



## Chemoprotective properties of rooibos (*Aspalathus linearis*), honeybush (*Cyclopia intermedia*) herbal and green and black (*Camellia sinensis*) teas against cancer promotion induced by fumonisin B<sub>1</sub> in rat liver

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### ABSTRACT

The chemoprotective properties of unfermented and fermented rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*) herbal teas, and green and black teas (*Camellia sinensis*) were investigated against fumonisin B<sub>1</sub> (FB<sub>1</sub>) promotion in rat liver utilizing diethylnitrosamine (DEN) as cancer initiator. The various teas differently affected the clinical chemical parameters associated with liver and kidney damage associated with FB<sub>1</sub> suggesting specific FB<sub>1</sub>/iron/polyphenolic interactions. Green tea enhanced ( $P < 0.05$ ) the FB<sub>1</sub>-induced reduction of the oxygen radical absorbance capacity, while fermented herbal teas and unfermented honeybush significantly ( $P < 0.05$ ) decreased FB<sub>1</sub>-induced lipid peroxidation in the liver. The teas exhibited varying effects on FB<sub>1</sub>-induced changes in the activities of catalase, glutathione peroxidase (GPx) glutathione reductase (GR) as well as the glutathione (GSH) status. Unfermented rooibos and honeybush significantly ( $P < 0.05$ ) to marginally ( $P < 0.1$ ) reduced the total number of foci ( $>10 \mu\text{m}$ ), respectively, while all the teas reduced the relative amount of the larger foci. Fermentation seems to reduce the protective effect of the herbal teas. Differences in the major polyphenolic components and certain FB<sub>1</sub>/polyphenolic/tissue interactions may explain the varying effects of the different teas on the oxidative parameters, hepatotoxic effects and cancer promotion in rat liver.

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### 1. Introduction

The popularity of the two South African indigenous herbal teas, rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*), is increasing as health beverages worldwide. This is mainly due to the absence of caffeine (Morton, 1983), as well as antioxidant (Von Gadow et al., 1997; Yoshikawa et al., 1990; Hubbe and Joubert, 2000a,b; Joubert et al., 2004, 2008), anticlastogenic (Sasaki et al., 1993; Shimoi et al., 1994) and antimutagenic properties (Marnewick et al., 2000; Van der Merwe et al., 2006; Snijman et al., 2007). Although the antioxidant and antimutagenic proper-

ties of rooibos and honeybush herbal teas have been investigated, little information is available about their potential cancer modulating properties *in vivo*.

A recent study indicated that the consumption of rooibos and honeybush herbal teas enhanced the activity of phase II detoxifying enzymes, as well as altering the oxidative status in the liver of rats (Marnewick et al., 2003). Cytosolic liver fractions of the herbal tea treated rats protected against the mutagenesis of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and 2-acetylaminofluorene (2-AAF). The microsomal bioactivation of AFB<sub>1</sub> was reduced, indicating the potential of the herbal tea components to modulate their metabolic fate *ex vivo* (Marnewick et al., 2004). These findings suggest that aqueous extracts of rooibos and honeybush are likely to alter the carcinogenic potency of hepatocarcinogens *in vivo*. A study in mouse skin showed that extracts of the herbal teas disrupt cancer promotion subsequently reduces the development of papillomas (Marnewick et al., 2005).

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Based on studies with green and black teas (*Camellia sinensis*), polyphenolic components have been considered as potential chemopreventive agents (Fujiki et al., 2002; Yang et al., 2000a). Tea polyphenols are known to modulate the metabolic fate of carcinogens in several ways to render them less active, thus protecting the target tissue against their adverse effects (Yang et al., 2000b). Several studies in animals showed that black and green teas modulate cancer development *in vivo* (Mukhtar et al., 1994; Steele et al., 1999). The consumption of tea polyphenols and tea pigments, comprising of the oxidized flavanol products, theaflavins and thearubigins, significantly reduced the number as well as the average area of glutathione-S-transferase placental form positive (GSTP<sup>+</sup>) foci in the liver (Gong et al., 2000; Jia et al., 2002). An aqueous extract of green tea inhibited both cancer initiation and promotion of AFB<sub>1</sub>- and carbon tetrachloride-induced hepatocarcinogenesis in male Fischer rats (Qin et al., 2000).

Whereas the major polyphenolic constituents of green and black tea are flavanols and oxidation products of flavanols, the main monomeric phenolic constituents of rooibos are the flavonoid aspalathin, a dihydrochalcone, and its flavone analogues (Joubert, 1996; Bramati et al., 2002). The major monomeric phenolic constituents of honeybush are the xanthone, mangiferin and the flavanone, hesperidin (Ferreira et al., 1998; Joubert et al., 2003; Kamara et al., 2003). Differences in the polyphenolic constituents could explain variations in the biological properties, e.g. when comparing the antioxidant and antimutagenic properties of the different teas (Van der Merwe et al., 2006; Joubert et al., 2008).

The fumonisin B (FB) mycotoxins, which are natural contaminants produced by *Fusarium verticillioides* on maize, are potent liver cancer promoters (Gelderblom et al., 1988) and were recently shown to synergistically interact with AFB<sub>1</sub> in the induction of liver nodules in a short-term carcinogenesis model (Gelderblom et al., 2002). As these mycotoxins co-occur naturally, the modulation of their carcinogenic characteristics is of importance (Zhang et al., 1997; Ali et al., 1998). Numerous studies reported on the modulation of the carcinogenicity of AFB<sub>1</sub> by natural occurring dietary constituents (Qin et al., 1997; Maxuitenko et al., 1998) whereas the modulation of the carcinogenic properties of the fumonisins has not been investigated. Carcinogenesis by FB<sub>1</sub>, the main fumonisin produced, is associated with a chronic hepatotoxic effect and the induction of oxidative damage (Abel and Gelderblom, 1998; Sahu et al., 1998; Lemmer et al., 1999). The role of reactive oxygen species in carcinogenesis is well established as it regulates critical events regarding cell proliferation and apoptosis (Klaunig and Kamendulis, 2004). The modulating role of antioxidants in the ROS-induced effects on the different cell survival parameters is therefore of interest with respect to fumonisin-induced hepatocarcinogenesis. The present study investigated the modulating properties of aqueous extracts of fermented and unfermented South African herbal teas, as well as green and black teas, against different oxidative parameters and cancer promoting activity induced by FB<sub>1</sub> in rat liver.

## 2. Materials and methods

### 2.1. Chemicals and diets

Diethylnitrosamine (DEN) was purchased from Sigma (Sigma-Aldrich, Cape Town, South Africa) and prepared in dimethylsulfoxide (DMSO). FB<sub>1</sub> was purified according to the method of Cawood et al. (1991) at the PROMEC Unit, Medical Research Council, Tygerberg, South Africa to a purity of 93–95%. The FB<sub>1</sub>-containing diet for the rats was prepared by evaporating FB<sub>1</sub>, dissolved in methanol (Gelderblom et al., 1994), on a sub sample (200 g) of Epol (Epol Ltd., Johannesburg, South Africa) rat mash diet and mixing it with the control diet to obtain the desired dietary level of 250 mg/kg diet. The diet was stored under nitrogen at 4 °C. Glutathione-S-transferase placental form (GSTP) antibody was obtained from Novacastra, Newcastle, UK. Reduced (GSH) and oxidized (GSSG) glutathione

were purchased from Roche (Cape Town, South Africa). Glutathione reductase, perchloric acid (PCA), trichloro acetic acid (TCA), 1-methyl-2-vinyl-pyridinium trifluoromethane sulfonate (M2VP), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), NADPH, EDTA, gallic acid, (+)-catechin, quercetin, mangiferin and rutin were purchased from Sigma (Sigma-Aldrich). Phycoerythrin (B-PE) was purchased from ProZyme and 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), Trolox, hesperidin and hesperetin were obtained from Aldrich Chemical Company (Cape Town, South Africa). Aspalathin and nothofagin, with >95% purity were isolated at the PROMEC Unit, while isovitexin, vitexin, isoquercitrin, orientin, iso-orientin were purchased from Extrasynthese (Genay, France). All other chemicals used were of analytical grade.

### 2.1.1. Plant material and tea preparation

Black tea (*C. sinensis* var *assamica*), a blend of locally produced African and Sri Lankan teas, was bought from a retail outlet in Cape Town, while green tea (*C. sinensis* var *sinensis*), imported from China, was a gift from Vital Health Foods (Kuilsriver, South Africa). Fermented ("oxidized") and unfermented ("green" or "unoxidized") rooibos (*A. linearis*) and honeybush (*C. intermedia*) herbal teas were obtained from Rooibos Ltd. (Clanwilliam, South Africa) and the Agriculture Research Council, Infruitec-Nietvoorbij (Stellenbosch, South Africa), respectively. Green and black tea and rooibos herbal tea were prepared at concentrations customarily used for tea making purposes, while for honeybush a higher ratio of plant material to water was used, to compensate for its relatively high stem content that lowers its extractable polyphenol content. Aqueous extracts of honeybush is known to exhibit lower antioxidant and antimutagenic properties than the other teas (Van der Merwe et al., 2006; Joubert et al., 2008). Freshly boiled tap water was added to the plant material at concentrations of 2 g/100 ml for fermented and unfermented rooibos herbal, green and black teas and at 4 g/100 ml for fermented and unfermented honeybush herbal tea. The aqueous extracts were allowed to cool to room temperature and fed to rats *ad libitum*. Fresh tea was prepared every second day.

