Caspase-3, Bcl-2, p53, CYP1A1 and COX -2 as a potential target in chemoprevention of Benzo (a) pyrene-induced lung carcinogenesis in mice: Role of thymoquinone

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ABSTRACT

Benzo(a)pyrene [B(a)P], a well-known environmental carcinogen, promotes oxidative stress and DNA damage. Thymoquinone (TQ) exhibits promising effects against inflammatory diseases and cancer. The possible protective and chemopreventive effects of TQ against [B(a)P] -induced lung cancer in mice were investigated. One hundred male Swiss albino mice divided into four equal groups. Group I: (Control) 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 + TQ treated) mice injected with [B(a)P] as in group II and treated with TQ (20 mg/kg b.wt/day, orally) from 22th week to 30th weeks. Group IV: (lung cancer + TQ protected) mice received TQ (20 mg/kg b.wt / Orally) on alternate days from 1 day prior to [B(a)P] injection and were treated continuously with TQ until 30th week. Blood samples and lung tissue for determination of serum CEA, Haptoglobin (HPT), ADA and for determination of serum CEA, HPT, ADA and metabolites of [B(a)P].

Keywords: Thymoquinone, Benzo(a)pyrene, Bd-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 it is increasing at a rapid rate [1,2]. 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 [3].

Mice lung tumor-genesis systems are valuable tools to study the process of chemical carcinogenesis induced by polycyclic aromatic hydrocarbons (PAHs) [4]. PAHs and N-nitrosamines are the two major classes of tobacco-related inhaled carcinogens [5]. Benzo(a)pyrene [B(a)P] is the archetypal PAH as it is the most intensely studied PAH, it is ubiquitous in the environment and it is a very potent carcinogen [6].
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 \([7, 8]\). Previous studies have proved that the toxicity of [B(a)P] behind its intermediate metabolites and the oxidative damage caused by reactive oxygen species (ROS) \([9]\).

Moreover, DNA damage has been recognized as the onset of many diseases, indicating cancer and could be a useful biomarker of the oxidative status and antioxidant defense system of an organism\([10]\). On the other hand, smoking is undoubtedly the main risk factor, to which 90\% of lung cancer cases are attributable \([11]\). 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\([12]\).

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 \([13,14]\).

Numerous studies have shown that the seeds and oil of this plant are characterized by a very low degree of toxicity \([15]\). Furthermore, TQ has been shown to exert anti-inflammatory, anti-oxidant and anti-neoplastic effects both in vitro and in vivo\([16]\). Many investigators have shown that the growth inhibitory effects of TQ are specific to cancer cells \([17,18]\). In addition TQ also exerts anti-oxidant effects and inhibits inflammation in animal models and cell culture systems \([19]\). Accordingly, the present study was designed to evaluated the chemo-preventive activity and the potential protective effect of TQ against [B(a)P] induced lung carcinogenesis in Swiss albino mice by determination of antioxidant parameters like 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, Beta cell lymphoma-2 (Bcl-2), Caspase 3, DNA fragmentation and Cycloxygenase -2 (COX-2) in lung tissues in addition to some serum tumor marker enzymes as Adenosine deaminase (ADA) and Gamma glutamyl transferase (γ GT) in addition to carcino-embryonic antigen (CEA) and Haptoglobin (HPT). Moreover, histopathological examination of lung tissue from all investigated groups were performed.

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 Thymoquinone

It is [2-isopropyl-5-methyl-1, 4 benzoquinone]. Thymoquinone yellow crystals, not soluble in water but dissolved in organic solvents (as ethyl alcohol).TQ have been purchased by Sigma Chemical Co. (St. Louis, Mo, USA) and purchased from Schnelldorf, Germany through the Egyptian International Center for Import Cairo, Egypt.

2.3 Preparation and Dosage of Thymoquinone

Thymoquinone was dissolved in few drops of ethyl alcohol and complete with distilled water and administered at dosage of (20 mg/kg.b.wt./ day, orally) all over the experimental period \([20]\).

2.4 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) \([21]\). [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.

2.5 Experimental design

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

**Group (1): Control Normal Group:**
Mice received no drugs, served as untreated control for all experimental groups.

**Group II: (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 + TQ treated group):**
Mice injected with [B(a)P] (100 mg/ kg b.wt, intraperitoneally)treated with TQ (20 mg/kg b.wt/day, Orally) from 22th week of the experiment and continued to 30th weeks(end of the experiment).

