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Protective effects of curcumin and quercetin during benzo(a)pyrene induced lung carcinogenesis in mice

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Abstract. – OBJECTIVE: Phytochemicals is one such family of chemopreventive agents that is being researched extensively the world over for its efficacies against several cancer pathways. Curcumin and quercetin belong to the family of phytochemicals and have anti-oxidative and anti-carcinogenic properties. In the present study, chemopreventive efficacy of curcumin and quercetin was investigated against benzo(a)pyrene (BP) induced lung carcinogenesis.

MATERIALS AND METHODS: The mice were segregated into five groups which included normal control, BP treated, BP+curcumin treated, BP+quercetin treated and BP+curcumin+quercetin treated groups. Lung carcinogenesis was induced by a single intra-peritoneal (IP) injection of BP (100 mg/kg body weight). Curcumin was supplemented to mice at a dose level of 60 mg/kg body weight in drinking water and quercetin was given at a dose level of 40 mg/kg body wt in drinking water.

RESULTS: The BP treatment resulted in a significant increase in LPO and ROS levels. GSH levels and the activities of SOD, GST as well as GR were found to be significantly decreased following BP treatment. Further, BP treatment brought about a significant increase in the activities of drug metabolizing enzymes (cytochrome P450 and b5). Curcumin and quercetin treatments to mice were able to decrease significantly the levels of LPO, ROS, as well as activities of SOD, GST. Also, the activities of drug metabolizing were markedly decreased by the administration of phytochemicals.

CONCLUSIONS: The results of this study suggest that combined treatment with curcumin and quercetin proved beneficial on antioxidant status and drug metabolizing enzymes during experimentally induced lung carcinogenesis in mice.

Key Words:
Mice, Curcumin, Quercetin, Benzo(a)pyrene, Lung cancer.

Introduction

Lung cancer is the cause for a large number of deaths in the world. According to estimates, 90% of lung cancers are caused by cigarette smoking[1]. It is the most common cancer amongst men in India, with increasing number of cases being reported every year[2]. Lung cancer in its early stages often produces no symptoms. As the disease progresses, symptoms may include a chronic cough with bloody sputum, repeated respiratory infections, and difficulty in breathing[3]. There are two major types of lung cancers namely non small cell lung cancer (NSCLC) and small cell lung cancer (SCLC)[4] However, prior to neoplastic transformation, the normal cell may be subjected to an assault either by carcinogen or by the hostile factors which include oxidative stress and hormonal imbalance.

In our study, benzo(a)pyrene (BP) was used to instigate lung carcinogenesis in mice. BP comes from the family of polycyclic aromatic hydrocarbons (PAH), which is a major carcinogenic pollutant[5] and has carcinogenic properties. BP was first isolated and characterized by Cook et al[6] in the year 1933 and is formed when gasoline, garbage or any plant or animal materials burn incompletely. BP is a mutagenic and has been shown to be a potent tumorigenic chemical in animal models[7]. For early-stage of the disease, surgical resection and radiation ablation is currently the mainstay of lung cancer therapy. Though, chemotherapy for treating cancerous cells is often successful in killing the malignant cells but it often causes intense side effects in the body. Researchers all over the world are busy in investi-

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gating several substances which have the potential to inhibit the molecular events leading to the occurrence of cancer and are called chemopreventive agents. The term 'chemoprevention' was coined by Sporn et al[8] in mid 1970s and is expressed as the use of chemicals or dietary components to block, inhibit, or reverse the development of cancer in normal or preneoplastic tissue. Curcumin and quercetin are the two phytochemicals used in the present study.

Curcumin has been accounted for exhibiting its anti-tumor role in cancer cells by modulating the de-regulated cell cycle[9]. Curcumin, an antioxidant is an active principle of turmeric and has been used for cooking in India for centuries. Curcumin has been recognized to retard the formation of adducts of carcinogen with DNA and delay the process of tumorigenesis in several animal models[10,11]. Various cellular as well as animal models have reported the chemopreventive effects of quercetin[12]. The antioxidant activity and the capacity to affect the expression of several detoxifying enzymes provide quercetin with ability to hamper particular stages of the carcinogenic process. In the present study, the response of curcumin and quercetin individually as well as in combination on key biochemical parameters and tumor marker enzymes involved during the progression stage of BP induced lung carcinogenesis was investigated.

