JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Antioxidant Activities of Grape (*Vitis vinifera*) Pomace Extracts

KOTAMBALLI N. CHIDAMBARA MURTHY,† RAVENDRA P. SINGH,‡ AND GUDDADARANGAVVANAHALLY K. JAYAPRAKASHA*,‡

Government College of Pharmacy, Bangalore 560 027, India, and Human Resource Development, Central Food Technological Research Institute, Mysore 570 013, India

Antioxidant-rich fractions were extracted from grape (*Vitis vinifera*) pomace using ethyl acetate, methanol, and water. The extracts were screened for their potential as antioxidants in different models. The ethyl acetate, methanol, and water extracts showed 76, 87.1, and 21.7% antioxidant activities at 100 ppm, respectively, using the 1,1-diphenyl-2-picrylhydrazyl model system. As the methanol extract of grape pomace showed maximum antioxidant activity among all of the extracts, it was selected to determine its effect on lipid peroxidation, hydroxyl radical scavenging activity, and human low-density lipoprotein (LDL) oxidation. The methanol extract showed 71.7, 73.6, and 91.2% inhibition using the thiobarbituric acid method, hydroxyl radical scavenging activity, and LDL oxidation, respectively, at 200 ppm. Treatment of albino rats of the Wistar strain with a single dose of CCl_4 at 1.25 mL/kg of body weight decreases the activities of catalase, superoxide dismutase (SOD), and peroxidase by 81, 49, and 89%, respectively, whereas the lipid peroxidation value increased nearly 3-fold. Pretreatment of the rats with the methanolic extract of grape pomace at 50 mg/kg (in terms of catechin equivalents) followed by CCl_4 treatment causes restoration of catalase, SOD, and peroxidase by 43.6, 73.2, and 54%, respectively, as compared with control, whereas lipid peroxidation was restored to values comparable with the control. Histopathological studies of the liver of different groups also support the protective effects exhibited by the methanol extract of grape pomace by restoring the normal hepatic architecture. Owing to this property, the studies on grape pomace can be further extended to exploit its possible application for the preservation of food products as well as a health supplement and neutraceutical.

KEYWORDS: ***Vitis vinifera;*** **antioxidant activity; DPPH; LDL; free radicals**

INTRODUCTION

The grape (*Vitis vinifera*) is one of the world's largest fruit crops, which approximates an annual production of 58 million metric tons (*1*). The predominant compounds reported are hexamers, but the structures of only some dimeric and trimeric procyanidins and their acylated derivatives have been elucidated. All of the acylated procyanidins found in grape seeds are esters of gallic acid (*2*). Phenolics in grapes and red wines have been reported to inhibit human low-density lipoprotein (LDL) oxidation in vitro (*3*). There are reports of the possible use of phenolics in grapes in preventing atherosclerosis (*4*). Phenolic compounds extracted from 12 different varieties of grapes showed antioxidant activity toward LDL oxidation in vitro (*5*). The major compounds are (+)-catechin (11%), epicatechin-(4β→8)-epicatechin (dimer B2) (6%), (−)-epicatechin (10%), epicatechin 3-*O*-gallate-(4β→8)-catechin (B1-3-*O*-gallate) (7%), and (−)-epicatechin 3-*O*-gallate (9%). Currently, a variety of health-promoting products obtained from byproducts of grape have been introduced on market, and a great many research efforts are being devoted to test the putative beneficial effects of grape polyphenols (*6*). Recently, Jayaprakasha et al. (*7*, *8*) have reported the antioxidant and antibacterial activities of grape extracts in in vitro model systems. In the present paper, an attempt has been made to elucidate the antioxidant properties of grape pomace different extracts using various in vitro and in vivo models.

MATERIALS AND METHODS

Materials. Catechin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and butylated hydroxyanisole (BHA) were obtained from Sigma Chemical Co. (St. Louis, MO). All solvents/chemicals used were of analytical grade and obtained from Merck, Mumbai, India. UV−visible spectra measurements were done using a Genesys-5 UV−visible spectrophotometer (Milton Roy, NY).

Extraction. *Vitis vinifera* (var. Bangalore blue) grapes are widely grown in the Indian States of Karnataka and Tamil Nadu (*9*). Grape pomace was collected from local juice-processing industries. Dried grape pomace was powdered and extracted in a Soxhlet with hexane for 6 h for the removal of fatty matter. The residue was extracted with different solvents (*10*).

* Author to whom correspondence should be addressed (telephone +91-0821-514 310; fax +91-0821-517 233; e-mail gkjp@yahooo.com).
† Government College of Pharmacy.
‡ Central Food Technological Research Institute.

