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Assessment of Lipid Profiles, Antioxidant Status and Liver Histopathology in Male Wistar Rats Following Dietary Intake of Rooibos (*Aspalathus linearis*)

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Abstract: Rooibos is a herbal tea which is known to contain a high amount of polyphenols. The lipid profiles, antioxidant status and liver histopathology in male rats fed with aqueous rooibos extract at different concentrations were studied. The rats were randomly divided into groups (A-D). Group A was the control group which received tap water only while groups B, C and D received 2, 4 and 6% aqueous rooibos extracts respectively orally for 7 weeks. The results showed no significant differences in the plasma and liver ferric reducing antioxidant powers (FRAP) in all the rooibos extracts fed groups. There were no significant ($p > 0.05$) decreases in total cholesterol, triglycerides, low density lipoprotein cholesterol and high density lipoprotein cholesterol levels in all the rooibos extracts fed groups. Liver catalase activity significantly ($p < 0.05$) increased in all of the rooibos extracts fed groups. There were no significant differences in glutathione peroxidase activities in the red blood cells and liver of rooibos extracts fed groups. Superoxide dismutase activities did not show any significant ($p > 0.05$) difference in both the red blood cells and liver of rooibos extracts fed groups. There was a significant increase in glutathione levels at 4 and 6% rooibos extracts when compared with the control group. Total protein, albumin and globulin levels were not significantly ($p > 0.05$) different in all the groups. Histopathological evaluations revealed no adverse effects in the structure of the liver in the rats. In conclusion, intake of rooibos did not have significant effects on lipid profiles while it contributed to the improvement of the body antioxidant system.

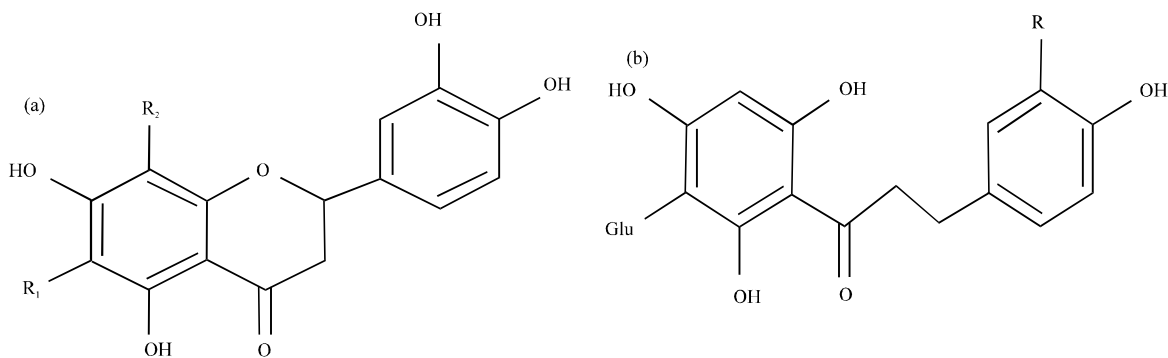
Key words: Lipid profiles, antioxidant, histopathology, wistar rats, rooibos

INTRODUCTION

Rooibos (*Aspalathus linearis*) is a herbal tea that can be found in the Cederberg mountain range area of the Western Cape, Republic of South Africa and it is known to contain a high and complex profile of antioxidants (polyphenols). Strong antioxidant activities in edible plants are due to the presence of phenolic and polyphenolics (Fang *et al.*, 2002). Free radicals scavenging effects of tea polyphenolic compounds and their involvement in the management of diseases such as coronary heart disease, hypertension, diabetes and cancer have been reported (Fang *et al.*, 2002; Sabu *et al.*, 2002; Weisburger, 2003; Negishi *et al.*, 2004; Baba *et al.*, 2012). The expression of antioxidant enzymes and other detoxifying enzymes can be regulated by oxidative stress and by low concentrations of a broad variety of chemical agents which includes antioxidants (Matsumoto *et al.*, 2009).

The protection against reactive oxidants that is produced during aerobic respiration is a complex process initiated by a system of antioxidant enzymes and antioxidant compounds (Szaleczky *et al.*, 1999). Superoxide radicals, the most abundant reactive oxygen species (ROS) generated in living systems is acted upon by superoxide dismutase (SOD) to produce hydrogen peroxide which in turn is inactivated by catalase and/or glutathione peroxidase (GPx) into water and oxygen (Narang *et al.*, 2004). Risk factors for coronary heart disease are the lipid profiles (Edem, 2002; Sherpa *et al.*, 2011). Cholesterol is moved through the blood by particles called lipoproteins and are categorised by their densities (Gotto, 2002). The high-density lipoprotein cholesterol (HDL-c) takes away lipids from the blood cells to the liver while the low density lipoproteins cholesterol (LDL-c) transports lipids to the cells and blood vessels (Owolabi *et al.*, 2010).

