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# *In vitro* Antioxidant, Antimutagenic and Genoprotective Activity of *Rosa roxburghii* Fruit Extract

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**The antioxidant properties of the fruit of the *Rosa roxburghii* (RR) plant have been associated with several putative health promoting effects. The possible cytotoxic, mutagenic/antimutagenic and genotoxic effects of RR fruit extract were investigated. The effect on antioxidant status and protection against induced oxidative stress were also investigated using primary rat hepatocytes. A RR fruit extract containing 45 g/L total ascorbic acid and 65 g/L total polyphenols was used in this study. Dilutions up to 0.08% (v/v) increased significantly the antioxidant status in primary rat hepatocytes. The glutathione redox state was decreased with RR treatment but was increased in Chang liver cells and MT-2 lymphoblast. No cyto- or genotoxicity were observed at levels of up to 5% (v/v) of the fruit extract. In addition, a significant protection against *t*-BHP induced oxidative stress was observed in primary rat hepatocytes. The Ames test revealed no mutagenic activity using the *Salmonella typhimurium* strains TA98, TA100 and TA102. A significant antimutagenic effect of the extract was observed against the metabolic activated mutagens 2-acetylaminofluorene and aflatoxin B1 and to a lesser extent against methyl methanesulfonate. It is concluded that these results support the associated health promoting potential of *Rosa Roxburghii* fruit and in particular against oxidative stress.**


*Keywords: Rosa roxburghii*; antioxidants; antimutagenic; COMET; cytotoxicity.



## INTRODUCTION

Although modern medicine is well developed in most countries of the world, large sections of the populations in developing countries still rely on medicinal plants and herbal medicines in primary health care. Moreover, recognition of the clinical, pharmaceutical and economic value of herbal medicine in natural therapies has increased greatly in industrialized countries (Elvin-Lewis, 2000). However, appropriate evaluation of these products and ensuring their safety and efficacy to patients presents important challenges. The dietary supplementation with an extract of the fruit (also called 'tratt') of the *Rosa roxburghii* plant is one such case that has recently been described to increase the antioxidant status in humans (Janse van Rensburg *et al*., 2005) and may be beneficial in diseases such as atherosclerosis, cancer and immunity stress, as well as limiting the effects of ageing (Zhang *et al*., 2003; Ma *et al*., 1997). However, there is limited published scientific information available with respect to its possible antimutagenic, antioxidant and possible toxicity *in vitro*. Although the relatively high content in ascorbic acid and flavonoids may suggest beneficial properties, it cannot be ignored that the presence of non-nutritional substances in the fruit of this plant may also have cytotoxic and genotoxic activities (Maron and Ames, 1982).

To better evaluate the health potential of this plant the putative protective effect of *Rosa roxburghii* fruit extract against oxidative stress was investigated *in vitro*, and possible antimutagenic and genoprotective activity while also evaluating the cytotoxicity. Since the liver is the major target organ of oxidative stress induced by xenobiotics, primary cultures of rat hepatocytes were used, which have been well described in pharmacological and toxicological literature (Lautraite *et al*., 2002; Richert *et al*., 2001; McKay *et al*., 2002). Oxidative damage in this study was induced by *tert*-butyl hydroperoxide (*t*-BHP) which is a short chain analogue of lipid hydroperoxide metabolized into free radical intermediates by the cytochrome P450 system of hepatocytes and initiates lipid peroxidation, glutathione depletion and cell damage (Wang *et al*., 1999; Yau *et al*., 2002; Tseng *et al*., 1997).

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## MATERIALS AND METHODS

**Materials.** *Rosa roxburghii* (RR sample) was kindly supplied by G. Joubert from Cili Health, Johannesburg, South Africa, who obtained the samples under a cooperative agreement with the Quangxi province, China. 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH), William's E medium, Hank's balanced salt solution, type VII collagen, type IV collagenase, *t*-BHP, reduced (GSH) and oxidised glutathione (GSSG), glutathione reductase, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), 2-acetylaminofluorene (2-AAF), aflatoxin $B_1$ ($AFB_1$), methyl-methane sulfonate (MMS), dimethylsulfoxide (DMSO), L-glutathione, and 5,5′dithiobis-(2-nitrobenzoic acid) (DTNB), 1-methyl-2-vinyl-pyridinium trifluoromethane sulfonate (M2VP), bicinchoninic acid (BCA) reagent and fluorescein sodium salt were all purchased from Sigma Chemical Co., St Louis, MO, USA. Diaphorase, 2-[4-Iodophenyl]-3-[4-nitrophenyl]-5-phenyl-tetrazolium (INT) and pyruvate were purchased from Roche, Penzberg, Germany. Difco agar and Oxoid nutrient broth no 2 was purchased from Rob Dyer Surgical (Johannesburg, South Africa), fetal calf serum, HEPES, PBS and antibiotics from Gibco, Invitrogen, Co., Auckland, New Zealand.