10.1021/jf0257042 CCC: $22.00
Published on Web 09/13/2002

Determination of Total Phenolics. The concentration of phenolics in the EtOAc, MeOH, and water extracts was determined according to the method of Jayaprakasha et al. (*8*), and results were expressed as catechin equivalents. The grape pomace extracts were dissolved in a mixture of methanol and water (6:4 v/v). Samples (0.2 mL) were mixed with 1.0 mL of 10-fold-diluted Folin−Ciocalteu reagent and 0.8 mL of 7.5% sodium carbonate solution. After the mixture had stood for 30 min at room temperature, the absorbance was measured at 765 nm using a Genesys-5 UV−visible spectrophotometer. The estimation of phenolics in the fractions was carried out in triplicate, and the results were averaged.

DPPH Radical Scavenging Assay. Different concentrations (25, 50, and 100 ppm) of grape pomace extracts and BHA were taken in different test tubes, and the volume was adjusted to 100 μL using MeOH. Five milliliters of 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand at 27 °C for 20 min (*11*). The control was prepared without any extract, and MeOH was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula

$$\%\ \text{radical scavenging activity} = (\text{control OD} - \text{sample OD/control OD}) \times 100$$

On the basis of the above results, the methanol extract of grape pomace was selected for further studies.

Lipid Peroxidation by Thiobarbituric Acid (TBA) Assay. TBA reacts with malondialdehyde (MDA) to form a diadduct, a pink chromogen, which can be detected spectrophotometrically at 532 nm (*12*). Normal albino rats of the Wister strain were used for the preparation of liver homogenate. The perfused liver was isolated, and 10% (w/v) homogenate was prepared using a Potter Elvehjem homogenizer at 0−4 °C with 0.15 M KCl. The homogenate was centrifuged at 800*g* for 15 min, and clear cell-free supernatant was used for the study of in vitro lipid peroxidation. Different concentrations (50, 100, and 200 ppm) of grape pomace extract (dissolved in EtOH) were taken in test tubes and evaporated to dryness. One milliliter of 0.15 M KCl and 0.5 mL of rat liver homogenates were added to the test tubes. Peroxidation was initiated by adding 100 μL of 0.2 mM ferric chloride. After incubation at 37 °C for 30 min, the reaction was stopped by adding 2 mL of ice-cold HCl (0.25 N) containing 15% trichloroacetic acid (TCA), 0.38% TBA, and 0.5% BHT. The reaction mixtures were heated at 80 °C for 60 min. The samples were cooled and centrifuged, and the absorbance of the supernatants was measured at 532 nm. An identical experiment was performed to determine the amount of lipid peroxidation obtained in the presence of inducing agents without any extract. The percentage inhibition of lipid peroxidation is calculated by the formula

$$\text{inhibition of lipid peroxidation (\%)} = 1 - (\text{sample OD/blank OD}) \times 100$$

Hydroxyl Radical Scavenging Activity. The hydroxyl radical scavenging activity was determined according to the method of Klein et al. (*13*). Various concentrations (50, 100, and 200 ppm) of extracts in EtOH were taken in different test tubes and evaporated to dryness. One milliliter of an iron−EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of EDTA (0.018%), and 1 mL of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5 mL of 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80−90 °C for 15 min. The reaction was terminated by the addition of 1 mL of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (75.0 g of ammonium acetate, 3 mL of glacial acetic acid, and 2 mL of acetyl acetone were mixed and made up to 1 L with distilled water) was added to all of the tubes and left at room temperature for 15 min for color development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against reagent blank. The percentage hydroxyl radical scavenging is calculated by the formula

$$\text{(\%) hydroxyl radical scavenging activity} = 1 - (\text{absorbance of sample/absorbance of blank}) \times 100$$

Inhibition of Human LDL Oxidation. Plasma was prepared from the blood drawn from human volunteers. The plasma collected was stored at 0−4 °C. The LDL was prepared from the plasma according to the method of Princen et al. (*14*) using a differential ultracentrifugation method. Various concentrations (100 and 200 ppm) of extract were taken in test tubes, 40 μL of copper sulfate (2 mM) was added, and the volume was made to 1.5 mL in all of the test tubes with phosphate buffer (50 mM, pH 7.4). A tube without extract and copper sulfate served as negative control, and another tube without extract served as positive control. All tubes were incubated at 37 °C. Aliquots of 0.5 mL from each tube were drawn at 2 h intervals, and 0.25 mL of TBA (1% in 50 mM NaOH) was added followed by 0.25 mL of TCA (2.8%). The tubes were incubated for 45 min at 95 °C. A pink chromogen was extracted by centrifugation (at 2000 rpm for 10 min) after the tubes had cooled to room temperature. TBA-reactive species in the pink chromogen were detected by fluorescence at 515 nm excitation and 553 nm emission. Data were expressed in terms of MDA equivalent, which was estimated by comparison with the standard graph drawn for 1,1,3,3-tetramethoxypropane, which gave the amount of oxidation. Protein was estimated by using the Folin−phenol method (*15*), and the results were expressed as protection per unit of protein concentration. Using the amount of MDA, the percentage protection was calculated using the formula

$$\text{protection (\%)} = (\text{oxidation in control} - \text{oxidation in experimental/oxidation in control}) \times 100$$