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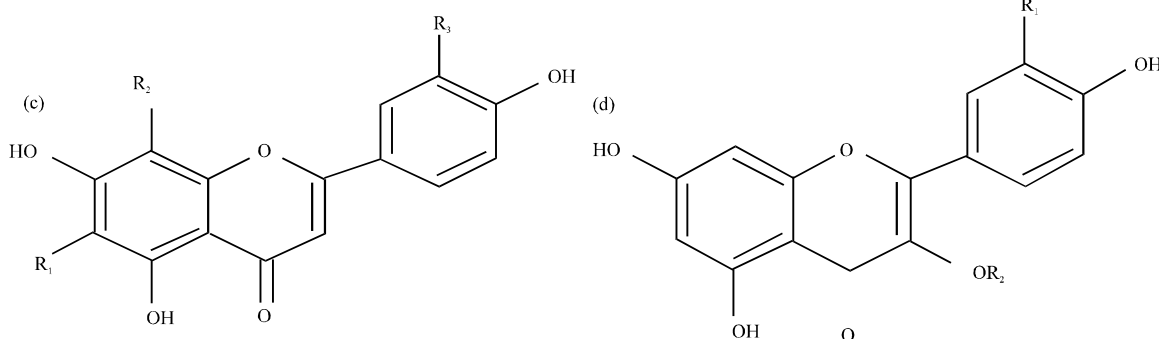


Flavanones

- (S)-Eriodictyol-6-C- β -D-glucopyranoside, $R_1 = \text{Glu}$, $R_2 = \text{H}$
- (S)-Eriodictyol-6-C- β -D-glucopyranoside, $R_1 = \text{H}$, $R_2 = \text{Glu}$
- (R)-Eriodictyol-6-C- β -D-glucopyranoside, $R_1 = \text{Glu}$, $R_2 = \text{H}$
- (R)-Eriodictyol-6-C- β -D-glucopyranoside, $R_1 = \text{H}$, $R_2 = \text{Glu}$

Dihydrochalcones

- Aspalathin, $R = \text{OH}$
- Nothofagin, $R = \text{H}$
- Rut-Rutinose, Gal-Galactose, Glu-Glucose



Flavones

- Orientin, $R_1 = \text{H}_2$, $R_2 = \text{Glu}$, $R_3 = \text{OH}$
- Isoorientin, $R_1 = \text{Glu}$, $R_2 = \text{H}$, $R_3 = \text{OH}$
- Vitexin, $R_1 = \text{H}$, $R_2 = \text{Glu}$, $R_3 = \text{H}$
- Isovitexin, $R_1 = \text{Glu}$, $R_2 = \text{H}$, $R_3 = \text{H}$
- Luteolin, $R_1 = \text{H}$, $R_2 = \text{H}$, $R_3 = \text{OH}$
- Chrysoeriol, $R_1 = \text{H}$, $R_2 = \text{H}$, $R_3 = \text{OCH}_3$

Flavonols

- Rutin, $R_1 = \text{OH}$, $R_2 = \text{Rut}$
- Hyperoside, $R_1 = \text{OH}$, $R_2 = \text{Gal}$
- Isoquercitrin, $R_1 = \text{OH}$, $R_2 = \text{Glu}$
- Ouerctin, $R_1 = \text{OH}$, $R_2 = \text{H}$

Fig. 1(a-d): Structures of the different classes of flavonoids present in rooibos (Krafczyk *et al.*, 2009), (a) Flavanones, (b) Dihydrochalcones, (c) Flavones and (d) Flavonols

Flavonoids preferentially enter the hydrophobic core of the membrane and exert a membrane-stabilizing effect by the modification of the lipid packing order and leads to a dramatic decrease in lipid fluidity in this region of the membrane (Arora *et al.*, 2000; Wojciech *et al.*, 2010). Tea and its components influence antioxidant capacity in biomembranes (Saija *et al.*, 1995; Wojciech *et al.*, 2010). The aim of this study was to investigate the biochemical effects of the intake of aqueous rooibos extract at different concentrations in male Wistar rats. Figure 1 shows the different structures of flavonoids that are present in rooibos.

