FEBS Letters 580 (2006) 533–538

Plant polyphenols mobilize endogenous copper in human peripheral lymphocytes leading to oxidative DNA breakage: A putative mechanism for anticancer properties

Asfar Sohail Azmi, Showket Hussain Bhat, Sarmad Hanif, S.M. Hadi*

Department of Biochemistry, Faculty of Life Sciences, A.M.U., Aligarh 202002, UP, India

Received 21 November 2005; revised 14 December 2005; accepted 19 December 2005

Available online 28 December 2005

Edited by Barry Halliwell

Abstract **Plant polyphenols are important components of human diet and a number of them are considered to possess chemopreventive and therapeutic properties against cancer. They are recognized as naturally occurring antioxidants but also act as prooxidants catalyzing DNA degradation in the presence of transition metal ions such as copper. Using human peripheral lymphocytes and Comet assay we have previously confirmed that resveratrol–Cu(II) is indeed capable of causing DNA degradation in cells. In this paper we show that the polyphenols alone (in the absence of added copper) are also capable of causing DNA breakage in cells. Incubation of lymphocytes with neocuproine inhibited the DNA degradation confirming that Cu(I) is an intermediate in the DNA cleavage reaction. Further, we have also shown that polyphenols generate oxidative stress in lymphocytes which is inhibited by scavengers of reactive oxygen species and neocuproine. These results are in further support of our hypothesis that anticancer mechanism of plant polyphenols involves mobilization of endogenous copper, possibly chromatin bound copper, and the consequent prooxidant action.**

Keywords: Plant polyphenols; Comet assay; Endogenous copper; Prooxidant action

1. Introduction

Plant-derived polyphenolic compounds such as flavonoids, tannins, curcumin and the stilbene resveratrol possess a wide range of pharmacological properties, the mechanisms of which have been the subject of considerable interest. They are recognized as naturally occurring antioxidants and have been implicated as anticancer compounds [1]. In recent years, several reports have documented that plant polyphenolics, including curcumin, resveratrol and gallocatechins such as gallic acid, epigallocatechin, epicatechin-3-gallate, and epigallocatechin-3-gallate (EGCG), induce apoptosis in various cancer cell lines [2–5]. Gallocatechins are constituents of green tea, the consumption of which is considered to reduce the risk of various cancers such as those of bladder, prostrate, oesophagus and stomach [5]. Resveratrol is present in human dietary material such as peanuts, grapes, mulberries and beverages such as red wine. Of particular interest is the observation that a number of these polyphenols including epigallocatechin-3-gallate, gallic acid and resveratrol induce apoptotic cell death in various cancer cell lines but not in normal cells [3–5].

Studies in our laboratory have shown that flavonoids [6], tannic acid and its structural constituent gallic acid [7], curcumin [8], gallocatechins [9] and resveratrol [10] cause oxidative strand breakage in DNA either alone or in the presence of transition metal ions such as copper. Copper is an important metal ion present in chromatin and is closely associated with DNA bases particularly guanine [11]. It is also one of the most redox active of the various metal ions present in cells. Most of the pharmacological properties of plant polyphenols are considered to reflect their ability to scavenge endogenously generated oxygen radicals or those free radicals formed by various xenobiotics, radiation etc. However, some data in the literature suggests that antioxidant properties of the polyphenolic compounds may not fully account for their chemopreventive effects [12,13]. Most of the plant polyphenols possess both antioxidant as well as prooxidant properties [4,6] and we have earlier proposed that the prooxidant action of polyphenolics may be an important mechanism of their anticancer and apoptosis inducing properties [13]. Such a mechanism for the cytotoxic action of these compounds against cancer cells would involve mobilization of endogenous copper ions and the consequent prooxidant action. Using a cellular system of peripheral lymphocytes isolated from human blood and alkaline single cell gel electrophoresis (Comet assay), we have confirmed that resveratrol in the presence of Cu(II) is indeed capable of causing DNA degradation in cells. Further, such DNA degradation in lymphocytes is inhibited by scavengers of reactive oxygen and neocuproine, a Cu(I) specific sequestering agent, indicating that the DNA breakage is caused by reactive oxygen species generated through the reduction of Cu(II)–Cu(I) by the polyphenols [14].

As a further confirmation of our hypothesis, in this paper we show that several plant polyphenols alone (in the absence of added Cu(II)) are also capable of lymphocyte DNA degradation and that such degradation is mediated through mobilization of endogenous copper ions.

*Corresponding author. Fax: +91 571 2706002/91 571 2507089.
E-mail addresses: smhadi@vsnl.com, smhadi@lycos.com (S.M. Hadi).

