Chemopreventive effect of thymoquinone on benzo(a)pyrene-induced lung cancer in male swiss albino mice

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A B S T R A C T

Benzo[a]pyrene [B(a)P], a well-known environmental carcinogen, promotes oxidative stress and DNA damage. Thymoquinone (TQ), the main active constituent of black seed essential oil, exhibits promising effects against inflammatory diseases and cancer. The present study was designed to investigate the possible protective effect of TQ on [B(a)P] -induced lung cancer in mice. One hundred male Swiss Albino mice were divided into four equal groups. Group I :(Control group) received no drugs. Group II :(lung cancer- induced group) mice administered with a single dose of [B(a)P] (100 mg/ kg b.wt, intraperitoneally). Group III :(lung cancer + TQ treated group) 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 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). Blood samples and lung tissue specimens were collected at the end of experimental period (30 week) for determination of serum carcino-embryonic antigen (CEA), Haptoglobin (HPT) and Gamma glutamyl transferase (ɤ-GT) in addition to catalase (CAT), Super oxide dismutase(SOD), L-Malondialdehyde (L-MDA), Caspase3, DNA fragmentation(DF), Cycloxygenase -2(COX-2) in lung tissues. The obtained results revealed that, [B(a)P] potentially increased serum ɤ- GT activity, HP and CEA levels in addition to lung tissues COX-2, Caspase 3 gene, L-MDA and DNA fragmentation. However, SOD and GST activities in lung tissues were significantly decreased. TQ treatment was able to mitigate lung cancer induced by [B(a)P] through enhanced the activity of SOD, CAT and attenuated the increased caspase 3 gene, DNA fragmentation, COX-2 and L-MDA in lung tissues and serum CEA, HP and ɤ- GT. It could be concluded that, TQ 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 lung tissues. These results suggest that, the possible efficiency of TQ as an distinct chemo-preventive agent in lung carcinogenesis.

KEYWORDS: thymoquinone, Benzo(a)pyrene, Lung cancer, DNA fragmentation, caspase-3 gene, Cycloxygenase -2.

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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 (Hecht et al., 2002; Osann et al., 2000).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. Mice lung tumor-genesis systems be valuable tools to study the process of chemical carcinogenesis induced by polycyclic aromatic hydrocarbons (PAHs). PAHs and *N*-nitrosamines are the two major classes of tobacco-related inhaled carcinogens (Kamaraj et al., 2007). 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 (Cavalieri et al .,1991).Who added that, [B(a)P] is typically selected as the standard against which the cancer potency of other PAHs are tested. Furthermore, PAH including [B(a)P], a potent tobacco carcinogen. 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 (Straif, 2005 and IARC, 1989 **)**.

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) **(**Wang et al., 2009; Wester et al., 2012) . Additionally, PAH is a significant pro-carcinogenic substance, which requires metabolic activation to electrophilic reactive metabolites for its carcinogenic activity(Gelboin et al .,1980).

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 On the other hand, smoking is undoubtedly the main risk factor, to which 90% of lung cancer cases are attributable (Hecht., 1999; Ruano-Ravina et al., 2003 and Winterhalder et al., 2004).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 (Panandiker et al., 1994).

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 (Hanspeter et al., 2000; Anto et al., 2002).

Numerous studies have shown that the seeds and oil of this plant are characterized by a very low degree of toxicity (Ali and Blunden, 2003). Furthermore, TQ has been shown to exert anti-inflammatory, anti-oxidant and anti-neoplastic effects both in vitro and in vivo (Pagola et al., 2004). Many investigators have shown that the growth inhibitory effects of TQ are specific to cancer cells (Gali-Muhtasib et al., 2004; Shoieb et al., 2003; Worthen et al., 1998). In addition TQ also exerts anti-oxidant effects and inhibits inflammation in animal models and cell culture systems (Mansour et al., 2002). 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), L-Malondialdehyde (L-MDA), Caspase 3, DNA fragmentation and Cycloxygenase -2 (COX-2) in lung tissues in addition to some serum parameters as carcino embryonic antigen (CEA), Haptoglobin (HP) and Gamma glutamyl transferase (ɤ GT).

2. MATERIAL 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.

2.1.1. 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.