Materials and Methods

Chemicals

BP (BP), curcumin and quercetin were procured from Sigma Aldrich company (St Louis, MO, USA). NADPH, GSH, NBT and DTNB were procured from Merck chemicals (Darmstadt, Germany).

Animals

Male laka mice in the weight range of 18-20 g were procured from the central animal facility, Southeast University, China. The animals were housed in polypropylene cages under hygienic conditions in the departmental animal house strictly in accordance with the guidelines as outlined by the Institutional Ethics Committee.

Experimental Design

Animals were segregated into five treatment groups. Animals in Group I served as normal controls and were administered with corn oil intraperitoneally (IP), which was used as a vehicle for the treatment of animals in BP group. Animals in Group II were given a single injection (IP) of BP at a dose level of 100 mg/kg body weight dissolved in corn oil[13] Group III animals were given curcumin orally in water at a dose level of 60 mg/kg/b.wt thrice a week. Animals in Group IV were given quercetin orally in water at a dose level of 40 mg/kg/b.wt in water thrice a week. Both the phytochemicals were given to animals using intubation gavage technique. Animals in group V animals were given a combined treatment of curcumin and quercetin in a similar manner as was given to group III and group IV animals respectively. The treatment with phytochemicals to the animal belonging to groups III to V was started 10 days prior to BP injection. All the animals had free access to the diet and water and the treatments continued for a total duration of 22 weeks.

Body Weight Changes

A careful record of body weight changes of normal control, BP, curcumin, quercetin and curcumin + quercetin treated animals was kept throughout the study. The animals were weighed at the beginning of the experiment, then weekly and finally before sacrificing them. A daily record of food as well as water intake was also maintained throughout the study period.

Lung Weight Changes

Following 22 weeks of BP treatment, animals were sacrificed under ether anesthesia, lung excised and washed in normal saline. Lung weights were measured using Shimadzu Digital weighing balance (Kyoto, Japan).

Collection of blood and Preparation of Lung Homogenate

At the end of the study, the animals were anaesthetized using mild ether anesthesia and the blood samples were drawn from the animals belonging to all the groups by puncturing the ocular vein (retro-orbital plexus), using fine sterilized capillaries. Thereafter, serum was separated by centrifugation and stored at –40°C until analyses for enzyme activities of alkaline phosphatase and lactate dehydrogenase. The mice were then sacrificed, lungs were removed immediately and washed with ice-chilled saline. 10% lung homogenates were prepared in ice cold Tris buffer (Sigma, ST. Louis, MO, USA), (pH 7.4) by using mechanically driven Teflon fitted Potter-Elve-

jhem type homogenizer for a few minutes till the total disruption of cells. Homogenates were centrifuged at 1.000 g for 10 minutes at 4°C. Pellets were discarded and the supernatants was used for the estimation of lipid peroxidation and reduced glutathione levels. A portion of the above supernatants were again centrifuged at 10.000 g for 20 minutes to obtain post mitochondrial fraction which were utilized for the rest of biochemical estimations.

Reactive Oxygen Species Levels (ROS)

Reactive oxygen species were estimated by using the method of Driver et al[14].

Antioxidant Defense Systems and Lipid Peroxidation

Lipid peroxidation assay was done according to the method of Wills[15].

Reduced Glutathione (GSH): Estimation of GSH was performed in the tissue homogenates of lung by using the method of Ellman[16].

Catalase: The method of Luck[17] was used for the estimation of catalase.

Superoxide Dismutase (SOD): The activity of SOD was estimated by using the method of Kono[18].