**Group IV: (lung cancer + TQ protected group):**
Mice received TQ (20 mg/kg b.wt. / Orally) on alternate days from 1 day prior to [B(a)P] injection and were treated continuously with TQ until 30th week (end of experiment).
2.6 Sampling
Blood samples and tissue specimens (lung tissues) were collected at the end of the experiment on 30th weeks from all animal groups (control and experimental groups).

2.7 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), \( \gamma \) GT, (ADA) and (CEA).

2.8 Tissue specimens (lung tissue)

a- For biochemical analysis:
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 freeze 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.

b- For Histopathological Examination:
Lung specimens were taken from different groups. The specimens were preserved in 10% neutral buffered formalin solution. The samples were dehydrated by using ascending grades concentrations of ethyl alcohol starting. The dehydrated samples were cleared in xylol. The samples were placed in a crucible containing soft paraffin and kept in an oven at 56°C for 12 hours. The samples were then blocked in hard paraffin and cut into sections of about 5 microns in thickness. Then the samples were subjected for histo-pathological examination following routine staining of the samples with haematoxylin and eosin (H&E) according to the technique described by [22].

2.9 Biochemical analysis
Serum CEA, HPT, \( \gamma \) GT, ADA and lung tissues SOD, CAT, L-MDA, GPX, NO, GR, GST, GSH, Bcl-2, CYP1A1, P53, COX-2, Caspase 3 and DNA fragmentation were analyzed according to the methods described by Bates, 1991 [23]; Cat. No. 6250; Sasz, 1976 [24]; Cloud-Clone Corp., assembled by Uscn Life Science Inc. ISO9001:2008: ISO13485:2003; Catalog Number.SEB390Hu ; Kakkar et al., 1984 [25]; Luck, 1974[26]; Mesbah et al., 2004[27]; Gross et al., 1967[28]; Vodovozt, 1996[29]; David and Richard, 1983[30]; Habig et al. 1974[31]; Moron et al., 1979 [32]; 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[33], respectively.

2.10 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

3.1 Effect of thymoquinone on some serum and lung tissues parameters of [B (a) P]-induced lung cancer in mice
The obtained results demonstrated in (Tables 1-4) 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, a significant increase in COX-2, Caspase 3, L-MDA, NO, Bcl-2, CYP1A1, P53 and DNA fragmentation in lung tissues and in serum HPT and CEA levels, \( \gamma \)-GT and ADA activities were observed in Benzo(a)pyrene treated mice when compared with normal control group.

Protection and treatment with TQ 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, TQ administration significantly decreased and attenuated the increased in COX-2, Caspase 3, L-MDA, NO, Bcl-2, CYP1A1, P53 and DNA fragmentation in lung tissues. Also, TQ administration significantly reduced elevated serum \( \gamma \)-GT, ADA activities, HPT and CEA concentrations when compared with [B(a)P]-induced lung cancer non-treated group.
Figure 1. Figures (A–D) are lung section of group 2 (B(a)P treated mice) showing, (A) infiltration of neoplastic cells in the wall of pulmonary blood vessels with perivascular mononuclear infiltration (H&E x 600), (B) extensive proliferation of alveolar epithelium forming adeno-carcinomatous masses with hyper chromatic nuclei (H&E x 600), (C) hyperplasia of the bronchial epithelium cellular with proliferation of neoplastic cellular areas beside the bronchial wall (H&E x 600), (D) large vesicular and hyper chromatic nuclei in the adenomatous proliferation (H&E x 600). (E) Lung of TQ post treated group showing hyperplasia of the bronchiolar epithelium with the presence of esinophilic debris in their lumen (H&E x 600). (F) Lung of TQ pre-treated group showing the presence of multiple neoplastic cells arised from the alveolar septa (H&E x 600).
### 3.2 Histopathological results

The microscopic examination of the lung tissue of control normal group revealed normal histological structure. Meanwhile, the lung of Benzo(a)pyrene treated mice showing severe congestion and dilatation of the pulmonary blood vessels and inter-alveolar blood capillaries. Moreover, proliferation of the endothelial cells lining of the pulmonary blood vessels with infiltration of the blood vessels wall by neoplastic malignant cells was detected (Fig. 1A). Moreover, extensive hyperplasia of the lining epithelium of the bronchioles with desquamation of epithelial cell lining was demonstrated (Fig. 1B). Marked peri-bronchial mononuclear leukocytic cellular infiltrations were observed in most cases. The neoplastic cells are enlarged vesicular or hyper chromatic nuclei. Additionally, the most common findings in this group are represented mainly by formation of well-developed neoplastic mass either in the form of adenoma or adeno-carciroma that arise mainly from the epithelial cell lining of the alveolar wall. Neoplastic foci of adenoma that characterized by complete loss of the alveolar structure and its replacement by glandular papillary or cell mass of hyper-chromatic or vesicular nuclei was demonstrated (Fig. 1C&D). Adeno-carciroma with multiple areas of dysplasia was seen among the neoplastic mass. Occasionally the neoplastic cells with hyper-chromatic nuclei were seen in the alveolar septa. Furthermore, scattered interstitial hemorrhage was also observed in most treated animals.

