Chemopreventive effect of anovel nanoomposite against Benzo[a]pyrene induced lung carcinogenesis

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A B S T R A C T

Benzo[a]pyrene induced lung cancer by mechanism, which interact with DNA and cause genetic changes; this mechanism accelerates the cell cycle progression and induces the abnormal cell proliferation. Selenium , N Acetyl cysteine and curcumin in nanocomposite have been shown to confer various biological effects, anticancer, enhance immune system and antioxidant properties. The present study was undertaken to evaluate the chemopreventive effect of nano (selenium ,acetyl cysteine, curcumin) (NSACC) and possess ability of SNACC with dose 4mg/kg.b.w against Benzo[a]pyrene carcinogenesis with dose 200mg\kg.bw at two doses the first at the 1st week of the experiment,the second after 20 week ,from the start time of the experiment.The results indicated that B[a]p induced lung cancer showed by histopathology in mice which cause significant decrease of SOD, GSH,CAT values and significant increase of NOx, LP ,over expression of p53,cas3 and cas9.While, treating with (NSACC) causes significant increase of SOD, GSH, GPx activities and significant decrease of CAT, lipid peroxidation, Nox, induction of p53, cas 3, cas 9 gradually then decrease to normal control values. From the obtained results, it could be concluded that inhibition of peroxidation and oxidative stress markers, enhanced antioxidant status, induction of p53 gene , caspase3 and 9 gene expressions in mice lung tissue by NSACC suggest the potential efficacy of NSACC as an addition chemopreventive agent in treatment of lung carcinogenesis.

Key Words: nanocomposite, caspase3, 9, tumor suppressor gene p53, superoxide dismutase.

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1. INTRODUCTION

Lung cancer has high incidence rate and currently has no effective treatment. It is therefore imperative to develop alternative approaches, such as chemoprevention, to help control this disease. Lung cancer kills more patients than any other malignancy in the world [1]. Early-stage disease can be treated with curative intent although the risk for relapse is notoriously high. Unfortunately, the majority of lung cancer patients present at an advanced stage. Despite an initial response to treatment, most of these late stage patients will eventually progress on standard therapy and die from their disease. Despite the complex nature of lung cancer biology, its molecular underpinnings are becoming increasingly clear [2]. Benzo[a]pyrene is considered a prototype polycyclic aromatic hydrocarbon (PAH), classic DNA damaging agent and carcinogen. Benzo[a]pyrene has been identified as a major risk factor for lung related cancer.Nanoparticles are small; they are able to penetrate through to the protein and DNA inside of cells of diseased tissues.Size and surface characteristics have long been recognized as key characteristics in the design and development of nanoparticles [3].So, we design our drug which composed of (selenium, n acetyl cysteine, curcumin) in

nano form to reduce toxicity and f selenium (Se) is a mineral trace element of fundamental importance to humans and animals. The role of Se as potential cancer chemotherapeutic and chemopreventive agents has been supported by many epidemiological, preclinical, and clinical studies. Se nanoparticles (SeNPs) have attracted increasing attention in the past decade because of their antioxidant activities and low toxicity. Compared to other nanoparticles that are currently most often studied, such as gold nanoparticles, SeNPs are superior, because Se is degradable in vivo and in vitro antioxidant novel in vitro and in vivo antioxidants activities through activation of selenoenzymes [4]. Degraded Se can be used as a nutrient for many kinds of normal cells or as an antiproliferative agent for many kinds of cancer cells. N Acetyl Cysteine is an antioxidant containing an acetylated form of the amino acid L-cysteine that functions as a precursor of glutathione synthesis. Glutathione is an important thiol involved in cellular detoxification [5]. The presence of sulfhydryl groups in NAC also enables the neutralisation of free radicals.curcumin as an excellent molecule among many naturally occurring compounds for cancer therapeutics. Curcumin is a hydrophobic molecule and passes easily through the plasma membrane into the cytosol [6].