**Preparation from *Rosa roxburghii* fruit extract.** Ripe fruit were collected and processed from wild growing *Rosa roxburghii* plants in the Leye County of the Quangxi province in China. Collection occurred in the seasonal period between August and November at average daily temperatures of 19 °C. Juice was prepared as follows: within 4 h after harvesting, the fruits were washed with sterilized water, and air dried after which they were crushed by milling and cold pressed. This process yielded approximately 1.17 mL juice/g fresh fruit. The seeds were removed and juice was pasteurized at 57 °C under vacuum, which resulted in an approximately 30% loss of water content. The resultant juice was stored at 2 °C until use.

**Total phenolic and antioxidant capacity analysis.** Selected components of the juice were re-evaluated to establish the levels in the concentrated sample. The amount of total phenolics in the sample was determined according to the Folin-Ciocalteu method (Singleton and Rossi, 1965) and expressed as gallic acid equivalents (GAE) in grams per liter (g/L). Corrections for the contribution of ascorbic acid and sugars to the assay were done as previously described (Asami *et al*., 2003; Singleton *et al*., 1999). Total and free ascorbic acid were determined spectrophotometrically at 578 nm (Beutler, 1984). A sugar content of 37.5% was determined via the standard Musnen Walker method (Clarke, 1996). An individual polyphenolic analysis of the same RR fruit extract was recently reported (Janse van Rensburg *et al*., 2005). The oxygen radical absorbance capacity (ORAC) method was used to determine the antioxidant capacity of both lipophilic and hydrophilic extractions of the RR fruit extract (Prior *et al*., 2003; Ou *et al*., 2001). For this analysis, the sample was neutralized with KOH and filtered through a 0.2 μm filter. In the assay, fluorescein (56 nM) was used in a final volume of 200 μL as a target for free radical attack by AAPH (240 mM). A BioTEK fluorescence plate reader (FL-600) was used to measure the fluorescence decay of fluorescein (excitation 485nm, emission 520 nm) every 5 min for 2 h. Trolox was used as the standard and the antioxidant capacity was expressed as μM trolox equivalents (TE).

**Mutagenic/antimutagenic activity of the juice extract.** The *Salmonella typhimurium* bacterial reverse mutation assay with standard plate incorporation procedure was used to determine the possible mutagenic/antimutagenic activity of the juice (Maron and Ames, 1982). The dilutions of the RR fruit extract were between 50% and 2% (v/v) in water or DMSO. The *S. typhimurium* strains TA98, TA100 and TA102 were kindly supplied by Dr B. N. Ames (Children Hospital, Oakland Research Institute, Berkeley, USA). For the mutagenicity assay, 0.1 mL of the various RR fruit extract dilutions, 0.5 mL of S9 activation mixture and 0.1 mL of an overnight bacterial culture were carefully mixed with 2 mL of molten top agar containing 0.05 mM biotin-histidine. This mixture was dispersed onto minimal glucose agar plates. A liver S9 homogenate (0.72 nmol cytochrome P450/mg protein) was prepared by inducing male Fischer rats (200 g) with aroclor-1245 as described by Maron and Ames (1982). The S9 homogenate was incorporated into the S9 activation mixture at 2 mg protein/mL. Mutagenicity was determined by counting the number of $His^+$ revertant colonies after incubation of the plates at 37 °C for 48 h using a Quebec Colony Counter (American Optical Corp., Buffalo, New York). Each diluted sample was assayed using five plates per treatment.

Antimutagenic properties of RR fruit extract dilutions against various known mutagens were assessed using the standard plate incorporation assay as described above, with the exception that 0.1 mL of each mutagen, 2-acetylaminofluorene (2-AAF, 10 μg per plate), aflatoxin $B_1$ ($AFB_1$, 20 and 50 ng per plate) and methyl methanesulfonate (MMS, 20 mM) was used. The same mutagens were used as positive controls in the various strains during the mutagenicity assays. In the presence of S9 activation mixture the effect of the metabolic activated mutagens was monitored against TA98 and TA100, respectively. The direct acting mutagen, MMS, was monitored using TA102 in the absence of the S9 activating mixture. Control plates containing only top agar, various *S. typhimurium* strains and solvents ($H_2O$ or DMSO) were used to estimate the background caused by spontaneous revertant bacteria.

