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Chemopreventive Role of Curcumin in benzo(A)pyrene induced Lung Carcinogenesis in mice via-modulation of Bcl-2, p53, Caspase-3, Cyp1A1, COX-2 and antioxidant defense system in Lung tissues

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ABSTRACT

Benzo(A)Pyrene [B(a)P] has been reported to cause lipid peroxidation and decrease antioxidant enzymes activities by inducing oxidative stress in lung carcinogenesis. Curcumin has exhibited chemopreventive potential against a variety of cancers, including lung cancer. The potential protective and chemopreventive effects of curcumin against ***[B(a)P]*** *-induced lung cancer in mice were investigated. One hundred male Swiss Albino mice were divided into four equal groups. Group I: (Control group) received no drugs. Group II: (lung cancer- induced) mice injected with a single dose of* ***[B(a)P]*** *(100 mg/ kg b.wt, i.p). Group III: (lung cancer + curcumin treated) mice injected with* ***[B(a)P]*** *and treated with curcumin (100 mg/kg b.wt/day, orally) from 22th to 30th weeks. Group IV: (lung cancer + curcumin protected) mice received curcumin on alternate days from 1 day prior to* ***[B(a)P]*** *injection and was treated continuously with curcumin until 30th week. Blood and lung tissue samples were collected for determination of serum CEA, Haptoglobin (HPT), adenosine deaminase (ADA) and γ-GT, antioxidants enzymes (CAT, SOD, GPx, GST and GR), L-MDA, NO, GSH, Bcl-2, CYP1A1, P53, Caspase 3, DNA fragmentation and COX-2 in lung tissues. The obtained results revealed that, [B(a)P] potentially increased serum CEA, HPT, ADA, γ- GT in addition to COX-2, Caspase 3, L-MDA, NO, Bcl-2, CYP1A1, P53, and DNA fragmentation in lung tissues. However, enzymatic antioxidants status and GSH were significantly decreased. It could be concluded that, curcumin may be effective in reducing lung cancer by its radical scavenging activity and anti-inflammatory effect, regenerating endogenous antioxidant mechanisms and decreased caspase-3 and DNA fragmentation and attenuate Bcl-2, P53, CYP1A1 and COX -2 in lung tissues. These results indicate the possible efficiency of curcumin as a distinct chemopreventive agent in lung carcinogenesis.*

Keywords: Curcumin, Benzo(a)pyrene, Bcl-2, COX-2, P53, Caspase-3, CYP1A1 Lung cancer.

1. INTRODUCTION

Lung cancer is one of the most lethal cancers of the 20th century and still the most common cancer in the world causing up to 3 million deaths annually, and is increasing at a rapid rate [1]. In Egypt, official statistics showed that lung cancer is the second most common cancer in men and second leading cause of cancer death, after bladder cancer [2]. Mice lung tumor-genesis systems are valuable tools to study the process of chemical carcinogenesis induced by polycyclic aromatic hydrocarbons (PAHs) [3].

Polycyclic aromatic hydrocarbons and *N*-nitrosamines are the two major classes of tobacco-related inhaled carcinogens [4]. [B(a)P] is the most intensely studied PAH, it is ubiquitous in the environment and it is a very potent carcinogen [5]. WHO added that, [B(a)P] is typically selected as the standard against which the cancer potency of other PAHs are tested. Moreover, [B(a)P] induces cancer in many species of rodents and [B(a)P] itself as well as [B(a)P]-containing complex

environmental mixtures are known human respiratory carcinogens [6].

Previous studies have proved the toxicity of [B(a)P] behind its intermediate metabolites and the oxidative damage caused by reactive oxygen species (ROS) [7]. Moreover, DNA damage has been recognized as the onset of many diseases, including cancer and could be a useful biomarker of the oxidative status and antioxidant defense system of an organism [8]. On the other hand, smoking is undoubtedly the main risk factor, to which 90% of lung cancer cases are attributable [9]. In fact, (ROS) and organic free radical intermediates formed from many carcinogens are suggested to be involved in the initiation and progression of carcinogenic transformation [10].

