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Induction of Cell Death in Caco-2 Human Colon Carcinoma Cells by Ellagic Acid Rich Fractions from Muscadine Grapes (*Vitis rotundifolia*)

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Possible anticancer mechanisms exerted by polyphenolic compounds contained in fruits and vegetables include antioxidant activity, the inhibition of proliferation, and the induction of apoptosis in cancer cells. This study examined the effects of four isolated polyphenolic extracts from red muscadine grapes (*Vitis rotundifolia*) on vital cell parameters and the induction of apoptosis in Caco-2 colon carcinoma cells. The magnitude of effects in cell culture was then correlated to polyphenolic composition and antioxidant capacity. Whereas anticancer effects of individual polyphenolic compounds have been demonstrated multiple times, information relating to anticancer effects of polyphenolic extracts is not available in abundance. All four extracts induced apoptosis, decreased cell number, and caused alterations in cell cycle kinetics in a concentration-dependent manner. The efficacy of the polyphenolics on vital cell parameters correlated well to the presence of ellagic acid glycosides and flavonoids and also to the antioxidant capacity. This study demonstrated the anticancer properties of ellagic acid rich extracts from red muscadine juice.

KEYWORDS: Polyphenols; anticancer; red wine; apoptosis

INTRODUCTION

The polyphenolic compound content of grapes has been the focus of increasing interest due to their potential health benefits from the consumption of fresh fruit, wine, and juice. Among fruits and vegetables, red grapes contain high polyphenolic compound concentrations, and their anticancer activity appears to be partially based on the ability to quench reactive oxygen species and the protection of critical cellular components such as DNA, proteins, and lipids from oxidative insult (*1*, *2*). Polyphenols and phenolic extracts from grape wine have been found to exert anticarcinogenic activity by decreasing growth, inducing apoptosis, altering cell cycle kinetics, and interfering with intracellular signal transduction events in cancer cells (*3*−*11*).

Specifically, the muscadine grape (*Vitis rotundifolia*) contains relatively high concentrations of polyphenolic compounds such as hydroxybenzoic acids, hydroxycinnamic acids, stilbenes, and flavonoids including anthocyanin 3,5-dilgucosides, quercetin, myricetin, and kaempferol when compared to other fruits and vegetables (*12*, *13*). The presence of ellagic acid and ellagic acid derivatives in muscadine grapes is unique among *Vitis* varieties, whereas it is a major compound in fruits such as blueberries, blackberries, raspberries, and cranberries (*12*, *14*, *15*). The anticancer properties of free ellagic acid and polyphenolic extracts that contain ellagic acid was demonstrated in several studies (*16*−*18*) and also enhanced the anticancer activity of other polyphenolic compounds (*19*, *20*).

Although muscadine grapes are predominantly cultivated in the South and Southeastern US in a limited quantity, interest in this fruit has been growing due to its unique phytochemical composition as recently identified for individual ellagic acid glycosides and ellagitannins (*21*). The antioxidant capacities of ellagic acid derivatives and muscadine grapes have been previously evaluated (*22*−*24*); however, more information is required to validate the anticancer properties of these compounds. The anticancer activities of ellagic acid as a pure compound and ellagitannins have previously been evaluated as well as extracts from fruits or plant materials (*25*−*27*). The contributions of different ellagic acid glycosides to the potential anticancer effects of muscadine grape juice have not been previously investigated.

Therefore, the objective of this study was to examine the effects of polyphenolic extracts isolated from red muscadine grapes that were rich in free ellagic acid or ellagic acid glycosides (**Figure 1**) on vital cellular parameters, apoptosis,

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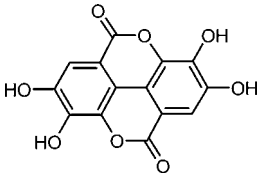

Figure 1. Chemical structure of ellagic acid.

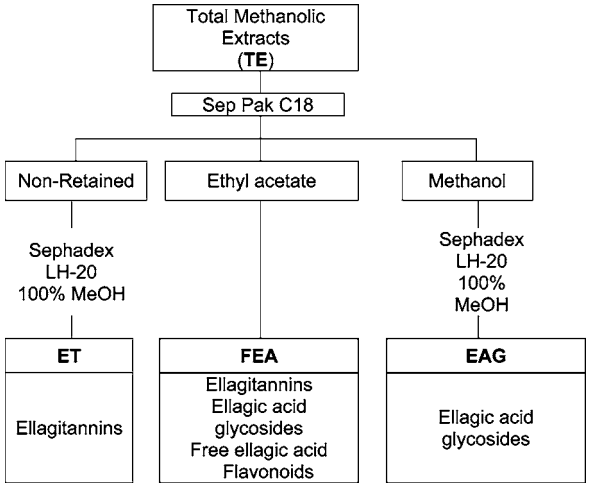

Figure 2. Flow diagram for the extraction and partition procedure utilized to isolate polyphenolic compounds present in muscadine grapes. TE = total extract; ET = ellagitannins; FEA = flavonoids and ellagic acid; EAG = ellagic acid glycosides.