2. MATERIAL AND METHODS

2.1. Chemicals :

Benzo[a]pyrene, N-Acetyl Cysteine purchased from (Sigma ,USA). Selenium:- used as selenious acid (assay 99% and MW 110.96) was purchased from international co. For trading chemical medicines and medical appliances, Egypt. Zinc cholride and sodium carbonate from Egypt, for chemical and drugs co. Natural materials:
Curcumin from sigma,USA.

2.2. Method and prepration of novel nano composide:

Preparation of nano composide for biochemical investigation of antitumor activity as anti cancer was prepared through the cursty of prof. Dr .Abdel fattah Mohsen Badwi after preparing ,we get some analysis as TEM, IR, NMR for conformation of nanocomposide size , shape and structure .

2.3. Induction of tumor:

By Benzo[a]pyrene at two doses of 100 mg\kg.bw. the first from the 1st week up to 20 week, the second after 20 weeks up to ten week for the last of experiment(30 week).

2.4. In vivo Expriment:

100 female swiss albino mice , 12-14 weeks old age and weighining about 20-25 gm recieved novel nanocomposide orally at different doses , mortality was reported.calculate Ld 50.

2.5. Exprimental design:

The present studied was carried out on 80 Female swiss albino mice divided into 4 groups:
Group (1): control group 20 mice not treated with anything negative control(-ve).
Group (2): Nanocomposide group, 20 mice treated only with nanocomposide at a dose of 0.02 mg three times in a week for 6 weeks and twice aweek for 6 weeks and finally one dose until the end of experiment.
Group (3): Benzo[a]pyrene group, 20 mice injected intra-pritoneally of benzo[a]pyrene at dose of 100 mg/kg body weight and the same other dose after 20 weeks.
Group (4): (Nanocomposide +Benzo [a] pyrene) group 20 mice injected by nanocomposide three times for aweek then injected by benzo[a]pyrene at adose of 100 mg/ kg B.W then complete injection orally with nanocomposide three times for 6 weeks, twice times for 6 weeks, only time

for rest of experiment completely 30 weeks.during this period Benzo[a]pyrene also injected for another time after 20 weeks from the beginning of experiment.

2.6. Tissue sampling:

After animals were anaesthetised , liver and lung tissue of each animal were dissected out, washed , and dried energy 30 weeks from the begining of treatment , and divided into two parts , part of them was homogenated and samples were prepared in ice-cold phosphate buffer (0.1 M, PH7-4) using a-potter - Elvehjem homogenizer to give a 10% homogenates which were used for determeation of antioxidant parameters and caspase 3, 9, p53 expressions and the other part of lung at the end of experiment (after 30 weeks of treatments) were dissected and kept in 10% formalin for histopathological examination.

2.7. Biochemical analysis:

SOD, GSH, GPx, CAT, NO, lipid peroxidase in Lung and liver tissue, Caspase 3,9 and p53 were analyzed according to the methods described previously [7,8,9,10,11,12,13]

2.8. Statistical Analysis:

The procedures were performed by using Anova .The Statistical Package for the Social Sciences (SPSS 17) statistical package (SPSS, Inc., Chicago, IL, USA).

3. RESULTS

The obtained results in (table 1) revealed that, administration of benzo[a]pyren induced lung carcinoma in mice exhibited a significant decrease in SOD activity in lung tissue , very highly significant decrease in GSH activity in lung tissue, non-significant change observed in GPx activity in lung tissue ,very highly significant increase in CAT activity in lung tissue and very highly significant increase in LP and NO comapred to control group. while, there was very highly significant increase in p53 gene expression and highly significant increase in cas -3 and cas- 9. Treatment with novel nanocomposide to Benzo[a]pyrene induced lung carcinoma in mices showed highly significant increase in SOD ,non-significant change observed in GSH content, significant increase in GPx activity ,highly significant decrease in CAT activity and non-significantly change observed in NO and LP in lung tissue compared to control group. While,there was significant increase observed in p53 gene expression and cas-3 but non-change observed in cas-9.