In Vivo Experiments. Male Albino rats of the Wistar strain, weighing 180−220 g, were used for the studies. The animals were grouped into three groups containing six animals in each group. The first group served as control, the second group was administered carbon tetrachloride (negative control), and the third group was administered a methanolic extract of grape pomace. The extract was suspended in 0.5% sodium carboxymethylcellulose and was fed to third group rats via an oral route at 50 mg (in terms of catechin equivalents)/kg of body weight for 14 days. The animals of the first and second groups were simultaneously administered saline until the 14th day. The animals of the second and third groups were given a single oral dose of carbon tetrachloride (1:1 in liquid paraffin) at 1.25 mL/kg of body weight 6 h after the time of the last dose of administration of extract/saline on the 14th day. After 24 h, animals were sacrificed, and the livers were isolated to prepare the liver homogenate. Five percent liver homogenate was prepared with 0.15 M KCl and centrifuged at 800*g* for 10 min. The cell-free supernatant was used for the estimation of lipid peroxidation, peroxidase, catalase, and superoxide dismutase (SOD).

Catalase Assay. The catalase assay was carried out as per the method of Aebi (*16*). One milliliter of liver homogenate from the first, second, and third groups was taken with 1.9 mL of phosphate buffer in different test tubes (50 mM, pH 7.4). The reaction was initiated by the addition of 1 mL of hydrogen peroxide (30 mM). The decrease in optical density due to decomposition of hydrogen peroxide measured at the end of 1 min was recorded against the blank (2.9 mL of phosphate buffer and 1.0 mL of hydrogen peroxide) at 240 nm. Units of catalase were expressed as the amount of enzyme, which decomposes 1 μM H_2O_2/min at 25 °C. The specific activity was expressed in terms of units per milligram of proteins.

Estimation of Superoxide Dismutase. The SOD assay was based on the reduction of yellow solution of nitro tetrazolium chloride (NBT) to water-insoluble purplish-blue diformazan as per the method of Beauchamp and Fridovich (*17*). Liver homogenate (0.5 mL) was taken, and 1 mL of 50 mM sodium carbonate, 0.4 mL of 24 μM NBT, and 0.2 mL of 0.1 mM EDTA were added. The reaction was initiated by adding 0.4 mL of 1 mM hydroxylamine hydrochloride. Zero-time absorbance was taken at 560 nm followed by recording the absorbance after 5 min at 25 °C. The control was simultaneously run without liver homogenate. Units of SOD activity were expressed as the amount of

Table 1. Percentage of Yield and Phenolics of Grape Pomace Extracts (w/w)

extraction medium	yield	phenolics
EtOAc	3.9 ± 0.40	27.9 ± 1.8
MeOH	5.6 ± 0.52	35.7 ± 4.4
water	1.1 ± 0.29	6.1 ± 0.8

enzyme required to inhibit the reduction of NBT by 50%. The specific activity was expressed in terms of units per milligram of proteins.

Estimation of Peroxidase. The peroxidase assay was carried out as per the method of Nicholos (*18*). Liver homogenate (0.5 mL) was taken; 1 mL of 10 mM KI solution and 1 mL of 40 mM sodium acetate solution were added to these tubes. The absorbance was read at 353 nm. Twenty microliters of hydrogen peroxide (15 mM) was added, and the change in the absorbance in 5 min was recorded. Units of peroxidase activity were expressed as the amount of enzyme required to change the optical density by 1 unit per minute. The specific activity was expressed in terms of units per milligram of proteins.

Lipid Peroxidation. Lipid peroxidation was carried out as per the method of Buege and Aust (*19*). Liver homogenate (0.5 mL) and 1 mL of 0.15 M KCl were taken. Peroxidation was initiated by adding 100 μL of 0.2 mM ferric chloride. The reaction was run at 37 °C for 30 min. The reaction was stopped by adding 2 mL of an ice-cold mixture of 0.25 N HCl containing 15% TCA, 0.30% TBA, and 0.05% butylated hydroxytoluene (BHT) and was heated at 80 °C for 60 min. The samples were cooled and centrifuged, and the absorbance of the supernatant was measured at 532 nm. The results were expressed as MDA equivalents, which was calculated by using an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$. Lipid peroxidation was expressed as MDA equivalents in nanomoles per milligram of protein of liver homogenate.

Determination of Proteins. The protein was determined using the method of Lowry et al. (*15*).

Histopathological Studies. Histopathological studies of liver of different groups of rats were carried out to see the effect of protection offered by feeding the methanolic extract of grape pomace against the toxic effects of CCl_4.

Statistical Analysis. The experiments were done in triplicate. Data were expressed as mean ± SD. *t* test was used for comparison of mean values. All tests were considered statistically significant at $p < 0.001$.