### 2.2. HPLC quantification of the major flavonoids in herbal tea aqueous extracts

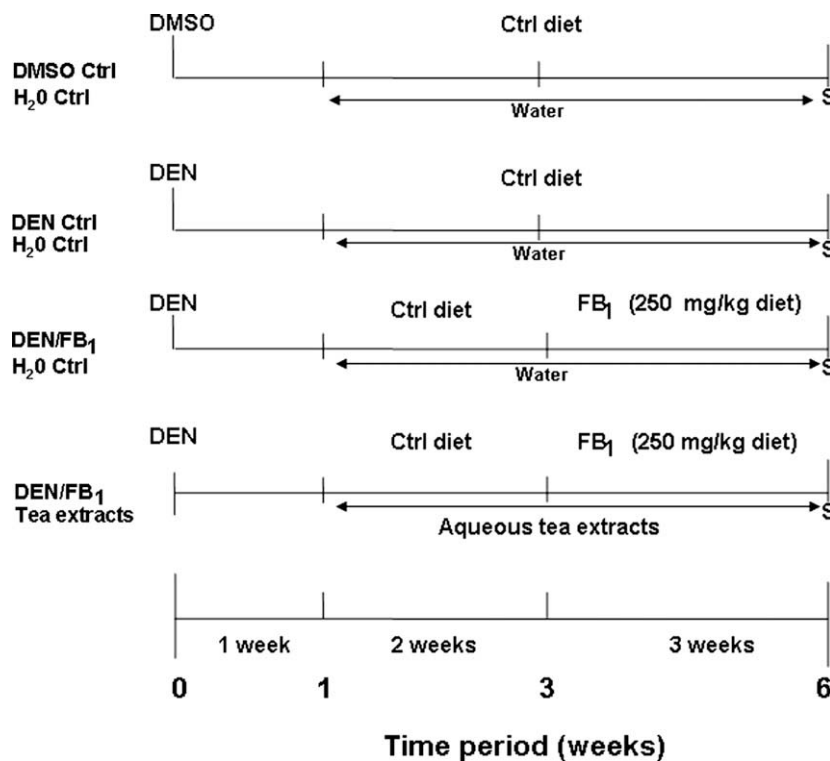
Freeze-dried samples of rooibos and honeybush aqueous extracts were reconstituted in purified water (Modulab Water Purification System from Continental Water System Corporation), filtered (Magna Nylon, 0.45 µm) and separated on a LiChrospher 100 RP-18 (5 µm, 250 × 4.6 mm) column (Joubert, 1996) and a Phenomenex Synergy Max-RP C12 column with TMS end capping (4 µm, 150 × 4.6 mm) (Joubert et al., 2003), respectively. A C<sub>18</sub> guard column was used in both cases and the HPLC system consisted of a Merck/Hitachi LaChrom system comprising of both a L-7400 UV and a L-7450 DAD detector, L-7100 pump, L-7200 autosampler, D-7000 HPLC system manager and interface module. The UV detector output was used for quantification, while the DAD detector was used to confirm peak identity, based on the spectra of the standards. Column temperature was maintained at 30 °C. The polyphenols were detected at 280 nm and quantified using calibration curves constructed for each flavonoid. Rutin co-eluted with isoquercitrin and the peak area was quantified in terms of quercetin equivalents.

### 2.2.1. Treatment of animals

Ninety male Fischer rats (150–170 g), obtained from the Primate Unit, MRC (Tygerberg, South Africa), had free access to Epol rat mash. They were housed in wired top and bottom cages, fitted with Perspex<sup>™</sup> houses and kept in a controlled environment of 23–24 °C, 50% humidity and a 12 h light/dark cycle. Rats were randomly divided into nine groups of ten rats each and caged individually. Initiation was effected by a single dose of diethylnitrosamine (DEN; 200 mg/kg body weight, i.p.). Tea or herbal teas were offered as the sole source of drinking fluid and commenced one week after initiation until the end of the experiment, while promotion commenced three weeks after initiation by giving the FB<sub>1</sub>-diet (250 mg/kg) for 21 days (Fig. 1). The DEN-initiated positive control group received the FB<sub>1</sub> promotion treatment (FB<sub>1</sub>) and tap water. The negative control groups either received DEN initiation or DMSO (carrier solvent) with the mash diet and tap water. All the rats were averaged fed during the FB<sub>1</sub> promotion treatment period, i.e., all the FB<sub>1</sub> treated rats received an equal amount of feed than the rats of the DEN-FB<sub>1</sub> positive control group. Their body weights were monitored on a weekly basis. The FB<sub>1</sub> and tea intake profiles over the 3 week period were calculated as a function of the body weight and expressed as mg FB<sub>1</sub> or ml tea per 100 g body weight. At termination, fasted (16 h) animals were euthanized by i.p. injection of sodium pentobarbital (0.15 mL/100 g bw) and blood collected from the abdominal aorta. Livers were excised, weighed and sections processed in buffered formalin for histological examination. The remaining liver tissue were immediately frozen in liquid nitrogen and stored at –80 °C for biochemical analyses.

### 2.2.2. Clinical chemistry

The clinical biochemical parameters including serum creatinine, total cholesterol, total iron, aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) were measured on a Technicon RA 1000 automated analyzer.



**Fig. 1.** Schematic diagram illustrating the experimental regimens utilized to investigate the modulating properties of the different teas on cancer promotion by FB<sub>1</sub> in rat liver. DEN = diethylnitrosamine (200 mg/kg body weight; i.p., single dose); FB<sub>1</sub> (250 mg/kg) = rat mash containing FB<sub>1</sub> (250 mg/kg diet for 3 weeks) as promotion regimen; s = sacrifice animals; ctrl = control.

### 2.2.3. Oxidative parameters

**2.2.3.1. Lipid peroxidation.** The thiobarbituric acid reacting substances (TBARS) were determined according to a modified method described by Esterbauer and Cheeseman (1990). Sub samples of the excised livers were homogenized on ice in 19 volumes of 0.01 M phosphate buffer (pH 7.4) and incubated with 15  $\mu$ M FeSO<sub>4</sub> for one hour at 37 °C. The incubated homogenate (1 mL) was mixed with 2 mL of cold TCA reagent (10% TCA, 0.01% BHT). The samples were centrifuged (3000 rpm) and 2 mL of the resultant supernatant was combined with 2 mL of 0.67% TBA solution and incubated at 90 °C for 20 min. The mixture was allowed to cool to room temperature and the absorbance was measured at 532 nm. Lipid peroxidation was expressed as nmole malondialdehyde (MDA) per mg protein using a mmolar extinction coefficient of 156 (Esterbauer and Cheeseman (1990)). Non specific lipid peroxidation was prevented by the incorporation of EDTA in the phosphate buffer and BHT in the reaction solution for the TBARS assay.

**2.2.3.2. Oxygen radical absorbance capacity (ORAC).** Sub samples of the stored livers were homogenized in 4 volumes of sodium phosphate buffer (75 mM, pH 7.0) in a Thomas homogenizer (10 strokes) and centrifuged at 12,000g for 10 min at 4 °C. The supernatant was deproteinised using 0.25 M perchloric acid (PCA), centrifuged at 16,000g for 15 min and the resulting supernatants stored at –80 °C prior to analysis. The ORAC assay was conducted according to the method of Cao and Prior, 1998 on a 96 well microtiter plate using a BioTEK Fluorescence plate reader (FL-600). The reaction consisted of 10  $\mu$ l of diluted sample (1:1) and fluorescein (56 nM), which was used as a target for free radical attack. The reaction was initiated by the addition of 20  $\mu$ l AAPH (240 mM) and the fluorescence (emission 590 nm, excitation 530 nm) recorded every 5 min until the reading had declined to less than 5% of the initial reading. The ORAC values were calculated and expressed as  $\mu$ M Trolox equivalents/g wet liver weight.

**2.2.3.3. Glutathione analysis.** The total glutathione (GSH and GSSG) was measured according to a modified method of Tietze (1969). Liver samples were homogenized (1:10) in 15% (w/v) TCA containing 1 mM EDTA for GSH determination and in 6% (v/v) PCA containing freshly prepared 3 mM M2VP and 1 mM EDTA for GSSG determination on ice. After centrifugation at 10,000g for 10 min, 50  $\mu$ l of supernatant was added to glutathione reductase (1U) and 75  $\mu$ M DTNB. The reaction was initiated by addition of 0.25 mM NADPH to a final volume of 200  $\mu$ l. The change in absorbance was monitored at 410 nm for 5 min and levels calculated using pure GSH and GSSG as standards.

**2.2.3.4. Activity of antioxidant enzymes.** Liver homogenates (10% m/v) were prepared in a phosphate buffer, centrifuged for 10 min at 15,000g at 4 °C and the supernatant preserved for enzyme analyses. Catalase (CAT) activity was determined spectrophotometrically at 240 nm by monitoring the decomposition of H<sub>2</sub>O<sub>2</sub> and expressed as  $\mu$ moles H<sub>2</sub>O<sub>2</sub>/min/ $\mu$ g protein while superoxide dismutase (SOD) activity was determined by the method of Ellerby and Bredesden (2000), modified for a microplate reader and expressed as the amount of protein (ng) required to produce a 50% inhibition of auto-oxidation of 6-hydroxydopamine. Glutathione peroxidase (GPx) activity was measured spectrophotometrically (340 nm) by the method of Flohe and Gunzler (1984), expressing activity as nmoles NADPH/min/ $\mu$ g protein using the mmolar extinction coefficient of 6.22. Glutathione reductase (GR) activity was measured spectrophotometrically (340 nm) by the method of Ellerby and Bredesden (2000), expressing activity as nmoles of NADPH utilised per min per  $\mu$ g protein using the mmolar extinction coefficient of 6.22.

### 2.2.4. GST-Pi immunohistochemical assay

Histochemical staining for the placental form of glutathione-S-transferase (GSTP) was conducted on dewaxed liver sections using a three-stage indirect streptavidin–biotin technique to identify GSTP<sup>+</sup> stained hepatocytes (Ogawa et al., 1980). The enzyme altered foci were quantified microscopically (10 $\times$  magnification), according to their number and size (internal diameter) and categorized according to the following sizes, 5–10 (mini foci), 11–20, 21–30, >30  $\mu$ m. Foci were expressed as number of foci/cm<sup>2</sup> of the liver section, the area of which was determined by image analyses. The relative amount of each focal size category was expressed as a % of the total GSTP<sup>+</sup> foci ( $\geq$  5  $\mu$ m). The total GSTP<sup>+</sup> foci ( $\geq$  10  $\mu$ m) excluding the mini foci were also determined.