Table (1) Effect of nanocomposite treatment on SOD & GSH & GPX & CA T & LP & Nox), p53 gene expression and caspase-3 ,9 in lung tissue of benzo[a]pyrene induced lung cancer in mice and their control.

Parameters\groups	Group I	Group II	Group III	Group IV
SOD(U/g tissue)	44.2±0.4	44.2±0.4	41.8±1.5*	47.4±2.2**
GSH(ng/g tissue)	1.7±0.13	1.7±0.12	1.5±0.17***	1.7±0.13
GPx(ng/g tissue)	10.6±0.6	9.9±0.5	10.4±1.4	12.6±2.1*
CAT (Mmol\L)	58.3±0.9	55.8±3.9	40.3±0.8***	54.1±2.6 **
LPx(Mmol\L)	121.7±3	119.8±3.3	134.7±3.5***	124.7±3.9
NOx(Mmol\L)	12.5±0.5	13.6±0.9	24±0.6***	13±0.4
P53 %	17.7±4.3	17.6±4.6	88.9±5.1***	34.6±5.1*
Cas-3%	13.8±5	11.35±5	49.31± 5.1**	17.94±5*
Cas-9%	9.6±5.1	7.7±5.1	31.2±5.1**	8.4±5.1

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 ($P<0.05$). Group I:(Control), Group II: (nanocomposide), Group III: (benzo[a]pyrene), Group IV: (nanocomposide+benzo[a]pyrene)

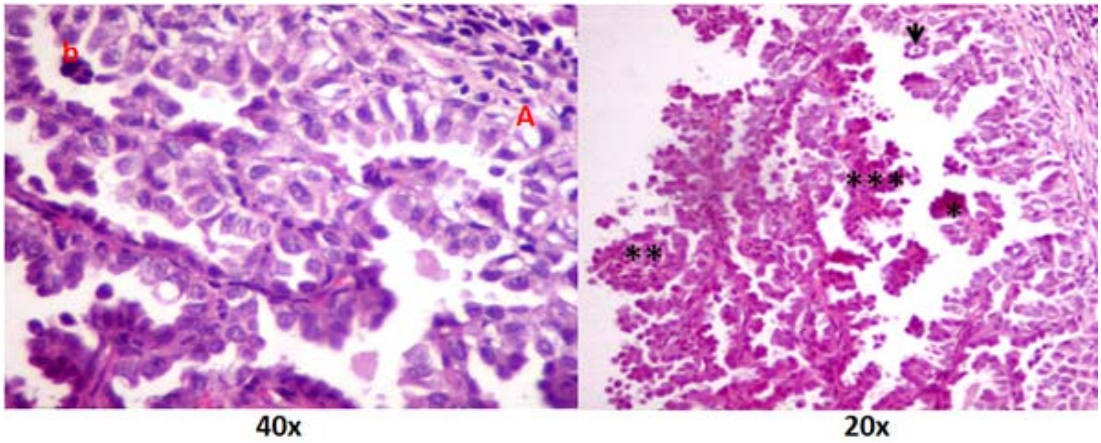

Figure (1): lung biopsy from benzo[a]pyrene induced mices showing adenocarcinoma. the tumor is formed of acinar (*)and papillary (**) structures lined by malignant epithelial cells exhibiting pleomorphism (***) , increased nuclear cytoplasmic ratio(A), hyperchromasia(b) , vesicular nuclei with prominent nucleoli and mitosis(→) (20x).

