Research Article

Olive oil protects rat liver microsomes against benzo(a)pyrene-induced oxidative damages: An *in vitro* study

Kasi Pandima Devi[1], Perumal Vijayaraman Kiruthiga[1], Shanmugiahthevar Karutha Pandian[1], Govindaraju Archunan[2] and Solayan Arun[3, 4]

[1] Department of Biotechnology, Alagappa University, Tamil Nadu, India
[2] Department of Animal Science, Bharathidasan University, Tamil Nadu, India
[3] Central Electrochemical Research Institute, Tamil Nadu, India
[4] Alagappa University, Tamil Nadu, India

Benzo(a)pyrene (B(a)P), a member of the polycyclic aromatic hydrocarbon family is present ubiquitously in the environment. One of its toxic effects is induction of oxidative stress (mediated by the enzyme B(a)P hydroxylase) which leads to various diseases like cancer. Olive oil (OO) that consists of many antioxidant compounds is reported to have many beneficial properties including protection against cancer. The objective of the present study is to evaluate the effect of OO on B(a)P hydroxylase enzyme and further elucidate the antioxidant capacity of OO against B(a)P-induced toxicity. Rat liver microsomes were divided into three groups: vehicle control, B(a)P treated group, and OO + B(a)P co-incubated group. Antioxidant enzymes which were decreased and protein carbonyl content and lipid peroxidation products which were increased on exposure to B(a)P was attenuated to near normal on OO exposure. B(a)P hydroxylase enzyme was very low in OO incubated group which may be due to inhibition of the enzyme by OO or high utilization for the metabolism of B(a)P. Further, no B(a)P metabolites (3-OH B(a)P and B(a)P 7,8-dihydrodiol) were identified in HPLC during B(a)P + OO exposure. The results prove the protective role of OO against B(a)P-induced oxidative damage.

Keywords: Antioxidant enzymes / B(a)P 7,8-dihydrodiol / B(a)P hydroxylase / B(a)P metabolites / 3-hydroxy B(a)P

Received: February 4, 2008; revised: April 21, 2008; accepted: April 21, 2008

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) like benzo(a)pyrene (B(a)P) are ubiquitous environmental contaminants which are activated by two major reactions (i) one-electron oxidation to form radical cations, and (ii) monooxygenations to form metabolites like quinone, phenol, diol epoxides, *etc*. [1–3]. The second reaction is the most important phenomenon that produces carcinogenic metabolites and paves for hazardous consequences to human health. Metabolism of B(a)P occurs primarily through the action of Cytochrome P 450 (CYP1) family of P450 monooxygenase enzymes, such as B(a)P hydroxylase, into electrophilic epoxides [4–6]. The effectiveness of B(a)P metabolites on the carcinogenicity and the binding of these metabolites with DNA and proteins are reported to be associated with the induction of B(a)P hydroxylase enzymes [7–9]. B(a)P also causes lipid peroxidation (LPO) either by producing the reactive oxygen species (ROS) or decreasing the activities of antioxidant enzymes that lead to cellular damage and cellular dysfunction [10]. Yan *et al*. [11] have reported that depletion of the intracellular antioxidant, glutathione significantly increased B(a)P-mediated induction of COX-2 which has been detected both in atherosclerotic lesions and in epithelial cancers. Results on acute neurotoxicity of B(a)P has suggested that B(a)P-induced acute neurobehavioral toxicity may occur through oxidative stress due to inhibition of the brain antioxidant scavenging system [12]. B(a)P-quinones (BPQs), which are the important metabolites of BaP have been associated with the production of

Correspondence: Dr. Kasi Pandima Devi, Department of Biotechnology, Alagappa University, Karaikudi 630 003, Tamil Nadu, India
E-mail: devikasi@yahoo.com
Fax: +914565-225202

Abbreviations: B(a)P, benzo(a)pyrene; **BPQ,** B(a)P-quinone; **CAT,** catalase; **CDNB,** 1-chloro-2,4-dinitrobenzene; **CYP1,** cytochrome P 450; **GR,** glutathione reductase; **GST,** glutathione *S*-transferase; **LPO,** lipid peroxidation; **OO,** olive oil; **PCC,** protein carbonyl content; **ROS,** reactive oxygen species; **SOD,** superoxide dismutase