### 2.2.5. Statistical analysis

Data were tested for normality using the Kolmogorov–Smirnov Test and Levene's Test for Equality of variances. One-way ANOVA's (two-tailed) was used to test for significant group differences followed by the Tukey–Cramér multiple comparison tests in order to establish which groups differed significantly. The Kruskal–Wallis Test, a non-parametric analogue to the one-way ANOVA, was used to test for group differences when the data was not normally distributed. Statistical significance was at the 5% level ( $P < 0.05$ ). Statistical comparisons were conducted between (1) the DEN–FB<sub>1</sub> treated rats with the DEN and DMSO control as well as the tea treated groups and (2) among the different tea treated groups in so-called inter tea comparisons.

### 3. Results

#### 3.1. Intake of selected herbal tea flavonoids

The dihydrochalcone, aspalathin, was the major flavonoid consumed by the rats that received unfermented rooibos. Substantially lower quantities of its structural flavone analogues, orientin and iso-orientin, the flavonol glycosides, rutin/isoquercitrin and the dihydrochalcone, nothofagin and its flavone analogues, vitexin and isovitexin, were consumed (Table 1). In the aqueous extract of fermented rooibos, aspalathin and nothofagin were present at much lower concentrations, resulting in an increased intake of iso-orientin, orientin, vitexin and isovitexin relatively to aspalathin and nothofagin. The xanthone mangiferin and the flavanone hesperidin were the major monomeric polyphenols in both the unfermented and fermented honeybush. The intake of mangiferin and hesperidin was reduced substantially when consuming fermented honeybush. Only trace amounts of hesperetin, the aglycone of hesperidin were detected.

#### 3.2. FB<sub>1</sub> and tea intake profiles

The average tea intake and feed intake were significantly ( $P < 0.05$ ) reduced during the second week due to FB<sub>1</sub>-induced toxicity. After the third week the tea intake profiles returned back to the levels monitored during the first three weeks (data not shown). During the second week, the black tea intake was significantly decreased (6.2 ml/100 g bw) when compared to the other teas which ranged between 8.6 and 10.2 ml/100 g bw. As a result the averaged black tea consumption was significantly ( $P < 0.05$ ) lowered during the FB<sub>1</sub> treatment (Table 2). The average intake of fermented honeybush was significantly higher when compared to the other teas. There was no significant difference between the FB<sub>1</sub> intake profiles between the different groups.

#### 3.3. Effect of FB<sub>1</sub> and tea treatments on body weight parameters

The total body weight gain (BWG) during the experimental period was significantly ( $P < 0.05$ ) reduced in the FB<sub>1</sub> treated rats compared to the DMSO and DEN control groups consuming water, while the different tea treatments showed no additional effect (Table 2). However, inter tea comparisons indicated that unfermented rooibos significantly ( $P < 0.05$ ) lowered the total BWG compared to fermented rooibos and green tea. Compared to the DMSO control treated rats, DEN and the DEN-FB<sub>1</sub> treatments significantly ( $P < 0.05$ ) increased and decreased the relative liver weight, respectively. Fermented honeybush counteracted ( $P < 0.05$ ) the FB<sub>1</sub>-induced reduction, while unfermented rooibos marginally ( $P < 0.1$ ) further enhanced the reduction in the relative liver weight. Inter tea comparisons showed that fermented and unfermented rooibos significantly ( $P < 0.05$ ) reduced the relative liver weights compared to the other teas.

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#### 3.4. Clinical chemical parameters

Serum levels of AST, ALT, ALP, creatinine and cholesterol were significantly ( $P < 0.05$ ) increased by FB<sub>1</sub> as compared to the DMSO control group (Table 3). The DEN treatment significantly increased the ALT and ALP serum parameters as compared to the DMSO control. Unfermented rooibos significantly ( $P < 0.05$ ) enhanced the FB<sub>1</sub>-induced increase of serum AST and ALT levels while unfermented honeybush significantly ( $P < 0.05$ ) increased the ALT level. No significant reduction of the FB<sub>1</sub>-induced increase in serum ALP levels was observed for any of the teas. Inter tea comparisons showed that both rooibos herbal teas significantly ( $P < 0.05$ ) increased AST compared to black and green teas. Unfermented rooibos significantly ( $P < 0.05$ ) increased ALT when compared to fermented honeybush, black and green teas. Fermented honeybush significantly ( $P < 0.05$ ) increased the ALP level compared to black tea.

Black tea and fermented honeybush significantly ( $P < 0.05$ ) and unfermented honeybush marginally ( $P < 0.1$ ) counteracted the FB<sub>1</sub>-induced increase in serum creatinine levels. A similar trend was noticed with the inter tea group comparison. With respect to FB<sub>1</sub>-induced increase of serum cholesterol both fermented and unfermented honeybush as well as green tea significantly ( $P < 0.05$ ) decreased the level. A marginal ( $P < 0.1$ ) reduction was also noticed with black tea. A similar trend was noticed for the inter tea comparison. The total iron levels were not significantly altered by the FB<sub>1</sub> treatment while the teas appeared not to have any additional effect. However, inter tea comparisons showed that fermented and unfermented honeybush and green tea significantly ( $P < 0.05$ ) increased the total iron levels compared to the rooibos herbal and black teas.

**Table 1**

Quantification of the major flavonoids in aqueous extracts of rooibos and honeybush teas consumed by rats during FB<sub>1</sub> promotion.

Soluble solids (mg/ml) <sup>a</sup>	Fermented		Unfermented	
	2.59 ± 0.44		5.36 ± 0.81	
Phenolic compounds	(%) of soluble solids	Daily intake (mg/100 g BW) <sup>**</sup>	(%) of soluble solids (%)	Daily intake (mg/100 g BW)
<i>Rooibos tea</i>				
Aspalathin	0.53 ± 0.02	0.15 ± 0.01	8.4 ± 1.38	4.96 ± 0.41
Nothofagin	0.05 ± 0.02	0.02 ± 0.01	0.36 ± 0.13	0.21 ± 0.02
Orientin	0.46 ± 0.01	0.13 ± 0.01	0.65 ± 0.18	0.38 ± 0.03
Iso-orientin	0.66 ± 0.04	0.19 ± 0.01	0.80 ± 0.18	0.47 ± 0.04
Vitexin	0.19 ± 0.01	0.05 ± 0.01	0.13 ± 0.02	0.08 ± 0.01
Isovitexin	0.18 ± 0.03	0.05 ± 0.01	0.29 ± 0.07	0.14 ± 0.01
Rutin/iso-quercitrin	0.45 ± 0.03	0.13 ± 0.01	0.60 ± 0.10	0.35 ± 0.03
	5.96 ± 0.78 <sup>b</sup>		11.78 ± 1.18 <sup>c</sup>	
	(%) of soluble solids	Daily intake (mg/100 g BW) <sup>**</sup>	(%) of soluble solids	Daily intake (mg/100 g BW)
<i>Honeybush tea</i>				
Mangiferin	1.11 ± 0.15	0.82 ± 0.06	6.68 ± 0.84	8.90 ± 0.79
Hesperidin	0.37 ± 0.08	0.27 ± 0.02	2.63 ± 0.16	3.49 ± 0.31
Hesperetin	Trace		Trace	

All samples used were aqueous extracts prepared as described in the methods. The values in columns represent the mean ± standard deviation of 2–3 repeats of each sample.

<sup>a</sup> Data from Marnewick et al., 2003. BW = body weight.

<sup>\*\*</sup> Mean daily tea intake during the 3 week FB<sub>1</sub> period (Table 2) was used.



**Table 2**  
Effect of DEN-FB<sub>1</sub> and various tea treatments on the rat body weight parameters.

Groups	Ave tea intake (ml/100 g BW/day)	Ave FB <sub>1</sub> intake (mg/100 g BW/day)	Body weight gain (g) <sup>B</sup>	Relative liver weight (%) <sup>A</sup>
DMSO	–	–	86.9 ± 8.2 <sup>a</sup>	2.9 ± 0.35 <sup>a</sup>
DEN	–	–	77.7 ± 25.1 <sup>a</sup>	3.51 ± 0.27 <sup>c</sup>
DEN-FB <sub>1</sub>	–	1.5 ± 0.3 <sup>a</sup>	39.8 ± 13.8 <sup>b</sup>	2.50 ± 0.16 <sup>b</sup>
DEN-FB <sub>1</sub> -Rf	11.0 ± 0.9 <sup>a</sup>	1.6 ± 0.3 <sup>a</sup>	44.0 ± 6.8 <sup>bc</sup>	2.36 ± 0.12 <sup>(b)d</sup>
DEN-FB <sub>1</sub> -Ru	10.8 ± 0.7 <sup>a</sup>	1.5 ± 0.4 <sup>a</sup>	29.6 ± 9.6 <sup>bd</sup>	2.44 ± 0.09 <sup>bd</sup>
DEN-FB <sub>1</sub> -Hf	12.4 ± 0.8 <sup>b</sup>	1.3 ± 0.3 <sup>a</sup>	37.9 ± 12.7 <sup>b</sup>	2.79 ± 0.05 <sup>ae</sup>
DEN-FB <sub>1</sub> -Hu	11.3 ± 1.0 <sup>a</sup>	1.5 ± 0.3 <sup>a</sup>	36.1 ± 16.4 <sup>b</sup>	2.58 ± 0.19 <sup>be</sup>
DEN-FB <sub>1</sub> -Bl	9.0 ± 0.9 <sup>c</sup>	1.5 ± 0.3 <sup>a</sup>	40.2 ± 19.8 <sup>b</sup>	2.58 ± 0.13 <sup>be</sup>

Values in columns represent the mean ± STD. Abbreviations: Rp = fermented rooibos, Rg = unfermented/“green” rooibos, Hp = fermented honeybush and Hg = unfermented/“green” honeybush tea extracts, Bl = black and Gr = green tea. Values followed by the same letters do not differ significantly. When letters differ then  $P < 0.05$ . Letters in parenthesis then  $P < 0.1$ . Inter tea comparison are indicated in bold.