2.1.2. 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) allover the experimental period (Gali-Muhtasib et al., 2006)

2.1.3. Induction of lung cancer :

] B(a)P]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) (Magesh et al., 2009). [B (a) P] has been purchased by Sigma Chemical Co. (St. Louis, Mo, USA) and purchased from Schnelldorf, Germany through the Egyptian International Center for Import Cairo, Egypt.

2.2. Experimental design :

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

Group (1): Control Normal Group:

Mice received no drugs, served as untreated 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 + TQ treated group:(

Mice injected with [B(a)P] (100 mg/ kg b.wt, intraperitoneally)and 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.(

2.3. Sampling :

Blood samples and tissue specimens (lung tissues) were collected at the end of the experiment on 30thweeks from all animal groups (control and experimental group)

2.3.1. 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 oC until used for subsequent biochemical analysis. All sera were analyzed for determination of (HPT), (ɤ GT) and (CEA)

2.3.2. 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, COX-2, Caspase 3 and DNA fragmentation.

2.4. Biochemical analysis:

Serum CEA, HPT, ɤ GT and lung tissues SOD, CAT, L-MDA, COX-2 , Caspase 3 and DNA fragmentation were analyzed.

2.5. 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

Protective and treatment Effects of TQ administration on some serum and lung tissues biochemical parameters of [B(a)P]-induced lung cancer in mice. The obtained results demonstrated in (Table 1) revealed that, administration of [B(a)P] induced lung cancer in mice exhibited a significant decrease in CAT and SOD activities and significantly increased COX-2, Caspase 3, L-MDA, and DNA fragmentation in lung tissues and in serum HP , CEA levels and ɤGT activity when compared with normal control group. protection and treatment with TQ in [B(a)P] induced lung cancer in mice significantly increased CAT and SOD activities and significantly decreased and attenuated the increased in COX-2, Caspase 3, L-MDA, and DNA fragmentation in lung tissues. Also, TQ administration significantly reduced elevated serum ɤ GT activity, HP and CEA concentrations when compared with [B(a)P]-induced lung cancer non-treated group.

4. DISCUSSION

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 (Ramakrishnan et al., 2007). 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 (Ren et al., 2003). 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(Ali and Blunden, 2003). In the present study, [B(a)P] treated mice showed a significant increase in the serum ɤ GT activity and CEA and HPT concentrations when compared with control group. However, TQ administration significantly reduced elevated serum ɤ GT activity, HPT and CEA concentrations when compared with [B (a) P] -induced lung cancer non-treated group. Similarly, a significant increase in the expression of serum CEA and serum marker enzyme ɤ GT activity were observed in the [B(a)P] administered group. Also, (Kassie et al., 2007) observed that, HPT 2004). These toxic radicals are involved in mediating tissue lipid peroxidation. Lipid peroxidation-induced tissue damage is the sensitive feature in the cancerous conditions any deterioration or destruction of the membrane can lead to the leakage of these enzymes from the tissues (Ramakrishnan et

Table 1: Protective and treatment Effects of TQ administration on some serum and lung tissues biochemical parameters of B(a)P-induced lung cancer in mice at the end o experimental period (30 days).

Experimental groups Parameters	Control Normal group	B(a)P group	B(a)P + TQ treated group	B(a)P + TQ protected group
CEA (ng/ml)	0.29±0.08^{b}	1.59±0.27^{a}	0.58±0.10^{b}	0.44±0.13^{b}
Haptoglobin(ng/ml)	2.05±0.21^{b}	7.40±1.31^{a}	2.67±0.37^{b}	2.17±0.60^{b}
GGT (U/L)	25.60±6.11^{b}	125.73±15.87^{a}	47.40±8.89^{b}	28.40±7.00^{b}
COX-2 (U/g.tissue)	4.88±0.48^{c,d}	12.42±1.04^{a}	5.62±0.41^{b,c}	3.05±0.84^{d}
Caspase-3 gene activity	0.59±0.05^{c}	2.44±0.03^{a}	1.19±0.19^{b}	0.77±0.11^{c}
SOD (U/g.tissue)	25.70±1.75^{d}	10.19±1.87^{e}	41.19±0.22^{b}	46.58±1.47^{a}
CAT(mmol/g.tissue)	59.04±3.33^{a}	19.06±1.92^{c}	47.75±5.11^{b}	59.26±2.87^{a}
MDA(mmole/g. tissue)	26.40±5.68^{c}	200.86±10.93^{a}	92.74±2.75^{b}	41.27±9.16^{c}
DNA fragmentation %	86.86±25.38^{c}	1477.57±159.42^{a}	144.45±38.71^{c}	115.37±32.69^{c}