**Preparation of rat primary hepatocytes.** Male Sprague Dawley rats (body weight ≈300 g) were housed in standard laboratory rodent cages in a thermally controlled environment with free access to water and standard rodent diet. All procedures were carried out with the necessary ethical approval (number 03D04 of the North-West University, Potchefstroom, South Africa). Hepatocytes were isolated by *in situ* perfusion of the liver with collagenase (Wang and Lautt, 1997). Only those preparations with cell viability greater than 90%, as determined by the trypan blue exclusion test, were used. Primary hepatocytes were cultured in William's E medium supplemented with 10 mM HEPES, 10% fetal calf serum (FCS), 0.1 U/mL insulin, 2 mM L-gluthamine and antibiotics (penicillin 250 U/mL and streptomycin 250 μg/mL) at 37 °C under 95% humidity and 5% $CO_2$.





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The cells were either seeded onto collagen coated 96-well plates (Nunc) at a count of $4 \times 10^4$ cells/well for cytotoxicity studies, or 6-well plates (Nunc) at a count of $1 \times 10^6$ cells/well for genotoxicity studies (Comet assay). The medium was substituted after 2 h of incubation with William's E medium, supplemented with 0.5% serum, 0.1 U/mL insulin, 2 mM L-gluthamine, 2 mM proline, 10 mM pyruvate and antibiotics. All subsequent assays were performed in this medium.

**Cytotoxicity of the *Rosa roxburghii* fruit extract.** Cytotoxicity was assessed using the LDH-release assay (Korzeniewski and Callewaert, 1983) and the MTT assay (Alley *et al*., 1998). Primary rat hepatocytes were incubated with several dilutions of the RR fruit extract ranging from 0 to 5% (v/v) for 3 or 24 h under standard culture conditions. Higher concentrations could not be used as the dark colour of the sample interferred with the assays. With each dilution a reagent blank was included to compensate for the contribution of the sample to background levels in the assays. In a parallel set of experiments, cells were incubated with the addition of 0.8 mM *t*-BHP for the final 2 h to evaluate the effect of RR treatment on increased oxidative stress. In both sets of experiments treatment with Triton X-100 (0.1%) were used as a positive control to denote 100% relative cytotoxicity. Statistical analyses were performed using the untreated cells (0% RR) as a reference point in both sets of experiments.

**Genoprotection evaluation using the Comet assay.** Measurement of DNA single- and double-strand breaks was assessed by single cell gel electrophoresis (Singh *et al*., 1988). Rat primary hepatocytes were plated onto collagen-coated 6-well plates at a density of $1 \times 10^6$ cells/well. The cells were incubated for 14 h in the presence of various RR concentrations with or without the addition of 0.8 mM *t*-BHP during the final 2 h. To evaluate the effect of RR on DNA repair, the cells were either assessed immediately after these incubations or the medium was replaced with new medium and the cells incubated for an additional period of 15 or 30 min. Following the incubation periods, the cells were washed twice with phosphate-buffered saline (PBS), detached by incubation with collagenase, collected by centrifugation ($600 \times$ **g**), washed and resuspended in PBS. The cells were dispersed in low-melting agarose and spread onto an agarose-precoated microscope slide. After solidification, the slides were immersed overnight in a lysing solution (5 M NaCl, 0.4 M EDTA, 10 mL Triton X-100 and 10% DMSO) at 4 °C. The slides were then washed and transferred to an electrophoresis chamber and soaked with an alkaline electrophoresis buffer (0.6 M NaOH and 0.05 M EDTA, pH 13) for 25 min at 30 V. Electrophoresis was performed for 25 min. After neutralization with a buffer (0.5 M Tris.HCl, pH 7.5) nuclei were stained with ethidium bromide (20 μg/mL), and the %DNA in the tail measured using an Olympus X70 fluorescence microscope and further analysed using the CASP software program. The extent of DNA damage was classified into five different classes based upon the %DNA in the tail (Singh *et al*., 1988).

***In vitro* antioxidant capacity and glutathione analyses.** Primary rat hepatocytes were prepared as described before. Chang liver cells (hepatocellular carcinoma), which were also used, were cultured in Dulbecco's Modified Eagle's medium supplemented with 10% inactivated FCS and penicillin (250 U/mL) and streptomycin (250 μg/mL). MT-2 cells (murine lymphoblast), which were a kind gift from Dr A. Puren from the NICD, South Africa, were cultured in RPMI 1640 medium with 10% inactivated FCS with penicillin (250 U/mL) and streptomycin (250 μg/mL). All cell lines were incubated at 37 °C in a humidified incubator with 5% $CO_2$. The cells were seeded at a density of approximately $5 \times 10^6$ cells per 75 $cm^2$ flask and incubated for 24 h with the various RR dilutions as described before, with or without the addition of 0.8 mM *t*-BHP.