<sup>A</sup> Relative liver weights equal liver weight/body weight × 100.

<sup>B</sup> Including DEN, Pre-FB<sub>1</sub> tea treatment and FB<sub>1</sub> treatment regimens.  $N = 7$ –10 rats per group.

**Table 3**  
Effect of DEN-FB<sub>1</sub> and various tea treatments on the serum clinical chemistry.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Creatinine (umol/L)	Cholesterol (mmol/L)	Total iron (μmol/L)
DMSO	88.1 ± 16.8 <sup>a</sup>	53.7 ± 7.3 <sup>a</sup>	168.9 ± 32.8 <sup>a</sup>	69.0 ± 6.0 <sup>a</sup>	1.6 ± 0.2 <sup>a</sup>	23.1 ± 5.8 <sup>a</sup>
DEN	102.8 ± 15.4 <sup>a</sup>	96.7 ± 11.5 <sup>c</sup>	272.4 ± 69.1 <sup>c</sup>	67.3 ± 3.7 <sup>a</sup>	1.4 ± 0.3 <sup>a</sup>	25.9 ± 2.6 <sup>a</sup>
DEN-FB <sub>1</sub>	258.7 ± 60.2 <sup>b</sup>	211.6 ± 48.0 <sup>b</sup>	282.4 ± 82.0 <sup>b</sup>	85.8 ± 4.7 <sup>b</sup>	3.5 ± 0.4 <sup>b</sup>	24.4 ± 7.4 <sup>a</sup>
DEN-FB <sub>1</sub> -Rf	296.8 ± 52.3 <sup>bd</sup>	256.62 ± 68.9 <sup>b</sup>	283.4 ± 61.3 <sup>b</sup>	85.3 ± 4.4 <sup>bd</sup>	3.3 ± 0.4 <sup>bc</sup>	21.9 ± 2.3 <sup>ab</sup>
DEN-FB <sub>1</sub> -Ru	341.1 ± 50.0 <sup>cd</sup>	321.3 ± 75.4 <sup>de</sup>	325.3 ± 86.8 <sup>b</sup>	83.5 ± 5.8 <sup>bd</sup>	2.9 ± 1.0 <sup>bc</sup>	22.6 ± 4.4 <sup>ab</sup>
DEN-FB <sub>1</sub> -Hf	242.9 ± 54.3 <sup>b</sup>	236.3 ± 63.9 <sup>bf</sup>	355.3 ± 77.8 <sup>bd</sup>	77.2 ± 3.8 <sup>ce</sup>	2.3 ± 0.8 <sup>ad</sup>	27.5 ± 2.2 <sup>ac</sup>
DEN-FB <sub>1</sub> -Hu	283.2 ± 52.1 <sup>b</sup>	280.9 ± 71.7 <sup>d</sup>	314.3 ± 57.9 <sup>b</sup>	80.8 ± 5.8 <sup>(b)d</sup>	2.1 ± 0.3 <sup>ad</sup>	28.3 ± 4.9 <sup>ac</sup>
DEN-FB <sub>1</sub> -Bl	207.1 ± 56.3 <sup>be</sup>	216.0 ± 90.5 <sup>bf</sup>	245.0 ± 58.4 <sup>be</sup>	78.4 ± 2.2 <sup>(c)d</sup>	3.0 ± 0.6 <sup>(b)c</sup>	24.1 ± 6.3 <sup>ab</sup>
DEN-FB <sub>1</sub> -Gr	226.4 ± 22.5 <sup>be</sup>	229.3 ± 41.4 <sup>bf</sup>	296.8 ± 62.1 <sup>bd</sup>	83.6 ± 3.0 <sup>bd</sup>	2.4 ± 0.5 <sup>ad</sup>	27.1 ± 4.4 <sup>ac</sup>

Values in columns represent the mean ± STD. Abbreviations: Rp = fermented rooibos, Rg = unfermented/“green” rooibos, Hp = fermented honeybush and Hg = unfermented/“green” honeybush tea extracts, Bl = black and Gr = green tea. Values followed by the same letters do not differ significantly. When letters differ then  $P < 0.05$ . Letters in parenthesis then  $P < 0.1$ . Inter tea comparison are indicated in bold.  $n = 7$ –10 rats per group.

### 3.5. Effect on hepatic oxidative status (Table 4)

#### 3.5.1. Oxygen radical absorbance capacity (ORAC)

The DEN treatment significantly ( $P < 0.05$ ) lowered the hepatic ORAC status compared to the DMSO control group. Of the teas, only green tea further decreased ( $P < 0.05$ ) the hepatic ORAC values while unfermented rooibos marginally ( $P < 0.1$ ) increased the ORAC status when compared to the DEN treatment group.

#### 3.5.2. Hepatic glutathione

Rats receiving the FB<sub>1</sub>-dietary treatment showed significantly ( $P < 0.05$ ) elevated GSH levels in the liver when compared to the DMSO and DEN treated control groups. Consumption of the rooibos herbal teas resulted in a significant ( $P < 0.05$ ) reduction in hepatic GSH concentration when compared to the DEN-FB<sub>1</sub> treatment group. A similar effect on the GSH concentration was noticed for the inter tea comparison. The GSSG levels were not affected by FB<sub>1</sub> resulting in a significantly ( $P < 0.05$ ) increased GSH:GSSG ratio in the liver when compared to the DMSO and DEN treated control groups. The GSSG level was significantly ( $P < 0.05$ ) elevated with the consumption of green, black, unfermented rooibos and honeybush herbal teas, resulting in a significantly ( $P < 0.05$ ) decreased GSH:GSSG ratio, while a marginal ( $P < 0.1$ ) decrease was noticed with fermented rooibos.

#### 3.5.3. Antioxidant enzyme activity

FB<sub>1</sub> treatment significantly ( $P < 0.05$ ) increased the activity of GPx (Table 3) while CAT activity was significantly ( $P < 0.05$ ) reduced. No effect was observed on the activity of SOD. Treatment with green, black and herbal teas did not alter the FB<sub>1</sub>-induced ef-

fect on GPx. Treatment with both honeybush herbal, green and black teas resulted in a significant ( $P < 0.05$ ) recovery in the FB<sub>1</sub>-induced decrease in the activity of CAT. FB<sub>1</sub> significantly increased the GR activity while none of the teas showed any modulating effect.

### 3.6. Lipid peroxidation

The TBARS levels were significantly ( $P < 0.05$ ) increased in the liver of the FB<sub>1</sub> treated rats (Table 4) when compared to the DMSO and DEN treated groups. The rats consuming fermented rooibos, and fermented and unfermented honeybush showed a significant ( $P < 0.05$ ) decrease in the TBARS levels. Consumption of unfermented rooibos, black and green teas showed no effect on the FB<sub>1</sub>-induced lipid peroxidation. Among the different teas, fermented rooibos and honeybush significantly ( $P < 0.05$ ) decreased lipid peroxidation compared to their unfermented counterparts, and unfermented honeybush significantly ( $P < 0.05$ ) reduced lipid peroxidation compared to black tea.

### 3.7. Effect of various tea treatments on FB<sub>1</sub>-induced promotion

FB<sub>1</sub> significantly ( $P < 0.05$ ) promoted the growth of GSTP<sup>+</sup> foci by increasing the number and relative number [(expressed as a % of the total foci (>5 μm))] of GSTP<sup>+</sup> foci in all size categories as well as the total number (>10 μm) when compared to the DEN treated control rats (Table 5). No GSTP<sup>+</sup> foci were detected in the liver of the DMSO control rats. When considering the positive control treatment (DEN-FB<sub>1</sub>), all the tea preparations significantly ( $P < 0.05$ ) to marginally ( $P < 0.1$ ) increased the number of

**Table 4**

Effect of unfermented and fermented herbal, green and black teas on reduced glutathione (GSH), oxidized glutathione (GSSG), the ratio GSH:GSSG, oxidative enzyme parameters and lipid peroxidation in livers of rats.

Groups	ORAC (umole Trolox eq/g wet liver)	GSH (umole/g wet liver)	GSSG	GSH: GSSG Ratio	GR (nmoleNADPH/ min/μg protein)	GPx (nmoleNADPH/ min/ μg protein)	CAT (μmole H <sub>2</sub> O <sub>2</sub> / min/μg protein)	SOD*	TBARS (nmol MDA /mg protein)
DMSO	15.5 ± 2.4a	5.3 ± 1.3a	0.41 ± 0.06a	12.8 ± 1.5a	0.025 ± 0.001a	0.024 ± 0.001a	0.21 ± 0.04a	413.6 ± 30.9a	0.08 ± 0.01a
DEN	12.8 ± 2.2b	5.3 ± 1.1a	0.50 ± 0.12a	11.1 ± 2.9a	Nd	Nd	Nd	Nd	0.07 ± 0.01a
DEN-FB <sub>1</sub>	13.6 ± 0.7b	8.8 ± 1.5b	0.41 ± 0.05a	21.6 ± 3.1b	0.032 ± 0.002b	0.032 ± 0.003b	0.14 ± 0.02b	450.2 ± 89.5a	0.32 ± 0.08b
DENFB <sub>1</sub> -Rf	14.5 ± 1.5b	6.0 ± 1.8a	0.40 ± 0.11a	15.9 ± 4.7(b)	0.031 ± 0.001b	0.033 ± 0.002b	0.17 ± 0.03b	454.0 ± 65.6a	0.21 ± 0.07a
DEN-FB <sub>1</sub> -Ru	14.7 ± 1.3(b)	5.2 ± 1.3a	0.54 ± 0.08b	9.9 ± 2.8a	0.032 ± 0.003b	0.035 ± 0.003b	0.15 ± 0.03b	418.5 ± 53.2a	0.30 ± 0.04b
DEN-FB <sub>1</sub> -Hf	14.4 ± 1.1b	8.5 ± 0.7b	0.46 ± 0.14a	20.5 ± 8.5b	0.029 ± 0.002b	0.033 ± 0.003b	0.20 ± 0.02a	381.4 ± 59.9a	0.16 ± 0.04ac
DEN-FB <sub>1</sub> -Hu	13.2 ± 1.1b	7.0 ± 0.9b	0.57 ± 0.13b	12.7 ± 2.4a	0.030 ± 0.002b	0.034 ± 0.004b	0.22 ± 0.03a	425.3 ± 67.3a	0.26 ± 0.06ad
DEN-FB <sub>1</sub> -Bl	13.6 ± 1.1b	7.8 ± 2.8b	0.6 ± 0.12b	13.1 ± 4.6a	0.030 ± 0.003b	0.036 ± 0.004b	0.22 ± 0.03a	400.3 ± 69.4a	0.35 ± 0.07be
DEN-FB <sub>1</sub> -Gr	10.0 ± 0.88c	7.4 ± 2.3b	0.71 ± 0.17b	11.1 ± 4.9a	0.030 ± 0.003b	0.038 ± 0.006b	0.20 ± 0.04a	420.9 ± 39.5a	0.31 ± 0.09bd