Glutathione Reductase (GR): The enzyme was assayed by following the method of Carlberg and Mannervik[19].

Glutathione-S-Transferase (GST): This enzyme was assayed by using the method of Habig et al[20].

Protein: Protein assay was done by the method of Lowry et al[21].

Drug Metabolizing Enzymes

Cytochrome P-450: Cytochrome P-450 was measured by the carbon monoxide difference spectrophotometry of dithionite-reduced samples by using the method of Omura and Sato[22].

Cytochrome b5: Cytochrome b5 activity was measured according to the method of Omura and Sato[23].

Statistical Analysis

The statistical significance of the data has been determined using one-way analysis of variance (ANOVA) followed by multiple post-hoc least significant difference (LSD) test. The results are represented as Means ± SD. $p < 0.05$ was considered statistically significant.

Results

Body Weight and Lung Weights Changes

BP treatment to all the treated groups caused a significant decrease in the body weights, when compared to the normal control mice (Table I). Supplementation of BP treated mice with curcumin and quercetin separately as well as in combination improved the body weight growth. However, no significant changes in food and water consumption were observed among various groups of animals. Further, BP treatment significantly increased (Table I) the lung weights of animals when compared with normal controls and showed an appreciable moderation following treatments of curcumin and quercetin separately as well as in combination.

Lipid Peroxidation and Reactive Oxygen Species Levels

A statistically significant increase in the levels of malondialdehyde (MDA) was observed in the lungs of mice subjected to BP treatment (Table II). Supplementation with curcumin and

Table I. Effects of curcumin and quercetin treatments on body and lung weights of mice subjected to benzo(a)pyrene treatment.

Groups	Body weights (g)	Lung weights (mg)
Normal control	36.60 ± 2.60	268.67 ± 15.50
Benzo(a)pyrene	24.40 ± 3.51^{c}	331.25 ± 14.36^{c}
Benzo(a)pyrene + Curcumin	29.40 ± 1.67^{c,y}	301.67 ± 4.73^{b,x}
Benzo(a)pyrene + Quercetin	29.50 ± 1.00^{c,y}	303.75 ± 16.52^{b,x}
Benzo(a)pyrene + Curcumin + Quercetin	30.00 ± 1.87^{c,z}	305.00 ± 18.71^{b,x}

Data are expressed in mean ± SD; $^{a}p \leq 0.05$, $^{b}p \leq 0.01$ and $^{c}p \leq 0.001$ by Least Significance Difference test when values are compared with normal control group; $^{x}p \leq 0.05$, $^{y}p \leq 0.01$, $^{z}p \leq 0.001$ by Least Significance Difference test when values of Groups III, IV and V are compared with Group II,

Table II. Effects of curcumin and quercetin treatments on lipid peroxidation (LPO) and reactive oxygen species (ROS) levels in lungs of mice subjected to benzo(a)pyrene treatment.

Groups	LPO (nano moles of MDA formed/min/mg protein)	ROS (DCF fluorescent intensity units/mg protein)
Normal control	14.87 ± 1.92	1.05 ± 0.20
Benzo(a)pyrene	19.88 ± 0.36^{c}	1.79 ± 0.27^{b}
Benzo(a)pyrene + Curcumin	18.42 ± 1.34^{b}	1.22 ± 0.39^{x}
Benzo(a)pyrene + Quercetin	15.98 ± 0.95^{y}	1.23 ± 0.31^{y}
Benzo(a)pyrene + Curcumin + Quercetin	15.07 ± 0.39^{y,p}	1.09 ± 0.07^{z}

Data are expressed in mean ± SD; $^{a}p \le 0.05$, $^{b}p \le 0.01$ and $^{c}p \le 0.001$ by Least Significance Difference test when values are compared with normal control group; $^{x}p \le 0.05$, $^{y}p \le 0.01$, $^{z}p \le 0.001$ by Least Significance Difference test when values of Groups III, IV and V are compared with Group II; $^{p}p \le 0.05$ by Least Significance Difference test when values of Groups V are compared with Group III.

quercetin separately and in combination to BP treated mice resulted in normalization in MDA levels. Moreover, combination treatment was more effective in lowering the MDA levels as compared to separate treatment with individual phytochemicals. The reactive oxygen species levels were observed to be significantly elevated in the lungs of BP treated mice (Table II). Supplementation with curcumin and quercetin separately as well as in combination moderated the levels of ROS and the moderation was pronounced in the combined treatment group thereby signifying the importance of combination therapy action.