The microscopic examination of pulmonary tissue obtained from mice in B(a)P+TQ treated group (group 3) revealed congestion of pulmonary blood vessels with hyperplasia of the bronchiolar epithelium (Fig. 1E). Moreover, the most of pulmonary blood vessels showing injury of the intema of blood vessels with thrombus formation. Additionally, necrotic changes were detected in the alveolar septa. Accidentially, neoplastic cells with either hyperchromatic or vesicular nuclei were seen in the alveolar septa. Multiple areas of atelecstasy in association with compensatory emphysema were observed scattered all over the pulmonary tissue.

The lung of mice of TQ protected group (group 4) showed degenerative changes in lining endothelial cells with perivesicular mononuclear cellular infiltration. Hyperplasia of the bronchiolar epithelium with neoplastic growth was demonstrated in some cases. Moreover, multiple focal mononuclear aggregations were seen scattered all over the pulmonary tissue. Additionally, multiple neoplastic cells arisen from the alveolar septa were demonstrated (Fig. 1F).
Lung cancer is currently a leading cause of death all over the world. It is well known that antitumor agents can block tumor initiation by blocking carcinogen activation, scavenging reactive carcinogens, enhancing DNA damage repair, inducing apoptosis of tumorinitiative cells or by suppressing the progression of initiated cells. 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 [34]. [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 [35]. 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 [34]. 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 chemopreventive agents [36]. The present study clearly demonstrates a potent inhibitory activity of TQ, the main constituent of the volatile oil of Nigella sativa (Black seed) 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. Numerous studies have shown that the seeds and oil of this plant are characterized by a very low degree of toxicity [15]. In this regard, earlier studies have demonstrated that TQ has a considerable protective effect against reactive oxygen species (ROS) generating agents including significant suppression of fore stomach tumor induced by [B(a)P] [37].

Histological mucosal changes in the large airways that may precede or accompany invasive carcinoma include hyperplasia, metaplasia, dysplasia and carcinoma in situ [38, 39, 40]. Therefore, a strategy to arrest or reverse pre-neoplastic changes in the bronchial epithelium by natural or synthetic agents before invasive cancer develops is a rational approach in reducing the burden of lung cancer [41].

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

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Control Normal group</th>
<th>B(a)P group</th>
<th>B(a)P + TQ treated group</th>
<th>B(a)P + TQ protected group</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA fragmentation %</td>
<td>86.8±6.25 38 9</td>
<td>1477.57±159.42 7</td>
<td>144.45±38.71 1</td>
<td>115.37±32.69 9</td>
</tr>
<tr>
<td>COX-2 (U/g tissue)</td>
<td>4.88±0.49 4</td>
<td>12.42±1.04 5</td>
<td>5.62±0.41 3</td>
<td>3.05±0.84 8</td>
</tr>
<tr>
<td>Caspase-3 (ng/g tissue)</td>
<td>0.59±0.05 4</td>
<td>2.44±0.03 2</td>
<td>1.19±0.19 1</td>
<td>0.77±0.11 3</td>
</tr>
</tbody>
</table>

Data are presented as (Mean ± SE). 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 thymoquinone on lung tissues P53, Bcl-2 and CyP1A1 concentrations of B(a)P-induced lung cancer in mice.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Control Normal group</th>
<th>B(a)P group</th>
<th>B(a)P + TQ treated group</th>
<th>B(a)P + TQ protected group</th>
</tr>
</thead>
<tbody>
<tr>
<td>P53 (pg/ml)</td>
<td>20.71±5.14 1</td>
<td>10.26±6.04 2</td>
<td>41.79±2.91 3</td>
<td>26.06±4.86 4</td>
</tr>
<tr>
<td>Bcl-2 (ng/g tissue)</td>
<td>0.45±0.05 1</td>
<td>0.87±0.01 2</td>
<td>0.52±0.12 3</td>
<td>0.35±0.02 4</td>
</tr>
<tr>
<td>CyP1A1 (ng/g tissue)</td>
<td>0.60±0.04 1</td>
<td>2.66±0.16 2</td>
<td>1.12±0.02 3</td>
<td>0.52±0.12 4</td>
</tr>
</tbody>
</table>