Values in columns represent average of 5–8 values per group ± STD. Means followed by the same letter do not differ significantly, when letters differ then  $P < 0.05$ . Letters in parenthesis indicated  $P < 0.1$ . Abbreviations: Rf = fermented rooibos, Ru = unfermented/"green" rooibos, Hp = fermented honeybush and Hg = unfermented/"green" honeybush tea extracts, Bl = black and Gr = green tea. Amount of protein (ng) required to produce a 50% inhibition of auto-oxidation of 6-hydroxydopamine. GR = glutathione reductase; GPx = glutathione peroxidase; CAT = catalase; SOD = superoxide dismutase.

**Table 5**

Effect of various tea treatments on the induction of GSTP<sup>+</sup> foci by combined treatment of DEN and FB<sub>1</sub>.

Treatment groups	GSTP <sup>+</sup> Liver Foci									
	No foci/cm <sup>2</sup> (5–10 μm)	% of the total no of foci	No foci/cm <sup>2</sup> (11–20 μm)	% of the total no of foci	No foci/cm <sup>2</sup> (21–30 μm)	% of the total no of foci	No foci/cm <sup>2</sup> (>30 μm/cm <sup>2</sup> )	% of the total no of foci	Total no of foci (>10 μm)	
DMSO	Nd	nd	nd	nd	nd	nd	nd	nd	nd	
DEN	4.7 ± 1.7a	58.7 ± 13.4a	1.9 ± 0.8a	32.9 ± 7.6a	0.5 ± 0.8a	6.5 ± 8.7a	0.14 ± 0.34a	2.0 ± 4.0a	2.6 ± 1.8a	
DEN-FB <sub>1</sub>	8.0 ± 2.8b	23.7 ± 7.5b	18.2 ± 5.6b	50.2 ± 8.4b	6.5 ± 1.4b	19.6 ± 5.0b	2.3 ± 1.4b	6.5 ± 3.9b	27.0 ± 11.1b	
DEN-FB <sub>1</sub> -Rf	15.9 ± 8.7(b)	39.2 ± 14.3c	15.5 ± 5.7b	39.1 ± 4.3a	6.5 ± 2.9b	16.1 ± 3.6b	2.3 ± 1.1b	6.0 ± 3.2b	24.2 ± 8.8b	
DEN-FB <sub>1</sub> -Ru	12.6 ± 4.9c	53.9 ± 17.1a	10.4 ± 5.3c	36.0 ± 9.0a	3.1 ± 2.4c	10.3 ± 6.6a	0.7 ± 1.1a	1.9 ± 2.8a	14.2 ± 7.8c	
DEN-FB <sub>1</sub> -Hf	17.9 ± 5.4c	42.3 ± 7.9a	15.1 ± 6.3b	33.9 ± 4.6a	7.6 ± 4.8b	16.4 ± 7.0b	3.5 ± 2.7b	7.5 ± 4.1b	26.3 ± 12.3b	
DEN-FB <sub>1</sub> -Hu	19.7 ± 8.5c	52.7 ± 14.7a	11.9 ± 5.9(b)	31.8 ± 6.2a	4.0 ± 2.7c	10.6 ± 5.1a	0.9 ± 1.7(b)	2.3 ± 4.1(b)	17.1 ± 14.2(b)	
DEN-FB <sub>1</sub> -Bl	30.0 ± 3.5c	54.0 ± 12.3a	17.3 ± 9.9b	27.9 ± 15.8a	7.7 ± 3.0b	13.8 ± 6.1(b)	2.5 ± 1.4b	4.3 ± 2.1b	27.5 ± 10.9b	
DEN-FB <sub>1</sub> -Gr	23.9 ± 11.3c	37.8 ± 13.3a	18.2 ± 6.2bd	33.3 ± 4.6a	10.4 ± 6.6b	17.5 ± 8.8b	5.9 ± 5.2b	9.7 ± 7.2b	33.7 ± 4.7b	

Values represent the average of ten rats per group ± STD. Means (column) followed by the same letter do not differ significantly. When letters differ then  $P < 0.05$ . Letters in parenthesis indicated  $P < 0.1$ . Abbreviations: Rf = fermented rooibos, Ru = unfermented rooibos, Hf = fermented honeybush and Hu = unfermented honeybush tea extracts, Bl = black and Gr = green tea, nd = not detected. Shaded areas; foci number expressed as a % of the total GSTP<sup>+</sup> foci (5 μm).

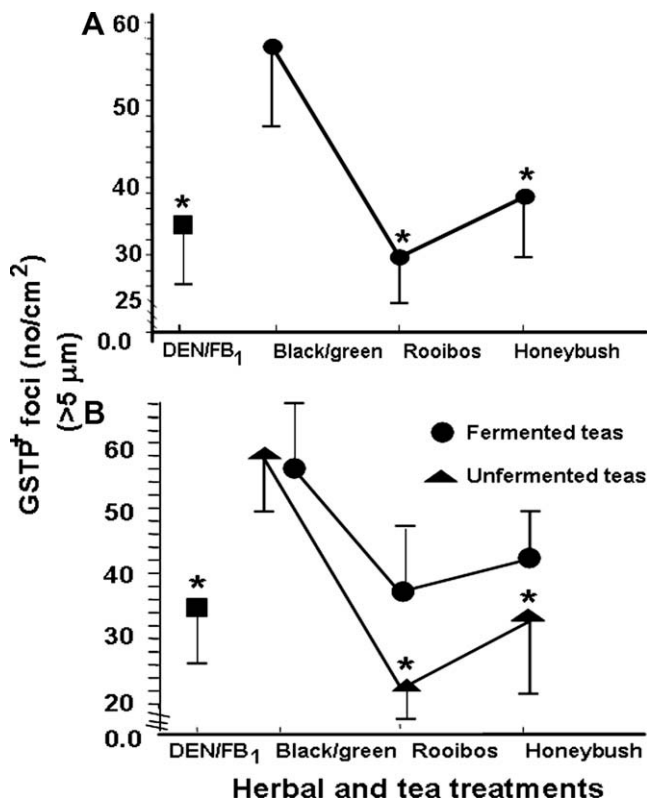
mini foci (5–10 μm) as well as the relative number. A similar effect was noticed when considering the relative amount of larger foci (11–20 μm) constituting 50% of the number of foci. The number of foci was significantly decreased by unfermented rooibos ( $P < 0.05$ ) and marginally by honeybush ( $P < 0.1$ ). The unfermented herbal teas significantly ( $P < 0.05$ ) decreased the number of foci (size 21–30 μm) constituting approximately 20% of the total number of foci in the positive control group. The unfermented herbal teas and black tea significantly ( $P < 0.05$ ) and marginally ( $P < 0.1$ ) decreased the relative amount of foci in this size category, respectively. When considering the focal size >30 μm, which constituted approximately 6% of the total amount of foci unfermented rooibos significantly ( $P < 0.05$ ) and unfermented honeybush marginally ( $P < 0.1$ ) decreased the number and relative amount. The total number of foci (>10 μm), excluding the mini foci, was significantly ( $P < 0.05$ ) and marginally ( $P < 0.1$ ) reduced by unfermented rooibos and honeybush, respectively.

Interactive plots showed that the herbal teas (combined effect) significantly ( $P < 0.05$ ) decreased the total amount of foci (>5 μm) as compared to the black and green teas (Fig. 2A). The separate effects of the different teas indicated that the unfermented rooibos and honeybush significantly ( $P < 0.05$ ) decreased the total number of foci when compared to the green and black teas (Fig. 2B). A similar effect was noticed with the fermented herbal teas, although differences were not significant, implying that fermentation reduced the protective effects of the herbal teas.

#### 4. Discussion

FB<sub>1</sub> was characterized as a non-genotoxic liver cancer promoter (Gelderblom et al., 1996) and shown to be hepato- and nephrocarcinogenic in rats (Gelderblom et al., 1991; Howard et al., 2001). In mice, FB<sub>1</sub> significantly increased the incidence of adenomas and carcinomas that developed spontaneously in the liver (Howard et al., 2001). The mechanism of cancer induction is not known at present but studies in rats indicate that FB<sub>1</sub> could effect both cancer initiation and promotion in the liver (Gelderblom et al., 1992, 1994, 1996, 2008). The disruption of growth-stimulatory responses in normal and genetically altered initiated cells is suggested to be important in establishing a growth differential whereby the preneoplastic cell populations are clonally expanded, subsequently leading to carcinogenesis by FB<sub>1</sub> in the liver. At a cellular level, FB<sub>1</sub> disrupts sphingolipid, phospholipid and fatty acid metabolism, which have been suggested to be the underlying mediators responsible for cancer promotion in the liver (Riley et al., 2001; Gelderblom et al., 2001; Burger et al., 2007).