0014-5793/$32.00
doi:10.1016/j.febslet.2005.12.059

2. Materials and methods

2.1. Materials

Resveratrol, piceatannol, *trans*-stilbene, gallic acid, syringic acid, neocuproine, superoxide dismutase (SOD), agarose, low melting point agarose (LMPA), RPMI 1640, Triton X-100, Trypan blue, Histopaque 1077 and phosphate buffered saline (PBS) Ca^{++} and Mg^{++} free were purchased from Sigma, (St. Louis, USA). All other chemicals were of analytical grade. Resveratrol, piceatannol and *trans*-stilbene were dissolved in 3 mM NaOH before use as a stock of 1 mM solution. All other polyphenols were dissolved in water. Upon addition to reaction mixtures, in the presence of buffers mentioned and at the concentrations used, all the polyphenols used remained in solution. The volumes of stock solutions added did not lead to any appreciable change in the pH of reaction mixtures.

2.2. Isolation of lymphocytes

Heparinized blood samples (2 ml) from healthy donors were obtained by venepuncture and diluted suitably in Ca^{++} and Mg^{++} free PBS. Lymphocytes were isolated from blood using Histopaque 1077 (Sigma), and the cells were finally suspended in RPMI 1640.

2.3. Viability assessment of lymphocytes

The lymphocytes were checked for their viability before the start and after the end of the reaction using Trypan Blue Exclusion test [15].

2.4. Lymphocyte treatment

Lymphocytes (1×10^5 cells) were exposed to different concentrations of polyphenols in a total reaction volume of 1 ml (400 μl RPMI, PBS Ca^{2+} and Mg^{2+} free and indicated concentrations of polyphenols). Incubation was performed at 37 °C for 2 h or as specified. In some experiments, scavengers of active oxygen were added at the final concentrations indicated. After the incubation, the mixture was centrifuged at 4000 rpm, the supernatant was discarded and pelleted lymphocytes were resuspended in 100 μl of PBS (Ca^{++} and Mg^{++} free) and processed further for Comet assay.

2.5. Comet assay

Comet assay was performed under alkaline conditions essentially according to the procedure of Singh et al. [16] and is described by us in detail previously [14].

2.6. Statistics

The statistical analysis was performed as described by Tice et al. [17] and is expressed as ±S.E.M. of three experiments. A student's *t*-test was used to examine statistically significant differences. Analysis of variance was performed using ANOVA. P values < 0.05 were considered statistically significant.

2.7. Determination of TBARS and H_2O_2

Thiobarbituric acid reactive substance was determined according to the method of Ramanathan et al. [18]. A cell suspension (1×10^5/ml) was incubated with different polyphenols (0–400 μM) at 37 °C for 1 h and then centrifuged at 1000 rpm. In some experiments the cells were pre-incubated with fixed concentrations of neocuproine and thiourea. The cell pellet was washed twice with phosphate buffered saline (Ca^{++} and Mg^{++} free) and suspended in 0.1 N NaOH. This cell suspension (1.4 ml) was further treated with 10% TCA and 0.6 M TBA (thiobarbituric acid) in boiling water bath for 10 min. The absorbance was read at 532 nm. H_2O_2 was determined in the incubation medium by FOX assay as described by Long et al. [19]. The reaction mixture was the same as mentioned in 2.4 above but without cells. After incubation for 2 h at 37 °C an aliquot of 100 μl was analyzed for H_2O_2 formation.