and the generation of reactive oxygen species (ROS) in a Caco-2 colon carcinoma cell culture model, with the goals of determining the anticarcinogenic effects of these extracts and to potentially relate the magnitude of effects to the extract composition. Results from this study significantly contribute to the knowledge regarding the potential of ellagic acid rich extracts from muscadine grapes in cancer prevention.

MATERIALS AND METHODS

Processing of Grapes and Chemical Analysis. Red muscadine grapes (cv. Albemarle) and isolated extracts used for the cell culture experiments were identical to those previously described (*22*) (**Figure 1**). In brief, polyphenolics were extracted from the skin and pulp with 100% methanol (0.01% 12 N hydrochloric acid). Extracts were filtered through Whatman No. 4 filter paper, solvent was removed under reduced pressure, and the polyphenolics were dissolved in either dimethyl sulfoxide (DMSO) resulting in the total methanolic extract (TE) or dissolved in 0.1 M citric acid buffer at pH 3.5 for further polyphenolic isolation. Those polyphenolics were then fractioned from Waters C18 Sep-Pak and Sephadex LH-20 cartridges and partitioned based on their solubility in ethyl acetate and/or methanol (**Figure 2**). The TE isolate was applied to a C18 cartridge, and the nonretained fraction was collected and subsequently partitioned from Sephadex LH-20 to obtain an ellagitannin-rich extract (ET). Those polyphenolics retained on the C18 cartridge were eluted first with ethyl acetate to obtain a fraction rich in free ellagic acid and flavonoids (FEA). Remaining compounds were eluted with 100% methanol and following solvent removal and dissolution in buffer were partitioned from a Sephadex LH-20 cartridge first with 10% (v/v) methanol to remove anthocyanins and then with 100% methanol to collect the remaining ellagic acid glycosides (EAG). Following solvent removal, polyphenolics were dissolved in DMSO for in vitro experiments. Phosphate-buffered saline was used as a diluent for all in vitro assays.

As previously described, polyphenolics were evaluated by HPLC−PDA and HPLC−MS^n analysis (±ESI ion source, ion trap) to identify free ellagic acid, flavonol glycosides, and several ellagic acid derivatives (*21*). Free and conjugated ellagic acids were quantified in ellagic acid equivalents. Total ellagic acid, representing free ellagic acid liberated from the ellagic acid derivatives, was assessed following acid hydrolysis as described by Lee and Talcott (*23*). Total soluble phenolics were additionally analyzed using the Folin-Ciocalteu assay (*28*), and antioxidant capacity (AOX) was measured using the oxygen radical absorbance capacity (ORAC) as previously described (*23*). Dilutions of all isolates are labeled as 0 = control without any extract, 1 = single strength, 10 = 10-fold dilution, 100 = 100-fold dilution, and 1000 = 1000-fold dilution. All dilutions are based on the concentrations initially present in the grape.

Cell Culture. Caco-2 colon carcinoma cells (American Type Culture Collection (ATCC), Manassas, VA), were maintained in DMEM medium containing 10% fetal bovine serum (FBS), 2 mmol/L L-glutamine, 100 000 units/L penicillin, 0.1 g/L streptomycin, 0.25 mg/L fungizone, 0.05 g/L gentamycin, 0.1 mM/L nonessential amino acids, and 1 mM/L sodium pyruvate (Gibco BRL, Grand Island, NY).

The extracts, which were dissolved in DMSO, were diluted with phosphate-buffered saline and added to 5×10^8 cells/L with a maximum final DMSO (Sigma Chemical Co., St. Louis, MO) concentration of 5 mL/L. A control with 5 mL/L DMSO was analyzed for all assays. Cell number and viability were assessed in a Neubauer hemacytometer by trypan blue dye exclusion.

Cell Cycle Kinetics. Cells were treated with different concentrations of extracts for 24 h. Cells were trypsinized, washed twice in phosphate-buffered saline (PBS), and fixed in 60% ethanol (v/v in PBS) at −20 °C for at least 30 min. Debris and ethanol were removed by underlying samples with FBS and centrifuging at 290*g* for 3 min. After centrifugation cells were incubated with 125 μL of 500 000 units/L ribonuclease in 38 mmol/L sodium citrate buffer for 15 min at 37 °C and stained with propidium iodide (0.05 g/L in 38 mmol/L sodium citrate buffer). Analysis was conducted by flow cytometry (488 nm excitation and 620 nm emission wavelengths) on a FACScan flow cytometer (BD Immunocytometry Systems, San Jose, CA). Histograms were quantitatively analyzed with the ModFit LT software (Verity Software House, Inc., Topsham, ME).