The present study confirmed the FB<sub>1</sub>-induced hepatotoxic and nephrotoxic effects (Gelderblom et al., 1991; Voss et al., 1995), which were manifested by a significant ( $P < 0.05$ ) increase in the clinical chemical parameters associated with liver and kidney function, decreased body weight gain and relative liver weight. Black tea and fermented honeybush counteracted the reduction in serum creatinine suggesting a protective effect against FB<sub>1</sub>-induced nephrotoxicity. Honeybush and green tea also protected against FB<sub>1</sub>-induced accumulation of serum cholesterol. Both hes-



**Fig. 2.** (A) Interactive plots illustrating the combined effects ( $n = 20$ ) of the various tea treatments on the induction of total number of hepatic GSTP<sup>+</sup> foci  $>5 \mu\text{m}$  by FB<sub>1</sub> and (B) separate effects ( $n = 10$ ) illustrating the affect of tea processing on the induction of GSTP<sup>+</sup> foci. Values are the means  $\pm$  STD. \*Significantly different ( $P < 0.05$ ) from black and green tea.

peridin and mangiferin, present in large quantities in unfermented honeybush, is of interest as the aglycone of hesperidin, hesperetin and mangiferin reduce the level of rat plasma cholesterol (Kim et al., 2003; Nair and Devi, 2006). FB<sub>1</sub> and the different tea treatments did not alter the total serum iron levels when compared to the control. However, fermented and unfermented rooibos and black teas significantly ( $P < 0.05$ ) decreased the levels when compared to green tea and fermented and unfermented honeybush. As rooibos did not affect serum iron after a 10 week feeding study (Marnewick et al., 2003) specific iron/polyphenolic interactions, the decreased feed intake profiles and associated hepatotoxic effects during the 3 week FB<sub>1</sub> feeding regimen seems to be involved in the reduction of serum iron levels as compared to the other teas.

Unfermented rooibos further enhanced the FB<sub>1</sub>-induced hepatotoxic effect when considering the increased liver function enzymes, reduced body weight gain and relative liver weight. Unfermented honeybush also increased the ALT levels but the body and liver weight parameters were not altered. Specific interactions between the tea polyphenolic constituents and FB<sub>1</sub>-induced toxic effects are likely to be involved as the tea consumption profiles, except for the lower black tea intake, and total FB<sub>1</sub> intake profiles were similar. One possibility could be the induction of a pro-oxidative effect by the unfermented herbal tea polyphenols in the presence of iron. Aspalathin-enriched extracts of unfermented rooibos exhibit pro-oxidative effects in a Fenton-type reaction *in vitro*, a property that was significantly reduced by fermentation (Joubert et al., 2005). It has been suggested that high levels of mangiferin, the major antioxidant of unfermented honeybush, could also result in pro-oxidant activity (Joubert et al., 2008). The rat intake profiles

of mangiferin given unfermented honeybush were almost double that of aspalathin receiving the unfermented rooibos, suggesting that under these conditions mangiferin could act as a pro-oxidant. However, studies regarding the bioavailability of these polyphenolic constituents in the herbal extracts that are currently under investigation will provide more information on their *in vivo* antioxidant potencies. FB<sub>1</sub>-induced hepatotoxicity was associated with an increased oxidative damage (Abel and Gelderblom, 1998) and the mobilization of iron (Lemmer et al., 1999) that create an ideal environment for the induction of pro-oxidant effects by aspalathin and mangiferin. In this regard, the antioxidant potency of specific tea polyphenols is likely to determine whether antioxidant and/or pro-oxidant effects prevail. Fermented rooibos and honeybush, exhibiting weaker antioxidant properties (Von Gadow et al., 1997; Hubbe and Joubert, 2000a,b) and containing far lower levels of aspalathin and mangiferin, reduced the FB<sub>1</sub>-induced lipid peroxidation. Similar to the major unfermented herbal tea polyphenols, the potent green and black tea antioxidants, epigallocatechingallate (EGCG) and theaflavins, are known to act as pro-oxidants in the presence of copper and/or iron (Azam et al., 2004; Schuck et al., 2008). It would appear that under the current conditions, the tea flavonoids with the highest antioxidant potency are likely to enhance free radical production via a Fenton-type reaction thereby potentiating the FB<sub>1</sub>-induced hepatotoxicity effects. The reason why green and black teas did not affect the FB<sub>1</sub>-induced hepatotoxicity could be related to the iron binding capacity of the tannins (Andrade et al., 2006) as well as the differential induction of antioxidant enzymes discussed below.

Protection against lipid peroxidation, proposed to be a secondary event to FB<sub>1</sub>-induced hepatotoxicity (Lemmer et al., 1999), will not necessarily reduce FB<sub>1</sub>-induced hepatotoxicity. The suggested pro-oxidative effect of unfermented rooibos and the potentiating effects on FB<sub>1</sub>-induced hepatotoxic effects vs the protection against the resultant lipid peroxidation appear to be two separate events. The FB<sub>1</sub>/polyphenolic interactions are further complicated when considering different oxidative parameters in the liver. FB<sub>1</sub> significantly ( $P < 0.05$ ) reduced the ORAC as a result of the increased oxidative stress which is in agreement with a recent study in glioblastoma cells showing that the FB<sub>1</sub>-induced lipid peroxidation and oxidative stress which was associated with a reduced GSH level (Stockmann-Juvala et al., 2008). In the present study, the herbal and black teas did not alter the reduction in ORAC, while green tea significantly ( $P < 0.05$ ) further reduced the level, implying that the antioxidant capacity is further depleted, which may lead to a depletion of GSH. This is in agreement with the findings of a previous study where green tea reduces ORAC in the liver although the GSH level was not altered (Marnewick et al., 2003). In contrast, FB<sub>1</sub> significantly increased the GSH level which was associated with the development of preneoplastic lesions known to stain positively for gamma glutamyl transpeptidase positive (GGT<sup>+</sup>) foci (Gelderblom et al., 1991). This is in agreement with the findings that these preneoplastic lesions had an increased level of GSH (Marinho et al., 1997; Abel and Gelderblom, 1998). The increased levels of GSH in preneoplastic lesions provide these lesions with a selective growth advantage when compared to the surrounding tissue (Hanigan and Pitot, 1985) and will therefore be more resistant to the oxidative stress induced by FB<sub>1</sub>. The reduction of the GSH level by unfermented rooibos and to some extent unfermented honeybush was associated with a decrease in the number of the preneoplastic foci. When considering the GSH:GSSG ratio unfermented rooibos, honeybush, black and green teas significantly ( $P < 0.05$ ) decreased the ratio mainly due to an increased GSSG level while GSH was reduced as a result of the decreased number and size of the GSTP<sup>+</sup> foci. It is known that, under stressed conditions, the GSH:GSSG ratio decreases either due to increased GSSG or decreased GSH

levels (Dickinson and Forman, 2002). The teas exhibiting the highest protective effect against FB<sub>1</sub>-induced lipid peroxidation, fermented rooibos and honeybush did not alter the GSSG level and the GSH:GSSG ratio although fermented rooibos marginally ( $P < 0.1$ ) reduced the ratio due to the reduction in the GSH level. The development of the preneoplastic lesion therefore complicated interpretations regarding the effect of the herbal teas on the GSH level and hence the oxidative status in the liver. However, unfermented rooibos and honeybush have been reported to significantly increase the GSH level in the liver after a 10 week feeding study (Marnewick et al., 2003), an aspect that is masked in the present study by the development of preneoplastic lesions.

None of the teas affected the FB<sub>1</sub>-induced activity of GR, implying that the increased GSH levels in the liver, as stated above, are more related to the induction of GSTP<sup>+</sup> foci. FB<sub>1</sub> increased and decreased the activities of GPx and CAT, respectively, implying that the production of hydrogen peroxide is involved in the FB<sub>1</sub>-induced oxidative stress. None of the teas affected the increased activity of the GPx while honeybush, green and black teas effectively counteracted the FB<sub>1</sub>-induced decrease in CAT. This could explain the above arguments that green tea, black tea and fermented honeybush did not enhance the FB<sub>1</sub>-induced hepatotoxic effects. The activity of SOD was not affected suggesting that the formation of superoxide is not a prominent feature during FB<sub>1</sub>-induced hepatotoxic effects as was suggested previously (Sahu et al., 1998).

Herbal tea consumption significantly ( $P < 0.05$ ) arrested the proliferation of GSTP<sup>+</sup> altered cells in the presence of the cancer promoter FB<sub>1</sub> as the relative number of foci (11–20 μm), constituting approximately 50% of the total amount of foci, were significantly ( $P < 0.05$ ) decreased, while the relative number of mini foci (5–10 μm) was significantly ( $P < 0.05$ ) increased. The reduction of the total number of foci (>10 μm) by unfermented rooibos and to a certain extent with unfermented honeybush, could be related to the increased oxidative stress, which could further enhance apoptosis known to be effected by FB<sub>1</sub> in the liver (Lemmer et al., 1999). It was reported that apoptosis, induced by FB<sub>1</sub>, delayed cancer induction by removing initiated cells from the liver (Gelderblom et al., 1992, 1994). Similar to the herbal teas, green and black teas significantly decreased and increased the relative number of foci (11–20 μm) and minifoci (5–10 μm), respectively. Inhibition of cell proliferation by green tea polyphenols during cancer promotion has been associated with a reduction of the number and size of enzyme altered foci (Gong et al., 2000). Green and black teas significantly enhanced the total number of foci (>5 μm) as compared to the herbal teas and the DEN-FB<sub>1</sub> treatments, mainly due to an increase in the number of mini foci, which could be related to the reduction in liver ORAC, in the case of green tea, and GSH:GSSG ratio suggestive of an increased oxidative stress. It is known that a dual role for oxidative stress exists that could either stimulate cell proliferation or inhibit cell proliferation thereby increasing cell depletion via the induction of apoptosis (Klaunig and Kamendulis, 2004). In this regard the green tea flavanol, (–)-epigallocatechin-3-gallate was shown to inhibit the proliferation of cancer cells through the induction of apoptosis (Yang et al., 1998; Kuo and Lin, 2003). However, the present study indicated that the polyphenolic constituents that differ between the teas selectively modulate oxidative stress and the cancer promoting potency of FB<sub>1</sub> in rat liver.