Cancer chemoprevention can be defined as the prevention, inhibition or reversal of carcinogenesis by administration of one or more chemical entities, either as individual drugs or as naturally occurring constituents of the diet [11]. Curcumin might have potential activity against symptoms related cancer treatment including neuropathic pain, depression, fatigue, decreased appetite, and sleep disturbance [12], There is extensive literature that suggests that curcumin has potential in the prevention and treatment of cancer [13]. It was shown to be a potent scavenger of a variety of ROS including superoxide anion radicals, hydroxyl radicals and nitrogen dioxide radicals [14]. The antioxidant mechanism of curcumin is attributed to its unique conjugated structure, which includes two methoxylated phenols and an enol form of β-diketone; the structure shows typical radical-trapping ability as a chain-breaking antioxidant [15]. Additionally, the phenol (-OH) is essential for both antioxidant activity and free radical kinetics, that the (-OH) hydrogen is more labile for abstraction compared to the ($-CH_2$) hydrogen in curcumin. Based on various experimental and theoretical results it is definitely concluded that the phenolic (-OH) plays a major role in the activity of curcumin [16].

Accordingly, the present study was designed to evaluate the chemo-preventive activity and the potential protective effect of Curcumin against [B(a)P] induced lung carcinogenesis in Swiss albino mice through determination of enzymatic antioxidant status resembling Catalase (CAT), Super oxide dismutase(SOD), Glutathione Peroxidase (GPx), Glutathione-S-transferase (GST), Glutathione Reductase (GR), non enzymatic antioxidants (GSH), L-Malondialdehyde (L-MDA), Nitric oxide (NO), Cytochrome p4501A1 (CYP1A1), Tumor suppressor protein-P53, Caspase 3, DNA fragmentation and Cycloxygenase -2 (COX-2) in lung tissues in addition to some serum tumor biomarkers as carcino embryonic antigen (CEA), Haptoglobin (HPT), Adenosine deaminase (ADA) and Gamma glutamyl transferase (ɤ GT).

2. MATERIALS AND METHODS

2.1 Experimental animals

One hundred male Swiss Albino mice of 6-8 weeks old and weighing 25-30 gm were used in the experimental investigation of this study. Mice were obtained from the Laboratory Animals Research Center, Faculty of Veterinary Medicine, Benha University. The animals were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of the experiment. Fresh and clean drinking water was supplied *ad-libitum.* The animals were left for 15 days for acclimatization prior to the beginning of the experiment.

2.2 Preparation and Dosage of Curcumin

Curcumin (purity~99%) was freshly prepared by dissolving in Dimethylsulfoxide (DMSO) solution, administered orally and daily at a dose level of 100 mg kg/ b.wt. [17]. Curcumin manufactured by Fluka Co. for Chemicals and purchased from El-goumhouria Co. for Trading Chemicals Medicines and Medical Appliance, Egypt.

2.3 Induction of lung cancer

Benzo(A)Pyrene was freshly dissolved in corn oil to ensure the stability of the chemical just prior to use. Lung cancer was induced in mice by a single intraperitoneal injection of [B(a)P] at a dose of (100 mg/kg body weight)[18]. [B(a)P] has been manufactured by Sigma Chemical Co. (St. Louis, Mo, USA) and purchased from Schnelldorf, Germany through the Egyptian International Center for Import Cairo, Egypt.

2.4 Experimental design

Mice were randomly divided into four main equal groups, 25 animals each, placed in individual cages and classified as follows:

Group I: Control Normal Group*:*
Mice received no drugs, served as control for all experimental groups.

Group Π: (lung cancer- induced group):
Mice administered with a single dose of [B(a)P] (100 mg/kg b.wt, intraperitoneally), served as carcinogenic non treated group.

Group III: (lung cancer + Curcumin treated group):
Mice injected with [B(a)P] (100 mg/kg b.wt, Intraperitoneally)and treated with Curcumin (100 mg/kg b.wt/day, Orally) from 22th week of the experiment and continued to 30th week (end of the experiment).

Group IV: (lung cancer + Curcumin protected group):
Mice received Curcumin (100 mg/kg b.wt., orally) on alternate days from 1 day prior to [B(a)P] injection and was treated continuously with Curcumin until 30th week (end of experiment).

2.5 Sampling:
Blood samples and tissue specimens (lung tissues) were collected at the end of the experiment on 30thweek from all animal groups (control and experimental groups).

2.5.1 Blood samples
Blood samples for serum separation were collected by ocular vein puncture at the end of each experimental period in dry, clean, and screw capped tubes and serum were separated by centrifugation at 2500 r.p.m for 15 minutes. The clean, clear serum was separated by automatic pipette and received in dry sterile samples tube and kept in a deep freeze at -20 °C until used for subsequent biochemical analysis.All sera were analyzed for determination of HPT, ɤ-GT, ADA and CEA.