Antioxidant Defence System

BP treatment to normal control animals resulted in a significant decrease in the levels of reduced glutathione (GSH) (Table III). Treatment with curcumin to BP treated animals significantly brought elevation in the levels of GSH and the levels were within normal range. However, quercetin treatment to BP treated mice was not able to show any increase in the GSH levels when compared with BP treated mice. Combined treatment of quercetin and curcumin also resulted in a significant elevation in the levels of GSH. Conversely, BP treatment caused a significant decrease in the activities of superoxide dismutase (SOD), glutathione S-transferase (GST) and glutathione reductase (GR) while there were no significant changes in the activity of catalase (CAT). Further, treatment with quercetin separately as well as in combination with curcumin significantly elevated the activities of SOD in BP treated animals. Moreover, curcumin and quercetin treatment alone as well as in combination to BP treated animals significantly increased the activi-

Table III. Effects of curcumin and quercetin treatments on antioxidant system in lungs of mice subjected to benzo(a)pyrene treatment.

Groups	GSH (µmoles/mg protein)	GST (nanomoles of CDNB conjugates/ min/mg protein)	SOD (Units/mg protein)	GR (nanomoles/ min/mg protein)	CAT (µmoles of H_2O_2 decomposed/ min/mg protein))
Normal control	3.33 ± 0.15	2.33 ± 0.12	2.64 ± 0.32	2.89 ± 0.58	35.22 ± 5.31
Benzo(a)pyrene	2.26 ± 0.13^{c}	1.72 ± 0.04^{c}	1.30 ± 0.35^{c}	1.87 ± 0.39^{c}	40.41 ± 1.39
Benzo(a)pyrene + Curcumin	2.91 ± 0.17^{a,x}	2.08 ± 0.01^{b,z}	1.68 ± 0.20^{c}	1.88 ± 0.34^{c}	31.93 ± 9.55
Benzo(a)pyrene + Quercetin	2.41 ± 0.36^{c}	1.99 ± 0.12^{c,y}	2.03 ± 0.36^{a,y}	1.75 ± 0.28^{c}	41.86 ± 7.43
Benzo(a)pyrene + Curcumin + Quercetin	2.97 ± 0.19^{y}	1.98 ± 0.04^{c,y}	2.10 ± 0.19^{a,y}	2.09 ± 0.30^{a}	39.13 ± 5.15

Data are expressed in mean ± SD; $^{a}p \le 0.05$, $^{b}p \le 0.01$ and $^{c}p \le 0.001$ by Least Significance Difference test when values are compared with normal control group; $^{x}p \le 0.05$, $^{y}p \le 0.01$, $^{z}p \le 0.001$ by Least Significance Difference test when values of Groups III, IV and V are compared with Group II.

ties of GST only when compared to BP treated mice and no significant change was observed in GR activity.

Drug Metabolizing Enzymes

A significant elevation in the enzyme activity of cytochrome p450 and b5 (Table IV) was observed in the lungs of the mice subjected to single dose of BP treatment. Administration of phytochemicals both separately as well as in combination for a period of 22 weeks to BP treated mice was able to lessen the enzyme activity significantly and the enzyme activity of P450 and b5 was restored to within normal limits.

Discussion

Chemoprevention, especially with non nutritive agents seems to be the cheapest and novel methods available which can help in combating the high mortality rates of lung cancer. Phytochemicals are one such class of non nutritive agents which have been reported in several studies to show a great potential in fighting cancer and other diseases[24]. In the present study, curcumin and quercetin were the phytochemicals of interest and were able to modulate the BP induced altered activities of drug metabolizing and antioxidant enzymes.