RESULTS AND DISCUSSION

The yields of extracts obtained from grape pomace using various solvents are shown in **Table 1**. It is evident from the table that extraction with methanol yields the highest amount of extracts and phenolic content followed by EtOAc and water. As the MeOH extract showed high antioxidant activity also, it may be directly correlated to the high phenolic content of the MeOH extract of grape pomace.

Free radical scavenging potentials of grape pomace extracts at different concentrations were tested by the DPPH method, and the results are shown in **Figure 1**. Antioxidant reacts with DPPH, which is a stable free radical, and converts it to 1,1-diphenyl-2-picrylhydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. At 50 ppm, EtOAc, MeOH, and water extracts of grape pomace exhibit 42.1, 67.3, and 9.1% free radical scavenging activities, respectively. The activity of the extracts is attributed to their hydrogen-donating ability (*20*). It is known that free radicals cause autoxidation of unsaturated lipids in food (*21*). On the other hand, antioxidants are believed to intercept the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate further oxidation of lipid (*22*). The data obtained reveal that the grape pomace extracts are free radical scavengers and primary antioxidants, which react with free radicals.

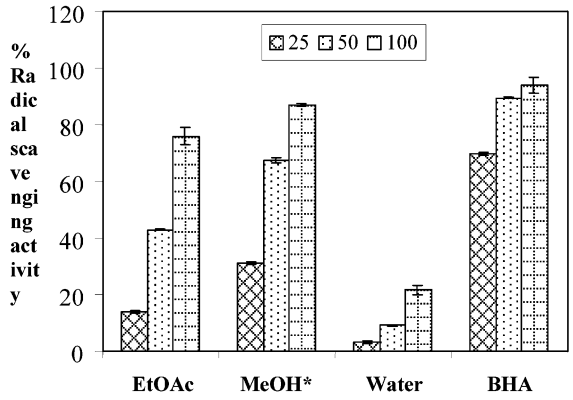

Figure 1. Radical scavenging activity of grape pomace extracts and BHA by DPPH method at different concentrations (ppm). An asterisk (*) indicates significance when compared to BHA.

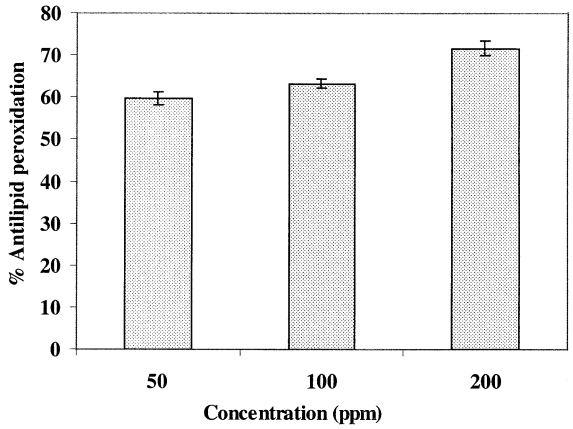

Figure 2. Inhibition of lipid peroxidation of grape pomace MeOH extract at different concentrations by TBA method.

The results of the effect of the MeOH extract of grape pomace to prevent lipid peroxidation are shown in **Figure 2**. At 200 ppm, the extract shows 71.74% scavenging activity by this method. In biological systems, MDA is a very reactive species and takes part in cross-linking of DNA with proteins and also damaging the liver cells (*23*, *24*). MDA, being the major product of lipid peroxidation, is used to study the lipid peroxidation process in rat liver homogenate by means of derivatizing with TBA at high temperature and acidic condition (*12*).

Hydroxyl radical is an extremely reactive species formed in biological systems and has been implicated as highly damaging in free radical pathology, capable of damaging almost every molecule found in living cells (*25*). This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis, and cytotoxicity. In addition, this species is considered to be one of the quick initiators of the lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids (*26*). Hydroxyl radical scavenging activity was estimated by generating the hydroxyl radicals using ascorbic acid−iron EDTA. The hydroxyl radicals formed by the oxidation react with DMSO to yield formaldehyde, which provides a convenient method for their detection by treatment with Nash reagent. The hydroxyl radical scavenging activity of the MeOH extract of grape pomace is shown in **Figure 3**. The methanol extract of grape pomace exhibits 73.65% hydroxyl radical scavenging activity at 200 ppm. The ability of the grape pomace extract to quench hydroxyl radicals

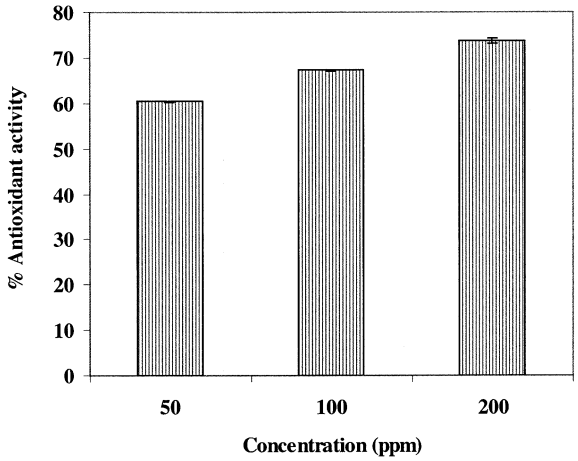

Figure 3. Hydroxyl radical scavenging activity of grape pomace MeOH extract at different concentrations.