Active Caspase-3 Assay. Caco-2 cells were trypsinized, washed twice in PBS, and stained with a PE-conjugated antibody that specifically targets the active form of caspase-3, according to the manufacturer's protocol (BD BioSciences, San Jose, CA). Analysis was performed by flow cytometry on a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Results were quantified as the percentage of active caspase-3-positive cells of the total cell population.

Generation of Reactive Oxygen Species (DCF Assay). The DCF (dichorofluorescein) assay was performed as previously described (*26*). Cells were passed into 96-well plates and at 70% confluency, washed twice with PBS, and incubated with 10 μmol/L dichorofluorescein diacetate (DCFH-DA) for 30 min at 37 °C in order to preload cells with DCFH-DA substrate. Cells were washed twice and incubated with different concentrations of the extracts. Fluorescence was determined after 20 min incubation with polyphenols using an F-max spectrofluorometer (Molecular Devices, Sunnyvale, CA), at 538 nm excitation and 485 nm emission wavelengths.

Statistical Analysis. Data from in vitro experiments were analyzed by one-way analysis of variance (ANOVA) with the JMP software (SAS Institute Inc., Cary, NC, 1996). Differences were deemed significant at $p \leq 0.05$ using a Tukey-Cramer HSD comparison for all pairs. The analysis of linear (pairwise) correlations was performed on chemical data prior to extract dilutions for cell culture assays and compared with results from the in vitro assays. Correlations with a *p*-value less than 0.05 were deemed significant.

RESULTS AND DISCUSSION

Chemical Analyses. Detailed phytochemical analyses of each fraction were previously described (*22*), with concentrations of ellagic acid and its glycosides (**Table 1**) presented herein. The antioxidant capacity of the four extracts was determined as 7.45, 0.8, 3.6, and 0.4 μmol/g Trolox equivalents for TE, ET, FEA, and EAG, respectively. The concentrations of total soluble phenolics were 1310, 188, 424, and 59.1 mg/L for TE, ET, FEA,

Table 1. Concentration of Ellagic Acid and Ellagic Acid Glycosides in Four Polyphenolic Fractions Obtained from Muscadine Grape (mg/L) (*22*)

extract[a]	free EA	EA glucoside	EA xyloside	EA rhamnoside	total EA
TE	32.9 ± 3.42a	7.80 ± 0.45a	20.0 ± 2.42a	37.6 ± 3.39a	912 ± 5.54a
ET	0.50 ± 0.12c	ND	ND	ND	53.7 ± 1.27c
FEA	27.2 ± 0.01b	ND	4.60 ± 0.01b	12.1 ± 0.01b	130 ± 2.75b
EAG	2.45 ± 0.13c	4.55 ± 0.11b	6.95 ± 0.65b	12.8 ± 1.57b	33.0 ± 0.05c

[a] Extracts were prepared by using Sep-Pak C18 and Sephadex LH-20 cartridges and elution with either methanol or ethyl acetate (see Materials and Methods). Values are means ± SD, $n = 2$; values within columns having the same letters are not significantly different (LSD test, $p \leq 0.05$). ND, concentrations were below the detection limit of 0.05 mg/L; EA, ellagic acid (*22*).

and EAG respectively (*22*). Significant differences in phytochemical composition existed among the four extracts, and each contained a broad range of polyphenolic compounds. The initial TE isolate consisted of numerous phenolic acids, flavonol glycosides (rhamnosides of quercetin, myricetin, and kaempferol), anthocyanins (3,5-diglucosides), free ellagic acid, ellagitannins (hexahydroxydiphenoyl derivatives), and ellagic acid glycosides (glucoside, xyloside, and rhamnoside). Subsequent fractions were then distinguished based on the presence or absence of these compounds. TE also contained the highest concentrations of ellagic acid and ellagic acid glycosides with the highest values for antioxidant capacity and total soluble phenolics followed the FEA extract that contained high concentrations of free ellagic acid and flavonol glycosides. Antioxidant capacity and concentrations of total soluble phenolics were similar between ET, with high ellagitannin concentrations (data not shown), and EAG with its high concentrations of free ellagic acid and ellagic acid glycosides. Due to varying concentrations of ellagic and ellagic acid derivatives among the fractions in relation to their respective antioxidant capacities, the extracts were normalized by calculation for the comparison to an equivalent of 100 mg/L total ellagic acid. The calculated antioxidant capacity of the constituents in TE, ET, and EAG was equal to 1 μM Trolox equivalent/mL, whereas that for FEA was 3 times higher. Differences were attributed to flavonols and other identified phenolic acids that were previously shown to be present in extracts of muscadine grapes.