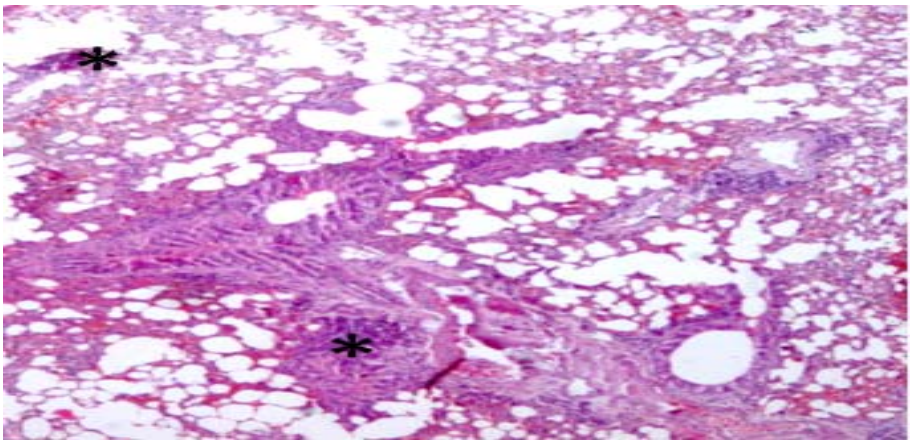

Figure (2): Lung biopsy from nanocomposite+benzo[a]pyrene. The lung parenchyma showed inflammatory cells infiltrate (*) at the site of the tumor with no evidence of residual tumor tissue.

4. DISSCUSION

Lung cancer kills more patients than any other malignancy in the world.Benzo[a]pyrene is considered a prototype polycyclic aromatic hydrocarbon (PAH), classic DNA damaging agent and carcinogen. It has been identified as a major risk factor for lung related cancer. During metabolism, benzo[a]pyrene are directly (or) indirectly metabolized into free radicals in liver and in lung tissue [14]. In our study results revealed that administration of Benzo[a]pyrene induced mice showed that exhibited a significant decrease in SOD activity in lung tissue table(1) these results are in agreement with previous[Emre15] who stated that SOD activity of lung tissue in benzo[a] pyrene group decreased significantly, compared to that in control group.And in disagreement previous with [Kumaraguruparan 16] who found that the activities of SOD and CAT in tumor tissues were elevated when compared to adjacent normal tissues. Glutathione a non enzymic antioxidant is a major low molecular weight non-protein thiol in living organisms. It plays a role in body's antioxidant defense against free radicals, peroxides and other toxic compounds [17]. GPx is an enzyme containing four selenium has a cofactors that catalyses the breakdown GPx is an enzyme containing four selenium has a cofactors that catalyses

the breakdown of H2O2 and organic hydro peroxides into H2O and O2. Thus, it plays a significant role in protecting cells against the free radicals and carcinogenic chemicals by scavenging the free radicals. Benzo[a]pyrene induced mice exhibited very highly significant decrease in GSH activity in lung tissue table (1). Similarly with previous [Anbarasi18]who reported that GSH level of the lung tissue in the experimental groups were lower when compared to the control group. Furthermore, non-significant change was reported in GPx activity in lung tissue of Benzo[a] pyrene induced mice. CAT is a hemoprotein, localized in peroxisomes or micropisomes or microperoxisomes. This also catalyses the decomposition of H2O2 to H2O and O2 thus protecting the cells from oxidative damage caused by H2O2.The mean value of CAT activity in lung tissue showed very highly significant decrease in Benzo [a] pyrene induced mices compared to control group. This results are in agreement with previous [Cigremis19] who observed a similar decrease in CAT activities of the liver and lung tissues on benzo [a] pyrene exposure. Thus both SOD and CAT activities are decreased on exposure to benzo [a] pyrene. Lipid peroxidation is an important event to cell death and has been reported to cause serve impairment of membrane functions through increased membrane permeability and membrane damage, cytotoxicity and eventually cell death. The free radicals reacts with lipids and generates LPO which is involved in the formation of tumors. The obtained data demonstrate of benzo[a]pyrene induced mice for lung cancer in table (1) revealed that very highly significant increase in level of LP activity in lung tissue. These results are in agreement with previous [Eijan20] who stated that Benzo[a] pyrene has been found to elevate lipid peroxidation in tissues. Nitric oxide , as a product of immune system cells, is implicated in the mechanism of carcinogensis.NO- is readily oxidized to nitrite and nitrate in biological systems . It can act either as a parameter or anti-tumor, depending on its concentration . The concentrations of NO- under non-pathological conditions, is in the nanomolar and under conditions of oxidant injury in micromolar range. Also, NO- reacts rapidly with superoxide anion to form peroxynitrite anion , which may be cytotoxic by itself or easily decompose to the highly reactive and toxic hydroxyl radical and nitrogen dioxide .nitric oxide(NOx) in lung tissue was very highly significant increase in B[a]p-induced lung cancer in mices compared to control group after 30 weeks. p53, a tumor suppressor protein, is believed to play an integral role in such cellular response pathways to DNA damage [21].The p53 gene is one of the most commonly mutated genes identified in various types of human tumors, and the results of numerous studies suggest that the inactivation or abnormality of p53 is a critical step leading to neoplastic transformation . It is important to maintain the integrity of the genome [22]. A loss of the p53 functions thus results in an enhanced frequency of genomic rearrangements or genomic instability and also eliminates the growth arrest response or apoptosis induced by DNA-damaging genotoxic insults . In accordance with this function, p53 is known to be recruited in response to various DNA-damaging agents such as UV, γ-irradiation, and anticancer drugs, and functions as a transcription factor to induce expression of various cellular genes involved in cell cycle control, such as a gene for p21, a cyclin-Cdk inhibitor that blocks cell cycle progression. The increased cellular p53 protein levels exposed to various genotoxic agents are due mainly to an increase in p53 protein stability rather than an increase in steady-state p53 mRNA levels. However, it has been suggested that an increase in p53 protein stability is not solely responsible for p53 recruitment in response to genotoxic stress, and it is more likely that p53 genotoxic stress response is a complex cellular process regulated at the