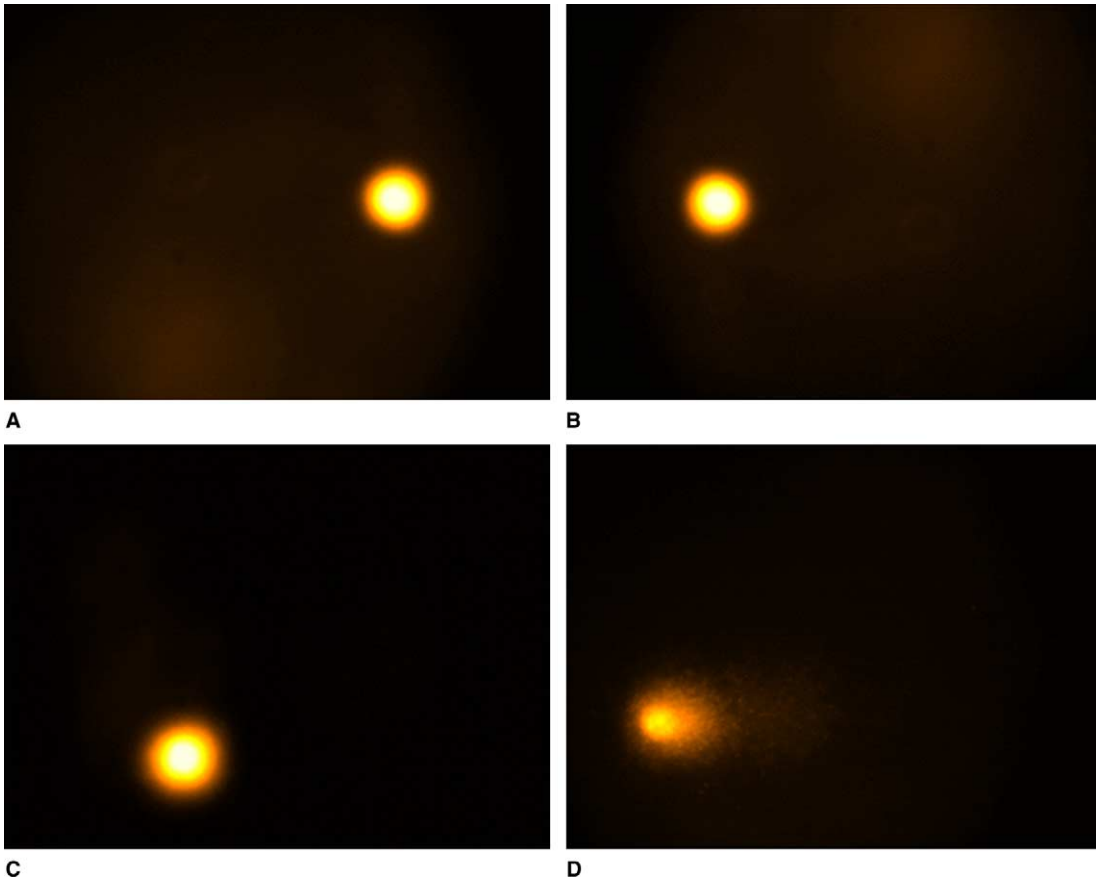

Fig. 1. Single cell gel electrophoresis of human peripheral lymphocytes showing Comets (100X) after treatment with different concentrations of resveratrol, (A) untreated, (B) resveratrol (50 μM), (C) resveratrol (100 μM) and (D) resveratrol (200 μM).

3. Results

3.1. DNA breakage by resveratrol in lymphocytes as measured by Comet assay

Increasing concentrations of resveratrol (50, 100, 200 μM) were tested for DNA breakage in isolated lymphocytes using the Comet assay. Photographs of Comets seen on treatment with these concentrations are shown in Fig 1. At 50 and 100 μM concentrations resveratrol did not damage the lymphocyte DNA to any significant extent whereas at 200 μM concentration a Comet with a tail indicative of DNA breakage was observed. In Fig. 2. the tail lengths obtained from the same experiment are plotted as a function of resveratrol concentration. The results clearly establish that resveratrol alone is capable of DNA breakage in lymphocytes. However, the minimum concentration required for such breakage (100–200 μM) is considerably greater than when resveratrol (10 μM) is used along with Cu(II) as was shown in our previous publication [14].

3.2. Effect of neocuproine, a Cu(I) specific sequestering agent, on resveratrol induced DNA breakage in lymphocytes

In the previous study [14] we had shown that the resveratrol–Cu(II) mediated degradation of lymphocyte DNA is inhibited by neocuproine which is a Cu(I) specific chelating agent and is membrane permeable [20]. Fig. 3 gives the results of an experiment where three progressively increasing concentrations of neocuproine were tested on resveratrol induced DNA breakage in lymphocytes. A progressive decrease in the tail length as a function of increasing neocuproine concentration was seen. From the results we can conclude that the DNA breakage by the polyphenol involves endogenous copper ions and that Cu(I) is an intermediate in the pathway that leads to DNA breakage.

3.3. Effect of active oxygen scavengers on resveratrol induced DNA breakage in lymphocytes

We have previously shown [14] that resveratrol–Cu(II) mediated degradation of lymphocyte DNA is inhibited to significant degrees by various scavengers of reactive oxygen species. Table 1 gives the results of an experiment where three such scavengers have been tested, namely: superoxide dismutase (SOD) and catalase which remove superoxide, and H_2O_2, respectively, and thiourea which is a scavenger of several reactive oxygen species. All three cause significant inhibition of DNA breakage as evidenced by decreased tail lengths. We conclude that superoxide anion and H_2O_2 are essential components in the pathway that leads to the formation of hydroxyl radical and other species which would be the proximal DNA cleaving agents. These results along with the results of Fig. 4 suggest that resveratrol–Cu(II) induced lymphocyte DNA breakage and DNA breakage by resveratrol alone are likely the result of the same mechanism.