Cytotoxicity and Total Cell Number. Total cell number was an indicator of the proliferative activity of the cell population and the cytotoxic effects of each extract. The total cell number was decreased by all extracts in a concentration-dependent manner (**Figure 3A**). TE was the most effective isolate across all dilutions in reducing the total cell number by 45% of the control at a 1:1000 dilution and by 89% at a 1:10 dilution. The high polyphenolic concentration and diversity of compounds present in TE correlated with the high efficacy in the reduction of cell growth, with the 1:10 dilution showing an equivalent reduction in cell number as the undiluted FEA and EAG extracts. This high efficacy may also be enhanced by the presence of anthocyanins that alone have significant anticancer properties or by synergistic effects among the polyphenolics as previously demonstrated through in vitro models (*18*, *19*, *26*, *29*, *30*). FEA reduced cell numbers by 11−98% over a dilution range from 1:1000 to undiluted, while EAG and ET also altered the cell number ratio to the control from 71% to 12% and from 108% to 31%, respectively, across the same dilution range. Overall, alterations in cell number induced by ET were lower than those induced by FEA and EAG. Conversely, EAG induced a higher effect on cell growth (undiluted and at the 1:1000 dilution) compared to the ET extract, though the ET extract contained more total soluble phenolics and total ellagic acid and had a higher ORAC value and therefore was expected to be more efficacious in the reduction of cell proliferation. This observation may in part be explained by the presence of ellagic acid glycosides in EAG, whereas ET contained predominantly hydrolyzable tannins such as gallotannins and ellagitannins. Ellagic acid glycosides were potentially more bioavailable (or more efficacious) in Caco-2 cells than the highly polar ellagitannins present in ET. The hydrolysis of glycosides esterified to polyphenolics by Caco-2 cells was previously demonstrated (*31*−*33*), and may have increased the uptake of ellagic acid glycosides and enhanced their effect on total cell numbers. The ability of individual polyphenolics to reduce cell growth and proliferation was previously investigated, and compounds such as resveratrol, quercetin, ellagic acid, catechin, and anthocyanins exhibited beneficial effects (*18*, *29*, *34*−*37*). Fewer studies investigated the influence of polyphenolic extracts, such as those found in red wine and other polyphenol-rich foods, on vital cellular parameters in cancer cell lines. Polyphenolic extracts from wine were found to inhibit proliferation in different cell lines in micromolar concentration ranges (*4*, *5*, *8*, *10*). These studies confirm that polyphenolic-rich extracts from grape products have the potential to reduce proliferative activity in cancer cells. This study also demonstrated similar effects for ellagic acid rich fractions from muscadine grapes applied to cell culture in concentration ranges equivalent to a micromolar range of total soluble phenolics

Apoptosis, Caspase-3 Activity. Caspase-3 activity was determined in order to quantify the effect of the polyphenolic isolates on apoptosis, since caspase-3 is one of the major executing enzymes in apoptosis. In previous studies single compounds such as quercetin, ellagic acid, resveratrol, and gallic acid have been shown to induce apoptosis in several cell lines at different concentrations (*38*−*44*). Also more complex mixtures of polyphenolics were found to induce apoptosis in vitro. Matito et al. found differences in the magnitude of induced apoptosis dependent on the polyphenolic composition of grape extracts (*45*). Anthocyanins from red wine were also found to induce apoptosis in MOLT-4B cells (*6*), and grape seed extract induced apoptosis in human prostate carcinoma cells (*46*, *47*). Moreover, extracts from ellagic acid containing fruits, such as pomegranate and blueberries, were found to induce apoptosis in cancer cells (*25*, *48*). In this study, all of the extracts induced caspase-3 activity in a concentration-dependent manner. Comparing among the single-strength extracts, FEA induced the highest percentage of caspase-3 activity at 42% followed by EAG at 34% and ET at 27% (**Figure 3B**). FEA had a higher antioxidant capacity, total soluble phenolics, and total ellagic acid concentration than ET and EAG, yet its concentration of ellagic acid glycosides (16.7 mg/L) was comparable to that of EAG (22.8 mg/L). These properties were likely responsible for the pro-apoptotic effect exhibited by FEA. Similar to the effect on total cell number EAG also demonstrated an appreciable pro-apoptotic effect when compared to ET, despite a higher antioxidant capacity, total soluble phenolics, and total ellagic acid present in ET. Therefore the occurrence of ellagic acid glycosides found in FEA and EAG was more influential on apoptotic activity in relation to the hydrolyzable tannins present