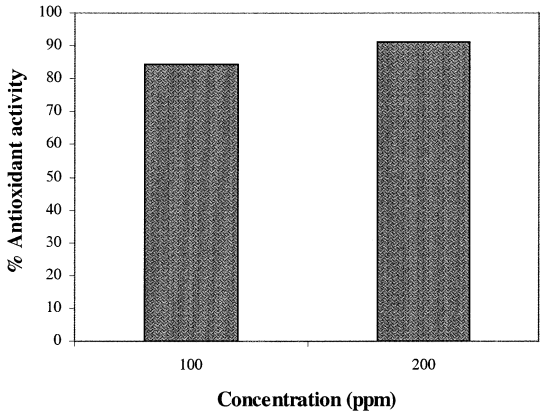

Figure 4. Inhibition of grape pomace MeOH extract on human LDL oxidation at different concentrations.

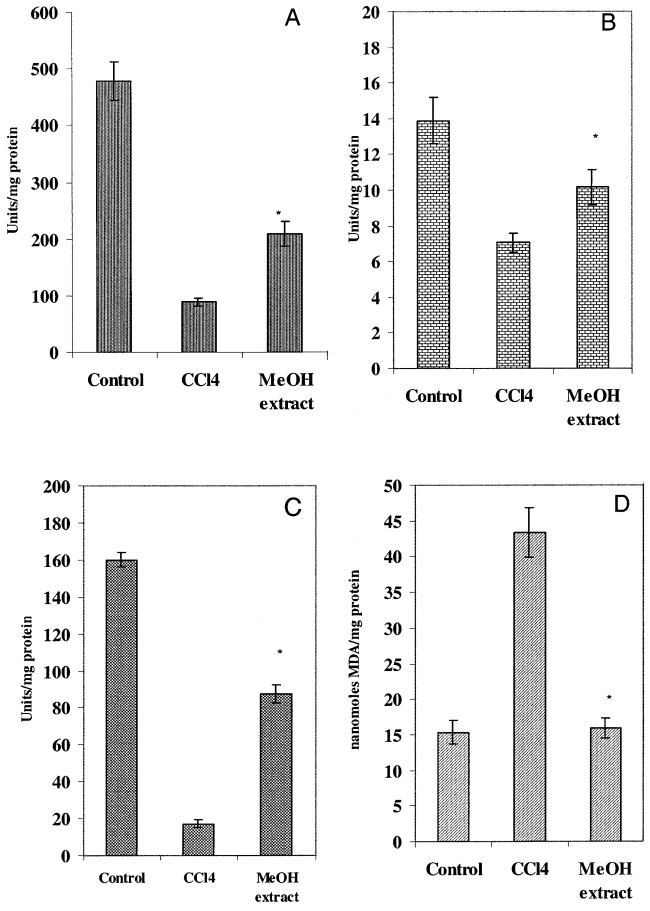

Figure 5. Effect of treatment of rats with MeOH extract of grape pomace followed by feeding carbon tetrachloride on the activity of various enzymes and lipid peroxidation of liver: (A) catalase; (B) SOD; (C) peroxidase; (D) inhibition of lipid peroxidation. Each data bar represents the mean ± SD of three replicates. An asterisk (∗) indicates significance when compared with carbon tetrachloride treatment ($p < 0.001$).

seems to directly relate to the prevention of propagation of the process of lipid peroxidation, and the extract seems to be a good scavenger of active oxygen species, thus reducing the rate of chain reaction.

The oxidative modification of LDL is known to play an important role in the pathogenesis of atherosclerosis and coronary heart diseases (*27*), and dietary antioxidants that protect LDL from oxidation may therefore reduce atherogenesis and coronary heart diseases (*28*). The inhibition of human LDL oxidation by grape pomace MeOH extracts is shown in **Figure 4**. The polyunsaturated fatty acids (PUFA) of human LDL were oxidized, and the MDA was estimated by the TBA method. The MeOH extract strongly protected LDL from oxidation, exhibiting 84.6 and 91.2% protection at 100 and 200 ppm, respectively, after 2 h.