The cellular content of reduced (GSH) and oxidized glutathione (GSSG) was determined using the recycling assay described by Tietze (1969). Briefly, cells were washed twice with PBS, scraped from the bottom, suspended in PBS and divided into two fractions for the GSHt (total GSH) and GSSG determinations. M2VP was used as GSH scavenger in the GSSG assay. Sample preparation and analysis were subsequently performed as described previously (Asensi *et al*., 1999). The protein content in the fractions was determined prior to acid precipitation using the BCA method (Smith *et al*., 1985). The total antioxidant capacity of the cellular samples prepared for glutathione analysis was also determined using the ORAC assay as described in the previous section (Ou *et al*., 2001).

**Data analysis.** The results were expressed as mean ± STD. Student's *t*-test was used to establish statistical significance between means and a value of $p < 0.05$ was regarded as statistically significant.

## RESULTS

### Total phenolic and antioxidant capacity of *Rosa roxburghii* fruit extract

Direct measurement of the antioxidant capacity of the concentrated fruit extract revealed the total hydrophilic antioxidant capacity as measured in the ORAC assay was significantly higher (1153 mM TE) than that of the lipophilic antioxidant activity (1.3 mM TE). Thus, the total antioxidant capacity of the concentrated fruit extract was 1154 mM TE. The total phenolic content was measured at 64.9 g of GAE/L and a total ascorbic acid of 45.4 g/L of which 20% was in the free acid form.

### *In vitro* antioxidant capacity and glutathione redox state

*In vitro* incubation of primary rat hepatocytes with various concentrations up to 0.08% (v/v) of the fruit extract resulted in a significant ($p < 0.05$) and more than two-fold increase in antioxidant capacity using the ORAC method (Table 1). Surprisingly, at 0.32% the capacity was lower, albeit still significantly higher than the untreated cells, compared with higher dilutions of the extract. This would suggest that the increase in antioxidant capacity was limited by dilutions greater than approximately 0.08% of the fruit extract.





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**Table 1. Effect of *Rosa roxburghii* fruit extract on the glutathione redox state and antioxidant capacity of rat primary hepatocytes**

| *Rosa roxburghii* fruit extract (v/v) | GSH (nmol/μg) | GSSG (nmol/μg) | GSH/GSSG | Antioxidant capacity (μM TE) |
|---|---|---|---|---|
| 0% | 16.4 ± 0.5 | 0.62 ± 0.04 | 26.7 ± 2.5 | 1081 ± 140 |
| 0.005% | 13.3 ± 0.6[a] | 0.61 ± 0.03 | 21.7 ± 0.2[a] | 1454 ± 95 |
| 0.02% | 18.4 ± 0.5[a] | 1.24 ± 0.12[a] | 15.0 ± 1.9[a] | 1759 ± 197[a] |
| 0.08% | 12.7 ± 0.2[a] | 0.98 ± 0.19[a] | 13.3 ± 2.7[a] | 2354 ± 230[a] |
| 0.32% | 9.0 ± 0.5[a] | 1.01 ± 0.15[a] | 9.0 ± 0.8[a] | 1385 ± 40 |

[a] $p < 0.05$. GSH and GSSG indicate reduced and oxidized glutathione, respectively. Values are the mean ± STD ($n = 3$). Antioxidant capacity is expressed as trolox equivalents (TE) in deproteinated homogenates of the same cellular preparations used for the glutathione analyses. Dilutions are expressed as volume per 100 mL $H_2O$.

**Table 2. Effect of *Rosa roxburghii* fruit extract on the glutathione redox state of Chang liver cells**

| *Rosa roxburghii* (v/v) | GSH (nmol/mg) | GSSG (nmol/mg) | GSH/GSSG |
|---|---|---|---|
| 0% | 7.37 ± 0.19 | 0.068 ± 0.006 | 108 ± 13 |
| 0.04% | 6.83 ± 0.17 | 0.048 ± 0.004 | 143 ± 16 |
| 0.08% | 6.93 ± 0.13 | 0.029 ± 0.002 | 238 ± 24 |
| 1.2% | 6.29 ± 0.1[a] | 0.020 ± 0.003[a] | 318 ± 50[a] |
| 2.0% | 6.55 ± 0.07 | 0.006 ± 0.0004[a] | 1149 ± 77[a] |

[a] $p < 0.05$. Values are mean ± STD ($n = 2$) and expressed relative to protein content. Dilutions are expressed as volume per 100 mL $H_2O$.