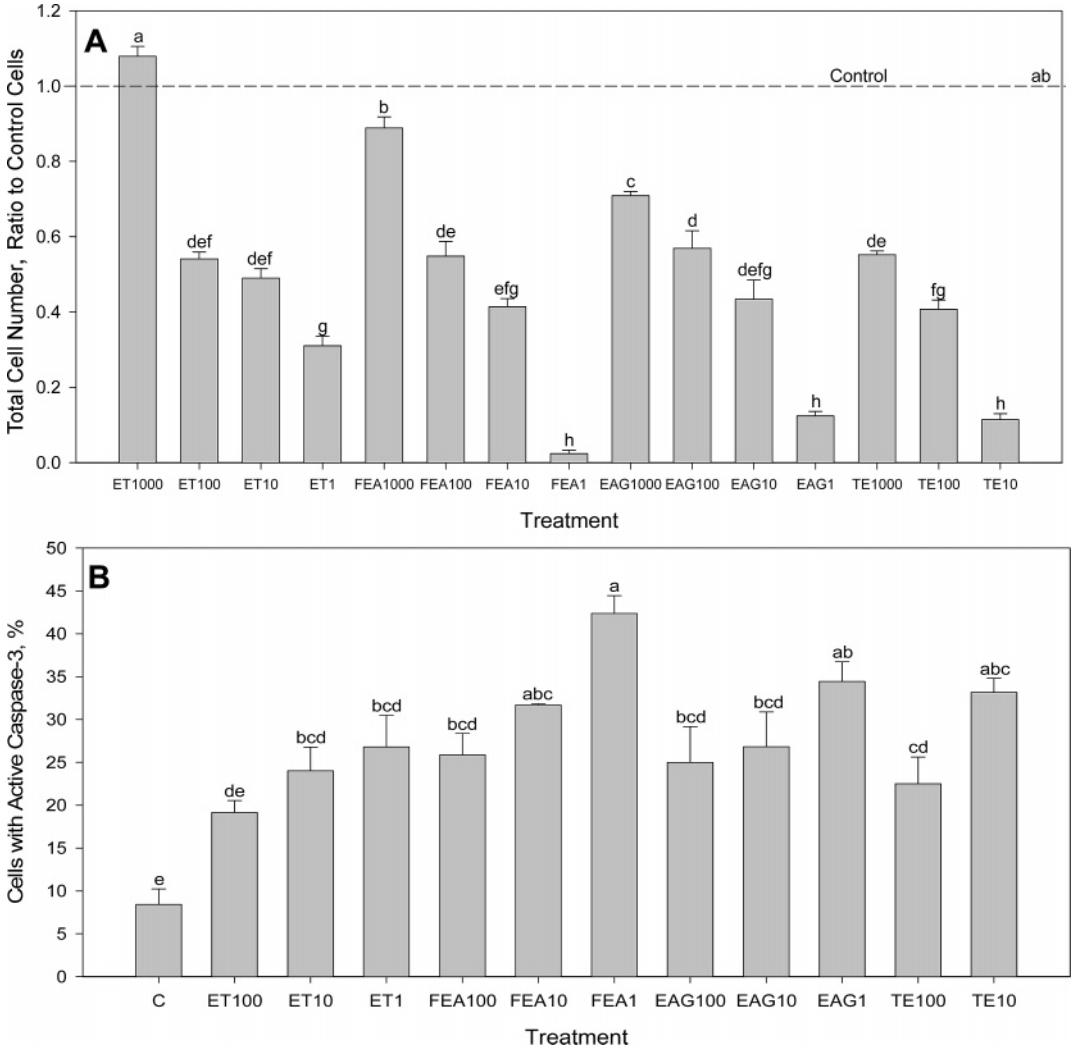

Figure 3. (**A**) Total cell number expressed as a ratio to control cells and viability as percentage of live cells of the total cell population. Caco-2 cells were treated with muscadine extract and fractions for 48 h. Values are means ± SEM, $n = 4$. Means with different letters differ significantly, $p < 0.05$. The dashed horizontal line indicates the control cells. (**B**) Caspase-3 activity in Caco-2 cells after 8 h of treatment with muscadine extract and fractions. Values are means ± SEM, $n = 3$. Means with different letters differ significantly, $p < 0.05$. The dashed horizontal line indicates control cells. TE = total extract; ET = ellagitannins; FEA = flavonoids and ellagic acid; EAG = ellagic acid glycosides; 1 = single strength; 10 = 10-fold dilution; 100 = 100-fold dilution; 1000 = 1000-fold dilution. All dilutions are based on the concentrations initially present in the grape.

in ET. The extent of caspase-3 activity induced by TE at 10- and 100-fold dilution was comparable to those of the other extracts at the same dilutions. Based on total cell numbers, where TE appeared to have a higher effect compared to the other extracts, TE appears to less potent in the induction of caspase-3.