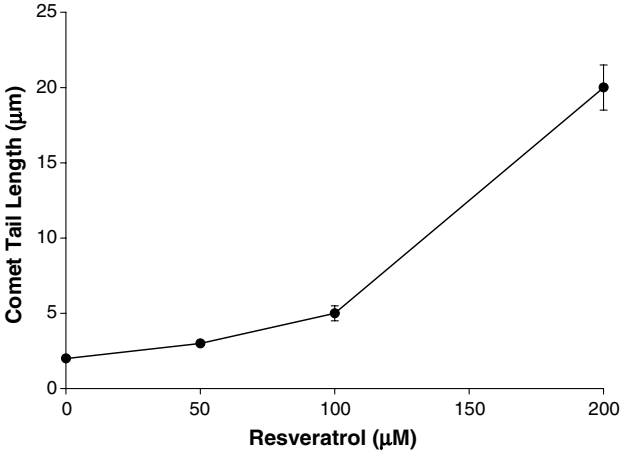

Fig. 2. Effect of increasing concentrations of resveratrol on DNA breakage in lymphocytes. The viability of cells after incubation was found to be greater than 93%. Values reported are ±S.E.M. of three independent experiments.

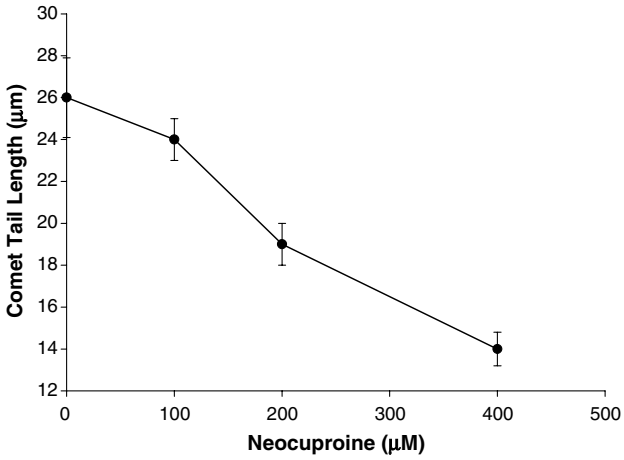

Fig. 3. Effect of increasing concentrations of neocuproine on resveratrol induced DNA breakage in human lymphocytes. Values reported are ±S.E.M. of three independent experiments.

3.4. Comparison of lymphocyte DNA breakage by various polyphenols

According to our hypothesis [13] mobilization of endogenous copper and the consequent degradation of cellular DNA is a general mechanism for anticancer properties of plant polyphenols. However, depending on the structure of the molecule there would be differences of efficiency between various polyphenols. We have therefore compared the lymphocyte DNA breakage efficiency of various polyphenols with different structures. Fig. 4 shows Comet tail lengths obtained using increasing concentrations of resveratrol and piceatannol whose parent compound *trans*-stilbene which does not have any hydroxyl group and gallic acid which is a structural constituent of tannins [7]. Syringic acid is a derivative of gallic acid where two of the hydroxyl groups are present as methoxy groups. It

Table 1
Effect of scavengers of active oxygen species on resveratrol induced lymphocyte DNA breakage

Dose	Tail length (μm)	% Inhibition
Untreated	1.22 ± 0.08 [a]	–
Resveratrol 200 μM	20.84 ± 1.28	–
+SOD 100 μg/ml	7.05 ± 0.25*	66%
+Catalase 100 μg/ml	8.86 ± 0.29 *	57%
+Thiourea 1 mM	11.93 ± 1.01*	45%

[a]All values represent S.E.M. of three independent experiments.
*P value < 0.05 when compared to control.

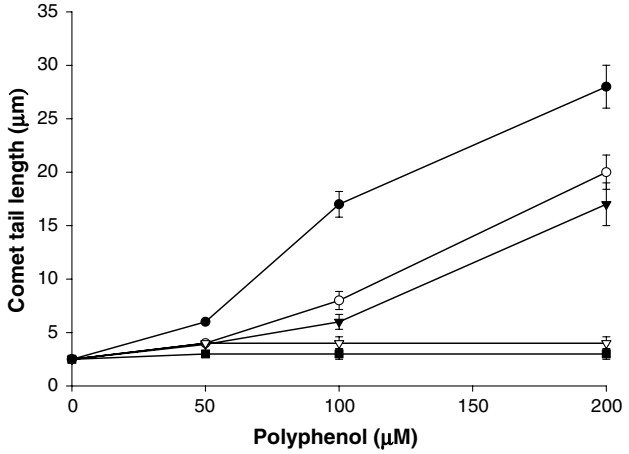

Fig. 4. Comparison of various polyphenols on the induction of DNA breakage in human lymphocytes. (●) gallic acid, (○) piceatannol, (▼) resveratrol, (△) *trans*-stilbene, (■) syringic acid. The viability of cells after treatment was found to be greater than 93%. Values reported are ±S.E.M. of three independent experiments.

is seen that except *trans*-stilbene and syringic acid all the other polyphenols are able to induce DNA breakage up to various degrees. It is worth mentioning that *trans*-stilbene and syringic acid are also ineffective as DNA cleaving agent in vitro [9] or lymphocyte DNA breakage in the presence of Cu(II) [14].