MATERIALS AND METHODS

Experimental rats: Male Wistar rats (192-240 g) were obtained from Stellenbosch University, Tygerberg, South Africa and used throughout the study. The study was conducted after obtaining Ethical Committee Clearance from Cape Peninsula University of Technology (CPUT/HAS-REC 2010/A002). The rats were individually housed in a well controlled environment set at $22^\circ\text{C} \pm 2$ with $50 \pm 5\%$ humidity and a 12 h light cycle. They were randomly placed in four groups. Group A (n = 6) received only tap water and served as the control while group

B (n = 6), C (n = 6) and D (n = 6) received 2, 4 and 6% aqueous rooibos extracts, respectively substituting the drinking water. All the groups received standard rat chow. The fermented rooibos was supplied by Rooibos Ltd (Clanwilliam, South Africa). At the end of the seven weeks, all the animals were sacrificed after overnight fasting. Blood samples were collected from the abdominal aorta and then centrifuged to obtain the serum, plasma and red blood cells for biochemical analysis. The liver was removed, frozen in liquid nitrogen and stored at 80°C until analysis.

Preparation of aqueous rooibos extracts: Aqueous extracts of fermented rooibos was prepared by the addition of freshly boiled tap water to the leaves and stems (2, 4 and 6 g 100 mL⁻¹). The mixture was allowed to stand for 30 min at room temperature, cooled, filtered and dispensed into water bottles.

Determination of total polyphenol, flavanol and flavonol content: The plasma was deproteinised using 0.5 M perchloric acid (PCA) (1:1 v/v). Folin-Ciocalteu method was used to determine the total polyphenol in the plasma and rooibos extracts according to the method of Singleton *et al.* (1999). The total polyphenols levels were expressed as mg gallic acid standard equivalents per litre. The flavanol content of the rooibos extracts was determined colorimetrically at 640 nm using p-dimethylaminocinnamaldehyde (DMACA) and expressed as mg catechin standard equivalents per litre extract (Delcour and Devarebeke, 1985; Treutter, 1989). The flavonol content of the rooibos extracts was determined spectrophotometrically at 360 nm and expressed as mg quercetin standard equivalents per litre extract (Mazza *et al.*, 1999).

Antioxidant enzymes assay: The activities of antioxidant enzymes in the liver and red blood cells were determined. Liver homogenates (10% w/v) were prepared in a phosphate buffer, centrifuged at 10,000 g (4°C) for 10 min and the supernatant kept at 80°C for enzyme analyses. Catalase (CAT) activity was determined spectrophotometrically at 240 nm by monitoring the decomposition of H₂O₂ and expressed as μmole H₂O₂ min⁻¹ μg⁻¹ protein according to the method of Aebi (1984). Superoxide dismutase (SOD) activity was determined by the method of Crosti *et al.* (1987) with slight modification for a microplate reader at 490 nm. SOD activity was expressed as the amount of protein (μg) 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 Ellerby and Bredesen (2000) and expressing activity as nmoles NADPH min⁻¹ μg⁻¹ protein.

Ferric Reducing Antioxidant Power (FRAP) assay: The ferric reducing antioxidant power was determined using the method described by Benzie and Strain (1996). The 10 μL of the plasma and liver homogenates was mixed with 300 μL FRAP reagent in a 96-well clear plate. The FRAP reagent was a mixture (10:1:1, v/v/v) of acetate buffer (300 mM, pH 3.6), tripyridyl triazine (TPTZ) (10 mM in 40 mM HCl) and FeCl₃·6H₂O (20 mM). After incubation at room temperature for 30 min, the plate was read at a wavelength of 593 nm in a Multiskan Spectrum plate reader (Thermo Fisher Scientific). Ascorbic acid (AA) was used as the standard and the results expressed as μmol AAE L⁻¹ for plasma and μmol AAE g⁻¹ tissue for liver homogenates.

Lipid profile determination: Triglycerides (TG), total Cholesterol (TC) and high density lipoprotein (HDL)-cholesterol were evaluated with kits using a clinical chemistry analyzer (Easyra medical, USA) to manufacturer's instructions. Very low density lipoprotein (VLDL)-cholesterol and LDL-cholesterol were calculated according to Friedewald's formula (Friedewald *et al.*, 1972).

$$\text{VLDL cholesterol} = \frac{\text{TG}}{5}$$

and

$\text{LDL cholesterol} = \text{TC} - \text{VLDL cholesterol} - \text{HDL cholesterol}$