Cell Cycle Kinetics. Alterations in cell cycle kinetics induced by single polyphenolic compounds from grapes have been extensively reported (*19*, *39*, *40*, *49*, *50*). However, few studies have examined the effect of wine extracts or any whole food extract on cell cycle kinetics. In an in vitro study by Kamai et al., flavonoid-rich extracts from red wine extract were found to induce cell cycle arrest in the S and G_2/M phases (*8*). In this study, cell cycle kinetics were altered by all muscadine grape extracts in a concentration-dependent manner (**Figure 4**), as observed for cell death and caspase-3 activation. Overall, each extract induced a significant decrease in the G_0/G_1 phase, with TE being the most effective with significant decreases even at a 1:100 dilution. FEA induced a significant decrease at 1:10 dilution, and ET and EAG induced a significant decrease at single strength. TE induced a significant increase in the S phase at 1:100 dilution, and FEA induced a significant decrease at single strength. The G_2/M phase only was increased by EAG at single strength. Overall, EAG induced the highest change at single strength decrease in the G_0/G_1 phase from 45% to 18%. TE was the only extract that induced a significant alteration in the cell cycle kinetics at a 1:100 dilution.

Generation of Reactive Oxygen Species (ROS) (DCF Assay). The activation of caspase-3 by muscadine extracts demonstrated the involvement of apoptosis in causing cell death. The DCF assay was performed in order to assess whether the generation of ROS had a significant role in the induction of apoptosis by the extracts. The influence of polyphenolic compounds from wine on the generation of ROS was previously reported, where quercetin induced an increase in the generation of ROS, while apoptosis was induced (*26*). In myeloid leukemic cells (−)-epigallocatechin-3-gallate has also been determined to increase the generation of ROS while apoptosis was induced (*51*).

In this study, a significant influence on the generation of ROS was observed for all extracts with the exception of FEA (**Figure 5**). ROS generation was induced by ET dilutions from 1:10 to

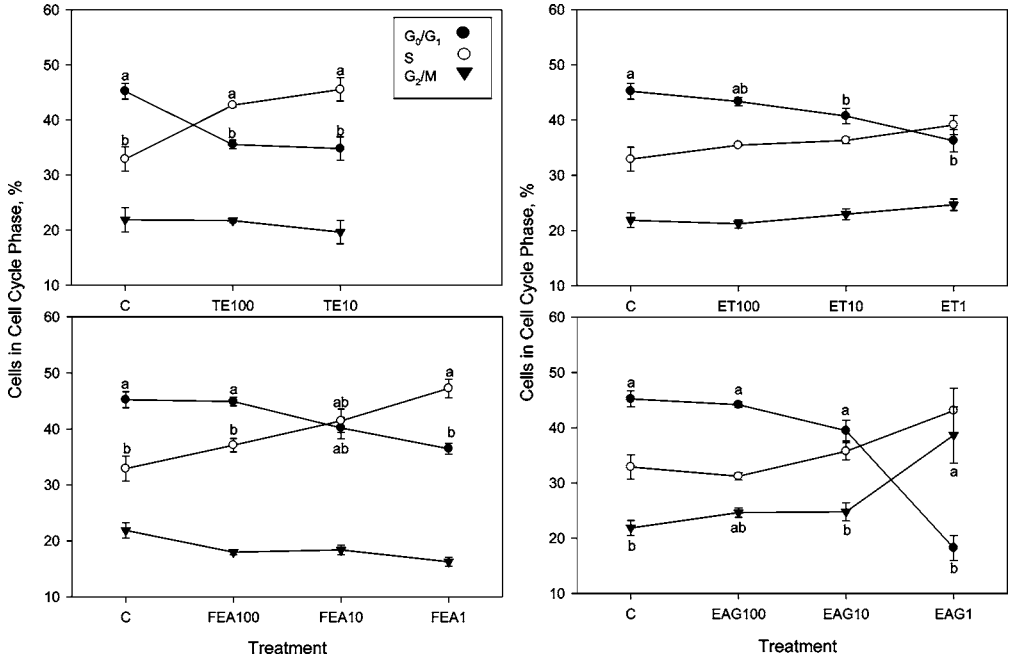

Figure 4. Cell cycle analysis of Caco-2 cells after 24 h of treatment with muscadine extract and fractions. Values are means ± SEM, $n = 4$. Means of the same cell cycle phase without a common letter are significantly different, $p \leq 0.05$. Cell cycle phases without any letters do not show any differences between treatment concentrations. TE = total extract; ET = ellagitannins; FEA = flavonoids and ellagic acid; EAG = ellagic acid glycosides; 1 = single strength; 10 = 10-fold dilution; 100 = 100-fold dilution; 1000 = 1000-fold dilution. All dilutions are based on the concentrations initially present in the grape.