3.5. Determination of TBARS as a measure of oxidative stress in lymphocytes by resveratrol and gallic acid in the presence of neocuproine and thiourea

As mentioned above we presume that lymphocyte DNA breakage is the result of the generation of hydroxyl radicals and other reactive oxygen species in situ. Oxygen radical damage to deoxyribose or DNA is considered to give rise to TBA reactive material [21,22] We have therefore determined the formation of TBA reactive substance (TBARS) as a measure of oxidative stress in lymphocytes with increasing concentrations of resveratrol and gallic acid. The effect of preincubating the cells with neocuproine and thiourea was also studied. Results given in Fig. 5(A) and (B) show that there is a dose dependant increase in the formation of TBA reactive substance in lymphocytes. However, when cells were preincubated with neocuproine and thiourea there was a considerable decrease in the rate of formation of TBA reactive substance by both resveratrol as well as gallic acid. These results indicate that both DNA breakage and oxidative stress in cells is inhibited by Cu(I) chelation and scavenging of reactive oxygen. Thus it can be safely concluded that the formation of reactive oxygen species by polyphenols in lymphocytes involves their interaction with intracellular copper as well as its reduction to Cu(I).

4. Discussion

As already mentioned, over the last several years, we have extensively characterized a DNA cleavage reaction mediated by a number of polyphenols in the presence of copper ions [6–9]. Subsequently, using human peripheral lymphocytes and Comet assay we confirmed that the polyphenol resveratrol in the presence of Cu(II) is indeed capable of DNA degradation in a cellular system [14]. Based on our own observations

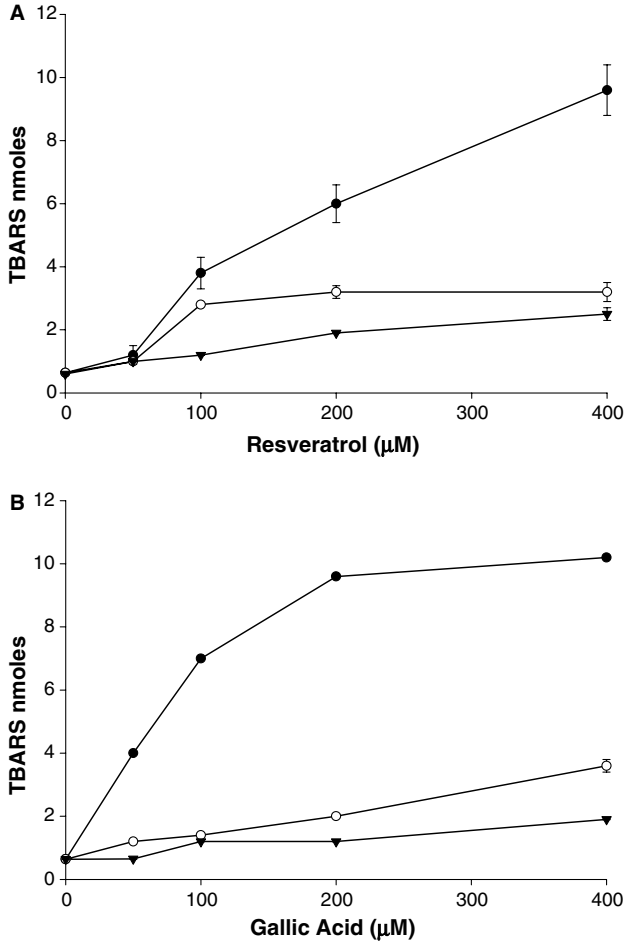

Fig. 5. Effect of pre-incubation of lymphocytes with neocuproine and thiourea on TBARS generated by increasing concentrations of resveratrol (A) and gallic acid (B). (A) Resveratrol alone (●), resveratrol + neocuproine (1 mM) (○), resveratrol + thiourea (1 mM) (▼). (B) Gallic acid alone (●), gallic acid + neocuproine (1 mM) (○), gallic acid + thiourea (1 mM) (▼). The isolated cells (1×10^5) suspended in RPMI 1640 were preincubated with the indicated concentrations of neocuproine and thiourea for 30 min at 37 °C. After pelleting the cells were washed twice with PBS (Ca^{2+} and Mg^{2+} free) before resuspension in RPMI and further incubation for 1 h in the presence of increasing polyphenol concentrations. Viability of lymphocytes after preincubation with neocuproine and thiourea was more than 90%. Values reported are ±S.E.M. of three independent experiments.