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

level was increased in carcinogenic-treated mice. A very highly significant increase in level of serum CEA was observed in [B(a)P] treated female mice as compared to control group (Shaymaa, 2014). Moreover, (Kamara et al., 2009) reported that, administration of [B(a)P]to mice exhibited significant increase in lung specific tumor marker (CEA) and ɤ GT. The transfer of ɣ- glutamyl groups from peptide donors to peptide receptors and aminoacids is the catalytic function of ɤ GT. ɤ GT is not only useful in diagnosis but also has prognostic value in malignancies such as lung cancer and malignant melanoma [Obrador et al., 2002]. [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(Selvendiran et al., and al.,2007).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 carcinogen enhancing oxidative stress and consequently inducing free radical formation, which in turn react with lipids in the cell membrane causing lipid peroxidation (Selvendiran et al., 2004). TQ 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] (Badary et al ., 1999) .

In the present study, SOD and CAT activities were significantly decreased and MDA level was significantly increased in lung tissues in [B (a) P] treated mice as compared to normal control mice. Similarly, (Ananda et al .,2013) 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. Also, (Wang et al., 2013) 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 (Panandiker et al., 1994). [B(a)P] has been reported to cause lipid peroxidation and decrease antioxidant enzymes levels by inducing oxidative stress in lung carcinogenesis (Sikkim and Mulee. 2000). Increased levels of LPO products play a major role in the early phases of tumor growth (Kim et al., 2000). Studies have also shown that SOD activity significantly decrease on [B(a)P] treatment, which may abet in inducing carcinogenesis(Emre et al., 2007). Hence, estimating lipid peroxidation and enzymatic antioxidants like SOD an useful tool for assessing oxidative damage induced carcinogenesis by [B(a)P].

The obtained results revealed that, protection and treatment with TQ in [B(a)P] induced lung cancer in mice significantly increased CAT and SOD activities and attenuated the increased in L-MDA level in lung tissues. This protective effect of TQ may be due to its potential free radical scavenging activity. TQ also exerts anti-oxidant effects and inhibits inflammation in animal models and cell culture systems (Mansour et al., 2002). It is assumed that, these probable anti-apoptogenic effects of TQ may be mediated by one or more of the following mechanisms: Antioxidant activity, immunomedulatory action and genoprotective effects (Rastogi et al., 2010; Gautam et al., 2008; Mousavi et al., 2010; Burits and Bucar; 2000). According to the previous studies, *N. sativa* (TQ source) protects lipids against free-radical damage (Burits and Bucar, 2000). 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) (Kanter et al., 2006).

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 reported by Shaymaa, (2014) She recorded that, [B(a)P] caused significant increase in levels of caspase 3,9 activities in lung tissue compared to control group**.** Also, Ashish_ et al., (2008) reported that, [B(a)P] increased the activation of caspase-3,7,8,9 and decreased cell viability. 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 (Anderson et al., 2002). 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. Phytochemicals such as curcumin and quercetin are such chemopreventive agents which have the potential to affect the COX-2 expression as well as its activity (Aggarwal, 2010).

Preclinical studies do suggest that COX-2 may be involved in the molecular pathogenesis of some types of lung cancer. In lung cancer, COX-2 expression is observed at the majority stages of tumor progression. 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 (Anderson et al., 2002). Moreover, increased COX-2 expression is associated with a poor prognosis in lung

cancer (Gardner et al., 2003). Consistent with the preclinical studies, [B(a)P] induced lung carcinogenesis in mice also showed an increase in the activity of COX-2 enzyme in the present study. As inflammation is linked with cancer development and progression (Mascaux et al., 2005), the resulting tumor incidence as well as multiplicity in the lungs of [B(a)P] treated mice can be correlated with the high COX-2 activity.