Fermentation of the herbal teas tended to reduce the inhibition of cancer promotion effected by FB<sub>1</sub>, implying that the chemopreventive properties of the unfermented herbal teas should be further evaluated. Fermentation significantly reduced the soluble solid content which was associated with a decrease in the major antioxidants constituents resulting in a reduction in the rat antioxidants intake profiles. It is known that the antioxidant potency of plant polyphenolic components may play a determining role in

maintaining the balance between cell growth and cell death in preneoplastic lesions (Lee et al., 2002). In the present study the involvement of antioxidant properties of the different teas modulates the oxidative stress during FB<sub>1</sub>-induced cancer promotion by selectively affecting the ORAC and GSH levels, antioxidant enzymes and the level of lipid peroxidation presumably due to differences in their polyphenolic constituents. The reduced protective effect of honeybush against FB<sub>1</sub>-induced cancer promotion further emphasized the role of antioxidants as rooibos extracts exhibit higher antioxidant properties than honeybush (Joubert et al., 2008). It would appear that at similar concentrations, honeybush would have exhibited a far weaker protection. However, the protection against FB<sub>1</sub>-induced cancer promotion by the different teas may not be entirely related to their antioxidative activities and other features, e.g. their pro-oxidant activity due to specific polyphenol/FB<sub>1</sub>/iron interactions, affecting cell regulatory processes related to cell survival need to be further investigated.

### Conflict of interest statement

The authors declare that there are no conflicts of interest.

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### References

- Abel, S., Gelderblom, W.C.A., 1998. Oxidative damage and fumonisin B<sub>1</sub>-induced toxic effects in primary rat hepatocytes and rat liver *in vivo*. *Toxicology* 131, 121–131.
- Ali, N., Sardjono, S., Yamashita, A., Yoshizawa, T., 1998. Natural co-occurrence of aflatoxins and *Fusarium* mycotoxins (fumonisins, deoxynivalenol, nivalenol and zearalenone) in corn from Indonesia. *Food Additives and Contaminants* 15, 377–384.
- Andrade, R.G., Ginami, J.S., Lopes, G.K.B., Dutra, F., Alonso, A., Hermes-Lima, L., 2006. Tannic acid inhibits *in vitro* iron-dependent free radical formation. *Biochimie* 88, 1287–1296.
- Azam, S., Hadi, N., Khan, N.U., Hadi, S.M., 2004. Prooxidant property of green tea polyphenols epicatechin and epigallocatechin-3-gallate: implications for anticancer properties. *Toxicology In Vitro* 18, 555–561.
- Burger, H.-M., Abel, S., Snijman, P., Swanevelder, S., Gelderblom, W.C.A., 2007. Altered lipid parameters in hepatic subcellular membrane fractions induced by fumonisin B<sub>1</sub>. *Lipids* 42, 249–261.
- Bramati, L., Minogio, M., Gardana, C., Simonetti, P., Mauri, P., Pietta, P., 2002. Quantitative characterization of flavonoid compounds in rooibos tea (*Aspalathus linearis*) by LC-UV/DAD. *Journal of Agricultural and Food Chemistry* 50, 5513–5519.
- Cao, G., Prior, R.L., 1998. Measurement of oxygen radical absorbance capacity in biological samples. *Methods in Enzymology* 299, 50–62.
- Cawood, M.E., Gelderblom, W.C.A., Vleggaar, R., Berhend, Y., Thiel, P.G., Marasas, W.F.O., 1991. Isolation of the fumonisin mycotoxin: a quantitative approach. *Journal of Agricultural and Food Chemistry* 39, 1958–1962.
- Dickinson, D.A., Forman, H.J., 2002. Glutathione in defense and signaling: lessons from a small thiol. *Annals of the New York Academy of Sciences* 973, 488–504.
- Ellerby, L.M., Bredesden, D.E., 2000. Measurement of cellular oxidation, reactive oxygen species, and antioxidant enzymes during apoptosis. *Methods in Enzymology* 322, 413–421.
- Esterbauer, H., Cheeseman, K.H., 1990. Determination of aldehydic lipid peroxidation product: malondialdehyde and 4-hydroxynonenol. *Methods in Enzymology* 186, 407–421.