and those of others in the literature we have proposed a mechanism for the cytotoxic action of plant polyphenolics against cancer cells that involves mobilization of endogenous copper and the consequent generation of reactive oxygen species particularly the hydroxyl radical [13]. These other observations in the literature include the fact that copper is the major metal ion present in the nucleus [23]. Further it has been shown that serum [24] and tissue [25,26] concentrations of copper are greatly increased in various malignancies. Copper ions from chromatin can be mobilized by metal chelating agents giving rise to internucleosomal DNA fragmentation a hallmark property of cells undergoing apoptosis [27]. Further, it has been proposed that most clinically used anticancer drugs can activate late events of apoptosis (DNA degradation and morphological changes) and the essential signaling pathways differ between pharmacological cell death and physiological induction of cell death [28].

Thus, we suggest that the conclusion of the present study is that polyphenols possessing anticancer or apoptosis inducing activity are able to mobilize endogenous copper ions possibly the copper bound to chromatin. It is realized that the above results do not categorically prove that lymphocyte DNA degradation described above involves mobilization of chromatin bound copper. However, there are a number of observations which suggest that this is indeed the case. The generation of hydroxyl radicals in the proximity of DNA is well established as a cause of strand scission. It is generally recognized that such reaction with DNA is preceded by the association of a ligand with DNA followed by the formation of hydroxyl radicals at that site. Among the oxygen radicals the hydroxyl radical is the most electrophilic with high reactivity and therefore possesses a small diffusion radius. Thus in order to cleave DNA it must be produced in the vicinity of the DNA [29]. The location of redox-active metals is of utmost importance for the ultimate effect because the hydroxyl radical, due to its extreme reactivity, interacts exclusively in the vicinity of the bound metal [30]. Indeed we have earlier shown that flavonoids are able to form a ternary complex with DNA and Cu(II) where Cu(II) is reduced to Cu(I) [31]. Polyphenols are known to reduce molecular oxygen to superoxide anion, an event that may occur even outside the cell, leading to the formation of H_2O_2 [6]. Superoxide can also be formed by reoxidation of Cu(I)–Cu(II) in the ternary complex [31]. Chromatin bound copper is understood to be present in the reduced form (Cu(I)) [32] and thus would be available for reoxidation to Cu(II) by H_2O_2 in the Fenton type reaction and binding to polyphenols and recycling. It is well known that polyphenols autooxidize in cell culture media to generate H_2O_2 and quinones that can enter cells causing damage to various macromolecules [19,33,34]. This may lead to extracellular production of reactive oxygen species that could account for lymphocytes DNA breakage. However, this does not appear to be the case in our system since we have previously shown that no lymphocyte DNA breakage is observed on preincubating the cells with resveratrol alone up to a concentration of 50 μM. DNA breakage could only be seen after incubating the pre-treated cells further in the presence of Cu(II) [14]. Further, we could not detect any H_2O_2 formation on incubating resveratrol (up to a concentration of 300 μM) in RPMI medium (results not shown).

Evidence suggests that polyphenolic compounds such as tannins and resveratrol are able to traverse cell membranes and may enter the cytoplasmic or nuclear space. Resveratrol is sufficiently hydrophobic and has been shown to be present in tissues such as heart, liver and kidney [35]. The question of bioavailability of polyphenols in mammalian systems also needs to be addressed. Relatively recent work by Asensi et al. [36] with resveratrol indicates that it may have a relatively low bioavailability due to its biotransformation and rapid elimination. It was reported that the highest concentration of resveratrol in plasma was reached within the first 5 min (2.6 ± 1 μM) after receiving 20 mg res/kg.b.w orally [36]. Nevertheless these authors further report that 5 μM resveratrol completely inhibited the growth of B-16 M murine melanoma cells. Because of higher intracellular copper levels it may be predicted that such concentrations of resveratrol for cytotoxic action against cancer cells would be considerably lower. Indeed it has been shown that ascorbate which also acts as a prooxidant in the presence of copper ions is cytotoxic to a leukemic cell line at a lower concentration than normal lymphocytes [37]. Most studies on anticancer mechanisms of plant polyphenols invoke the induction of cell cycle arrest at the S/G2 phase transition brought about by an increase in cyclins A and E and inactivation of cdc 2. Other mechanisms have also been proposed [36]. Based on our work we would like to propose that mobilization of endogenous copper ions by polyphenols and the consequent prooxidant action could be one of the important mechanisms for their anticancer and chemopreventive properties. Indeed such a common mechanism would better explain the anticancer effects of polyphenols with diverse chemical structures as also the preferential cytotoxicity towards cancer cells.