Total glutathione, total protein, albumin and globulin analysis: The levels of total glutathione (GSht) in the liver and red blood cells were determined according to the method of Asensi *et al.* (1999). Red blood cells were deproteinised using 5% metaphosphoric acid (MPA) solution. Liver samples were homogenized (1:10) in 15% TCA containing 1 mM EDTA. The homogenates were centrifuged at 15,000 g for 10 min and the supernatant collected. Total glutathione in the red blood cells and liver homogenates extracts was done by adding 50 μL of the samples into plate wells and 50 μL of 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) was added, followed by 50 μL of glutathione reductase. The reaction was initiated by the addition of 50 μL of nicotinamide adenine dinucleotide phosphate (NADPH) to a final volume of 200 μL. The change in absorbance was monitored at 412 nm for 5 min and levels of GSht calculated using pure glutathione (GSH) as a standard and expressed as μmol mg⁻¹ protein for red blood cells and μmol g⁻¹ tissue for liver homogenates. Total protein and albumin levels in the serum were measured with kits using an automated chemistry analyzer

(Easy RA Medical, USA) according to manufacturer's instructions. Globulin level was determined by using the equation:

$$(\text{Globulin} = \text{Total protein} - \text{Albumin})$$

Histopathological evaluations: At the end of treatment, the animals were sacrificed in order to collect the liver. The liver was blotted and freed from excess blood, fixed in 10% neutral formalin, trimmed, processed for paraffin embedment and 5 µm thick tissue sections were stained with haematoxylin and eosin (H&E). Histopathological structures of liver were examined using light microscopy at 20x magnification.

Statistical analysis: Data were expressed as the Means±standard deviations. Significant differences between mean values of different groups were determined by one-way analysis of variance (ANOVA) with MedCalc software. Data not normally distributed was log transformed and analyzed using the Kruskal-Wallis one-way ANOVA on ranks hypotheses. Differences were considered significant at $p < 0.05$.

RESULTS

The results showed no significant ($p < 0.05$) differences in the body weights of the rats fed with different concentrations of rooibos extracts in comparison with the control group (Table 1). Similarly, there were no significant ($p < 0.05$) differences in the percentage liver weight of the rooibos extracts fed rats when compared with the control group as shown in Table 1.

From the results it was observed that the higher the concentrations of the rooibos extracts, the more the

antioxidants consumed (Table 2). The daily intake of rooibos extracts at 2, 4 and 6% were 38.96 ± 3.78 , 35.39 ± 3.54 and 33.72 ± 3.44 mL day⁻¹ and total polyphenol consumed daily were 21.05 ± 2.04 , 36.09 ± 3.61 and 47.64 ± 4.87 mg day⁻¹, respectively.

The results showed no significant ($p > 0.05$) differences in the serum total cholesterol (TC), triglycerides (TG), HDL-cholesterol, VLDL-cholesterol and LDL-cholesterol of all the rooibos extracts fed groups when compared with the control group (Table 3).

In this study, there were no significant ($p > 0.05$) differences in the serum total protein, albumin and globulin levels of all the rooibos extracts fed groups when compared with the control group (Table 4).

The results showed no significant ($p > 0.05$) differences in both the liver and plasma FRAP status of all the groups as shown in Table 5. Plasma total polyphenol levels in the rats fed with 2 and 4% rooibos extracts were not significantly different when compared with the control group (Table 5). However, a significant increase ($p < 0.05$) in the total polyphenol level at 6% rooibos extracts was shown with a value of 151.23 ± 21.84 mg L⁻¹ when compared with the control group that has a value of 108.45 ± 8.38 mg L⁻¹ (Table 5).

The results showed a significant ($p < 0.05$) increase in the activity of catalase (CAT) in the liver at 2, 4 and 6% rooibos extracts with the values (0.78 ± 0.05 , 0.87 ± 0.11 and 0.94 ± 0.08), respectively when compared with the control group with a value of 0.55 ± 0.09 as represented in Fig. 2a. CAT activity in the red blood cells (RBCs) was not significantly ($p > 0.05$) different in all the rooibos fed groups when compared with the control groups (Fig. 2b). There were no significant ($p > 0.05$) differences in the activities of glutathione peroxidase (GPx) in both the liver

Table 1: Percentage body weight gain and liver weight in rats fed with the different concentrations of rooibos extracts

Rooibos extracts (%)	Body weight gain (%)	Liver weight (%)
0	55.77±6.87	3.15±0.14
2	65.00±11.68	3.22±0.13
4	61.37±18.70	3.21±0.15
6	51.15±8.73	3.18±0.19

Table 2: Daily intake of rooibos and antioxidant profile of the rooibos extracts at different concentrations