Data are presented as (Mean ± SE). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at (P<0.05).
covalently binding to nucleic acid [53]. Furthermore, ROS generated during the metabolic process are capable of causing damage to DNA [54]. [B(a)P] administration resulted in elevated levels of oxidative DNA lesion, (8-oxo-dG) in both liver and lungs of mice [55]. Moreover, oral treatment of [B(a)P] leads to oxidative DNA damages (8-oxo-dG) in the liver, kidney and lung [56]. Increased formation of DNA–protein cross links reduces DNA repair capacity and results in DNA-strand breaks and other genetic effects [57].

3.4 Effect of thymoquinone on lung tissues SOD, GST, GPX, GR and CAT activities and GSH, MDA and NO concentrations of B(a)P-induced lung cancer in mice

Thymoquinone is a well-known scavenger of ROS such as superoxide anions, hydroxyl radicals, and peroxynitrite anion. In this regard, earlier studies have demonstrated that TQ has a considerable protective effect against reactive oxygen species (ROS) generating agents including significant suppression of fore stomach tumor induced by [B(a)P] [37]. In the present study, SOD, GST, GPX, GR and CAT activities and GSH concentration were significantly decreased while MDA and NO level were significantly increased in lung tissues in [B(a)P] treated mice as compared to normal control mice. Similarly, Anandakumar et al., (2013) [65] reported that, [B(a)P] treated mice showed significant increase in the MDA level with significant decrease of SOD activity in lung tissue as compared to control mice. These reports have cited decreased activities of SOD and CAT in various carcinogenic conditions that may be due to the increased LPO [66]. Also, Wang et al., (2013) [67] reported that, compared with normal group the value of MDA level, a classic indicator of oxidative stress, was significantly increased in lung tissues of [B(a)P] treated group. Meanwhile, the activity of SOD in [B(a)P] administered group was significantly decreased. Carcinogen induced reactive oxidative species and free radical intermediates have been suggested to have a role in the initiation and development of cancer [12]. [B(a)P] has been reported to cause lipid peroxidation and decrease antioxidant enzymes levels by inducing oxidative stress in lung carcinogenesis [68]. Increased levels of LPO products play a major role in the early phases of tumor growth [69]. Studies have also shown that SOD activity significantly decrease on [B(a)P] treatment, which may abet in inducing carcinogenesis [70]. Hence, estimating lipid peroxidation and enzymatic antioxidants like SOD a useful tool for assessing oxidative damage induced carcinogenesis by [B(a)P]. Similarly, Kamaraj et al., (2007) [5] found that, highly significant reductions in the activities of enzymic and non-enzymic antioxidants (SOD, CAT, GPx, GST, GR and GSH) were observed in the cancer bearing animals. Moreover, Sankaranarayanan and Pari, (2011) [71] showed that, the decrease of CAT, GPx, GST and GSH in kidney and liver tissues in the streptozotocin diabetic rat could be reversed by thymoquinone. Moreover, El-Mahmoudy et al., (2002) [72] found that, TQ reduced the NO production in supernatants of lipo-polysaccharide (LPS)-stimulated macrophages, without affecting their cell viability. Furthermore, the inducible nitric oxide synthase (iNOS) is responsible for the production of NO.

On the other hand, the present work neglected some studies that suggest no alteration in ADA activity in lung cancer patients [63]. Also, high amounts of HPT in plasma and locally in tumoral tissue have been observed in diverse types of malignancies including, lung, bladder, breast cancer, leukemia, glioblastoma, malignant lymphoma, and ovarian cancer [64]. Furthermore, the level of HPT increased in lung tissues from carcigen-treated compared with untreated mice [59]. The marked elevation in such serum parameters observed in [B(a)P] treated group may be due to the genotoxic property of [B(a)P], which is a very effective carcigen enhancing oxidative stress and consequently inducing free radical formation, which in turn react with lipids in the cell membrane causing lipid peroxidation [35].

http://ijcn.saiseonpublishers.net/content/2016/3/ijcns430-441.pdf
inflammation in animal models and cell culture systems (Mansour et al., 2002) [19]. It is assumed that, these probable anti-apoptogenic effects of TQ may be mediated by one or more of the following mechanisms: Antioxidant activity, immunomodulatory action and genoprotective effects [73, 74]. According to the previous studies, N. sativa (TQ source) protects lipids against free-radical damage [75]. Decreased tissue malondialdehyde(MDA), protein carbonyl levels and prevented inhibition of superoxide dismutase (SOD) and catalase (CAT) enzyme activities following experimental spinal cord injury in rats were seen following treatment with N. sativa (TQ source) [76].