The body weights of mice recorded after 22 weeks of single injection of BP were observed to be decreased significantly. BP and certain polycyclic hydrocarbons have been reported to cause similar decrease in the body weights[25]. In our study, the decrease in body weights might be due to cachexia in which the tumor induces metabolic changes in the host leading to loss of adipose tissue and skeletal muscle mass[26]. When curcumin and quercetin were administered to BP treated mice separately as well as in combination, a significant improvement in the body weights was observed. Curcumin has been reported to reduce muscle wasting by inhibiting multiple proteolytic pathways[27]. Quercetin along with other polyphenols, through its activating of SIRT1 has been reported to be beneficial in calorie restriction, metabolism and skeletal muscle function[28].

The lung weights of BP treated mice were found to be significantly higher than those of normal controls. The increase in the lung weights might be due to inflammation as it plays a major contributor role in tumor development and progression[29,30]. We observed that simultaneous as well as separate administration of curcumin and quercetin was able to significantly decrease the lung weights as compared to BP treated mice. Curcumin is a known anti-inflammatory agent and it exhibits its anti-inflammatory effect in part, through inhibition of the NF-κB pathway and COX-2 enzyme which is responsible for the production of certain proinflammatory prostaglandins[31]. Also, quercetin was able to inhibit inflammation as its anti-inflammatory role was reported in chronic prostatitis patients[32].

BP metabolism results in the production of ROS 33 that in turn cause mutations to the DNA which if not repaired helps in promotion of carcinogenesis. Certain forms of ROS also act as essential intracellular second messengers for several cytokines and growth factors which may ultimately lead to the process of tumor promotion[34]. Further, ROS initiates lipid peroxidation (LPO) directly by reacting with the lipids of membranes or by acting as second messengers for the primary free radicals[35]. In the present study, we also observed a significant rise in the LPO levels as well as ROS levels upon BP treatment. Combined as well as individual treatments of BP treated mice with curcumin and quercetin signifi-

Table IV. Effects of curcumin and quercetin treatments on the activity of cytochrome P450 and cytochrome b5 in lungs of mice subjected to benzo(a)pyrene treatment (nanomoles/mg protein).

Groups	Cytochrome P450	Cytochrome b5
Normal control	1.42 ± 0.25	5.27 ± 0.31
Benzo(a)pyrene	1.78 ± 0.08^{b}	6.87 ± 0.36^{c}
Benzo(a)pyrene + Curcumin	1.38 ± 0.06^{y}	5.51 ± 0.39^{z}
Benzo(a)pyrene + Quercetin	1.44 ± 0.17^{x}	5.31 ± 0.55^{z}
Benzo(a)pyrene + Curcumin + Quercetin	1.42 ± 0.20^{y}	5.75 ± 0.30^{y}

Data are expressed in mean ± SD; $^{a}p \leq 0.05$, $^{b}p \leq 0.01$ and $^{c}p \leq 0.001$ by Least Significance Difference test when values are compared with normal control group; $^{x}p \leq 0.05$, $^{y}p \leq 0.01$, $^{z}p \leq 0.001$ by Least Significance Difference test when values of Groups III, IV and V are compared with Group II.

cantly reduced the LPO as well as ROS levels, thereby, depicting their protective role. The ameliorating effects on the LPO and ROS levels due to curcumin and quercetin may be attributed to their antioxidant and free radical scavenging properties[36-37].