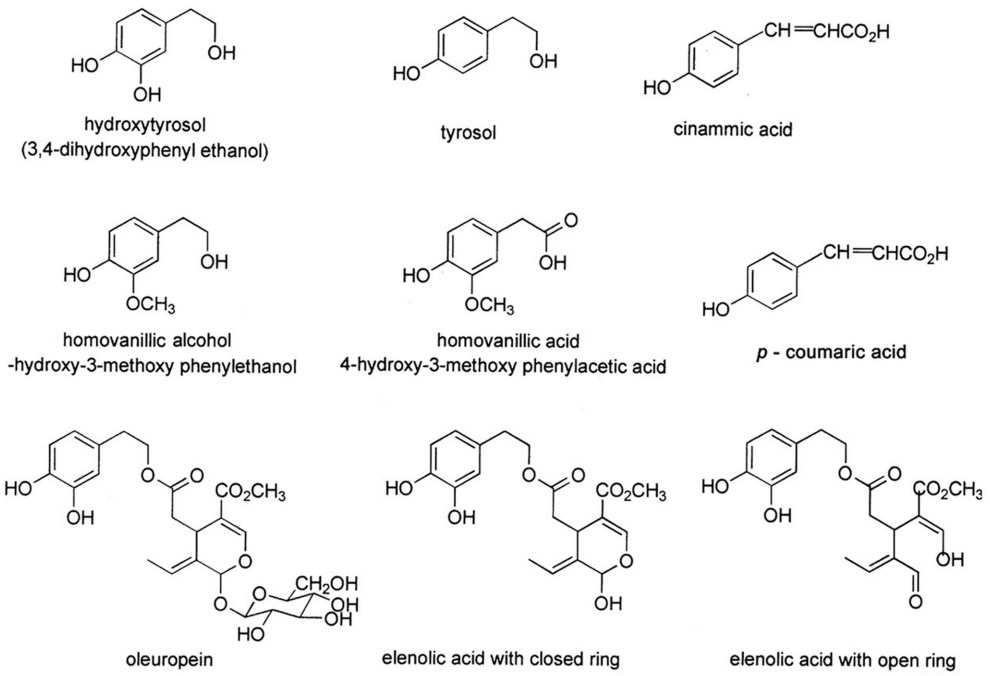

Figure 1. The major constituents of OO.

ROS. The BPQs – 1,6-BPQ and 3,6-BPQ produce superoxide anion and hydrogen peroxide in breast epithelial cells [13]. Our earlier report also proves that the toxic potential of B(a)P is ensued by production of oxidative stress [14]. Consequently, B(a)P-induced toxicity can be attributed to both induction of B(a)P hydroxylase and oxidative stress. Though several synthetic antioxidants are available to combat such toxicities, a growing trend has been targeted toward the use of natural products as antioxidants in view of toxicity condemn [15]. It has also been reported that some plant products, nutrients, and food additives are involved in the alteration of CYP450 dependent monooxygenase system and finally alter the toxicity and carcinogenicity of environmental carcinogens [16–18].

The olive tree, *Olea europaea*, is native to the Mediterranean basin and parts of Asia Minor. The fruit and compression extracted oil have a wide range of therapeutic and culinary applications. Olive oil (OO) also constitutes a major component of the "Mediterranean diet." Although the composition of OO is complex, the major groups of compounds thought to contribute to its observed health benefits include oleic acid, squalene, and phenolics like hydroxytyrosol, tyrosol, and oleuropein (Fig. 1), all of which have been found to inhibit oxidative stress [19, 20]. Hydroxytyrosol and oleuropein scavenge-free radicals and inhibit low density lipoprotein (LDL) oxidation. These two phenols are considered potent antioxidants, demonstrating activity in the micromolar range. Both are more potent at scavenging free radicals than the endogenous antioxidant vitamin E and the exogenous antioxidants DMSO and butylated hydroxytoluene (BHT). These two catechols have been shown to scavenge a variety of endogenous and exogenous free radicals and oxidants, including those generated by hydrogen peroxide, hypochlorous acid, and xanthine/xanthine oxidase [21]. Reports describing the beneficial properties of OO as an antioxidant have dramatically increased nowadays [22] and it has been postulated that the lower incidence of coronary heart disease and prostate and colon cancers in Greece, Italy, and Spain is due to the Mediterranean diet, which has OO. Based on earlier reports, it has been assumed that, OO may have a potential role in lowering the risk of cancer [23–25]. However, there are no reports on the protective potential of OO against toxicity induced by environmental carcinogens like B(a)P.

Hence, the aim of our study is to investigate the oxidative damage in microsomes during B(a)P *in vitro* incubation and to further elucidate the protective antioxidant potential of OO against this environmental toxin. In order to detect the ability of B(a)P metabolism in microsomes we made an attempt to find out the metabolites of B(a)P during *in vitro* incubation. In addition, we have estimated oxidative stress-induced changes in some essential biochemical parameters important for the correct functioning of microsomes, *i.e.*, the effect of OO and B(a)P on lipid peroxidation product, protein carbonyl content (PCC), and the activities of B(a)P hydroxylase and antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione *S*-transferase (GST), and glutathione reductase (GR) together with the

quantification and identification of B(a)P metabolites. Our results demonstrate that OO is a potent antioxidant against B(a)P-induced oxidative stress damages in microsomes.

2 Materials and methods

2.1 Chemicals and reagents

OO was purchased from Sd fine Chem (Mumbai, India). B(a)P was purchased from Alfa Aesar (MA, USA). B(a)P metabolites 3-OH B(a)P and B(a)P 7,8-dihydrodiol were obtained from the National Cancer Institute's Chemical Repository, USA. All the other chemicals and reagents used were of analytical grade.

2.2 Preparation of hepatic microsomes

Male Wistar Albino Rats were anesthetized with pentobarbital sodium (0.2 g/kg body weight), and the liver was removed immediately after death of animal without any macroscopic alterations. The rat liver microsomal fractions were prepared by calcium-aggregation method [26]. Briefly excised livers (20 g) were thawed, finely chopped with a pair of scissors, and homogenized with four volumes (80 mL) of ice-cold 10 mM Tris-HCl buffer containing 0.25 M sucrose, pH 7.4, in a glass homogenizer equipped with a Teflon pestle. The homogenate was centrifuged at $600 \times g$ for 5 min to remove pellet with debris and then take supernatant. It was centrifuged at $13\,000 \times g$ for 10 min at 4°C in a refrigerated centrifuge (Hitachi Koki, Japan), and the precipitate was discarded. To the supernatant, calcium chloride was added to yield a final concentration of 8 mM. The solution was stirred for 15–20 min, and then centrifuged at $25\,000 \times g$ for 10 min at 4°C. The firmly packed pellets of microsomes were resuspended by homogenization in 100 mM Tris-HCl buffer containing 20% w/v glycerol and 10 mM EDTA, pH 7.4. The protein concentration of the microsomal fraction was determined by Lowry's method, 1951 [27] and the microsomes were finally stored at −80°C until required for assay.