Rooibos extracts (%)	Rooibos intake (mL day ⁻¹)	Total Polyphenol intake (mg day ⁻¹)	Flavonol intake (mg day ⁻¹)	Flavanol intake (mg day ⁻¹)	FRAP status (µmol day ⁻¹)
0	ND	ND	ND	ND	ND
2	38.96±3.78	21.05±2.04	0.88±0.09	0.43±0.04	97.21±9.43
4	35.39±3.54	36.09±3.61	1.48±0.15	0.93±0.09	180.24±18.02
6	33.72±3.44	47.64±4.87	2.19±0.22	1.37±0.14	238.16±24.32

ND: Not detected

Table 3: Effect of rooibos extracts on lipid profiles in rats at different concentrations

Rooibos extracts (%)	TC (mmol L ⁻¹)	TG (mmol L ⁻¹)	HDL cholesterol (mmol L ⁻¹)	VLDL cholesterol (mmol L ⁻¹)	LDL cholesterol (mmol L ⁻¹)
0	1.72±0.11	0.40±0.11	0.52±0.03	0.08±0.02	1.12±0.06
2	1.54±0.10	0.33±0.12	0.45±0.04	0.07±0.07	1.02±0.04
4	1.64±0.19	0.30±0.03	0.47±0.03	0.06±0.01	1.11±1.08
6	1.65±0.13	0.32±0.04	0.51±0.04	0.06±0.01	1.08±0.08

TC: Total cholesterol; TG: Triglycerides; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein

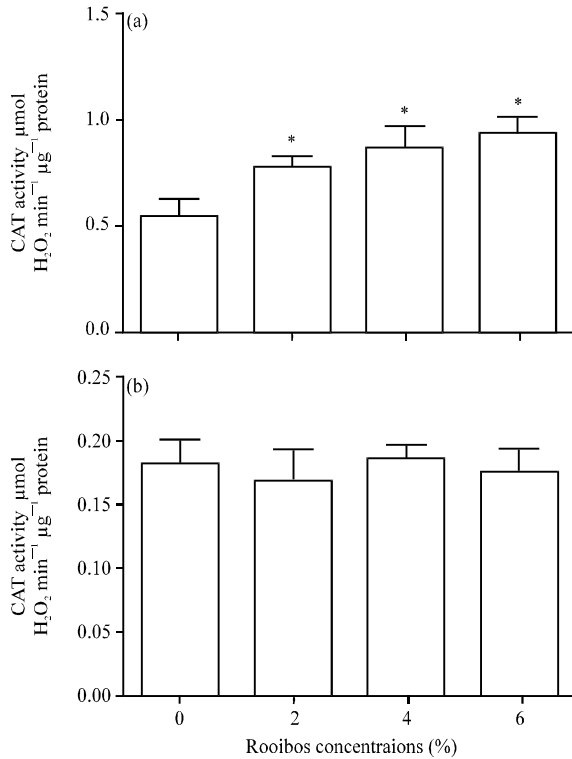


Fig. 2(a-b): Effect of rooibos extracts on the activity of catalase (CAT) in the, (a) Liver and (b) Red blood cells, *: Indicates significant difference from control group at $p < 0.05$

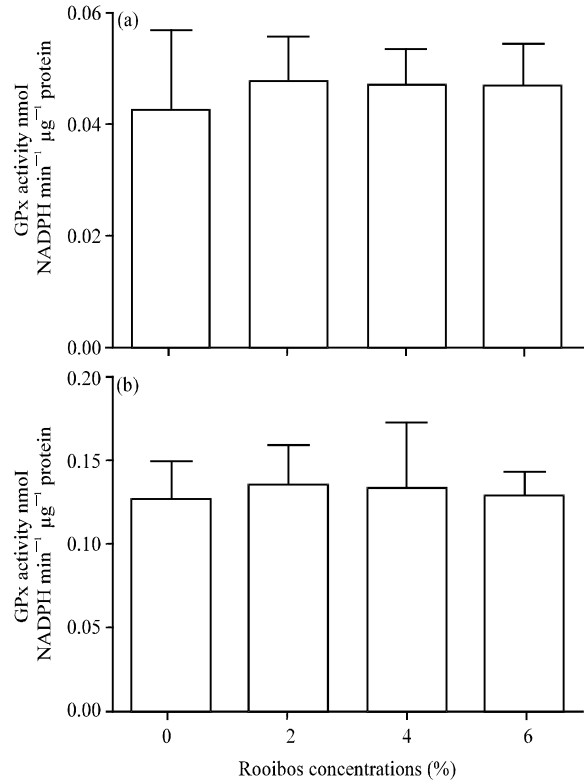


Fig. 3(a-b): Effect of rooibos extracts on the activity of glutathione peroxidase (Gpx) in the, (a) Liver and (b) Red blood cells, *: Indicates significant difference from control group at $p < 0.05$