Acknowledgment: The authors acknowledge the financial assistance provided by the University Grants Commission, New Delhi under the DRS programme.

References

[1] Mukhtar, H., Das, M., Khan, W.A., Wang, Z.Y., Bik, D.P. and Bickers, D.R. (1988) Exceptional activity of tannic acid among naturally occurring plant polyphenols protecting against 7,12 dimethyl benz(a)anthracene, benzo(a)pyrene, 3-methyl cholanthrene and *N*-methyl-*N*-nitrosourea-induced skin tumorigenesis in mice. Cancer Res. 48, 2361–2365.

[2] Jaruga, E., Salvioli, S., Dobrucki, J., Chrul, S., Bandorowicz-Pikula, J., Sikora, E., Franceschi, C. and Cossarizza, A. (1998) Apoptosis like reversible changes in plasma membrane asymmetry and permeability and transient modifications in mitochondrial membrane potential induced by curcumin in rat hepatocytes. FEBS Lett. 433, 287–293.

[3] Clement, M., Hirpara, J.L., Chawdhury, S.H. and Pervaiz, S. (1998) Chemopreventive agent resveratrol, a natural product derived from grapes, triggers CD95 signalling dependant apoptosis in human tumor cells. Blood 92, 996–1002.

[4] Inoue, M., Suzuki, R., Koide, T., Sakaguchi, N., Ogihara, Y. and Yabu, Y. (1994) Antioxidant, gallic acid induces apoptosis in HL 60 R cells. Biochem. Biophys. Res. Commun. 204, 898–904.

[5] Ahmad, N., Feyes, D.K., Nieminen, A.L., Agarwal, R. and Mukhtar, H. (1997) Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. J. Natl. Cancer Inst. 89, 1881–1886.

[6] Ahmad, M.S., Fazal, F., Rahman, A., Hadi, S.M. and Parish, J.H. (1992) Activities of flavonoids for the cleavage of DNA in the presence of Cu(II): correlation with the generation of active oxygen species. Carcinogenesis 13, 605–608.

[7] Khan, N.S. and Hadi, S.M. (1998) Structural features of tannic acid important for DNA degradation in the presence of Cu(II). Mutagenesis 13, 271–274.

[8] Ahsan, H. and Hadi, S.M. (1998) Strand scission in DNA induced by curcumin in the presence of Cu(II). Cancer Lett. 124, 23–30.

[9] Malik, A., Azam, S., Hadi, N. and Hadi, S.M. (2003) DNA degradation by water extract of green tea in the presence of copper ions: implications for anticancer properties. Phytother. Res. 17, 358–363.

[10] Ahmad, A., Asad, S.F., Singh, S. and Hadi, S.M. (2000) DNA breakage by resveratrol and Cu(II): reaction mechanism and bacteriophage inactivation. Cancer Lett. 154, 24–27.

[11] Kagawa, T.F., Geierstanger, B.H., Wang, A.H.J. and Ho, P.S. (1991) Covalent modification of guanine bases in double stranded DNA: the 1:2-AZ-DNA structure of dc(cacacg) in the presence of $CuCl_2$. J. Biol. Chem. 266, 20175–20184.

[12] Gali, H.U., Perchellet, E.M., Klish, D.S., Johnson, J.M. and Perchellet, J.P. (1992) Hydrolysable tannins: potent inhibitors of hydroperoxide production and tumor promotion in mouse skin treated with 12-*O*-tetradecanoyl phorbol-13-acetate. Int. J. Cancer 51, 425–432.

[13] Hadi, S.M., Asad, S.F., Singh, S. and Ahmad, A. (2000) Putative mechanism for anticancer and apoptosis inducing properties of plant-derived polyphenolic compounds. IUBMB Life 50, 1–5.