2.3 Microsomal incubation for *in vitro* experiments

For *in vitro* incubation study, microsomal mixture was divided into three groups.

Group 1

Vehicle control — 1 mL of the reaction mixture containing 1.25 mg of microsomal protein, 100 mM $MgCl_2$, 0.2 mg BSA, and 2 mM NADPH in 100 mM Tris-HCl buffer pH 7.4

Group 2

B(a)P treatment — 1 mL of the reaction mixture containing 1.25 mg of microsomal protein, 100 mM $MgCl_2$, 0.2 mg BSA, 2 mM NADPH, and 25 µM of B(a)P in 100 mM Tris-HCl buffer pH 7.4

Group 3

OO cotreatment — 1 mL of the reaction mixture containing 1.25 mg of microsomal protein, 100 mM $MgCl_2$, 0.2 mg BSA, 2 mM NADPH, 25 µM of B(a)P, and 10 µL of OO in 100 mM Tris-HCl buffer pH 7.4

The treatment of microsomes with OO *per se* had a similar value as that of control in all the parameters. So the values are not given in the manuscript. Incubations were carried out for 1 h at 37°C in a shaker and the reaction was terminated by chilling the mixture on ice. Following this, the enzyme assays were carried out at 37°C.

2.4 Assay of CAT activity

CAT activity was measured according to Aebi *et al*. [28] by using UV/Vis spectrophotometer. At 37°C, 25 µL of microsomes from the different groups and 1 mL of H_2O_2 (30 mM) were added to 1.950 mL phosphate buffer pH 7.4. The rapid decomposition of H_2O_2 was determined for every 30 s from the decrease in absorbance at 240 nm for 3 min. Enzyme activity was expressed as mM H_2O_2 decomposed/min/mg protein using a molar extinction coefficient for H_2O_2 of 0.0394 $mM^{-1} \cdot cm^{-1}$.

2.5 Assay of GST activity

GST was assayed by measuring at 340 nm the rate of formation of GSH conjugate with 1-chloro-2,4-dinitrobenzene (CDNB) [29]. The reaction mixture contained 50 µL of microsomes from the different groups, 20 mM CDNB (in ethanol), and 0.1 M phosphate buffer, pH 7.4, in a final volume of 1 mL. Phosphate buffer–CDNB mixture was preincubated for 10 min at 37°C and the reaction was started by adding 20 mM GSH. The activity was expressed as nM of GSH decomposed /min/mg protein using an extinction coefficient of GSH 9.6 $M^{-1} \cdot cm^{-1}$.

2.6 Assay of GR activity

Activity of the GR was determined as described by Carlberg and Mannervik [30]. The reaction mixture consisted of 0.1 M phosphate buffer (pH 7.6), containing 0.5 mM EDTA, 1.0 mM GSSG, and 50 µL of microsomes from the different groups in a final volume of 1.0 mL. The reaction was started by adding 0.1 mM NADPH. The consumption of NADPH was monitored spectrophotometrically at 340 nm. The enzyme activity was expressed as nM of NAPDH oxidized /min/mg of protein, using an extinction coefficient of 6.22 $mM^{-1} \cdot cm^{-1}$.

2.7 Assay of SOD

SOD was assayed according to the method of Paoletti and Mocali [31], based on NADPH oxidation. The method consists of a purely chemical reaction sequence, which generates superoxide anion from molecular oxygen in the presence of EDTA, manganese(II) chloride, and mercaptoethanol. The reaction mixture in a final volume of 1 mL contained 1.9 mL of 100 mM triethanolamine diethanolamine – HCl buffer (TDB), 10 μL of 2 mM NADPH, 50 μL of 100 mM EDTA – MnCl, and 0.2 mL of microsomes from the different groups. The contents were mixed and allowed to stand for 5 min for a stable baseline. The reaction was initiated by the addition of 0.2 mL of 10 mM mercaptoethanol. The contents of the cuvette were mixed and the decrease in absorbance at 340 nm was followed for 10 min to allow NADPH oxidation. The enzyme activity was expressed as units/mg protein (1 U = mM of NAPDH oxidized /min using an extinction coefficient of 6.22 $mM^{-1} \cdot cm^{-1}$).

2.8 Measurement of LPO

Microsomes from the different groups were mixed with 20% TCA (1:1) to precipitate the protein. The samples were centrifuged (3000 rpm for 15 min), 15% TBA was added to the supernatant and the samples were heated to 100°C for 15 min in a water bath. The absorbance of the supernatant was measured at 532 nm and LPO was expressed as nM of TBARS/mg of protein which was determined using the calibration curve prepared with different concentrations of MDA standards.

2.9 Determination of protein carbonyl content

Carbonyl content was measured by the method of Levine *et al*. [32]. Briefly, 100 μL of microsomes from the different groups were taken in two tubes labeled as "test" and "control." Equal volume of 10 mM 2,4-dinitrophenylhydrazine (DNPH) prepared in 2.5 M HCl was added to the test tube and equal volume of 2.5 M HCl alone was added to the control tube. The contents were mixed thoroughly and incubated in the dark (room temperature) for 1 h, with intermittent shaking for every 15 min. After 1 h, equal volume of 10% TCA w/v was added to both the tubes and centrifuged at 3500 rpm for 20 min to obtain the protein pellet. The supernatant was carefully aspirated and discarded. This was followed by a second wash with 10% TCA as described above. Further, the precipitates were washed three times with of ethanol/ethyl acetate (1:1 v/v) to remove unreacted DNPH and lipid remnants. The final protein pellet was dissolved in 2 mL of 6 M guanidine hydrochloride and incubated at 37°C for 10 min. The insoluble materials were removed by centrifugation and the carbonyl content was determined by taking the absorbance of the representative samples at 370 nm. The test sample was read against the corresponding control sample and the carbonyl content was calculated using an absorption coefficient of 22 000 $M^{-1} \cdot cm^{-1}$. The PCC was expressed as μM/mg of protein.