3.5 Effect of thymoquinone on lung cancers COX-2, Caspase 3 and DNA fragmentation of B(a)P-induced lung cancer in mice:

The obtained results revealed that, administration of B(a)P induced lung cancer in mice significantly increased COX-2, Caspase 3 and DNA fragmentation in lung tissues when compared with normal control group. This elevation may be due to the genotoxic property of [B(a)P]. The obtained results are nearly similar with those recorded by Shaymaa, (2014) [60]. She recorded that, [B(a)P] caused significant increase in levels of caspase 3,9 activities in lung tissue compared to control group. The present results supported by Chunga et al., (2007) [77] who shown that caspase-3 activation was clearly observed in B(a)P-treated cells. These 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 (Burbach et al., 1992) [78].

Furthermore, COX-2 has been shown to regulate some aspects of tumor-associated angiogenesis and its expression has been previously reported to be present in elevated levels as compared to normal lung tissue. Certain chemo-preventive agents have the capability to affect the COX-2 expression as one of their many functions thereby paving the way for cancer chemoprevention. There are certain agents which have the capability of inhibiting COX-2 and thus have the potential to impart antitumor effects against lung cancer. Clinical studies have demonstrated high levels of expression of COX-2 in almost all non small cell lung cancer pre-invasive precursor lesions as well as invasive lung carcinomas, when compared to normal lung tissue [79]. Moreover, increased COX-2 expression is associated with a poor prognosis in lung cancer [80]. As inflammation is linked with cancer development and progression [81]. Also, Zhu et al., (2008) [82] reported that, COX-2 is over expressed in up to 85% of lung cancers and is associated with advanced clinical stage and distant. Protection and treatment with TQ in B(a)P-induced lung cancer in mice attenuated the increased in COX-2, Caspase 3 and DNA fragmentation in lung tissues when compared with B(a)P -induced lung cancer non-treated group. On contrary, Banerjee et al., (2009) [83] indicated that, increased caspase-3 activity was observed in the tumor tissues treated with the TQ.

3.6 Effect of thymoquinone on lung tissues P53, Bcl-2 and CyP1A1 of B(a)P-induced lung cancer in mice:

The obtained results shown an increased level of P53, Bcl-2 and CyP1A1 in [B(a)P] induced lung cancer in mice. These results are nearly similar to those recorded by, Solhaug et al., (2004) [84] who reported that, marked increases in p53 protein levels activity were observed after exposure to B(a)P in Clara cells compared with the other lung cells. Also, Chung et al., (2007) [77] recorded that, p53 activation was clearly observed in [B(a)P]-treated cells. These suggested that [B(a)P]-induced cell death is mediated by p53-dependent apoptotic process. 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 [85]. Like [B(a)P] several PAH metabolites are highly genotoxic and elicit an accumulation of p53 [86]. [B(a)P] treatment of murine 3T3 cells have been shown to result in DNA damage associated with elevated levels of nuclear p53 [87]. Rotter et al., (1993) [88] found that, a relationship between p53 and the incidence of apoptosis. Similarly, the observed increase in p53 expression is a natural defense to prevent uncontrolled proliferation of transformed cells [89].This suggesting that, the p53 tumour suppressor is a tightly regulated protein that acts by inhibiting cell-cycle progression or by promoting apoptosis whenever cells encounter DNA damage, oncogene activation or during carcinogenesis. 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 [90].