The production of ROS initiates the synthesis of superoxide dismutase at the transcriptional level which is responsible for hunting down the superoxide radicals[38]. However, the activity of superoxide dismutase was significantly reduced upon BP treatment, which seems to be the natural response of the body tissues to constant onslaught of free radicals. It has been stated that increased LPO levels have a bearing on the activities of SOD which are ultimately found to be decreased in different carcinogenic states[39]. Further, BP treatment caused a significant reduction in the levels of GSH that might be due to its consumption in detoxifying the peroxides produced by increased LPO levels. Curcumin and quercetin supplementation to BP treated mice have been able to bring improvement in the levels of both SOD and GSH. Both the phytochemicals have been reported earlier to increase the concentration of GSH as well as the gene expression of SOD[40]. Also in the present study glutathione-S-transferase (GST) activity was found to be reduced in the BP treated mice at 22 weeks time duration. BP administration has been reported to deplete the levels of GST in several studies[41]. However, supplementation with phytochemicals in individual and combined form resulted in significant increase in the GST activity in the lungs indicating the effectiveness of the phytochemicals. Valentine et al[42] reported that there was a 20% increase in the activity of GST upon curcumin administration to female Swiss Webster mice. Also, Garg et al[43] showed that dietary curcumin was able to increase the activity of GST in BP treated mice. Thus, increased activity of GST by curcumin and quercetin administration might be linked to an improvement in the detoxification system which is indicative of protection against damaging effects of carcinogen.

A significant elevation in the enzyme activities of cytochrome P450 as well as cytochrome b5 was noticed in the lungs of the mice subjected to single dose of BP treatment. A growing body of research worldwide has recognized the fact that certain individual forms of cytochrome P450 enzymes overexpress themselves in tumor[44-45]. Further, cytochrome b5 has been shown to be an electron transport hemoprotein for the cytochrome P450 enzyme thereby playing a significant role in cytochrome P450 associated drug metabolism.46 Increase in cytochrome P450 enzyme activity as discussed earlier, can be correlated with the increase in the activity of cytochrome b5. Administration of phytochemicals both separately as well as in combination for a period of 22 weeks to BP treated mice were able to moderate enzyme activities of both the enzymes significantly.

Conclusions

We observed that curcumin and quercetin exhibit inhibiting effects probably by modulating regulators of phase I and phase II enzymes and thereby adversely affecting the carcinogenic process to the benefit of the biological system. Therefore, curcumin and quercetin show great prospects in dealing with the condition of lung carcinogenesis. All the observations discussed clearly suggest that these phytochemicals hold great potential to be used as an effective preventive measure against the occurrence of lung cancer in a section of human population who have a family history of lung cancer as well those who are constantly exposed to carcinogens from different sources.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Shi WY, Liu KD, Xu SG, Zhang JT, Yu LL, Xu KQ, Zhang TF. Gene expression analysis of lung cancer. Eur Rev Med Pharmacol Sci 2014; 18: 217-228.
2) Dinshaw KA, Shastri SS, Patil SS. Cancer control programme in India: Challenges for the new millennium. Health Administrator 2005; 17: 10-13.
3) Wu QP, Min JX, Jiang L, Li JM, Yao K. Screening of biomarkers for lung cancer with gene expression profiling data. Eur Rev Med Pharmacol Sci 2013; 17: 3221-3228.
4) Eisen T, Matakidou A, Houlston R, GELCAPS Consortium. Identification of low penetrance alleles for lung cancer: the genetic lung cancer predisposition study (GELCAPS). BMC Cancer 2008; 8:244.
5) Van Agen B, Maas LM, Zwingmann IH, Van Schooten FJ, Kleinjans JC. B[a]P-DNA adduct for-

mation and induction of human epithelial lung cell transformation. Environ Mol Mutagen 1997; 30: 287-292.

6) Cook JW, Hewett CL, Hieger IJ. The isolation of a cancer-producing hydrocarbon from coal tar. Parts I, II, and III. Chem Soc 1933; 1: 395-405.

7) Karle Yagi H, Sayer, Jerina DM. Crystal and molecular structure of a benzo[a]pyrene 7,8-diol 9,10-epoxide N2-deoxyguanosine adduct: Absolute configuration and conformation. PNAS 2004; 101: 1433-1438.

8) Sporn MB, Dunlop NM, Newton DL, Smith JM. Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). Fed Proc 1976; 35: 1332-1338.