2.10 Assay of B(a)P hydroxylase activity

B(a)P hydroxylase enzyme was measured by the formation of phenolic metabolites using fluorescent spectrophotometer (excitation 467 nm; emission 525 nm) [33]. Quinine sulfate (QS) and 3-OH B(a)P were used as standards for B(a)P metabolites. In brief, final assay conditions in a volume of 1 mL contained 50 mM triethanolamine – HCl pH 7.25, 0.2 μM NADPH, 100 μM B(a)P, 100 μL of microsomal homogenate, and an incubation time of 10 min. Arbitrary fluorescence units were converted into picomoles of B(a)P-phenols formed using intercalibration between QS and 3-hydroxyB(a)P.

2.11 Detection of B(a)P metabolites using HPLC

After incubating the microsomes with B(a)P and OO, the reaction was terminated with 1 mL of ice-cold acetone. B(a)P metabolites were extracted with ethyl acetate, dried with Na_2SO_4, and evaporated. Metabolite residue was redissolved in 60 μL methanol. *In vitro* metabolism of B(a)P in microsomes were investigated with the help of HPLC. B(a)P and B(a)P metabolites (3-hydroxy B(a)P and 7,8-B(a)P dihydrodiol) were separated by HPLC using C-18 column with a linear solvent methanol in 15 min at a flow rate of 1 mL/min and monitored at 254 nm. Retention time of these metabolites was compared to authentic standards obtained from the National Cancer Institute's Chemical Repository, USA.

2.12 Statistical significance

All the experiments were performed individually in triplicates and the statistical analysis of the data was determined by Student's *t*-test, expressed as *p* (probability) values, and comparisons were made between group 1 *versus* 2 and group 2 *versus* 3.

3 Results and discussion

The importance of nutrition in protecting the living organisms from the toxic effects of environmental carcinogens has recently been realized [34, 35] and foods with antioxidants play a significant role in protecting living organisms from the toxic effects of carcinogenic chemical substances present in the environment. In this study, we have investigated the antioxidant property of OO (which consists of many antioxidant phenolic components) against B(a)P-

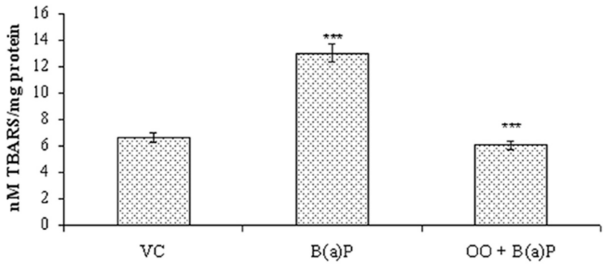

Figure 2. Effect of OO on LPO against B(a)P-induced oxidative damage in rat liver microsomes. Values are mean ± SD for three individual determinations (***$p < 0.05$).

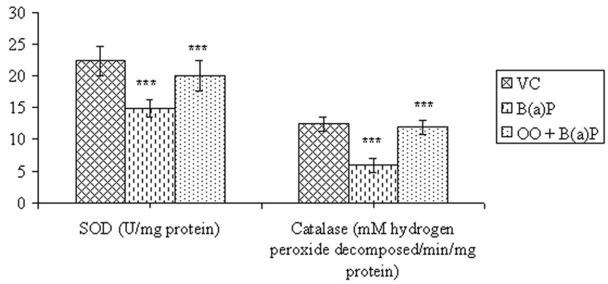

Figure 3. Effect of OO on activities of SOD and CAT against B(a)P-induced oxidative damage in rat liver microsomes. Values are mean ± SD for three individual determinations (***$p < 0.05$).

induced toxicity. In addition, the effect of OO in scavenging the reactive metabolites of B(a)P were also evaluated.

B(a)P treatment increased the levels of LPO products significantly ($p < 0.05$) which indicated the imposition of oxidative stress in the microsomes (Fig. 2) B(a)P is reported to cause LPO either by producing the ROS or decrease in the activities of antioxidant enzymes that lead to cellular damage and cellular dysfunction. Emre *et al*. [10] have shown that MDA levels of lung and brain tissues in B(a)P treated animals were significantly higher than that in the control group. Kim *et al*. [36] have also observed a significantly increased MDA levels in sera of experimental animals 12 h after B(a)P treatment which persisted up to 96 h. The time-dependent pattern of serum LPO was shown to be related to the concentrations of the BPQ metabolites. These results suggest that BaP treatment, probably *via* the formation of BPQs, oxidatively altered lipids, and might be associated with BaP-related carcinogenesis. Treatment of microsomes with OO normalized the levels of LPO, which indicates that the components of OO might effectively remove the metabolites and thus protects the lipids from peroxidation.

