	0.15 ± 0.05b	8.10 ± 0.85a	7.29 ± 0.19a	63.09 ± 3.09a	3.73 ± 1.04a
Hg tea	11.78 ± 1.18c	0.23 ± 0.04ab	8.58 ± 0.92a	22.90 ± 0.36c	74.86 ± 1.39b	19.07 ± 2.37c
black tea	8.37 ± 0.66c	0.26 ± 0.03a	8.03 ± 0.52a	17.30 ± 0.11b	92.9 ± 2.35c	15.14 ± 2.09c
green tea	7.21 ± 0.82b	0.30 ± 0.04a	7.92 ± 0.35a	17.02 ± 0.06b	92.89 ± 0.7c	16.48 ± 1.91c

[a] Parameters of tea intake are mean of 16 determinations. Values in columns are means ± STD of 10 rats per group. Means followed by the same letter are not significantly different. If the letters differ, then $P < 0.001$. [b] Aqueous solutions (2%) were prepared of rooibos processed (Rp), unprocessed (Rg), green and black teas, and 4% (w/v) of honeybush processed (Hp) and unprocessed (Hg). [c] Calculation of the total phenolic intake was based on the soluble solids intake obtained from the average liquid intake/day. [d] Flavonoids were determined after subtracting the nonflavonoid content from the total phenolic content (Materials and Methods).

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[a]

treatment	GST-μ (mu) (pmol/min/mg protein)	GST-α (alpha) (pmol/min/mg protein)	UDP-GT (nmol/min/mg protein)
water	2.41 ± 0.39a	2.39 ± 0.40a	31.55 ± 3.97a
rooibos processed tea	2.96 ± 0.77a	4.60 ± 1.10b	29.32 ± 3.09a
rooibos unprocessed tea	3.04 ± 0.64a	4.34 ± 1.03b	45.09 ± 4.49b
honeybush processed tea	2.50 ± 0.43a	3.87 ± 0.63b	37.7 ± 5.88ab
honeybush unprocessed tea	2.43 ± 0.74a	3.45 ± 1.13b	40.81 ± 6.56(b)
black tea	2.15 ± 0.47a	2.39 ± 0.66a	33.09 ± 3.67a
green tea	1.96 ± 0.38a	2.06 ± 0.46a	24.55 ± 2.57aa

[a] Values in columns are means ± STD of 10 rats per group. Means followed by the same letter are not significantly different ($P > 0.05$). If letters differ, then $P < 0.05$. If letters in parentheses differ, then $P < 0.1$. DCNB was used as substrate for GST-μ determination and CDNB for GST-α,

livers of rats fed the South African herbal teas when compared to the control and green and black tea treated groups (**Table 3**).

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**). Although green and black teas markedly increased the GSH/GSSG ratio when compared to the control group, this increase was not statistically significant.

Oxygen 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[a]

treatment	GSH (μM/mg protein)	GSSG (μM/mg protein)	GSH/GSSG ratio	ORAC (μM Trolox equivs/mg liver)
water	9.35 ± 2.00a	1.23 ± 0.32a	7.98 ± 2.50a	10.49 ± 1.91a
rooibos processed tea	15.06 ± 5.38a	0.42 ± 0.13b	38.89 ± 12.19b	11.47 ± 1.11a
rooibos unprocessed tea	16.39 ± 5.33a	0.40 ± 0.13b	42.1 ± 9.66b	10.74 ± 1.98a
honeybush processed tea	13.6 ± 2.67a	0.70 ± 0.16c	20.99 ± 7.01(b)	11.28 ± 1.84a
honeybush unprocessed tea	18.56 ± 6.2a	0.51 ± 0.18c	43.73 ± 12.73b	10.10 ± 1.20a
black tea	10.67 ± 2.91a	0.87 ± 0.29c	12.9 ± 4.11a	7.99 ± 0.83b
green tea	9.2 ± 2.12a	0.76 ± 0.15c	13.3 ± 1.77a	8.72 ± 1.96ab

[a] Values in columns are means ± STD 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 α-tocopherol from its radical, maintaining the levels of α-tocopherol at a level to protect the cell membranes against lipid peroxidation. In this regard, flavonoids mimic the antioxidant activity of α-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 α-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-α and UDP-GT. Rats treated with coumarin, a natural benzopyrone, were protected from developing hepatic AFB_1-induced preneoplastic lesions (*27*). The induction of class GST-A5, a subunit of GST-α, was implied to play an important protective role. In the present study, the activity of GST-α 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-α and GST-μ 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-methylumbelliferone 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 AFB_1 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-phycoerythrin; ORAC, oxygen radical absorbance capacity; DPPH, 1,1-diphenyl-2-picrylhydrazyl.

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