The levels of GSH in hepatocytes exposed to increasing concentrations of the fruit extract was significantly decreased compared with untreated cells (Table 1). Inversely, a significant increase in GSSG levels was noted at all the fruit extract concentrations, except at 0.005% where the levels remained unchanged. Due to these changes in GSH and GSSG levels, the glutathione redox ratio decreased dose-dependently and significantly from 26.7 to 9.0 (Table 1). This was contrary to what was found in the two transformed cell lines that were investigated before. In Chang liver cells (hepatocellular carcinoma) the ratio increased significantly from 108 to 1149 at RR sample dilutions ranging from 0.04% to 2% (v/v) (Table 2). Similarly, a 0.8% RR sample dilution also increased the GSH/GSSG ratio in MT-2 lymphoblasts from a baseline ratio of 62 to 440 (results not shown). Total glutathione levels remained relatively constant in the *in vitro* analyses, suggesting that changes occur only in redox state and not in the total glutathione pool.

## Cytotoxicity

Both cytotoxicity assays (MTT and LDH-release) indicated that concentrations of up to 5% (v/v) of the RR fruit extract exhibited no enhanced toxic effect in the cells after 3 and 24 h exposure (Fig. 1). The oxidative stress inducer, *t*-BHP, increased toxicity in general and effectively reduced cell viability in both MTT (Fig. 1A-B) and LDH-release (Fig. 1C-D) assays relative to the Triton X-100-treated positive control. Co-incubation with various dilutions of RR fruit extract significantly protected the cells against *t*-BHP-induced toxicity in a dose-dependent manner. This effect could, however, be demonstrated much better in the MTT assay where a significant reduction of toxicity could be demonstrated at concentrations higher than 0.008% at 3 and 24 h. In both assays complete protection against *t*-BHP was achieved after both 3 and 24 h pre-incubation with 0.2% of the RR fruit extract.

## Genotoxicity

Untreated primary rat hepatocytes displayed baseline DNA damage with approximately 18% of cells falling into classes 4–5, which is an indication of major DNA damage (Fig. 2A). Co-incubation with RR fruit extract at 0.008% (v/v) reduced the percentage of cells in these two classes to 11% and almost 0% at higher concentrations. With the inclusion of *t*-BHP (Fig. 2B) DNA damage clearly increased when compared with the baseline, with 15% of the comets falling into class 5 and a notable decrease in class 1 (undamaged cells). With co-incubation of 0.008% (v/v) RR fruit extract, class 5 comets decreased markedly (±15%), while at higher concentrations the *t*-BHP-induced damage was not detected. Comparison of DNA repair over a 15 and 30 min time period after *t*-BHP-induced oxidative damage (Fig. 2C and -D) showed a notable decrease of DNA tail% in classes 2–5 when 0.2% (v/v) of the RR fruit extract was included in the culture medium during the two repair periods (Fig. 2D).

## Mutagenic and antimutagenic activity of *Rosa roxburghii* fruit extract

Table 3 summarizes the results of the *Salmonella typhimurium* mutagenicity and antimutagenicity assays. No toxicity or mutagenic activity of the different RR