Table 4: Effect of rooibos extracts on total protein, albumin and globulin in rats at different concentrations

Rooibos extracts (%)	Total protein (g L ⁻¹)	Albumin (g L ⁻¹)	Globulin (g L ⁻¹)
0	56.58±1.88	33.98±0.96	22.61±0.92
2	54.17±2.50	32.30±1.03	21.87±1.47
4	54.25±3.17	32.63±1.91	21.63±1.27
6	54.33±1.75	32.73±0.88	21.60±0.87

and RBCs of rooibos extracts fed groups when compared with their respective control groups (Fig. 3a and b). Similarly, the activities of superoxide dismutase (SOD) in both the liver and RBCs of rooibos extracts fed groups were not significantly ($p > 0.05$) different in comparison to their respective control groups (Fig. 4a and b). The total glutathione (GSHt) levels were significantly ($p < 0.05$) increased in the liver at 4 and 6% rooibos extracts with the values (5.20±0.32 and 5.77±0.65) when compared with the control group with a value of 4.74±0.32 as represented in Fig. 5a. Though not significant ($p > 0.05$), an increase in GSHt was also shown at 2% rooibos extract with a value of 5.21±0.33 when compared with the control group. GSHt levels did not show significant ($p > 0.05$)

differences in the RBCs of all the rooibos extracts fed groups when compared with the control group (Fig. 5b).

The results from the histopathology evaluations of the liver of the control group and rooibos extracts fed groups revealed normal hepatic structures which contain hepatocytes that are arranged into plates and separated by vascular channels known as sinusoids (Fig. 6a-d).

DISCUSSION

Rooibos is consumed for enjoyment and traditionally it has been used to alleviate infantile colic, asthma, allergies and dermatological problems as well as certain malignancies and inflammatory disorders (Sinisalo *et al.*, 2010). There was no obvious toxicity found such as a significant decrease in body and organ weights in the rats that received rooibos extracts at different concentrations. Rooibos contains many polyphenol antioxidants that are potent free radical scavengers. Polyphenols present in rooibos include aspalathin, nothofagin, orientin,

Table 5: Effects of different concentrations of rooibos extracts on FRAP status and total polyphenols in the rats

Rooibos extracts (%)	FRAP ($\mu\text{mol L}^{-1} \mu\text{mol g}^{-1} \text{ tissue}$)		Total polyphenols (mg L^{-1})
	Plasma	Liver	Plasma
0	313.80±34.98	2.48±0.27	108.45±8.380
2	248.18±23.32	2.39±0.53	123.48±32.01
4	254.39±59.95	2.48±0.17	110.06±12.70
6	276.10±40.09	2.43±0.10	151.23±21.84*

(*) Indicates significant difference from control group at $p < 0.05$. FRAP, ferric reducing antioxidant power

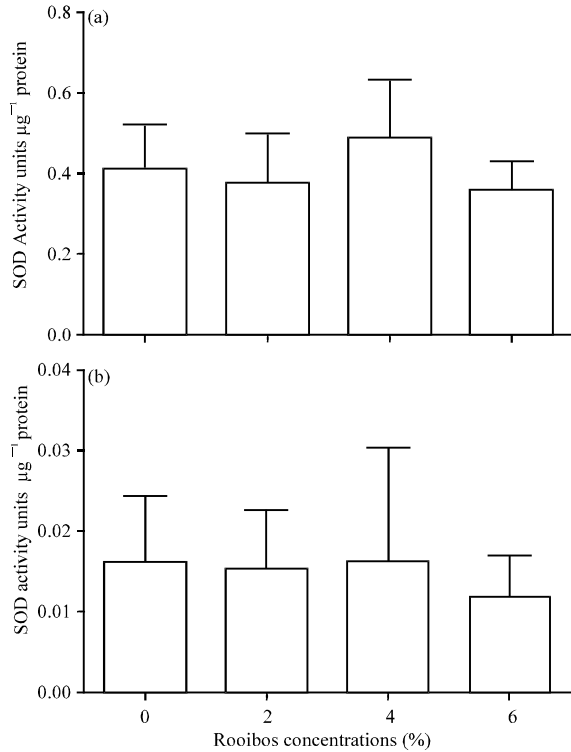


Fig. 4(a-b): Effect of rooibos extracts on the activity of superoxide dismutase (SOD) in the, (a) Liver and (b) Red blood cells