9) Kumaravel M, Sankar P, Rukkumani R. Antiproliferative effect of an analog of curcumin bis-1,7-(2-hydroxyphenyl)-hepta-1,6-diene-3,5-dione in human breast cancer cells. Eur Rev Med Pharmacol Sci 2012; 16: 1900-1907.

10) Conney AH, Lysz T, Ferraro T, Abidi TF, Manchand PS, Laskin JD, Huang MT. Inhibitory effect of curcumin and some related dietary compounds on tumor promotion and arachidonic acid metabolism in mouse skin. Adv Enzyme Regulation 2008; 31:385-96.

11) Lin JK, Lin-Shiau SY. Mechanisms of cancer chemoprevention by curcumin. Proc Natl Sci Counc Repub China B 2001; 2: 59-66.

12) Annapurna A, Ansari MA, Manjunath PM. Partial role of multiple pathways in infarct size limiting effect of quercetin and rutin against cerebral ischemia-reperfusion injury in rats. Eur Rev Med Pharmacol Sci 2013; 17: 491-500.

13) Gunning WT, Kramer PM, Lubet RA, Steele VE, End DW, Wouters W, Pereira MA. Chemoprevention of benzo(a)pyrene induced lung tumors in mice by the farnesyltransferase inhibitor R1157771. Clin Cancer Res 2003; 9: 1927-1930.

14) Driver AS, Kodavanti PR, Mundi WR. Age related changes in reactive oxygen species in rat brain homogenates. Neurotoxicol Teratol 2000; 22: 175-181.

15) Wills ED. Mechanism of lipid peroxide formation in animal tissues; Biochem J 1966; 99: 667-676.

16) Ellman GL. Tissue sulphydryl groups; Archievs of Biochemistry Biophysics 1959; 82: 70-77.

17) Luck H. In: methods of enzymatic analysis, Ed.: H.U. Bergmeyer, Academic Press, New York, 1971; pp. 885-893.

18) Kono Y. Generation of superoxide radical during auto oxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys 1978; 186: 189-195.

19) Carlberg I, Mannervik B. Glutathione reductase; Methods Enzymology 1985; 113: 484-490.

20) Habig WH, Pabst MJ, Jacoby WB. Glutathione-S-Transferase: The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249: 7130-7139.

21) Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Follin-phenol reagent. J Biol Chem 1951; 193: 265-275.

22) Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes. II. Solubilisation, purification and properties. J Biol Chem 1964; 239: 2379-2385.

23) Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J Biol Chem 1964b; 239: 2370-2378.

24) Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. J Nutr 2004; 134: 3479S-3485S.

25) Kamaraj S, Vinodhkumar R, Anandakumar P, Jagan S, Ramakrishnan G, Devaki T. The effects of quercetin on antioxidant status and tumor markers in the lung and serum of mice treated with benzo(a)pyrene. Biol Pharm Bull 2007; 30: 2268-2273.

26) Mantovani G. Randomised phase III clinical trial of 5 different arms of treatment on 332 patients with cancer cachexia. Eur Rev Med Pharmacol Sci 2010; 14: 292-301.

27) Zhou GZ, Cao FL, Chang JM, Sun GC, Chen XB. Mechanism of curcumin analog MHMD-induced cell death in A549 lung cancer cells. Eur Rev Med Pharmacol Sci 2014; 18: 3134-3138

28) Chung S, Yao H, Caito S, Hwang JW, Arunachalam G, Rahman I. Regulation of SIRT1 in cellular functions: Role of polyphenols. Arch Biochem Biophys 2010; 501: 79-90.

29) Mueller MM. Inflammation in epithelial skin tumours: old stories and new ideas. Eur J Cancer 2006; 42: 735-744.

30) Farron B, Evers BM. Inflammation and the development of pancreatic cancer. Surgical Oncol 2002; 10: 153-169.