Furthermore, Li et al., (2008) [91] showed that, the ability of a chemotherapeutic drug to induce apoptosis is an important factor in determining its effectiveness in cancer treatment. TQ was shown to induce apoptosis in p53 dependent or p53-independent pathway. It is widely accepted that the mutation of the p53 tumor suppressor gene plays a critical role somewhere in the multitstage process of the carcinogenesis of lung cancers. Also, the up regulation of CYP1A1 protein expression by B(a)P exposure has been shown in Hepa1c1c7 cells [92]. These findings are consistent with previous studies suggesting that CYP1A1 expression in lung tissue from smokers and ex-smokers is significantly greater than in non-smokers [93, 94]. The current study supported by Solhaug et al., (2004) [84] shown that marked increases in CYP1A1 activity was observed after exposure to B(a)P in Clara cells compared with the other lung cells. Moreover, Chung et al., (2007) [77] demonstrated that, the abundance of AhR and subsequent expression of CYP1A1 are prerequisites for the induction of apoptosis after B(a)P
exposure. After B(a)P treatment, CYP1A1 was found to be significantly induced. Several P450 enzymes are involved in key steps in the oxidation of B(a)P, and CYP1A1 has been demonstrated to be the most active in these oxidations in mammals [95].

Regarding lung tissues Bcl-2 in B(a)P treated mice the obtained results are nearly similar to those reported by Anandakumar et al., (2013) [65] 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. Who also added 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. Expression of Bcl-2 family proteins the Bcl-2 family proteins are important regulators of apoptotic pathways [28] and p53 has been suggested to cause apoptosis through changes in the level of the Bcl-2. Apoptosis is an active and physiological mode of cell death characterized by condensation of chromatin in the nucleus and DNA fragmentation. Mitochondria, Bcl-2 protein, cytochrome c and caspases, a family of intracellular cysteine proteases, are considered to be essential components of the intracellular apoptotic signalling pathways. It is well known that PAH may cause an accumulation of the tumour suppressor protein p53 [96] and more recent studies have indicated that the resulting accumulation of p53 may be important for the induction of apoptosis [97]. Also, Gali-Muhtasib, (2004) [17] reported that, up-regulation of p53 and p21 together with Bcl-2 inhibition were also observed. Additionally, in the xenograft tumor mouse model, TQ augmented the anti-cancer effects of carcinogen. In the xenograft tumor mouse model, TQ augmented the anti-cancer effects of carcinogen. TQ treatment led to a decrease in bcl-2 expression, an increase in bax protein expression and the release of cyt-c from the mitochondria into the cytoplasm [98]. Hence in the present study we have assessed TQ induced apoptosis through its ability to modulate apoptosis related proteins like CyP1A1. The Phase I enzymes, CyP 1A1 is some of the enzymes responsible in metabolism of B(a)P. The decreased Bcl-2 observed after treatment further indicated that the promoted cell growth/survival could be regulated by the Bcl-2 family in addition to the other signaling molecules such as p53 [99]. Drop in Bcl-2/Bax ratio plays a key role in the release of proteins from the inter-membrane space of mitochondria by enhancing the mitochondrial permeabilization. For this event, Bax must be activated and it has been proposed that p53 activates Bax, resulting in oligomerization of Bax and increasing mitochondrial permeabilization, which, in turn, releases cytochrome c and other proteins of mitochondrial inter-membrane space leading to the activation of caspase and cell death [65]. Cellular proliferation and apoptosis were found to occur in opposite directions during B(a)P-induced lung carcinogenesis and that the interplay of p53 and bcl-2 genes has an important role in the initiation of lung carcinogenesis[100].

4. CONCLUSION

The present study demonstrated that, TQ administration provided an effective protection and treatment in B(a)P induced lung carcinogenesis in Swiss Albino mice. In conclusion, protection and treatment of TQ effectively decrease oxidative stress, ameliorate serum tumor and inflammatory markers, enzymatic antioxidant defense system in lung tissue and protected lung cells via inhibition of caspase 3 and modulating pro-inflammatory enzyme COX-2 activity. This study establishes the role of TQ as a chemo-preventive and chemotherapeutic agent and also, provides the possible mechanism of TQ modulating caspase 3, DNA fragmentation and inhibiting the activity of COX-2 and attenuate Bcl-2, P53, CYP1A1 in lung tissues in B(a)P induced lung carcinogenesis. Moreover, inhibition of peroxidation and oxidative stress markers and enhanced antioxidant status and decreased caspase-3 gene activity and DNA fragmentation percent in mice lung tissues by TQ suggest a protective effect and the potential efficacy of TQ as chemo-preventive agent on lung carcinogenesis induced by B(a)P in mice. So we recommended that, supplementation of diet rich in the natural agents (TQ) is extremely essential for protection of different body organs from cancer incidence. Also, we strongly support the use of TQ as pure active ingredients in pharmaceutical industry for manufacture of new drugs used as therapeutics for protection and treatment of cancer particularly lung cancer.

5. REFERENCES


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