transcriptional, mRNA stability level as well as the protein stability level [23] . Thus, administration of B[a]p in our study revealed very highly significant increase compared to control group as shown in table (1). We are similarly with previous[Venkatachalam24] who stated that After metabolic activation, carcinogens, such as PAHs (benzo[a]pyrene) bind to DNA and form predominantly covalent carcinogen-DNA adduct, and thus express their carcinogenic and genotoxic activity .Which resulted in a rapid accumulation of the p53 gene product in human and mouse cells . The BPDE-induced apoptosis , release of cytochrome c from mitochondria to the cytosol without a change in mitochondrial membrane potential or mitochondrial morphology (electron microscopy), and cleavage of caspase-8, -9, and -3 [25].caspase 3 and caspase 9 activity in lung tissue of Benzo[a]pyrene induced mices were highly significant increase compared to control group as shown in table (1).we are in Similarly with previous[Chen 26] who stated that B[a]P increased the activation of caspase-3/7, -8, -9, and -12 and decreased cell viability . Activation of caspase-3/7 represents a commitment for cell disassembly and is a hallmark for apoptosis. Treatment with novel nanocomposide (selenium, N acetyl cysteine, curcumin) to inhibit Benzo[a]pyrene induced lung mice by understanding the effect of each compound alone then reaching the idea of combination in addition to use it in nanoform to understand the fully mechanism for prevention and treatment. Selenium nanoparticles (Nano –se) is a novel se species with novel biological activities and low toxicity .It has been reported that Nano- se has assize dependent effect in scavenging various free radical ; small-size Nano-se has greater ability to transfer electrons to radicals . selenium detoxifies direct-acting mutagens such as superoxides, hydrogen peroxides , and singlet oxygen due to antioxidant activity . The mechanisms being proposed for the anti –cancer activity of se includes antioxidant protection (via selenoproteins) , altered carcinogen metabolism , enhanced immune surveillance , regulation of cell proliferation (cell cycle and apoptosis) [27], tumor cell invasion , inhibition of neo angiogenesis and disruption of protein signaling path way. The higher intake of selenium can induce phase 2 enzymes, such as glutathione –S –transferase (GST). On the other hand, high expression of GST has been shown to be protective against tumor development. N acetyl cysteine detoxifies direct-acting mutagens such as superoxides, hydrogen peroxides, and singlet oxygen due to antioxidant activity . NAC also inhibits the mutagenicity of procarcinogens such as cigarette smoking condensate, benzo [a]pyrene, and aflatoxin by binding with their metabolites [28]. Inside cells, NAC rapidly converted to cysteine and glutathione. As a result, NAC enhances the detoxification of carcinogens inside cells. The glutathione formed from NAC effectively blocks electrophilic compounds and metabolites, as well as efficiently scavenging reactive oxygen species.Glutathione also protects against the down regulation of nuclear enzymes that is produced by carcinogens, decrease carcinogen-induced DNA damage, and prevents the ultimate formation of carcinogen – DNA adducts .NAC to prevent carcinogen- DNA adducts offers hope for more than preventing cancer. For instance , multiple DNA adducts were found not only in the lung , but also in the heart and aorta in cigarette smoke exposed rats. Inhibition of oxidative DNA damage in experimental animals and smoking humans. Curcumin have broad antioxidant properties . Curcumin is a potent “scavenger” of the superoxide radical, a free radical that initiates potentially harmful oxidative processes such as lipid peroxidation [29].The presented results in (table 1) revealed that very highly significant increase in SOD activity , non-