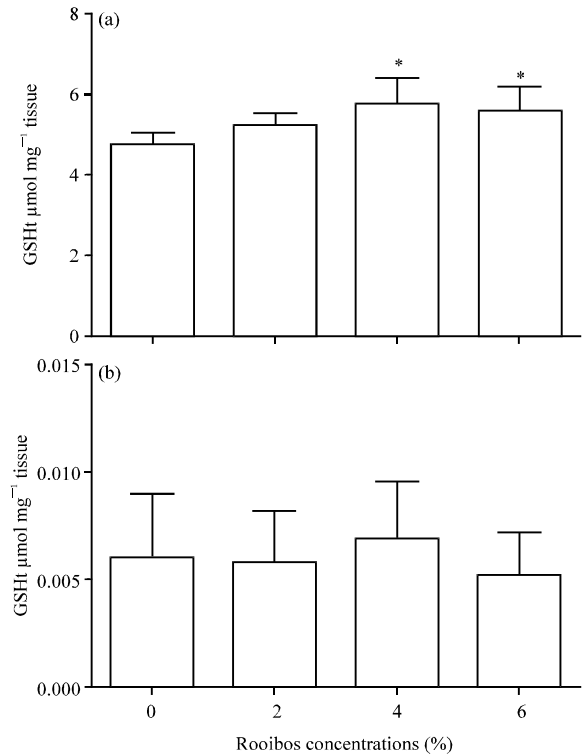


Fig. 5(a-b): Effect of rooibos extract on the levels of total glutathione (GSht) in the, (a) Liver and (b) Whole blood

isoorientin, vitexin, isovitexin, isoquercitrin, hyperoside, rutin, quercetin, luteolin and chrysoeriol (Fig. 1). The ability of rooibos to boost the liver antioxidant status and provide hepatoprotective effects on liver damage have been demonstrated (Ulicna *et al.*, 2003; Kucharska *et al.*, 2004). Antioxidant enzymes as well as some non-enzymatic enzymes such as vitamin C, vitamin E and flavonoid have anti-oxidative activity to prevent oxidative reactions which has been implicated in various diseases (Baba *et al.*, 2009).

In this study, there were no significant differences in the activities of GPx and SOD in both the RBCs and liver in the rats when compared with the control. The activity of CAT was significantly increased in rats receiving aqueous rooibos extracts. Similarly, there was a significant

increase in glutathione levels in the liver at 4 and 6% rooibos extracts. The results suggest that rooibos contains phytochemicals that could induce the activation of CAT at the intracellular level as well as increasing liver glutathione levels. It further confirms that the administration of rooibos or its polyphenolic constituents could help to prevent or attenuate decreases in antioxidant enzyme activities in oxidative stress mediated diseases such as diabetes, cancer, cardiovascular diseases. The modulatory roles of rooibos consumption on antioxidant enzymes have been shown by several studies (Baba *et al.*, 2009; Marnewick *et al.*, 2009; Awoniyi *et al.*, 2011, 2012). Similarly its preventive roles on induced-oxidative stress using animal models have been reported (Marnewick *et al.*, 2003; Ulicna *et al.*, 2006; Awoniyi *et al.*, 2011, 2012).