2.5.2 Tissue samples (lung tissue)
At the end of the experimental period, the animals were sacrificed by cervical decapitation. The lungs were dissected out, quickly removed and were rinsed in ice-cold physiological saline, then blotted between 2 filter papers and quickly stored in a deep freezer at -20 °C for further biochemical analysis**.** Briefly, lung tissue was subsequently minced into small pieces and 10% homogenate was prepared in cold phosphate buffer (pH 7.4). The homogenate was centrifuged at 1000 × g for 10 min at 4°C, and the supernatant was used directly for the determination of CAT, SOD, L-MDA, GPX, NO, GR, GST, GSH, Bcl-2, CYP1A1, P53, COX-2, Caspase 3 and DNA fragmentation.

2.6 Biochemical analysis
Serum CEA, HPT, ɤ GT, ADA and lung tissues SOD, CAT, L-MDA,, GPX, NO, GR, GST, GSH, Bcl2, CYP1A1, P53, COX-2, Caspase 3 and DNA fragmentation were analyzed according to the methods described by Bates, 1991[19]; Cat. No. 6250 ; Szasz, (1976)[20]; Cloud-Clone Corp., assembled by Uscn Life Science Inc. ISO9001:2008: ISO13485:2003, Catalog Number.SEB390Hu ; Kakkar *et al*., (1984)[21]; Luck, (1974)[22]; Mesbah *et al.,* (2004) [23]; Gross *et al*., (1967)[24]; Vodovotz, (1996)[25]; David and Richard, (1983)[26]; Habig *et al.,* (1974) [27]; Moron *et al*., (1979)[28]; CUSABIO BIOTECH Company, Catalog Number.CSB-EL002611BO; Cloud-Clone Corp., assembled by Uscn Life Science Inc. ISO9001:2008: ISO13485:2003, Catalog Number. SED295Ra; CUSABIO BIOTECH Company, Catalog No.CSB-E08335m; Cat.No.MBS160196; CUSABIO BIOTECH CO., LTD, Cat.No.CSB-E08857r and Shi *et al*., (1996)[29], respectively.

2.7 Statistical analysis
The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple test. All analyses were performed using the statistical package for social science (SPSS, 13.0 software, 2009). Values of $P<0.05$ were considered to be significant.

3. RESULTS AND DISCUSSION
Effect of Curcumin administration on some serum and lung tissues parameters of [B(a)P]-induced lung cancer in mice is presented in (Tables 1-4). The obtained results revealed that, administration of [B(a)P] induced lung cancer in mice exhibited a significant decrease in GSH level and CAT, GST, GR, GPX and SOD activities. On the other hand, administration of [B(a)P] exhibited a significant increase in COX-2, Caspase 3, L-MDA, NO, Bcl2, CYP1A1, P53 and DNA fragmentation in lung tissues and in serum HP, CEA levels and ɤ GT, ADA activity when compared with normal control group. Protection and treatment with Curcumin in [B(a)P] induced lung cancer in mice significantly increased GSH level and CAT, GST, GR, GPX and SOD activities. On the other hand, Curcumin administration significantly decreased and attenuated the increased in COX-2, Caspase 3, L-MDA, NO, Bcl2, CYP1A1, P53 and DNA fragmentation in lung tissues. Also, Curcumin administration significantly reduced elevated serum ɤ GT, ADA activities and HP, CEA concentrations when compared with [B(a)P]-induced lung cancer non-treated group.

Table 1: Effect of curcumin on serum CEA and HPT concentrations, GGT and ADA activities of B(a)P-induced lung cancer in mice.

Experimental groups / Parameters	Control Normal group	B(a)P group	B(a)P + Curcumin treated group	B(a)P + Curcumin protected group
CEA (ng/ml)	0.29±0.08^{b}	1.59±0.27^{a}	0.49±0.59^{b}	0.73±0.10^{b}
Haptoglobin(ng/ml)	2.05±0.21^{b}	7.40±1.31^{a}	3.84±0.45^{b}	2.85±0.67^{b}
GGT (U/L)	25.60±6.11^{b}	125.73±15.87^{a}	52.61±5.73^{b}	42.69±5.67^{b}
ADA (pg/ml)	5.52±1.27^{c,d}	24.09±1.62^{a}	13.24±2.19^{b}	9.10±1.26^{b,c}

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at ($P<0.05$).

Table 2: Effect of curcumin on lung tissues SOD, CAT, GR, GPX, and GST activities, GSH, L-MDA and NO concentrations of B(a)P-induced lung cancer in mice.