significant change in GSH , highly significant increase in GPx activity and highly significant increase in CAT activity in lung tissue of treated of benzo[a]pyrene induced mices by a novel nanocomposide compared to control group. Our findings are in accordance to previous [Ithayarasi 30] who reported that GSH-px, SOD and CAT activities were higher and MDA was lower than in se supplemented group (sodium selenite, Se-yeast or nano-Se) than control ($p<0.05$). GSH-px, SOD and CAT activities notably increased in elemental nano-selenium compared with the other two Se supplementation groups.(NSACC) treatment in benzo[a]pyrene induced lung cancer in mice normalized the value of nitric oxide and lipid peroxidase in lung tissue as shown in table (1) . These results are in good accordance with those obtained by previous [El-Demerdash 31], who found that Se maintained the levels of antioxidants, membrane-bound enzymes and the activities of antioxidant enzymes near normal levels. Several investigators have reported that Se compounds preferentially inhibit growth and induce apoptosis in cancer cells compared with normal cells [32]. several forms of selenium have inhibited carcinogen-induced covalent DNA adduct formation, retarded oxidative damage to DNA, lipids and proteins, inhibited tumor cell growth, altered DNA, RNA, and protein synthesis, increased apoptosis, changed cell cycle, and p53 and COX-2 expression, modified transcriptional factors activator protein P and nuclear factor κB, decreased aberrant crypt foci, and decreased Mtase activity . DNA hypermethylation, increased COX-2 expression, and decreased apoptosis are three possible mechanisms that have been implicated in lung carcinogenesis. At present, there is evidence, primarily from animal and in vitro systems, that selenium modulates these biomarkers, which may explain the effect that selenium supplementation has role on lung cancer incidence [33]. NAC was capable of inducing apoptosis in several transformed cell lines and transformed primary cultures but not in normal cells [34].curcumin at first was maximally distributed in the cell membrane and the nucleus. Second, the glutathione levels in tumor cells tend to be lower than normal cells, thus enhancing the sensitivity of tumor cells to curcumin [35] .Third, most tumor cells, but not normal cells, express constitutively active NF-κB and mediate their survival. Curcumin has been shown to inhibit COX-2 expression by repressing degradation of the inhibitory unit I-kappaB alpha and hindering the nuclear translocation of the functionally active subunit of NF-kappaB, thereby blocking improper NF-kappa B activation [36]. Curcumin also induces apoptosis in tumor cells by activating caspase-8, which leads to cleavage of Bid, thus resulting in sequential release of mitochondrial cytochrome C and activation of caspase-9 and caspase-3, which leads to activation of poly ADP ribose polymerase (PARP) and apoptosis of tumor cells. Curcumin also suppresses the activation of several transcription factors that are implicated in carcinogenesis . It suppresses the activation of nuclear factor kappa B (NF-KB), activator protein 1(AP-1), and at least two of the signal transducer and activator of transcription proteins (STAT3, STAT5), and modulates the expression of early growth response protein 1 (Egr-1), peroxisome proliferator associated receptor gamma (PPAR-Y), B-catenin, and Nrf-2. Curcumin also modulates expression of genes involved in cell proliferation, cell invasion, metastasis, angiogenesis, and resistance to chemotherapy .Curcumin inhibits multiple levels within transcriptional network to restrict cell proliferation. It induces p53-dependent apoptosis in various cancers of colon, breast, bladder, neuron, lung, ovary etc., although both p53-dependent and -independent G2/M phase arrest by curcumin .caspase-3 is essential for DNA fragmentation and the morphological changes associated with apoptosis.