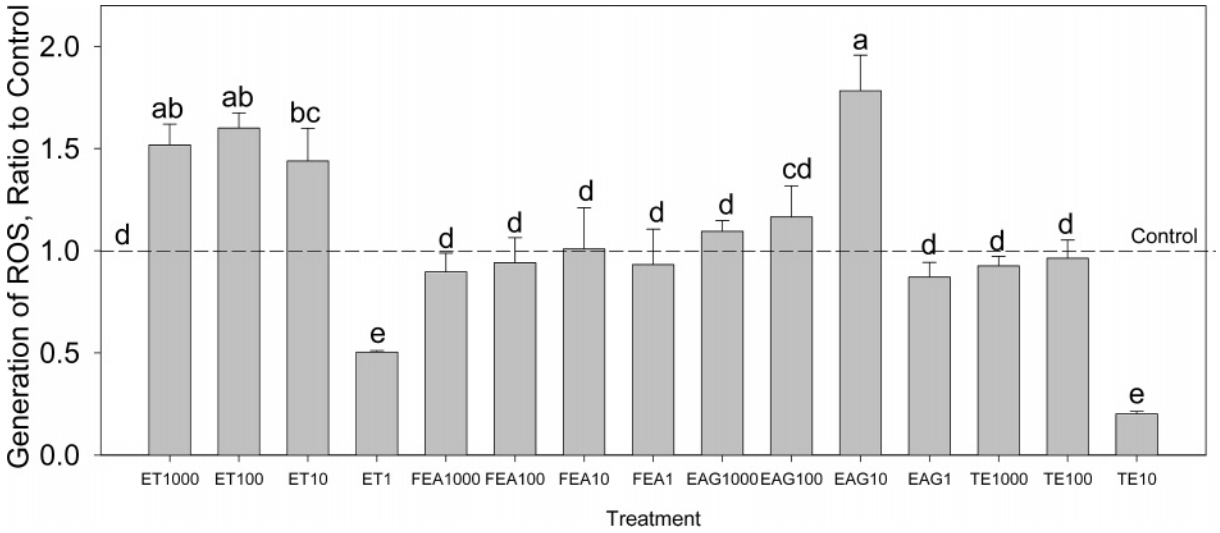

Figure 5. Intracellular levels of ROS in Caco-2 cells 25 min after treatment with muscadine extract and fractions. Values are means ± SEM, $n = 3$. Values with different letters are significantly different, $p \leq 0.05$. The dashed line indicates control cells. TE = total extract; ET = ellagitannins; FEA = flavonoids and ellagic acid; EAG = ellagic acid glycosides; 1 = single strength; 10 = 10-fold dilution; 100 = 100-fold dilution; 1000 = 1000-fold dilution. All dilutions are based on the concentrations initially present in the grape.

1:1000, but was reduced at single strength of this extract. A similar trend was observed for EAG, where the inductive effect was lower at single strength than at higher dilutions. Similarly, the 1:100 and 1:1000 dilutions of TE did not alter the generation of ROS, whereas the 1:10 dilution decreased the generation of ROS significantly compared to the control. FEA and TE did not show an inductive effect on the generation of ROS at any concentration, potentially due to the presence of flavonol glycosides that may have prevented an increase in the production of ROS through their high antioxidant potential. In this study only ET and EAG increased the generation of ROS at several concentrations, but neither extract had an inductive effect. Potentially, sufficient amounts of polyphenolic compounds that prevented or reduced the generation of ROS caused by other polyphenolics in that extract were present at higher concentrations. However, the observation that samples treated with the highest concentrations of each extract reduced the generation of ROS to a extent less than or equal to the control indicates that this parameter is not likely to be responsible for the induction of apoptosis and cell cycle arrest and the reduction of cell number.