Experimental groups / Parameters	Control Normal group	B(a)P group	B(a)P + Curcumin treated group	B(a)P +Curcumin protected group
SOD (U/g.tissue)	25.70±1.75^{d}	10.19±1.87^{e}	28.86±1.81^{c,d}	31.00±0.83^{c}
CAT(mmol/g.tissue)	59.04±3.33^{a}	19.06±1.92^{c}	45.82±0.95^{b}	66.95±4.74^{a}
GR (ng/g. tissue)	2.33±0.12^{b}	1.52±0.08^{c}	2.68±0.10^{a,b}	2.98±0.45^{a,b}
GPx(ng/g. tissue)	23.62±0.29^{b,c}	11.00±1.95^{d}	22.09±1.21^{b,c}	28.74±1.91^{a}
GST (ng/g tissue)	0.36±0.03^{c}	0.17±0.01^{d}	0.37±0.03^{b,c}	0.45±0.03^{a,b}
GSH(ng/g tissue)	7.18±0.76^{a}	1.54±0.17^{c}	4.37±0.19^{b}	3.46±0.29^{b}
MDA(mmole/g. tissue)	26.40±5.68^{c}	200.86±10.93^{a}	71.85±4.01^{b}	43.08±4.67^{c}
NO (ng/g. tissue)	62.14±5.32^{b}	108.08±12.40^{a}	77.49±3.56^{b}	102.38±7.52^{a}

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at (P<0.05).

Table 3: Effect of curcumin on lung tissues DNA fragmentation percent, COX-2 and Caspase - 3 activities of B(a)P-induced lung cancer in mice.

Experimental groups / Parameters	Control Normal group	B(a)P group	B(a)P + Curcumin treated group	B(a)P +Curcumin protected group
DNA fragmentation %	86.86±25.38^{c}	1477.57±159.42^{a}	439.97±3.13^{b}	229.50±72.75^{b,c}
COX-2 (U/g.tissue)	4.88±0.48^{c,d}	12.42±1.04^{a}	7.31±0.12^{b}	4.22±0.94^{c,d}
Caspase-3 (ng/g.tissue)	0.59±0.05^{c}	2.44±0.03^{a}	0.81±0.15^{b,c}	0.93±0.12^{b,c}

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at (P<0.05).

Table 4: Effect of curcumin on lung tissues P53, Bcl-2 and CyP1A1 concentrations of B(a)P-induced lung cancer in mice.

Experimental groups / Parameters	Control Normal group	B(a)P group	B(a)P+ Curcumin treated group	B(a)P +Curcumin protected group
P53 (pg/ml)	20.71±5.14^{d}	102.96±6.04^{a}	50.84±3.62^{b}	25.53±3.93^{c,d}
Bcl-2 (ng/g. tissue)	0.45±0.05^{b,c,d}	0.87±0.01^{a}	0.62±0.02^{b}	0.31±0.05^{d}
CyP1A1(ng/g. tissue)	0.60±0.04^{c}	2.66±0.16^{a}	1.15±1.13^{b}	0.99±0.28^{b,c}

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at (P<0.05).

Lung cancer is currently a leading cause of death all over the world. In recent years, considerable attention has been given to increased dietary intake of phytochemicals, since numerous epidemiological as well as experimental studies gave positive correlation between reduced risk of cancer and intake of phytochemicals[30]. **[B(a)P]**, a well-identified environmental carcinogen is known to produce enormous amounts of free radicals and these free radicals and non-radical oxidizing species are highly reactive, toxic and mutagenic[31]. These toxic radicals are involved in mediating tissue lipid peroxidation. Lipid peroxidation-induced tissue damage is the sensitive feature in the cancerous conditions and any deterioration or destruction of the membrane can lead to the leakage of these enzymes from the tissues [30].