The results shown above indicate that the extraction with methanol not only gives a high yield of the extract but also gives a high antioxidant activity, which was confirmed by various methods used for the antioxidant assay. Thus, the selective extraction medium is very important in obtaining the fraction with potent antioxidant activity from natural sources. The antioxidant activities of the individual phenolic compounds may depend on structural factors, such as the number of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups, and other structural features. Dihydroxylation in both rings and in the 3-postion in catechin, myricetin, quercetin, and epicatechin is required for antioxidant activity as reported in various lipid systems (*29*). Although the phenolic compounds have similar chemical properties, their reduction capacity is not a very precise predictor of their antioxidant activity. In the LDL oxidation assay and other tests for antioxidant activity, the system is typically heterogeneous, and physical properties such as lipophilicity, solubility, and partition between the aqueous and lipid phases of LDL can become important in determining antioxidant activity (*30*).

Figure 5 depicts the effect of treatment of rats with CCl_4 and pretreatment with grape pomace extracts followed by CCl_4 treatment on the activities of catalase, superoxide dismutase, and peroxidase enzymes and lipid peroxidation activity in liver homogenates. Treatment of rats with a single dose of CCl_4 at 1.25 mL/kg of body weight significantly reduces the activities of catalase, SOD, and peroxidase by 81, 49, and 89%, respectively. On the other hand, the lipid peroxidation increases to ∼3-fold as compared to control due to CCl_4 treatment. However, pretreatment of the rats with the methanolic extract of grape pomace at 50 mg/kg (in terms of catechin equivalents) causes restoration of catalase, SOD, and peroxidase activities by 43.6, 73.2, and 54% of the control values, respectively. This shows the protection provided by feeding of grape pomace extract to the rats by protecting and maintaining the activities of these enzymes even after CCl_4 treatment. It was found that the hydrogen peroxide-scavenging capacity of the MeOH extract of grape pomace was highly efficient, which may be attributed to the presence of phenolic compounds in the extract. These phenolics could donate electrons to hydrogen peroxide to neutralize it to water (*31*). Increase in the catalase activity with

respect to CCl_4 treatment indicates that grape pomace can play an important role in scavenging hydrogen peroxide. Restoration of SOD activity indicates that pomace can help in cellular defense mechanisms by preventing cell membrane oxidation. Similarly, an increase in peroxidase activity indicates that grape pomace also helps in the restoration of vital molecules such as NAD, cytochromes, and glutathione.

It has been observed that the lipid peroxidation was restored at the values close to the control showing the effects of the components of grape pomace extract. The major effect of free radical on the mean liver detoxificant enzymes (catalase, SOD, and peroxidase) is reduction of enzyme activity due to enzyme inactivation during the catalytic cycle. However, feeding the grape pomace extract containing gallic acid, catechin, epicatechin, and procyanidins, which act as potent free radical scavengers, reduces the activity of hydrogen peroxide and superoxide anion, consequently reducing the lipid peroxidation and enzyme inactivation and restoring enzyme activity (**Figure 5**). This may indicate the possible de novo synthesis of these enzymes induced by the components of grape pomace extract (*32*, *33*).

CCl_4 and its metabolites, such as the trichloromethyl radical ($CCl_3^{\bullet}$) and the trichloromethyl peroxy radical ($CCl_3O_2^{\bullet}$), are involved in the pathogenesis of liver (*34*) and kidney damage (*35*). CCl_4 is also shown to cause changes around the liver central vein and other oxidative damages with the leakage of marker enzymes such as GOT and GPT in the serum. Histopathological studies showed that in the case of controls, hepatocytes with normal architecture and portal triad, portal veins, and hepatic artery and vein are visible. However, CCl_4-treated rats showed total loss of hepatic architecture, areas of hemorrhage, and necrosis. In the case of rats pretreated with grape pomace extract followed by exposure to CCl_4, the liver is shown to retain the normal hepatic architecture with few areas of hemorrhage between the columns of hepatocytes, indicating the protection provided by the grape pomace extract. Massive generation of free radical in the CCl_4 liver induced damage provokes a sharp increase of lipid peroxidation in liver. When free radical generation is massive, in the CCl_4-induced damage, the cytotoxic effect is not located, but can be propagated intracellularly and extracellularly, increasing the interaction of these radicals with phopholipid structures, inducing peroxidation processes that destroy organ structure. The studies showed that in the case of control, hepatocytes having normal architecture with portal triad, portal veins, and hepatic artery and vein were visible. However, in the case of CCl_4-treated rats, total loss of hepatic architecture, areas of hemorrhage, and necrosis were seen. In the case of rats pretreated with grape pomace extract followed by exposure to CCl_4, the liver was shown to retain the normal hepatic architecture with few areas of hemorrhage between the columns of hepatocytes. These results clearly indicate the protection provided by the grape pomace extract. Lin et al. (*36*) also showed the liver protective and antioxidative effects of certain plant extracts against CCl_4-induced liver injury and proposed that it may possibly involve the mechanisms related to free radical scavenging effects. The effects of various phenolic compounds such as caffeic acid, chlorogenic acid, cyanarin, and cyanaroside to protect the rat hepatocytes against the *tert*-butyl hydroperoxide toxicity has also been demonstrated (*37*).