31) Aggarwal BB. Targeting inflammation-induced obesity and metabolic diseases by curcumin and other nutraceuticals. Annu Rev Nutr 2010; 21:173-199.

32) Khanna D, Sethi G, Ahn KS, Pandey MK, Kunnumakkara AB, Sung B, Aggarwal A, Aggarwal BB. Natural products as a gold mine for arthritis treatment. Curr Opin Pharmacol 2007; 7: 344-351.

33) Samarth RM, Panwar M, Kumar A. Modulatory effects of Mentha piperita on lung tumor incidence, genotoxicity, and oxidative stress in benzo[a]pyrene-treated Swiss albino mice. Environ Mol Mutagen 2006; 47: 192-198.

34) Sundaresan M, Yu ZX, Ferrans VJ, Irani K, Fuikel T. Requirement for generation of hydrogen peroxide for platelet derived growth factor signal transduction. Science 1995; 270: 296-299.

35) Rajendran P, Venugopal R, Ekambaram G, Aadithya A, Sakthisekaran D. Rehabilitating activity of mangiferin in benzo(a)pyrene induced lung carcinogenesis. Asian J Biochem 2008; 3: 118-125.

36) Kohli K, Ali J, Ansari M, Raheman Z. Curcumin: a natural antiinflammatory agent. Indian J Pharmacol 2005; 37: 141-147.

37) Ruiz P, Braune A, Lzlwimmer G, Quintanilla-Fend L, Haller D. Quercetin inhibits TNF-induced NF-kB transcription factor recruitment to proinflammatory gene promoters in murine intestinal epithelial cells. J Nutr 2007; 137: 1208-1215.

38) Jyoti A, Sethi P, Sharma D. Bacopa monniera prevents from aluminium neurotoxicity in the cerebral cortex of rat brain. J Ethnophramacol 2007; 111: 56-62.

39) Ramakrishnan G, Raghavendran HR, Vinodhkumar R, Devaki T. Suppression of N-nitrosodiethylamine induced hepatocarcinogenesis by silymarin in rats. Chem Biol Interact 2006; 161: 104-114.

40) Alia M, Mateos R , Ramos S, Lecumberri E, Bravo L, Goya L. Influence of quercetin and rutin on growth and antioxidant defense system of a human hepatoma cell line (HepG2). Eur J Nutr 2006; 45: 19-28.

41) Jahangir T, Sultana S. Benzo(a)pyrene-induced genotoxicity: Attenuation by farnesol in a mouse model. J Enzyme Inhib Med Chem 2008; 23: 888-894.

42) Valentine SP, Le Nedelec MJ, Menzies AR, Scandlyn MJ, Goodin MG, Rosengren RJ. Curcumin modulates drug metabolizing enzymes in the female Swiss Webster mouse. Life Sci 2006; 78: 2391-2398.

43) Garg R, Gupta S, Maru GB. Dietary curcumin modulates transcriptional regulators of phase I and phase II enzymes in benzo[a]pyrene-treated mice: mechanism of its anti-initiating action. Carcinogenesis 2008; 29: 1022-1032.

44) Su JM, Lin P, Wang CK, Chang H. Overexpression of cytochrome P450 1B1 in advanced non-small cell lung cancer: a potential therapeutic target. Anticancer Res 2009; 29: 509-515.

45) Leclerc J, Tournel G, Courcot-Ngoubo Ngangue E, Pottier N, Lafitte JJ, Jaillard S, Mensier E, Lhermitte M, Broly F, Lo-Guidice JM. Profiling gene expression of whole cytochrome P450 superfamily in human bronchial and peripheral lung tissues: Differential expression in non-small cell lung cancers. Biochimie 2010; 92: 292-306.

46) Finn RD, McLaughlin LA, Ronseaux S, Rosewell I, Houston JB, Henderson CJ, Wolf CR. Defining the in Vivo Role for cytochrome b5 in cytochrome P450 function through the conditional hepatic deletion of microsomal cytochrome b5. J Biol Chem 2008; 283: 31385-31393.