[14] Azmi, A.S., Bhat, S.H. and Hadi, S.M. (2005) Resveratrol–Cu(II) induced DNA breakage in human peripheral lymphocytes: implications for anticancer properties. FEBS Lett. 579, 3131–3135.
[15] Renner, H.W. and Schmezer, P. (1993) Assessment of genotoxic effects of lindane. Food Chem. Toxicol. 31, 184–191.
[16] Singh, N.P., McCoy, M.T., Tice, R.R and Schneider, E.L. (1998) A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. 175, 184–191.
[17] Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.C. and Sasaki, Y.F. (2000) Single cell gel electrophoresis/Comet assay; guidelines for in vitro and in vivo genetic toxicology testing. Environ. Mol. Mutagen. 35, 206–221.
[18] Ramanathan, A., Das, N.P. and Tan, C.H. (1994) Effects of γ-linolenic acid, flavonoids and vitamins on cytotoxicity and lipid peroxidation. Free Radical Biol. Med. 16, 43–48.
[19] Long, L.H., Clement, M.V. and Halliwell, B. (2000) Artifacts in cell culture: rapid generation of hydrogen peroxide on addition of (−)-epigallocatechin, (−)-epigallocatechin (+)-catechin and quercetin to commonly used cell culture media. Biochem. Biophys. Res. Commun. 273, 50–53.
[20] Barbouti, A., Doulias, P.E., Zhu, B.Z., Frei, B. and Galaris, D. (2001) Intracellular iron, but not copper plays a critical role in hydrogen peroxide-induced DNA damage. Free Radical Biol. Med. 31, 490–498.
[21] Smith, C., Halliwell, B. and Aruoma, O.I. (1992) Protection by albumin against the prooxidant action of phenolic dietary components. Food Chem. Toxicol. 30, 483–489.
[22] Quinlan, G.J. and Gutteridge, M.C. (1987) Oxygen radical damage to DNA by rifamycin SV and copper ions. Biochem. Pharmacol. 36 (21), 3629–3633.
[23] Bryan, S.E. (1979) Metal Ions in Biological Systems, Marcel Dekker, New York.
[24] Ebadi, E. and Swanson, S. (1998) The status of zinc, copper and metallothionine in cancer patients. Prog. Clin. Biol. Res. 259, 167–175.
[25] Yoshida, D., Ikada, Y. and Nakayama, S. (1993) Quantitative analysis of copper, zinc and copper/zinc ration in selective human brain tumors. J. Neuroncol. 16, 109–115.
[26] Nasulewis, A., Mazur, A. and Opolski, A. (2004) Role of copper in angiogenesis: clinical-implication. J. Trace Elem. Med. Biol. 18, 1–8.
[27] Burkitt, M.J., Milne, L., Nicotera, P. and Orrenius, S. (1996) 1–10-Phenanthroline stimulates internucleosomal DNA fragmentation in isolated rat liver nuclei by promoting redox activity of endogenous copper ions. Biochem. J. 313, 163–169.
[28] Smets, K.A. (1994) Programmed cell death (apoptosis) and the response to anticancer drugs. Anticancer Drugs 5, 3–9.
[29] Pryor, W.A. (1988) Why is hydroxyl radical the only radical that commonly binds to DNA? Hypothesis: it has rare combination of high electrophilicity, thermochemical reactivity and a mode of production near DNA. Free Radical Biol. Med. 4, 219–233.
[30] Chevion, M. (1988) Site specific mechanism for free radical induced biological damage. The essential role of redox-active transition metals. Free Radical Biol. Med. 5, 27–37.
[31] Rahman, A., Shahabuddin, S., Hadi, S.M., Parish, J.H. and Ainley, K. (1989) Strand scission in DNA induced by quercetin and Cu(II) and oxygen free radicals. Carcinogenesis 10, 1833–1839.
[32] Lewis, C.D. and Laemelli, U.K. (1982) Higher order metaphase chromosome structure: evidence for metalloproteins interactions. Cell 29, 171–181.
[33] Halliwell, B. (2003) Oxidative stress in cell culture: an underappreciated problem? FEBS Lett. 540, 3–6.
[34] Clement, M.V., Long, L.H., Ramalingam, J. and Halliwell, B. (2002) The cytotoxicity of dopamine may be an artefact of cell culture. J. Neurochem. 81 (3), 414–421.
[35] Bertilli, A.A., Giovannini, L., Stradi, R., Bertelli, A. and Tillement, J.P. (1996) Plasma, urine and tissue levels of *trans* and *cis*-resveratrol (3,4′,5-trihydroxystilbene) after short-term or prolonged administration of red wine to rats. Int. J. Tissue React. 18, 67–71.
[36] Asensi, M., Medina, I., Ortega, A., Corretera, J., Carmen-Bano, M., Obrador, E. and Estrela, M.J. (2002) Inhibition of cancer growth by resveratrol is related to its low bioavailability. Free Radical Biol. Med. 33, 387–398.
[37] Singh, N.P. (1997) Sodium ascorbate induces DNA single-strand breaks in human cells in vitro. Mutat. Res. 375, 195–203.