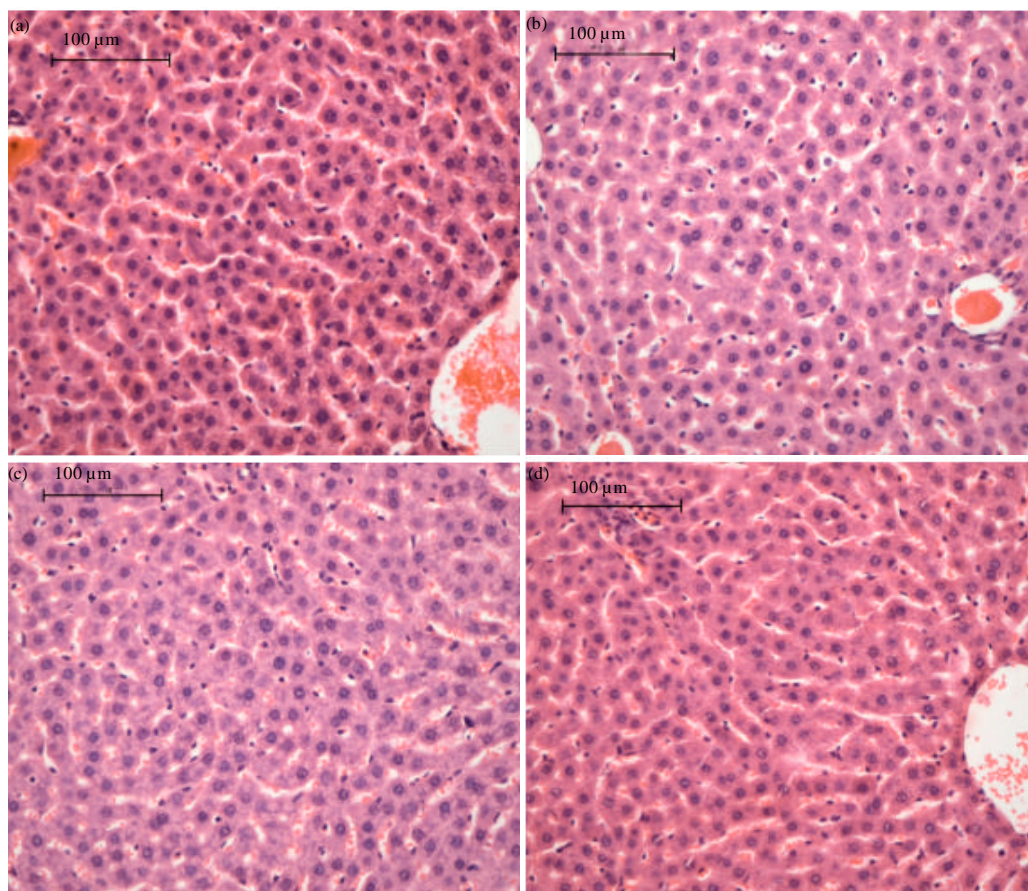


Fig. 6(a-d): Histopathology of the liver of (a) Control group, (b) 2% aqueous rooibos extract, (c) 4% aqueous rooibos extract and (d) 6% aqueous rooibos extract

Fang *et al.* (2002) reported that the dietary supplementation of tea polyphenols decreased serum concentrations of total cholesterol and malondialdehyde (an indicator of lipid peroxidation) and increased serum concentrations of high density lipoprotein in humans. Though, not significantly different, the results from this study showed a decrease in the levels of cholesterol and triglycerides. Owolabi *et al.* (2010) reported that lipids and other substances are accumulated on the arterial wall and form plaque which occlude the vascular lumen and hinder the flow of blood to vital organs such as the heart, brain, liver, or kidney. A good connection between increased plasma total cholesterol, low density lipoprotein cholesterol and increase in the occurrence of coronary heart disease has been documented (Edionwe and Kies, 2001; Kamisah *et al.*, 2005; Yakubu *et al.*, 2008). Elevated levels of all lipids except the high density lipoprotein (HDL) are associated with increased risk of atherosclerosis (Yakubu and Afolayan, 2009).

No significant differences in the levels of HDL-cholesterol and LDL-cholesterol were shown in all the rooibos fed rats when compared with the normal control group. In cholesterol homeostasis, HDL-cholesterol plays an important role (Wang and Peng, 2011). HDL protective effects are most widely attributed to its major role in mediating the reverse cholesterol transport from the peripheral tissues to the liver for reutilization (Von Eckardstein *et al.*, 2002). It is broadly known that low plasma HDL-cholesterol levels are inversely related to the risk of cardiovascular diseases (CVD) independent of other risk factors (Wang and Peng, 2011). It has also been reported that HDL-cholesterols are also carriers of enzymes that destroy the lipid hydroperoxides that oxidize LDL phospholipids (Navab *et al.*, 2001).

Albumin, the most abundant circulating protein in the plasma exerts important antioxidant activities and it acts through its multiple-binding sites and free radical-trapping

properties (Roche *et al.*, 2008). Albumin is largely responsible for the antioxidant properties of the serum (Roche *et al.*, 2008). This study showed no significant differences in the levels of total protein, globulin and albumin in rooibos receiving groups in comparison to the control group. Albumin which consists of more than 60% of free serum proteins is synthesized and secreted by the liver and it has many vital functions such as maintaining plasma colloid osmotic pressure, anti-oxidation and substances transfer (Shi *et al.*, 2010). Serum concentrations of proteins, bilirubin and albumin can help to show the condition of the liver and also ascertain the different types of liver damage (Yakubu *et al.*, 2003). The exposure of liver to xenobiotic-induced damage is due to its central role in xenobiotic metabolism and its portal location within the circulatory system (Jones, 1996; Avwioro *et al.*, 2010). In this study, the results showed no adverse effects on histopathology of the liver of rats subjected to different concentrations of rooibos extracts and this indicates that rooibos consumption can be well tolerated in the body.

CONCLUSION

In conclusion, the results from the present study indicated that rooibos consumption improved the antioxidant defence system while other biochemical indices measured did not show significant changes. Further studies on the mechanisms of induction of antioxidant defence system by rooibos intake are also needed.

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