The antioxidant systems present in our system prevent the uncontrolled formation of free radicals and activated oxygen species, or inhibit their reactions with biological structures [37]. It is discernible from the result that, incubation with 25 μM B(a)P for one hour significantly ($p < 0.05$) reduced SOD and CAT in rat liver microsomes (Fig. 3). SOD, which was discovered in the late 1960s, catalyzes the

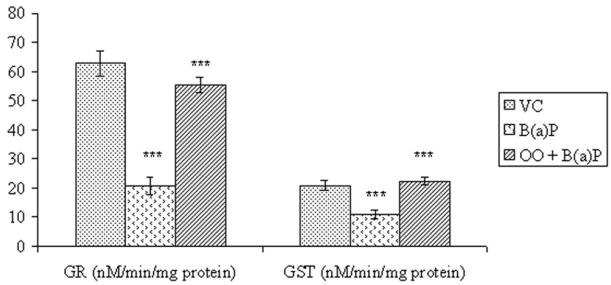

Figure 4. Effect of OO on activities of GR and GST against B(a)P-induced oxidative damage in rat liver microsomes. Values are mean ± SD for three individual determinations (***$p < 0.05$).

transformation of the superoxide radical into hydrogen peroxide, which can then be further transformed by the enzyme CAT into water and molecular oxygen. SOD acts in the aqueous phase to trap superoxide free radicals. Emre *et al*. [10] have reported that SOD activities of lung and brain tissues in B(a)P treated rats decreased significantly, compared to that in control group. Kim and Lee [38] have reported that the activities of SOD and CAT activities in the tissues of rats (liver, kidney, and lung) were decreased after B(a)P treatment which suggests that B(a)P oxidatively alters antioxidant enzymes which might be associated with B(a)P carcinogenesis. The levels of SOD and CAT were normalized by treatment with OO.

The levels of GR and the conjugation enzyme GST also significantly reduced ($p < 0.05$) on B(a)P treatment (Fig. 4). Kim *et al*. [39] observed that nearly 0.5 – 4 h after B(a)P treatment, GR expression was decreased in human bronchial epithelial cells. Selvendiran *et al*. [40] have observed a decrease in the levels of GST in B(a)P-induced experimental lung carcinogenesis. GST, the major cellular phase II detoxification enzymes catalyzes the detoxification of electrophilic diol epoxides produced by the metabolism of B(a)P. In contrast, B(a)P incubation did not alter the levels of the above enzymes when co-incubated with OO. This may due to removal of ROS by the components of OO. Masella *et al*. [35] found that biophenols of OO inhibit the oxidation of LDL by way of increase in the transcription of glutathione-related enzymes. Ruiz-Gutierrez *et al*. [41] observed a significant increase in the activities of the antioxidant enzymes CAT, GPx, and SOD in OO fed rats. These results of OO treatment on SOD to rats has suggest that OO has modulatory effects on the expression of CuZnSOD and MnSOD activity in the liver of female rats [42]. High levels of GST was found to be effective in reducing B(a)P-diolepoxide (BPDE)-induced DNA damage in cell lines [43]. Hence, the normalized levels of GST on OO treatment in our present study may play an important role in lowering the incidence of BPDE-induced DNA damage.

The levels PCC which is measured as an index of protein oxidation was significantly increased ($p < 0.05$) in the

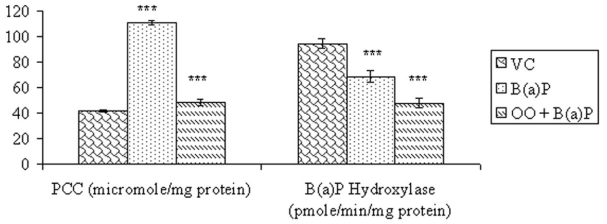

Figure 5. Effect of OO on PCC and B(a)P hydroxylase activity in rat liver microsomes. Values are mean ± SD for three individual determinations (***$p < 0.05$).

microsomes after treatment with B(a)P (Fig. 5). This indicates an increase in oxidatively modified proteins during B(a)P treatment. ROS are known to convert amino groups of protein to carbonyl moieties. Oxidative modification of proteins leads to increased recognition and degradation by proteases and loss of enzymatic activity [44]. Kim and Lee [38] have reported an increase in PCC 6 h after treatment of B(a)P to experimental animals. In our present study, treatment with OO decreased PCC to a level that was statistically indistinguishable from that of the control, which further elucidates the protective potential of OO against B(a)P-induced toxicity.

Earlier reports affirm that certain plant extracts have the ability to inhibit microsomal CYP1A activity [45]. To ascertain whether the components of OO alter/inhibit the activity of B(a)P hydroxylase (CYP1A) enzyme and thus reduce the production of ROS, we measured the B(a)P hydroxylase enzyme in rat liver microsomes that were co-incubated with 10 μL/mL of OO and 25 μM of B(a)P for 1 h. At the end of the incubation period B(a)P hydroxylase enzyme was measured, the results of which are depicted in Fig. 5. In B(a)P alone incubated reaction mixture the utilized enzyme level was very low (68.5 pmol/min/mg protein). After 1 h incubation period, B(a)P hydroxylase enzyme was very low in OO incubated reaction mixture (48 ± 3.5 pmol/min/mg protein) when compared to control (94.5 ± 4 pmol/min/mg protein) which denotes two possible reasons. One is inhibition of hydroxylase enzyme by OO and the other is high utilization of hydroxylase enzyme for the metabolism of B(a)P (control – 94.5 ± 4 pmol/min/mg protein; OO co-incubation – 48 ± 3.5 pmol/min/mg protein). Zhu *et al*. [16] also found an increased level of liver microsomal oxidation in mice when fed with rosemary plant extract. The results could mean that OO constituents can also be oxidized by BaP hydroxylase noncompetitively and therefore radical scavenging enzymes were not altered, as B(a)P was not converted to the hydroxyl products.