Analysis of Linear Correlation. Linear correlation analysis was conducted between phytochemical analyses and cell culture effects to determine whether these specific chemical parameters

Table 2. Linear Correlation of Chemical and Biological End Points[a]

chemical end point	biological end point	TE		EAG		ET	
		R^2	p	R^2	p	R^2	p
ORAC	G_0/G_1			0.99*	0.01		
	S			0.92*	0.04		
	G_2/M	0.99*	0.02	0.98*	0.01		
	DCF	0.99*	0.01				
TSP	G_0/G_1			0.99*	0.01		
	S			0.92*	0.04		
	G_2/M	0.99*	0.02	0.98*	0.01		
	DCF	0.99*	0.01				
total EA	G_0/G_1			0.99*	0.01		
	S			0.92*	0.04		
	G_2/M	0.99*	0.02	0.98*	0.01		
	DCF	0.99*	0.01				
free EA	G_0/G_1	0.99*	<0.01	0.99	0.06		
	S	0.99*	<0.01				
	G_2/M	0.99*	<0.01	0.99*	0.05		
	casp	0.99*	<0.01	0.99	0.06		
	DCF					0.99*	<0.01
EA-*O*-glucosides	G_0/G_1	0.99*	<0.01	0.99	0.06	ND	ND
	S	0.99*	<0.01			ND	ND
	G_2/M	0.99*	<0.01	0.99*	0.05	ND	ND
	casp	0.99*	<0.01	0.99	0.06	ND	ND
EA-*O*-xyloside	G_0/G_1	0.99*	<0.01	0.99	0.06	ND	ND
	S	0.99*	<0.01			ND	ND
	G_2/M	0.99*	<0.01	0.99*	0.05	ND	ND
	casp	0.99*	<0.01	0.99	0.06	ND	ND
EA-*O*-rhamnoside	G_0/G_1	0.99*	<0.01	0.99	0.06	ND	ND
	S	0.99*	<0.01			ND	ND
	G2/M	0.99*	<0.01	0.99*	0.05	ND	ND
	casp	0.99*	<0.01	0.99	0.06	ND	ND

[a] Correlation performed for the following: TE, total extract; ET, ellagitannin extract; EAG, ellagic acid glycoside extract; TSP, total soluble phenolics. Correlations with an R^2 value larger than 0.90 are shown for $p \leq 0.06$. Correlations with $p \leq 0.05$ were deemed significant and are marked with an asterisk. ND, concentrations of ellagic acid glycosides were below the detection limit of 0.05 mg/kg; EA, ellagic acid (*21*).

maybe useful in the prediction of biological outcomes (**Table 2**). In the TE extract, values for antioxidant capacity, total soluble phenolics, and total ellagic acid were correlated to the G_2/M phase and the formation of reactive oxygen species ($R^2 \geq 0.99$, $p \leq 0.02$), and free ellagic acid and the glycosides showed a high correlation to all cell cycle phases and caspase-3 activity ($R^2 \geq 0.99$, $p \leq 0.01$). For EAG, values from the antioxidant capacity, total soluble phenolics, and total ellagic acid analysis were correlated to all cell cycle phases ($R^2 \geq 0.92$, $p \leq 0.04$). Free ellagic acid and its glycosides were correlated to the G_2/M phases ($R^2 \geq 0.99$, $p \leq 0.05$) and to the G_0/G_1 phase and caspase-3 activity with $R^2 \geq 0.99$, $p \leq 0.06$. In cell culture samples treated with ET, the content of free ellagic acid showed a significant correlation to the ROS assay ($R^2 = 0.99$, $p \leq 0.01$). For FEA no significant correlations were assessed.

Overall, cell cycle phases and caspase-3 activity showed higher correlations to chemical/antioxidant parameters than the other bioassays. It appears that ellagic acid and its glycosides contained in the extract preparations significantly contributed to the alterations in cell cycle phases and caspase activity in all ellagic acid glycoside containing extracts, whereas the antioxidant capacity and soluble polyphenolic concentration had a less significant role in the biological parameters in this study.

Conclusions. Ellagic acid rich extracts were prepared from muscadine grapes in order to determine the anticancer effects of these extracts in Caco-2 colon carcinoma cells. Overall, FEA at a 1:1 and TE at 1:10 dilution appeared to be most effective in their influence on vital cellular parameters. For TE this may be due to the fact that compounds from all three subextracts and additionally the anthocyanins are contained in this extract. FEA contained flavonoid glycosides in addition to the ellagic acid derivatives, which may be the cause for the overall higher magnitude of anticancer effects in Caco-2 cells. The analysis of linear correlation indicated that the presence of ellagic acid and ellagic acid glycosides may be linked to a higher anticancer efficacy, more so than the overall antioxidant potential and concentration of soluble phenolics. The extracts from muscadine grape demonstrated a high potential in the induction of cell death in colonic cancer cells, where the involvement of apoptosis was demonstrated by the presence of active caspase-3. These findings may indicate a possible potential of this grape variety and other fruits rich in ellagic acid and its glycosides in cancer prevention.

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