Experimental studies have discovered that the process of carcinogenesis can be modulated. One of the approaches is chemoprevention by administrating or consuming foods and drinks containing chemo-preventive agents[32]. The present study clearly demonstrates a potent inhibitory activity of Curcumin against **[B(a)P]**-induced mutagenic effect in lung tissue in male mice. The use of cytotoxic agents plays an important role in the management of intermediate and high-risk tumors in addition to delayed surgery. The obtained data revealed that, there was an increase in tissue NO and LMDA level upon [B(a)P] treatment but these were found to be reversed in curcumin. In this regard, earlier studies have demonstrated that treatment with curcumin in 1,2 –dimethylhydrazine (DMH)induced colon cancer in rats resulted in a significant decrease in the colon tissue NO and LMDA concentrations [33]. Also, curcumin co-treatment with DMH treated rats caused a significant decrease in the colon tissue NO concentration when compared with DMH non- treated rats. Moreover, in the present study, the lung cancer bearing animals showed decrease in the activities of enzymatic and non enzymatic antioxidants SOD, CAT, GPx, GST, GR and GSH but these were found to be reversed in curcumin. Similarly, Kamaraj *et al.,* (2007) [4] found that, a highly significant reductions in the activities of (SOD, CAT, GPx, GST, GR) and GSH concentration were observed in the cancer bearing animals. These results are nearly similar to those recorded by, Hussein *et al*., (2014) [33] who reported that, a significant decrease in the colon tissue GSH concentration and enzymatic antioxidant status (GST, GPX, SOD, CAT and GR) activities were observed in DMH-induced colon cancer in rats when

compared with normal control group. Treatment with curcumin resulted in a non-significant increase in the colon tissue GSH concentration. However, curcumin co-treatment with DMH administered rats resulted in a significant increase in the colon tissue GSH concentration and enzymatic antioxidant status(GST, GPX, SOD, CAT and GR) activities when compared with DMH non-treated group.

The obtained results showed an elevation in lung tissues COX-2, P53, Caspase3, DNA fragmentation, Bcl-2, CyP1A1 in [B(a)P] treated group. Upon curcumin treatment, the concentrations of these markers was brought back to near normal high-lighting the anti-tumour property of curcumin. These results are nearly similar to those recorded by (Nair *et al .,* 2012) [34] who found that, [B(a)P] treatment to mice brought about a statistically significant increase in the activity of COX-2 in the lungs tissue. Curcumin administration to [B(a)P] treated mice result in a significant decrease in the activity of the pro-inflammatory enzyme (COX-2). Also, when phytochemicals was supplemented simultaneously to [B(a)P] treated mice, the enzyme activity of COX-2 was restored to within normal limits. Moreover, Aggarwal *et al.,* (2003) [17] shown that, curcumin down regulate the expression of anti-apoptotic (Bcl-2) and (COX-2). Additionally, several studies have suggested that COX-2 and other proteins in the same pathway serve as modulators of tumor growth[35]. The present study was supported by the finding of Sengupta and Harris, (2005) [36] who mention that, accumulation and activation of p53 occur in-response to various cellular stresses, including DNA damage, and may lead to the activation of several genes whose products trigger cell cycle arrest, DNA repair, or apoptosis . Like [B(a)P], several PAH metabolites are highly genotoxic and elicit an accumulation of p53 [37]. [B(a)P] treatment of murine 3T3 cells have been shown to result in DNA damage associated with elevated levels of nuclear p53 [38]. On the other hand, Shankar *et al*., (2007) [39] stated that, curcumin has been reported to induce the expression of p53 in various cell lines such as glioma and prostate cancer. Also, Anandakumar *et al*., (2013) [40] shown that, [B(a)P]-induced lung cancer animals exhibited significantly decreased expression of p53 when compared with control animals. Protected animals showed markedly increased expressions of p53 when compared with [B(a)P] administered lung cancer animals. Moreover, Rotter *et al*., (1993) [41] found that, a relationship between p53 and the incidence of apoptosis was observed. Previous studies confirmed that [B(a)P] induced apoptosis in Hepa1c1c7 cells requires p53 accumulation followed by caspase-3 activation. Furthermore, Caspases are key enzymes in apoptosis and their activation is a cascade of reactions elemental to cell death [42]. On the other hand, Malhotra *et al.,* (2014) [43]shown that, a significant decline in the enzyme activities of caspase 3 (apoptotic marker enzyme) was observed in the lungs of mice treated with [B [a] P]. Also, Sen *et al.,* (2005) [44] suggested that, stimulation of caspases by phytochemicals treatment in lung cancer cells. According to [45] caspase-3 is suggested to be essential for DNA fragmentation and for morphological changes of cell during apoptosis. The obtained results was supported by Chunga *et al.,* (2007) [46] who showed that, caspase-3 activation was clearly observed in B(a)P-treated cells. Who suggested that [B(a)P]-induced cell death is mediated by caspase-dependent apoptotic process. Previous studies confirmed that B(a)P-induced apoptosis in Hepa1c1c7 cells requires p53 accumulation followed by caspase-3 activation. It was further shown that cellular abundance of endogenous and potentially hazardous agents [47]. Moreover, Chunga *et al.,* (2007) [46]demonstrated that, the abundance of AhR and subsequent expression of CYP1A1 are pre-requisites for the induction of apoptosis after B(a)P exposure, After B(a)P treatment, CYP1A1 was found to be significantly induced. Furthermore, Clara cells were found to have higher CYP1A1 expression compared with macrophages and type 2 cells. Also, the upregulation of CYP1A1 protein expression by B (a) P exposure has been shown in Hepa1c1c7 cells [48]. The obtained results agree with Anandakumar *et al.,* (2013) [40] who showed that, [B(a)P]-induced lung cancer animals exhibited significantly increased levels/expressions of Bcl-2 protein when compared with control animals. Protected animals showed remarkable decrease in the levels of anti-apoptotic protein Bcl-2, when compared with [B(a)P]-administered lung cancer animals. Curcumin showed no adverse effects but provided protection against [B(a)P]-induced cytotoxicity through reducing ROS and DNA damage, and/or altering p53/Bcl-2 expression/activation in lung BEAS-2B cells. Although, this study revealed that, curcumin to be promising chemoprotective agents [49]. On the other hand, (Solhaug *et al.,* 2004) [50] established that, no changes in the level of Bcl-2 protein was observed in response to [B(a)P]. Also, Anandakumar *et al.,* (2013) [40] reported that, a significant increase in the level of Bcl-2 was observed in [B(a)P] treated animals as compared to normal vehicle treated animals. However, curcumin has been shown to down regulate the expression of ant-apoptotic (Bcl-2) [17]. In the present study, we have assessed curcumin induced apoptosis through its ability to modulate apoptosis related proteins like p53, Bcl-2, CyP1A1, DNA fragmentation and caspase-3. This might be due to significant reduction in the expression of proliferation-associated genes and induction in the expression of apoptosis-associated genes (p53) noted.