During B(a)P hydroxylase (CYP1A)-mediated B(a)P metabolism, generation of free radicals and carcinogenic metabolites (epoxides and diols) have been reported [46]. Several metabolites of B(a)P can induce mutations, transform cells and/or bind to cellular macromolecules; however, only a 7,8-diol-9,10-epoxide is presently considered to be an ultimate carcinogenic metabolite [47]. Using HPLC method (ODS-C-18 column) we made an attempt to find out the levels of B(a)P metabolites in rat liver microsomes incubated with B(a)P and B(a)P + OO. After 1 h incubation we could detect 3-OH B(a)P and 7,8-dihydrodiol in 25 μM B(a)P treated group (Fig. 6A). In contrast, in OO co-incubated reaction mixture the metabolites were not detectable (Fig. 6B). The reason may be due to the inhibition of hydroxylase enzyme by OO which has prevented the convertion of BaP to its respective metabolites.

4 Concluding remarks

OO is rich in phenolic components that have potent antioxidant properties. From the above results, it has been discernible that components of OO could have the potential to pre-

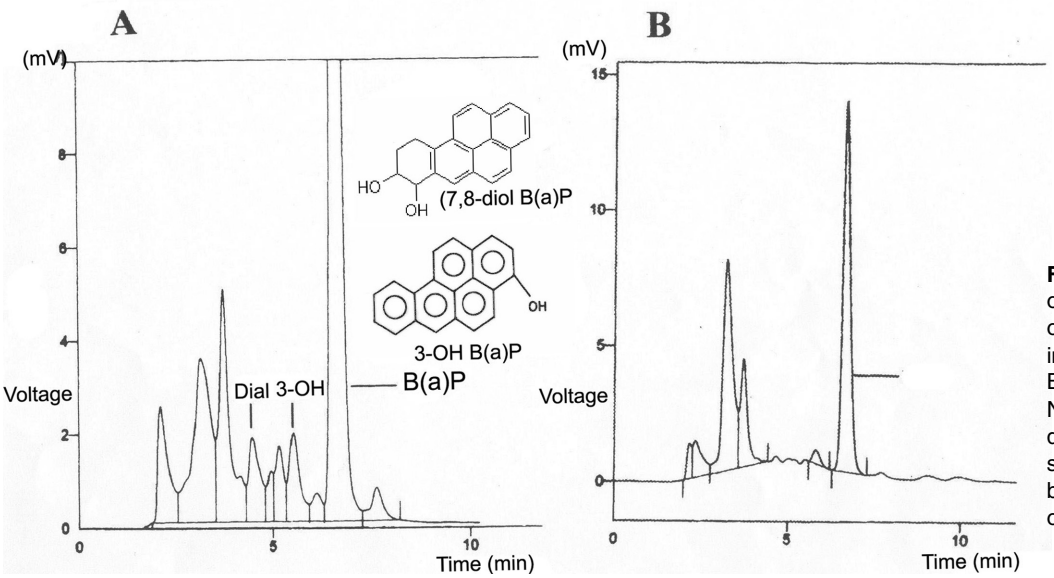

Figure 6. (A) Detection of 3-OH and diol metabolites in microsomes incubated with 25 μl of B(a)P for one hour. (B) No metabolite was detected when microsomes were co-incubated with 10 μl of olive oil.

vent the production of B(a)P metabolites and afford considerable protection against B(a)P-induced toxicity through its antioxidative property. In *in vitro* and *ex vivo* models, OO phenolics have shown to have antioxidant properties, higher than that of vitamin E, on lipids and DNA oxidation [48]. The protective effect of OO can also be attributable to the effect of OO to inhibit the levels of CYP2C11 protein [49], since this major isoenzyme of CYP in liver, metabolizes a host of xenobiotics including B(a)P [50]. Inhibition of CYP3A activity by OO phenols [51], could also account for the protective effect of OO.

The authors K. P. D. and S. A. wish to thank DST SERC – (Fast Track Scheme), India for financial support. The authors also gratefully acknowledge the use of the Bioinformatics Infrastructure Facility, Alagappa University funded by the Department of Biotechnology, Ministry of Science and Technology, Government of India (no. BT/BI/04/055/2001).

The authors have declared no conflict of interest.

5 References

[1] Cavalieri, E.-L., Rogan, E.-G., The approach to understanding aromatic hydrocarbon carcinogenesis. The central role of radical cations in metabolic activation. *Pharmacol. Ther.* 1992, *55*, 183 – 199.

[2] Devanesan, P.-D., Ramakrishna, N. V.-S., Todorovic, R., Rogan, E.-G., *et al*., Identification and quantitation of benzo(a)pyrene DNA adducts formed by rat liver microsomes in vitro. *Chem. Res. Toxicol.* 1992, *5*, 302 – 309.

[3] Chen, L., Devanesan, P.-D., Higginbotham, S., Ariese, F., *et al*., Expanded analysis of benzo(a)pyrene- DNA adducts formed in vitro and in mouse skin: Their significance in tumor initiation. *Chem. Res. Toxicol.* 1996, *9*, 897 – 903.

[4] Gelboin, H.-V., Benzo(a)pyrene metabolism, activation, and carcinogenesis: Role and regulation of mixed-function oxidases and related enzymes. *Physiol. Rev.* 1980, *60*, 1107 – 1166.

[5] Guengerich, F.-P., Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. *Chem. Res. Toxicol.* 1991, *4*, 391 – 407.

[6] Brauze, D., Mikstacka, R., Baer-Dubowka, W., Formation and presence of benzo(a)pyrene-DNA adducts in different tissues of C57BL/10 and DBA/2 mice. *Carcinogenesis* 1991, *12*, 1607 – 1611.