Antioxidant activity is also dependent on the structure of the free radical scavenging compounds, the substituents present on the rings of the flavonoids, and the degree of polymerization. Although there is some debate as to whether the degree of polymerization increases the antioxidant capacity, it appears that epicatechin and epicatechin polymers and the B procyanidins are better antioxidants than catechin and catechin polymers and the B procyanidins (*38*, *39*). The structural criteria for the potent free radical scavengers are that these should possess either (i) a 3-hydroxy group on an unsaturated C ring or (ii) a 2,3-double bond with the 3-OH group and 4-one in the C ring or (iii) an ortho-OH substitution pattern in the B ring where the OH groups are not glycated (*40*, *41*). The major polyphenolic components in grapes are catechin, epicatechin, and procyanidins (*7*), which fulfill the first and third structural criteria for being a good antioxidant.

In conclusion, the results of the present studies indicate that the grape pomace MeOH extract is capable of protecting the activities of hepatic enzymes, which play important roles in combating the reactive oxygen species. Also, feeding of the grape pomace extract provides protection against CCl_4 toxicity as shown by histopathological studies of liver. Grape pomace is rich in phenolic compounds, and different activities of the grape extract can be ascribed to their different phenolic compositions. Further studies with individual phenolic compounds of grape pomace may be undertaken to elucidate the mechanisms involved in the enhancement of enzyme activity and protection provided to the liver and also to explore the possible synergism, which may potentiate the protective effects against reactive oxygen species.

ACKNOWLEDGMENT

We thank Dr. V. Prakash, Director, and Dr. K. K. Sakariah, Head, Human Resource Development, CFTRI, for their keen interest in this work. K.N.C.M. is thankful to them for giving permission to work at this institute for the partial fulfillment of his M.Pharm. degree. He is also thankful to Prof. Uma D. Murthy, Government College of Pharmacy, Bangalore, for constant support and guidance for the dissertation work.

LITERATURE CITED

(1) *FAO Production Year Book*; FAO Statistics 51; Food and Agriculture Organization of the United Nations: Rome, Italy, 1997; p 151.

(2) Lee, C. Y.; Jaworski, A. W. Identification of some phenolics in white grapes. *Am. J. Enol. Vitic.* **1990**, *41*, 87.

(3) Teissedre, P. L.; Frankel, E. N.; Waterhouse, A. L.; Peleg, H.; German, J. B. Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. *J. Sci. Food Agric.* **1996**, *70*, 55−61.

(4) Kovac, V.; Pekic, B. Proanthocyanidols from grape and wine. *Contemp. Agric.* **1991**, *39*, 5−17.

(5) Mayer, A. S.; Ock-Sook, Yi.; Person, D. A.; Waterhouse, A. L.; Franke, E. N. Inhibition of human low-density lipoprotein oxidation in relation to composition of phenolic antioxidants in grapes (*Vitis vinifera*). *J. Agric. Food Chem.* **1997**, *45*, 1638−1643.

(6) Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. Wine as a biological fluid: History, production and role in disease prevention. *J. Clin. Lab. Anal.* **1997**, *11*, 287−313.

(7) Jayaprakasha, G. K.; Singh, R. P.; Sakariah, K. K. Antioxidant activity of grape seed (*Vitis vinefera*) extracts on peroxidation models *in vitro*. *Food Chem.* **2001**, *73*, 285−290.

(8) Jayaprakasha, G. K.; Tamil Selvi; Sakariah, K. K. Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. *Food Res. Int.* **2002**, in press.

(9) *The Wealth of India: A Dictionary of Indian Raw Materials*; Publication and Information Directorate, CSIR: New Delhi, India, 1976; p 529.