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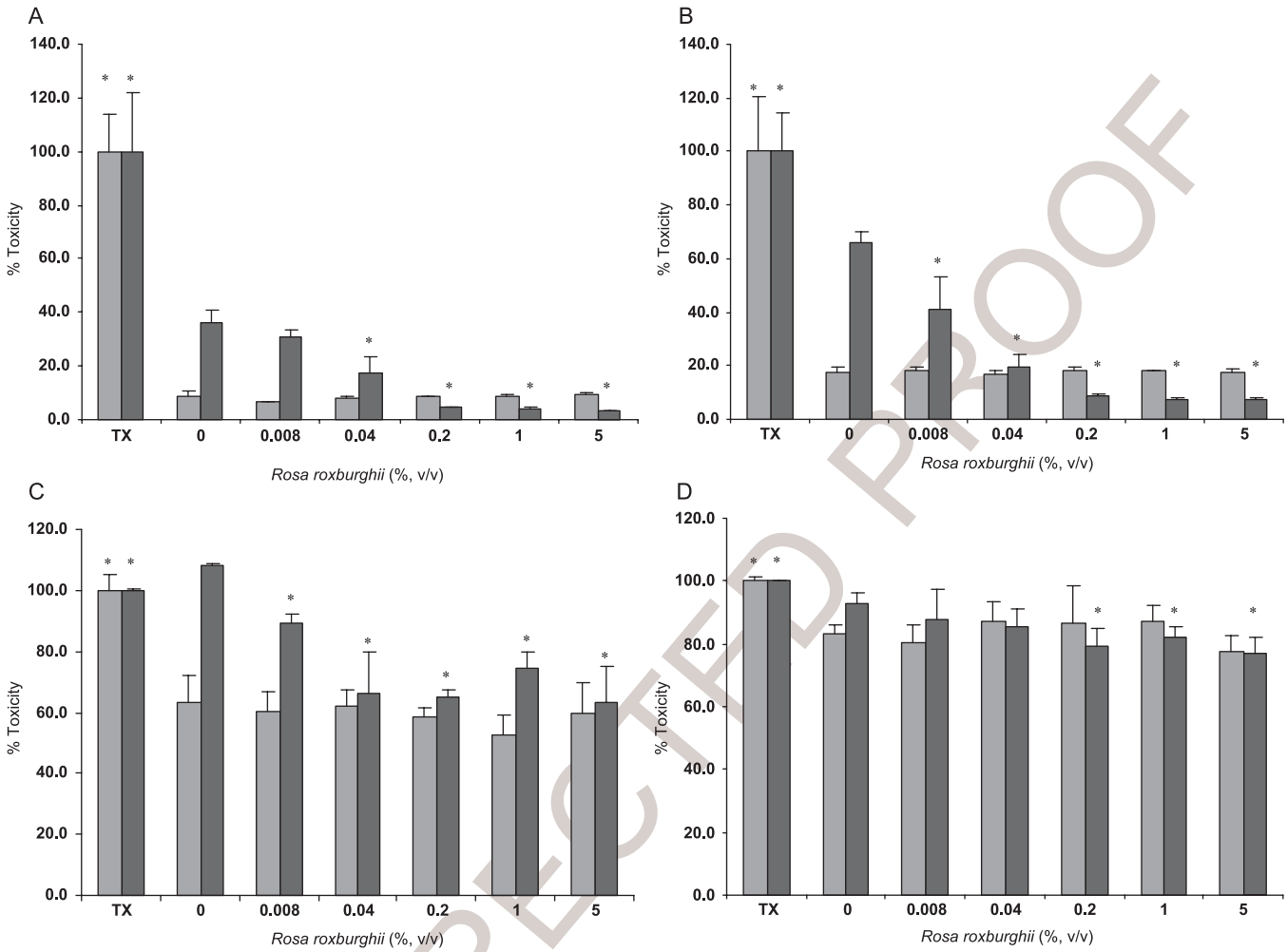


**Figure 1.** Cytotoxicity analyses of *Rosa roxburghii* fruit extract. Dilutions of a *Rosa roxburghii* fruit extract (volume per 100 mL $H_2O$, %) were incubated with primary rat hepatocytes for 3 h (A and C) and 24 h (B and D) for the MTT assay (A and B) and LDH-release assay (C and D), respectively. Triton X-100 (TX) was used as a positive control in incubations at 0.1% (v/v) and denotes 100% toxicity. Light grey bars indicate RR treatment only and dark grey bars indicate a parallel series of experiments where additions of 0.8 mM *t*-BHP were also included for the final 2 h of the incubation with RR. All statistically significant changes ($p < 0.05$) compared with untreated cells (0% RR) in each series of experiments are indicated by the asterisk (*). Values are given as a mean of triplicate analyses with standard deviation.

**Table 3. Antimutagenic activity of *Rosa roxburghii* fruit extract against various carcinogens used in the Salmonella assay**

| | Histidine revertants per plate | | | | | |
|---|---|---|---|---|---|---|
| Strain | TA98 | | TA100 | | TA102 | |
| Mutagen | – | +2-AAF (10 ng/plate) | – | +AFB (20 ng/plate) | – | +MMS (20 mM/plate) |
| Control (–) | 25 ± 7 | 27 ± 4[a] | 91 ± 7 | 123 ± 5[a] | 154 ± 10 | 189 ± 9[a] |
| Control (+) | Nd | 626 ± 128 | Nd | 333 ± 42 | Nd | 750 ± 16 |
| RR (2%) | 29 ± 7 | 382 ± 27[a] | Nd | Nd | Nd | Nd |
| RR (10%) | 34 ± 10 | 108 ± 32[a] | 86 ± 12 | 101 ± 24[a] | 169 ± 8 | 740 ± 76 |
| RR (20%) | 34 ± 4 | 80 ± 21[a] | 88 ± 9 | 68 ± 16[a] | 193 ± 14 | 732 ± 32 |
| RR (50%) | 42 ± 9 | 27 ± 7[a] | 81 ± 12 | 54 ± 28[a] | 176 ± 5 | 608 ± 48[a] |

[a] $p < 0.05$.

Nd, not done. RR, *Rosa roxburghii* fruit extract dilutions expressed as volume per 100 mL $H_2O$. Columns indicate the mean ± STD ($n = 3$) of bacterial revertants without (for mutagenicity test) or with specific mutagens (for antimutagenicity test). All controls had no RR added and in the case of the negative control for antimutagenicity, no mutagen was added either.

fruit extracts was shown in any of the three test strains in the presence and/or absence of metabolic activation. The spontaneous revertant counts were in the range of published values (Maron and Ames, 1983). The most concentrated RR fruit extract (50%, v/v) used in the assay did not significantly increase the number of bacterial revertants.