[7] Conney, A.-H., Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture. *Cancer Res.* 1982, *42*, 4875 – 4917.

[8] Gooderham, N.-J., Mannering, G.-J., Depression of the hepatic cytochrome P450 monooxygenase system by treatment of mice with anti-neoplastic agent 5-azacytidine. *Cancer Res.* 1985, *45*, 1569 – 1572.

[9] Sugihara, N., James, M.-O., Binding of 3-Hydroxybenzo(a)-pyrene to bovine hemoglobin and albumin. *J. Biochem. Mol. Toxicol.* 2003, *7*, 4.

[10] Emre, M.-H., Aktay, G., Polat, A., Vardt, N., Effects of benzo(a)pyrene and ethanol on oxidative stress of brain, lung tissues and lung morphology in rats. *Chin. J. Physiol.* 2007, *50*, 143 – 148.

[11] Yan, Z., Subbaramaiah, K., Camilli, T., Zhang, F., *et al*., Benzo(a)pyrene induces the transcription of cyclooxygenase-2 in vascular smooth muscle cells. Evidence for the involvement of extracellular signal-regulated kinase and NF-kappaB. *J. Biol. Chem.* 2000, *275*, 4949 – 4955.

[12] Saunders, C.-R., Das, S. K., Ramesh, A., Shockley, D.-C., Mukherjee, S., Benzo(a)pyrene-induced acute neurotoxicity in the F-344 rat: Role of oxidative stress. *J. Appl. Toxicol.* 2006, *26*, 427 – 438.

[13] Burdick, A.-D., Davis, J.-W., Liu, K.-J., Hudson, L.-G., *et al*., Benzo(a)pyrene quinones increase cell proliferation, generate reactive oxygen species, and transactivate the epidermal growth factor receptor in breast epithelial cells. *Cancer. Res.* 2003, *63*, 7825 – 7833.

[14] Kiruthiga, P.-V., Shafreen, R.-B., Pandian, S.-K., Arun, S., Govindu, S., Devi, K.-P., Protective effect of silymarin on erythrocyte haemolysate against benzo(a)pyrene and exogenous reactive oxygen species (H_2O_2) induced oxidative stress. *Chemosphere* 2007, *68*, 1511 – 1518.

[15] Zeisel, S. H., Antioxidant suppress apoptosis. *J. Nutr.* 2004, *134*, 3179S – 3180S.

[16] Zhu, B.-T., Loder, D.-P., Cai, M.-X., Ho, C.-T., *et al*., Dietary administration of an extract from rosemary leaves enhances the liver microsomal metabolism of endogenous estrogens and decreases their uterotropic action in CD-1 mice. *Carcinogenesis* 1998, *19*, 1821 – 1827.

[17] Wargovich, M., Diallyl sulfide, a flavor component of garlic (*Allium acti_um*), inhibits dimethylhydrazine-induced colon cancer. *Carcinogenesis* 1987, *8*, 487 – 489.

[18] Wargovich, M., Lmada, O., Stephens, L., Initiation and post-initiation chemopreventive effects of diallyl sulfide in esophageal carcinogensis. *Cancer Lett.* 1992, *64*, 39 – 42.

[19] Waterman, E., Lockwood, B., Active components and clinical applications of olive oil. *Altern. Med. Rev.* 2007, *12*, 331 – 340.

[20] Owen, R.-W., Giacosa, A., Hull, W.-E., Haubner, R., *et al*., Olive oil consumption and health: The possible role of antioxidants. *Lancet Oncol.* 2000, *1*, 107 – 112.

[21] Visioli, F., Poli, A., Gall, C., Antioxidant and other biological activities of phenols from olives and olive oil. *Med. Res. Rev.* 2002, *22*, 65 – 75.

[22] Galvano, F., La Fauci, L., Graziani, G., Ferracane, R., *et al*., Phenolic compounds and antioxidant activity of italian extra virgin olive oil monti iblei. *J. Med. Food* 2007, *10*, 650 – 656.

[23] Martin-Moreno, J.-M., Willett, W.-C., Gorgojo, L., Banegas, J.-R., *et al*., Dietary fat, olive oil intake and breast cancer risk. *Int. J. Cancer* 1994, *58*, 774 – 780.

[24] Trichopoulou, A., Katsouyanni, K., Stuver, S., Tzala, L., *et al*., Consumption of olive oil and specific food groups in relation to breast cancer risk in Greece. *J. Natl. Cancer Inst.* 1995, *87*, 110 – 116.

[25] Landa, M.-C., Fargo, N., Tres, A., Diet and the risk of breast cancer in Spain. *Eur. J. Cancer Prev.* 1994, *3*, 313 – 320.

[26] Walawalkar, P. S., Serai, P. S., Iyer, K. R., Isolation and catalytic competence of different animal liver microsomal fractions prepared by calcium-aggregation method. *Indian J. Pharm. Sci.* 2006, *68*, 262 – 265.

[27] Lowry, O.-H., Rosebrough, N.-J., Farr, A.-L., Randall, R.-J., Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 1951, *193*, 265 – 275.

[28] Aebi, H., Catalase in vitro. *Meth. Enzymol.* 1984, *105*, 121 – 126.

[29] Habig, W.-H., Jakoby, W.-B., Assays for the differentiation of glutathione S-transferase. *Meth. Enzymol.* 1981, *77*, 398 – 405.

[30] Carlberg, J., Mannervik, B., Purification and characterization of flavoenzyme glutathione reductase from rat liver. *J. Nutr. Biochem.* 1975, *250*, 5475 – 5480.