(10) Jayaprakasha, G. K.; Singh, R. P.; Sakariah, K. K. An improved process for the extraction of antioxidants from grape seeds. Indian Patent Appl. 218/Del/2001, dated Feb 28, 2001.
(11) Singh, R. P.; Chidamabara Murthy, K. N.; Jayaprakasha, G. K. Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models. *J. Agric. Food Chem.* **2002**, *50*, 81−86.
(12) Halliwell, B.; Guttridge, J. M. C. In *Free Radicals in Biology and Medicine*, 2nd ed.; Japan Scientific Societies Press: Tokyo, Japan, 1989.
(13) Klein, S. M.; Cohen, G.; Cederbaum, A. I. Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system. *Biochemistry* **1991**, *20*, 6006−6012.
(14) Princen, H. M. G.; Van Poppel, G.; Vogelezang, C.; Buytenhek, R.; Kok, F. J. Supplementation with vitamin E but not β-carotene in vivo protects low-density lipoprotein from lipid peroxidation in vitro. *Arterioscler. Thromb.* **1992**, *12*, 554−562.
(15) Lowry, O. H.; Rosebrough, N. I.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.
(16) Aebi, H. Catalyse *in vitro*. *Methods Enzymol.* **1984**, *105*, 121−125.
(17) Beauchamp, C.; Fridovich. Superoxide dismutase: improved assay and an assay applicable to to acrylamide gel. *Anal. Biochem.* **1971**, *10*, 276−287.
(18) Nicholos, M. A. A spectrophotometric assay for iodide oxidation by thyroid peroxidase. *Anal. Biochem.* **1962**, *4*, 311−345.
(19) Buege, J. A.; Aust, S. D. Microsomal lipid peroxidation. *Methods Enzymol.* **1978**, *52*, 302−310.
(20) Shimada, K. K.; Fujikawa, K. Y.; Nakamura, T. Antioxidative properties of xanthan on autoxidation of soybean oil in cyclodextrin. *J. Agric. Food Chem.* **1992**, *40*, 945−948.
(21) Kaur, H.; Perkins, J. The free radical chemistry of food additives, In *Free Radicals and Food Additives*; Aruoma, O. I., Halliwell, B., Eds.; Taylor and Francis: London, U.K., 1991; pp 17−35.
(22) Sherwin, E. R. Oxidation and antioxidants in fat and oil processing. *J. Am. Oil Chem. Soc.* **1978**, *55*, 809−814.
(23) Kubow, S. Toxicity of dietary lipid peroxidation products. *Trends Food Sci. Technol.* **1990**, *1*, 67−71.
(24) Jadhav, S. J.; Nimbalkar Kulkarni, A. D.; Madhavi, D. L. Lipid oxidation in biological and food systems. In *Food Antioxidants*; Madhavi, D. L., Deshpande, S. S., Salunkhe, D. K., Eds.; Dekker: New York, 1996; pp 5−63.
(25) Hochestein, P.; Atallah, A. S. The nature of oxidant and antioxidant systems in the inhibition of mutation and cancer. *Mutat. Res.* **1988**, *202*, 363−375.
(26) Kappus, H. Lipid peroxidation−Mechanism and biological relevance. In *Free Radicals and Food Additives*; Aruoma, O. I., Halliwell, B., Eds.; Taylor and Francis: London, U.K., 1991; pp 59−75.
(27) Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witzum, J. L. Beyond Cholesterol−Modification of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* **1989**, *320*, 915−924.
(28) Kinsella, J. E.; Frankel, E.; German, B.; Kanner, J. Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technol.* **1993**, *47*, 85−89.
(29) Pratt, D. E.; Hudson, B. J. F. Natural antioxidants not exploited commercially. In *Food Antioxidants*; Hudson, B. J. F., Ed.; Elsevier Applied Science: London, U.K., 1990; pp 171−191.
(30) Frankl, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. Inhibition of human low-density lipoproteins by phenolic substances in red wine. *Lancet* **1993**, *341*, 454−457.
(31) Halliwell, B.; Gutteridge, J. M. C. *Free Radicals in Biology and Medicine*; Oxford University Press: Oxford, U.K., 1985.
(32) Halliwell, B. How to characterize a biological antioxidant. *Free Radicals Res. Commun.* **1990**, *9*, 1−32.
(33) Aruoma, O. I. Nutrition and health aspects of free radicals and antioxidants. *Food Chem.* **1994**, *32*, 671−683.
(34) Singh, K.; Khanna, A. K.; Chander, R. Hepatoprotective activity of ellagic acid against carbon tetrachloride induced hepatotoxicity in rats. *Indian J. Exp. Biol.* **1999**, *37*, 1025−1026.
(35) U.S. EPA. http://www.epa.gov/ttn/uatw/hlthef/carbonte.html.
(36) Lin, C. C.; Yen, M. H.; Lo, T. S.; Lin, J. M. Evaluation of the hepatoprotective and antioxidant activity of *Boehmeria nivea* var. *nivea* and *B. nivea* var. *tenacissima*. *J. Ethnopharmacol.* **1998**, *60*, 9−17.
(37) Gebhardt, R.; Fausel, M. Antioxidant and hepatoprotective effects of artichoke extracts and constituents in cultured rat hepatocytes. *Toxicol In Vitro* **1997**, *11*, 669−672.
(38) Ricardo da Silva, J. M.; Darmon, N.; Fernandez, Y.; Mitjavila, S. Oxygen free radical scavengers capacity in aqeous models of different procyanidins from grape seeds. *J. Agric. Food Chem.* **1991**, *39*, 1549−1522.
(39) Saint-Crizq de Gaulejac, N.; Provost, C.; Vivas, N. Comparative study of polyphenol scavenging activities assesses by different methods. *J. Agric. Food Chem.* **1991**, *47*, 425−431.
(40) Rice-Evans, C. A.; Miller, N. J.; Bolwell, P. G.; Bramley, P. M. J. B. The relative antioxidant activites of plant derived polyphenolic flavonoids. *Free Radical Res.* **1995**, *22*, 375−383.
(41) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.

Received for review May 29, 2002. Revised manuscript received August 14, 2002. Accepted August 14, 2002.

JF0257042