In the present study, there was an increase in serum CEA, ADA, HPT and GGT levels upon [B(a)P] treatment. The activity of these markers was brought back to near normalcy high-lighting the anti-tumor property of curcumin. The obtained results supported by Amal *et al.,* (2013) [51]who showed significant elevation in the level of serum CEA in cancer induced rats group as compared to normal control. While cancer induced rats treated with turmeric (Curcumin) showed significant reduction in this parameter as compared to untreated cancer induced group. Recently Hussein *et al.,* (2014)

[33] revealed that, a significant increase in serum CEA concentration and GGT activity were observed in DMH-induced colon cancer in rats when compared with normal control group. Who added that, treatment with curcumin in DMH-induced colon cancer in rats resulted in significant decrease in serum CEA and GGT level. Furthermore, the level of HPT increased in lung tissues from carcinogen-treated compared with untreated mice [52]. On the other hand, Fournier et *al.,* (2000) [53] suggested that,[B(a)P] failed to affect the levels of HPT, This is contrary to previously reported results where [B(a)P] slightly increased HPT release from Dark Agouti rat liver. The suggested functions of HPT in cancer are as a biomarker of malignancy, as a regulator of the immune response against tumor cells, and as a facilitator of metastasis, since HPT seems to participate in cell migration and angiogenesis [54]. Similarly, the activity of γ-GT was found to be significantly increased in carcinogen-treated animals when compared with the control animals [54], brought about a decrease in the level of γ-GT and ADA activities in the carcinogen-treated animals, a feature that would indicate cells protective response. Additionally, ADA production by neoplastic cells is increased [55]. Investigation of the marker enzymes such as γ-GT and ADA are specific indicators of lung damage [56]. GGT activity serves as a specific marker for the prognosis of carcinogenic events. Curcumin treated animals showed a decreased level of γ-GT, CEA, HPT and ADA compared with the cancer-bearing animals. The observed reduction in the levels of γ-GT and ADA activities in curcumin pre and post-treated animals was presumably due to the anti-tumor effect of curcumin.

4. CONCLUSION

It could be concluded that, curcumin may be effective in reducing lung cancer by its radical scavenging activity and anti-inflammatory effect, regenerating endogenous antioxidant mechanisms and decreased caspase-3 gene and DNA fragmentation in addition to modulation of Bcl-2, P53, CYP1A1, COX -2 in lung tissues. These results suggest that, the possible efficiency of curcumin as a distinct chemopreventive agent in lung carcinogenesis.

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