[31] Paoletti, F., Mocali, A., Determination of superoxide dismutase activity by purely chemical system based on NAD(P)H oxidation. *Meth. Enzymol.* 1990, *186*, 209 – 220.

[32] Levine, R.-L., Garland, D., Oliver, C.-N., Amici, A., *et al*., Determination of carbonyl content in oxidatively modified proteins. *Meth. Enzymol.* 1990, *186*, 464 – 78.

[33] Nebert, D.-W., Gelboin, H.-V., Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. I Assay and properties of induced enzyme. *J. Biol. Chem.* 1968, *243*, 6242 – 6249.

[34] Coni, E., Di Benedetto, R., Di Pasquale, M., Masella, R., *et al*., Protective effect of oleuropein, an olive oil biophenol, on low density lipoprotein oxidizability in rabbits. *Lipids* 2000, *35*, 45 – 54.

[35] Masella, R., Varì, R., D'Archivio, M., Di Benedetto, R., *et al*., Extra virgin olive oil biophenols inhibit cell-mediated oxidation of LDL by increasing the mRNA transcription of glutathione-related enzymes. *J. Nutr.* 2004, *134*, 785 – 791.

[36] Kim, H.-S., Kwack, S.-J., Lee, B.-M., Lipid peroxidation, antioxidant enzymes, and benzo[a]pyrene-quinones in the blood of rats treated with benzo[a]pyrene. *Chem. Biol. Interact.* 2000, *127*, 139 – 150.

[37] Somogyi, A., Rosta, K., Pusztai, P., Tulassay, Z., Nagy, G., Antioxidant measurements. *Physiol. Meas.* 2007, *28*, R41 – R55.

[38] Kim, K.-B., Lee, B.-M., Oxidative stress to DNA, protein, and antioxidant enzymes (superoxide dismutase and catalase) in rats treated with benzo(a)pyrene. *Cancer Lett.* 1997, *113*, 205 – 212.

[39] Kim, Y.-S., Lee, J.-H., Kim, S.-H., Kim, T.-H., *et al*., Effects of nicotine, cotinine and benzopyrene as smoke components on the expression of antioxidants in human bronchial epithelial cells. *Tuberc. Respir. Dis.* 2007, *62*, 197 – 202.

[40] Selvendiran, K., Banu, S. M., Sakthisekaran, D., Oral supplementation of piperine leads to altered phase II enzymes and reduced DNA damage and DNA-protein cross links in Benzo(a)pyrene induced experimental lung carcinogenesis. *Mol. Cell. Biochem.* 2005, *268*, 141 – 147.

[41] Ruiz-Gutierrez, R., Perez-Espinosa, A., Vazquez, C.-M., Santa-Maria, C., Effects of dietary fats (fish, olive and high-oleic-acid sunflower oils) on lipid composition and antioxidant enzymes in rat liver. *Br. J. Nutr.* 1999, *82*, 233 – 241.

[42] Pajovic, S. B., Kasapovic, J., Martinovic, J., Superoxide dismutase activities in different tissues of female rats treated with olive oil. *Physiol. Res.* 1997, *46*, 381 – 384.

[43] Weng, M.-W., Hsiao, Y.-M., Chiou, H.-L., Yang, S.-F., *et al*., Alleviation of benzo[a]pyrene-diolepoxide-DNA damage in human lung carcinoma by glutathione S-transferase M2. *DNA Repair. (Amst)* 2005, *4*, 493 – 502.

[44] Parihar, M.-S., Pandit, M.-K., Free radical induced increase in protein carbonyl is attenuated by low dose of adenosine in hippocampus and mid brain: Implication in neurodegenerative disorders. *Gen. Physiol. Biophys.* 2003, *22*, 29 – 39.

[45] Obach, S., Inhibition of human Cytochrome P450 enzymes by constituents of St. John's Wort, a herbal preparation used in the treatment of depression. *J. Pharmacol. Exp. Ther.* 2000, *294*, 88 – 95.

[46] Shimada, T., Yun, C. H., Yamazaki, H., Gautier, J. C., *et al*., Characterization of human lung microsomal cytochrome P-450 1A1 and its role in the oxidation of chemical carcinogens. *Mol. Pharmacol.* 1992, *41*, 856 – 864.

[47] Irwin, R.-J., VanMouwerik, M., Stevens, L., Seese, M.-D., Basham, W., *Environmental Contaminants Encyclopedia. Benzo(a)pyrene (BaP)*, National Park Service, Water Resources Division, Fort Collins, Colorado. Distributed within the Federal Government as an Electronic Document, 1998 (Projected public availability on the internet or NTIS).

[48] Fitó, M., Torre, R., Farré-Albaladejo, M., Khymenetz, O., *et al*., Bioavailability and antioxidant effects of olive oil phenolic compounds in humans: A review. *Ann. Ist. Super. Sanita* 2007, *43*, 375 – 381.

[49] Brunner, L.-J., Bai, S., Effect of dietary oil intake on hepatic cytochrome P450 activity in the rat. *J. Pharm. Sci.* 2000, *89*, 1022 – 1027.

[50] Chen, G.-F., Ronis, M.-J., Thomas, P.-E., Flint, D.-J., Badger, T.-M., Hormonal regulation of microsomal cytochrome P450 2C11 in rat liver and kidney. *J. Pharmacol. Exp. Ther.* 1997, *283*, 1486 – 1494.

[51] Stupans, I., Stretch, G., Hayball, P., Olive oil phenols inhibit human hepatic microsomal activity. *J. Nutr.* 2000, *130*, 2367 – 2370.