- Ferreira, D., Kamara, B.I., Brand, E.V., Joubert, E., 1998. Phenolic compounds from *Cyclopia intermedia* (Honeybush tea). *Journal of Agricultural and Food Chemistry* 46, 3406–3410.
- Flohe, L., Gunzler, W.A., 1984. Assays of glutathione peroxidase. *Methods in Enzymology* 105, 114–121.
- Fujiki, H., Suganuma, M., Imai, K., Nakachi, K., 2002. Green tea: cancer preventive beverage and/or drug. *Cancer Letters* 188, 9–13.
- Gelderblom, W.C.A., Jaskiewicz, K., Marasas, W.F.O., Thiel, P.G., Horak, R.M., Vlegaar, R., Kriek, N.P.J., 1988. Fumonisin- novel mycotoxins with cancer promoting activity produced by *Fusarium moniliforme*. *Applied and Environmental Microbiology* 54, 1806–1811.
- Gelderblom, W.C.A., Kriek, N.P.J., Marasas, W.F.O., Thiel, P.G., 1991. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B<sub>1</sub> in rats. *Carcinogenesis* 12, 1247–1251.
- Gelderblom, W.C.A., Semple, E., Marasas, W.F.O., Farber, E., 1992. The cancer initiating potential of the fumonisin mycotoxins produced by *Fusarium moniliforme*. *Carcinogenesis* 13, 433–437.
- Gelderblom, W.C.A., Cawood, M.E., Snyman, S.D., Marasas, W.F.O., 1994. Fumonisin B<sub>1</sub> dosimetry in relation to cancer initiation in rat liver. *Carcinogenesis* 15, 209–214.
- Gelderblom, W.C.A., Snyman, S.D., Lebepe-Mazur, S., Van der Westhuizen, L., Kriek, N.P.J., Marasas, W.F.O., 1996. The cancer-promoting potential of fumonisin B<sub>1</sub> in rat liver using diethylnitrosamine as a cancer initiator. *Cancer Letters* 109, 101–108.
- Gelderblom, W.C.A., Abel, S., Smuts, C.M., Marnewick, J.L., Marasas, W.F.O., Lemmer, E.R., Ramljak, D., 2001. Fumonisin-induced hepatocarcinogenesis: mechanisms related to cancer initiation and promotion. *Environmental Health Perspectives* 109, 291–300.
- Gelderblom, W.C.A., Marasas, W.F.O., Lebepe-Mazur, S., Swanevelder, S., Vessey, C.J., Hall, P., de la, M., 2002. Interaction of fumonisin B<sub>1</sub> and aflatoxin B<sub>1</sub> in a short term carcinogenesis model in rat liver. *Toxicology* 171, 161–173.
- Gelderblom, W.C.A., Marasas, W.F.O., Lebepe-Mazur, S., Swanevelder, S., Abel, S., 2008. Cancer initiating properties of fumonisin B<sub>1</sub> in a short-term rat liver carcinogenesis assay. *Toxicology* 250, 89–95.
- Gong, Y., Han, C., Chen, J., 2000. Effect of tea polyphenols and tea pigments on the inhibition of precancerous liver lesions in rats. *Nutrition and Cancer* 38, 81–86.
- Hanigan, M.H., Pitot, H.C., 1985. Gamma-glutamyl transpeptidase – its role in hepatocarcinogenesis. *Carcinogenesis* 6, 165–172.
- Howard, P.C., Eppley, R.M., Stack, M.E., Warbritton, A., Voss, K.A., Lorentzen, R.J., Kovach, R.M., Buccini, T.J., 2001. Fumonisin B<sub>1</sub> carcinogenicity in a two-year feeding study using F344 rats and B6C3F<sub>1</sub> mice. *Environmental Health Perspectives* 109, 277–282.
- Hubbe, M.E., Joubert, E., 2000a. *In vitro* superoxide anion radical scavenging activity of honeybush tea (*Cyclopia*). In: Johnson, I.T., Fenwick, G.R. (Eds.), *Dietary anticarcinogens and antimutagens – chemical and biological aspects*. The Royal Society of Chemistry, Cambridge, UK, pp. 242–244.
- Hubbe, M.E., Joubert, E., 2000b. Hydrogen donating ability of honeybush tea (*Cyclopia intermedia*) as a measure of antioxidant activity. In: Martens, S., Treutter, D., Forkmann, G. (Eds.), *Polyphenol Communications*. TUM, pp. 361–362.
- Jia, X., Han, C., Chen, J., 2002. Effects of tea on preneoplastic lesions and cell cycle regulators in rat liver. *Cancer Epidemiology and Biomarkers Prevention* 11, 1663–1667.
- Joubert, E., 1996. HPLC quantification of the dihydrochalcones, aspalathin and nothofagin in rooibos tea (*Aspalathus linearis*) as affected by processing. *Food Chemistry* 55, 403–411.
- Joubert, E., Otto, F., Grüner, S., Weinreich, B., 2003. Reversed-phase HPLC determination of mangiferin, isomangiferin and hesperidin in *Cyclopia* and the effect of harvesting date on the phenolic composition of *C. genistoides*. *European Food Research Technology* 216, 270–273.
- Joubert, E., Winterton, P., Britz, T.J., Ferreira, D., 2004. Superoxide anion and  $\alpha,\alpha$ -diphenyl- $\beta$ -picrylhydrazyl radical scavenging capacity of rooibos (*Aspalathus linearis*) aqueous extracts, crude phenolic fractions, tannin and flavonoids. *Food Research International* 37, 133–138.
- Joubert, E., Winterton, P., Britz, T.J., Gelderblom, W.C.A., 2005. Antioxidant and pro-oxidant activities of aqueous extracts and crude polyphenolic fractions of rooibos (*Aspalathus linearis*). *Journal of Agricultural and Food Chemistry* 53, 10260–10267.
- Joubert, E., Richards, E.S., Van der Merwe, J.D., de Beer, D., Manley, M., Gelderblom, W.C.A., 2008. Effect of species variation and processing on phenolic composition and *in vitro* antioxidant activity of aqueous extracts of *Cyclopia* spp. (honeybush tea). *Journal of Agriculture and Food Chemistry* 56, 954–963.
- Kamara, B.I., Brandt, E.V., Ferreira, D., Joubert, E., 2003. Polyphenols from honeybush tea (*Cyclopia intermedia*). *Journal of Agricultural and Food Chemistry* 51, 3874–3879.
- Klaunig, J.E., Kamendulis, L.M., 2004. The role of oxidative stress in carcinogenesis. *Annual Reviews of Pharmacological Toxicology* 44, 239–267.
- Kim, H.K., Jeong, T.-S., Lee, M.-K., Park, Y.B., Choi, M.S., 2003. Lipid-lowering efficacy of hesperetin metabolites in high-cholesterol fed rats. *Clinical Chemical Acta* 327, 129–137.
- Kuo, P.-L., Lin, C.-C., 2003. Green tea constituent (–)-epigallocatechin-3-gallate inhibits HepG2 cell proliferation and induces apoptosis through p53-dependent and Fas-mediated pathways. *Journal of Biomedical Science* 10, 219–227.
- Lee, S.E., Ju, E.M., Kim, J.H., 2002. Antioxidant activity of extracts from *Euryale ferox* seed. *Experimental Molecular Medicine* 40, 100–106.
- Lemmer, E.R., Gelderblom, W.C.A., Shephard, E.G., Abel, S., Seymour, B.L., Cruse, J.P., Kirsch, R.E., Marasas, W.F.O., Hall, P.M., 1999. The effects of dietary iron overload on fumonisin B<sub>1</sub>-induced lipid peroxidation and cancer induction in rat liver. *Cancer Letters* 146, 207–215.
- Marinho, H.S., Baptista, M., Pinto, R.E., 1997. Glutathione metabolism in hepatomas liver of rats treated with diethylnitrosamine. *Biochemical Biophysical Acta* 1360, 157–168.
- Marnewick, J.L., Gelderblom, W.C.A., Joubert, E., 2000. An investigation on the antimutagenic properties of South African herbal teas. *Mutation Research* 471, 157–166.
- Marnewick, J.L., Joubert, E., Swart, P., Van der Westhuizen, F., Gelderblom, W.C.A., 2003. Modulation of hepatic drug metabolising enzymes and oxidative status by green and black (*Camellia sinensis*), rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*) teas in rats. *Journal of Agricultural and Food Chemistry* 51, 8113–8119.
- Marnewick, J.L., Batenburg, W., Swart, P., Joubert, E., Swanevelder, S., Gelderblom, W.C.A., 2004. *Ex vivo* modulation of chemical-induced mutagenesis by subcellular liver fraction of rats treated with rooibos (*Aspalathus linearis*) tea, honeybush (*Cyclopia intermedia*) tea, as well as green and black (*Camellia sinensis*) teas. *Mutation Research* 558, 145–154.
- Marnewick, J.L., Joubert, E., Joseph, S., Swanevelder, S., Swart, P., Gelderblom, W.C.A., 2005. Inhibition of tumour promotion in mouse skin by extracts of rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*) unique South African herbal teas. *Cancer Letters* 224, 193–202.
- Maxuitenko, Y.Y., Libby, A.H., Joyner, H.H., Curphey, T.J., MacMillan, D.L., Kensler, T.W., Roebuck, B.D., 1998. Identification of dithiolethiones with better chemopreventive properties than oltipraz. *Carcinogenesis* 19, 1609–1615.
- Morton, J., 1983. Rooibos tea, *Aspalathus linearis*, a caffeineless, low-tannin beverage. *Economic Botany* 37, 164–173.
- Mukhtar, H., Katiyar, S.K., Agarwal, R., 1994. Green tea and skin – anticarcinogenic effects. *Journal of Investigative Dermatology* 102, 3–7.
- Nair, P.S., Devi, C.S.S., 2006. Efficacy of mangiferin on serum and heart tissue lipids in rats subjected to isoproterenol induced cardiotoxicity. *Toxicology* 228, 135–139.
- Ogawa, K., Solt, D., Farber, E., 1980. Phenotypic diversity as an early property of putative preneoplastic cells in liver carcinogenesis. *Cancer Research* 40, 725–733.
- Qin, G., Gopalan-Kriczky, P., Su, J., Ning, Y., Lotlikar, P.D., 1997. Inhibition of aflatoxin B<sub>1</sub>-induced initiation of hepatocarcinogenesis in the rat by green tea. *Cancer Letters* 112, 149–154.
- Qin, G., Ning, Y., Lotlikar, P.D., 2000. Chemoprevention of aflatoxin B<sub>1</sub>-initiated and carbon tetrachloride-promoted hepatocarcinogenesis in the rat by green tea. *Nutrition and Cancer* 38, 215–222.
- Riley, R.T., Enongene, E., Voss, K.A., Norred, W.P., Meredith, F.I., Sharma, R.P., Williams, D.E., Carlson, D.B., Spitsbergen, J., Merrill Jr., A.H., 2001. Sphingolipid perturbations as mechanisms for fumonisin carcinogenesis. *Environmental Health Perspectives* 109, 301–308.
- Sahu, A.C., Epply, R.M., Page, S.W., Gray, G.G., Barton, C.N., O'Donnell, M.W., 1998. Peroxidation of membrane lipids and oxidative DNA damage by fumonisin B<sub>1</sub> in isolated rat liver nuclei. *Cancer Letters* 125, 117–121.
- Sasaki, Y.F., Yamada, H., Shimoi, K., Kator, K., Kinai, N., 1993. The clastogen-suppressing effects of green tea, Po-lei tea and Rooibos tea in CHO cells and mice. *Mutation Research* 286, 221–232.
- Schuck, A.G., Ausubel, M.B., Zuckerbraun, H.L., Babich, H., 2008. Theaflavin-3,3'-digallate, a component of black tea: an inducer of oxidative stress and apoptosis. *Toxicology In Vitro* 22, 598–609.
- Shimoi, K., Hokabe, Y.F., Yamada, H., Kator, K., Kinai, N., 1994. Inhibitory effects of Rooibos tea (*Aspalathus linearis*) on the induction of chromosome aberrations *in vivo* and *in vitro*. In: How, C.-T., Osawa, T., Sheng, M.-T., Rosen, R.T. (Eds.), *Food Phytochemicals for Cancer Prevention II Teas Spices and Herbs*. American Chemical Society Symposium Series No. 547, 12, pp. 104–113.
- Snijman, P.W., Swanevelder, S., Joubert, E., Green, I.R., Gelderblom, W.C.A., 2007. The antimutagenic activity of the major flavonoids of rooibos (*Aspalathus linearis*) – some dose-response effects on mutagen activation-flavonoid interactions. *Mutation Research* 613, 111–123.
- Steele, V.E., Bagheri, D., Balentine, D.A., Boone, C.W., Rajendra, M., Morse, M.A., Sharma, S., Sigman, C.C., Stoner, G.D., Wargovich, M.J., Weisburger, J.H., Zhu, S., Kelloff, G.J., 1999. Preclinical efficacy studies of green and black tea extracts. *Proceedings Society of Experimental Biological Medical* 220, 210–212.
- Stockmann-Juvala, H., Mikkola, J., Naarala, J., Loikkanen, J., Elovaara, E., Savolainen, K., 2008. Oxidative stress induced by fumonisin B<sub>1</sub>-induced in continuous human and rodent neural cell cultures. *Free Radical Research* 38, 933–942.
- Tietze, F., 1969. Enzymatic method for quantitative determination of nanogram amounts of total and oxidised glutathione: applications to mammalian blood and other tissues. *Analytical Biochemical* 27, 502–522.
- Van der Merwe, J.D., Joubert, E., Richards, E.S., Manley, M., Snijman, P.W., Marnewick, J.L., Gelderblom, W.C.A., 2006. A comparative study on the antimutagenic properties of aqueous extracts of *Aspalathus linearis* (rooibos) different *Cyclopia* spp. (honeybush) and *Camellia sinensis* teas. *Mutation Research* 611, 42–53.
- Von Gadow, A., Joubert, E., Hansmann, C.F., 1997. Comparison of the antioxidant activity of rooibos tea (*Aspalathus linearis*) with green, oolong and black tea. *Food Chemistry* 60, 73–77.
- Voss, K.A., Chamberlain, W.J., Bacon, C.W., Herbert, R.A., Walters, D.B., Norred, W.P., 1995. Sub chronic feeding study of the mycotoxin fumonisin B<sub>1</sub> in B6C3F<sub>1</sub> Mice and Fischer 344 Rats. *Fundamental and Applied Toxicology* 24, 102–110.

- Yang, G.Y., Liao, J., Kim, K.M., Yurkow, E.J., Yang, C.S., 1998. Inhibition of growth and induction of apoptosis in human cancer cell lines by tea polyphenols. *Carcinogenesis* 19, 611–616.
- Yang, C.S., Chung, J.Y., Li, C., Meng, X., Lee, M.J., 2000a. Mechanisms of inhibition of carcinogenesis by tea. *Biofactors* 13, 73–79.
- Yang, C.S., Chung, J.Y., Yang, G-Y., Chhabra, S.K., Lee, M.-J., 2000b. Tea and tea polyphenols in cancer prevention. *Journal of Nutrition* 130, 472S–478S.
- Yoshikawa, T., Natio, Y., Oyamada, H., Ueda, S., Tanigawa, T., Takemura, T., Sugino, S., Kondo, M., 1990. Scavenging affects of *Aspalathus linearis* (rooibos tea) on active oxygen species. *Advances in Experimental Medical Biology* 264, 171–174.
- Zhang, H., Nagashima, H., Goto, T., 1997. Natural occurrence of mycotoxins in corn samples from high and low risk areas for human esophageal cancer in China. *Mycotoxins* 44, 29–35.