In fact, the same dilution significantly protected against mutagenicity induced by 2-AAF, $AFB_1$ and MMS. The positive control plates showed a 23-fold increase of



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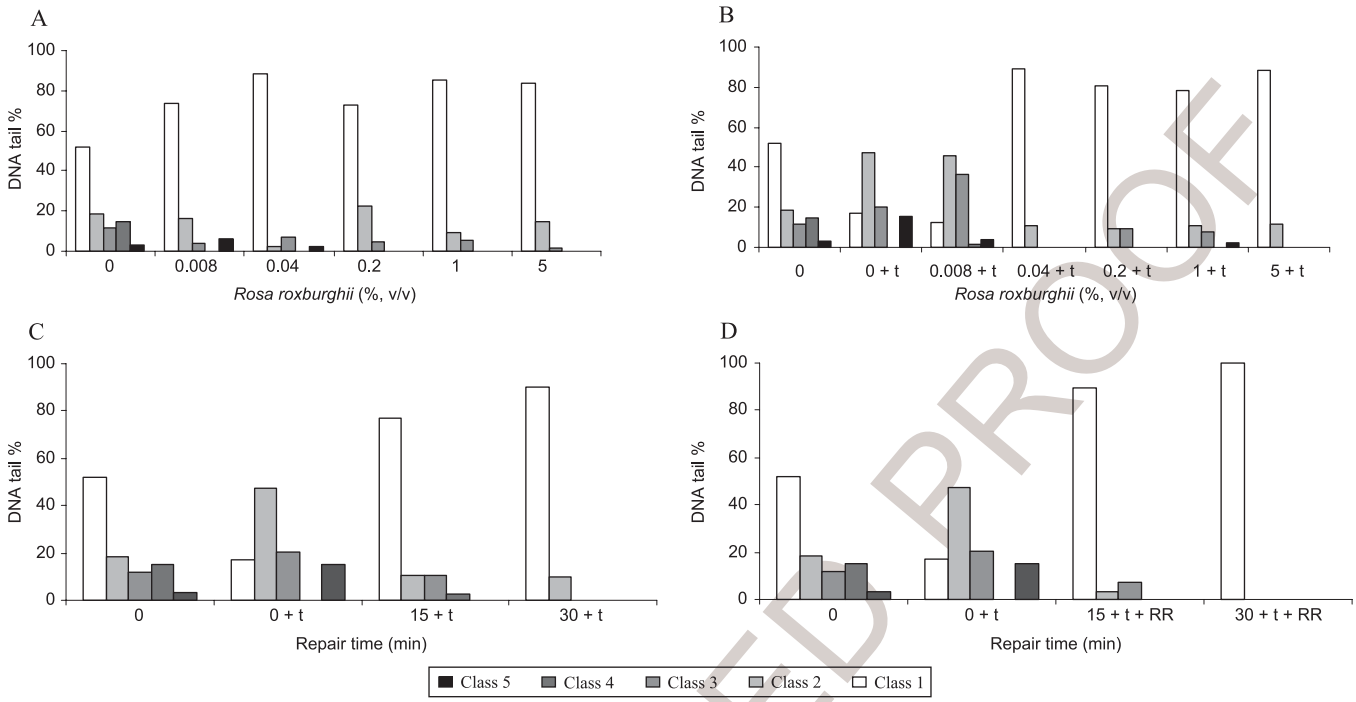


**Figure 2.** Protective effect of *Rosa roxburghii* fruit extract on oxidative stress-induced DNA damage in rat primary hepatocytes. Single- and double-strand DNA breaks were estimated using single cell gel electrophoresis (comet assay) in the absence (A) or presence (B) of 0.8 mM *t*-BHP (+t) at various RR dilutions (volume per 100 mL $H_2O$, %). DNA repair in cells in the absence of RR fruit extract after *t*-BHP-induced DNA damage was also compared over 15 and 30 min (C) as well as in the presence of 0.2% (v/v) RR fruit extract (D). The extent of DNA damage was expressed as the %DNA in the tail and the comets were grouped in the following classes according to the extent of DNA damage (Singh *et al.*, 1988). This information was generated by the CASP scoring programme: class 1, < 6%; class 2, 6.1–17%; class 3, 17.1–34%; class 4, 34.1–60%, class 5 > 60%.

mutagenicity for TA98, 37-fold for TA100 and four-fold for TA102 strain when compared with the spontaneous revertant plates. Mutagenicity of $AFB_1$, which requires metabolic activation, was significantly decreased in all dilutions of the fruit extract. At both 20 ng and 50 ng per plate (results not shown) of $AFB_1$, all fruit extract dilutions protected against $AFB_1$-induced mutagenicity. The RR fruit extract showed a significant dose-dependent protection against 2-AAF-induced mutagenicity in the tester strain TA98. In this regard the 2% fruit extract effectively reduced the number of $His^+$ revertants by half. Complete protection against 2-AAF-induced mutagenicity was achieved at 50% dilution. When using the direct acting mutagen, MMS, only the 50% (v/v) RR fruit extract showed a significant protective effect.