Similarly, previous work found that, [B(a)P] treatment to mice brought about a statistically significant increase in the activity of COX-2 in the lung tissues of mice. Also, (Zhu et al., 2008) 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. Supplementation of phytochemicals significantly reduced elevated activity of COX-2 and further normalized the lung weights as compared to normal controls. Curcumin exhibits its anti-inflammatory effect in part, through inhibition of the NF-kappaB pathway and cyclo-oxygenase 2 (COX-2) enzymes and thus plays a pivotal role in suppressing tumor cell growth (Aggarwal, 2010). On contrary, (Banerjee et al., 2009) indicated that, increased caspase-3 activity was observed in the tumor tissues treated with the TQ.

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 in [B(a)P] induced lung carcinogenesis.

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التأثير الكيميائي الوقائى للثيموكينون على سرطان الرئة المحدث بالبنزو(أ)بيرين في ذكور الجرذان

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الملخص العربي

يعتبر الثيموكينون من المركبات التي لها تأثيرات مضادة للاكسده والالتهابات ضد العديد من الأمراض مثل مرض السكر والربو والتهاب المخ وسرطان الرئة. في هذه الدراسة تم دراسة تأثير الثيموكينون كمضاد للاكسده والالتهابات على العديد من العوامل الكيميائية كما تم دراسة استخدامه كعلاج للالتهابات وسرطان الرئة . في هذه الدراسة تم استخدام 100 من ذكور الجرذان حيث تم تقسيمها إلى أربع مجموعات رئيسيه . ألمجموعه الأولى (ألمجموعه الضابطة):حيث تركت بلا علاج ، ألمجموعه الثانية (ألمجموعه المعالجة باستخدام البنزو(ا) بيرين) حيث تم إعطاءها المادة المسرطنه (البنزو(ا)بيرين)عند جرعة 100 مجم /كيلوجرام من وزن الجسم وذالك لإحداث سرطان الرئة في ذكور الجرذان ، أما ألمجموعه الثالثة (مجموعة سرطان الرئة والمعالجة باستخدام الثيموكينون) حيث تم إعطائها الثيموكينون عند جرعة 20 مجم / كيلوجرام من وزن الجرذان يوميا عن طريق الفم لمدة 8 أسابيع ، أما ألمجموعه الرابعة (مجموعه سرطان الرئة التي تم وقايتها باستخدام الثيموكينون) حيث تم إعطائها الثيموكينون عند جرعة 20 مجم /كجم من وزن الجرذان يوم بعد يوم عن طريق الفم لمدة 30 أسبوع خلال فترة التجربة . تم اخذ عينات من الدم وأنسجة جسم الجرذان (الرئة) وقد أسفرت النتائج عن وجود انخفاض معنوي في إنزيم السوبر أكسيد ديسميوتيز والكتاليز ووجود زيادة في إل – مالون داى الدهيد بالاضافه إلى زيادة الهيبتاجلوبين وإنزيمات السيكلواوكسيجيناز-2 والجاما جليوتوميل ترانسفيراز و تجزئة الحمض النووى دى ان ايه ودلالات الأورام كرسينوجينك امبريونك انتجين وأخيرا الكسباس 3 وقد أظهرت نتائج هذه الدراسة ان الثيموكينون كان له القدرة على علاج سرطان الرئة المحدث باستخدام البنزو(ا)بيرين وذالك من خلال الزيادة المعنوية في مضادات الاكسده السوبر أكسيد ديسميوتيز والكتاليز بالاضافه إلى النقص المعنوي في في إل – مالون داى الدهيد بالاضافه إلى زيادة الهيبتاجلوبين وإنزيمات السيكلواوكسيجيناز-2 والجاما جليوتوميل ترانسفيراز تجزئة الحمض النووى دى ان ايه ودلالات الأورام كرسينوجينك امبريونك انتيجين وأخيرا الكسباس3. يمكن القول أن الثيموكينون قد يكون فعالا في الحد من سرطان الرئة بواسطة نشاطها وتأثيرها كمضاد للالتهابات، وتجديد آليات مضادة للأكسدة الذاتية وانخفاض كاسباس 3 وتجزئة الحمض النووي في أنسجة الرئة. وتشير هذه النتائج أن كفاءة الثيموكينون كعامل وقائى متميز في الحد من سرطان الرئه.

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