## DISCUSSION

The putative health promoting effects of *Rosa roxburghii* fruit are mainly attributed to its antioxidant properties (Janse van Rensburg *et al.*, 2005; Zhang *et al.*, 2003; Ma *et al.*, 1997). This study was conducted to establish whether these properties contribute to the antioxidant status and afford protection against oxidative stress. In addition, possible toxicity and mutagenicity activity were also assessed *in vitro*. The RR fruit extract had a remarkably high total antioxidant capacity of 1154 mM TE, considering values of commonly known fruit juices that range between 3 and 30 mM TE (Prior *et al.*, 2003). This is most likely due to the exceptionally high amount of polyphenols (64.9 g of GAE/L fruit juice) and ascorbic acid (45.4 g/L), all contributing to the antioxidant capacity of the sample. Although it is not accurate to compare these values of the concentrated RR fruit extract to other fruit products, it remains to be remarkably high when taking into account the polyphenol content of blueberry juice amounts of 2.6 g GAE/L (Pedersen *et al.*, 2000) and the ascorbic acid content of orange juice amounts to only 0.78 g/L (Rapisarda *et al.*, 1999).

Data from the current study indicate that the RR fruit extract not only enhances the antioxidant capacity (as measured by the ORAC assay) but also significantly protects against induced oxidative stress in primary rat hepatocytes. In this *in vitro* system no cytotoxic activity was noted at RR fruit extract concentrations that far exceed that needed for effective protection against *t*-BHP-induced oxidative stress. Furthermore, the genotoxicity investigation (COMET assay) indicated that the RR fruit extract lowered baseline DNA damage in primary rat hepatocytes and also protected against *t*-BHP-induced oxidative DNA damage. Similar observations have been described for several natural plant products containing diverse nutritional content (Mayne, 2003; Duthie *et al.*, 1996; Pool-Zobel *et al.*, 1997).

Elevated antioxidant status has been linked to a modulation of the glutathione redox state, which is probably due to a protective effect of the reduced form of glutathione during oxidative stress, rather than an increase in the GSH pool (Griffith, 1999). An increase in the glutathione redox state, however, was not observed when treating primary rat hepatocytes with RR. It was, however, previously reported that several key enzymes, including glutathione reductase, catalase





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and cytochrome P450 were significantly inhibited by perfusion and isolation (Richert *et al*., 2001; Fahrig *et al*., 1998). This suggests that primary hepatocytes are not the ideal cellular system to investigate the contribution of a putative modulator to GSH metabolism and redox fluxes *in vitro*. An RR-induced increase in glutathione redox state was, however, demonstrated in two carcinoma cell lines, Chang liver cells and MT-2 lymphoblasts.

The RR fruit extract was not mutagenic using three *S. typhimurium* strains. These included TA98, monitoring frame shift mutations, TA100 monitoring base-pair substitutions and TA102 which is sensitive to both transitions and transversions and by mutagens that cause oxidative damage (Mortelmans and Zeiger, 2000). In addition, the RR fruit extract dose-dependently protected against the metabolically activated carcinogens, 2-AAF and $AFB_1$ in T98 and TA100, respectively. In TA102 only the most concentrated form of the fruit extract (50%, v/v) protected significantly, although slightly, against the direct acting mutagen, MMS. Herbal products containing high levels of polyphenols such as herbal teas also cause similar weak protection against this direct acting mutagen (Marnewick *et al*., 2000; Yamada and Tomita, 1994).

The relatively high ascorbic acid and polyphenol content of *Rosa roxburghii* fruit makes it a very attractive and interesting consideration as a natural antioxidant supplement. Previous studies have indeed concluded that it has beneficial or health promoting properties in humans (Janse van Rensburg *et al*., 2005; Zhang *et al*., 2003; Ma *et al*., 1997) which can most likely be linked to an increased antioxidant status. Although further investigations are warranted, these *in vitro* results using an unrefined RR fruit extract support these putative health promoting properties and specifically those relating to protection against cellular oxidative stress.

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