Caspase-3 activation and site-specific proteolysis of PARP were evident in cells treated with curcumin. Curcumin promotes caspase-3-mediated cleavage of β-catenin, decreases β-catenin/Tcf-Lef transactivation capacity for c-Myc and cyclin D1.Nano(selenium, acetyl cysteine, curcumin)treatment for lung cancer mice induced by benzo[a]pyrene significantly attenuated the increased level of p53 expression in lung tissue compared to control mice as shown in table (1). These results are in agreement with previous [Aggarwal 37]. Who stated that n acetyl cysteine modulates growth of tumor cells through regulation of multiple cell signaling pathways including cell proliferation pathway (cyclin D1, c-myc), cell survival pathway (Bcl-2, Bcl-xL, cFLIP, XIAP, c-IAP1), caspase activation pathway (caspase-8, 3, 9), tumor suppressor pathway (p53, p21). In addition these results are in agreement with previous [Choudhuri 38] , who showed that curcumin selectively increases p53 expression at G2 phase of carcinoma cells and releases cytochrome c from mitochondria, which is an essential requirement for apoptosis.Meanwhile, the level of caspase 3 in lung tissue of benzo[a]pyrene induced mice which treated with NSACC showed highly significant increase but non-significant change observed in the level of cas-9 as indicated in table(1).These results are in agreement with previous [Thayyullathil 39] who stated that Curcumin induces the release of cytochrome c from mitochondria, causing activation of caspase 3 and concomitant PARP cleavage, which is the hallmark of caspase-dependent apoptosis .While, this results for cas-9 in lung tissue of NSACC treated mice showed non-significant change compared to control group as shown in table (1).Our results are in agreement with previous[Bush 40] suggested that curcumin activates caspases-3 and -8 but not caspase-9, supporting the rationale that apoptosis occur via a membrane-mediated mechanism. Both a caspase-8 and broad-based caspase inhibitor, but not a caspase-9 specific inhibitor, suppressed curcumin-induced cell death. Conclusions: The findings of the present study demonstrated that treatment with NSACC provided an effective protection against lung cancer induced by Benzo[a]pyrene in mice since nanocomposide drug was able to inhibit free radical scavenging by preventing lipid peroxidation and diminshed nitric oxide to normal value.Also, increasing seleno enzymes, targeting p53 expression , increasing apoptotic path way as cas-3 and cas-9 gradually then decreased to be close to near value.In addation to histopathology examination we observed fully removal of adenocarcinoma of lung cancer, exept residual of inflamattion, so, we could uses this nanocomposide drug as chemopreventive and treated drug.

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التأثير الكيميائي الوقائي لمركب نانوتركيبى جديد على مادة البنزوالفابيرين المحدثة لسرطان الرئة

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الملخص العربي

تحد ث مادة البنزو الفابيرين سرطان الرئة بميكانيكية تداخلها مع الخمض النووى والتى تسبب تغيير جينى يؤدي الي تطور انقسام الخلية بشكل مضاعف. بخلط كلا من السيلينيوم والاسيتيل سيستاين والكركمين وتصغيرهم فى صورة نانو وبدورهم الفعال فى التاثيرات الحيوية كمضادات للاكسدة وللأورام وتقوية جهاز المناعة. تم حقن مادة النانوالتركيبى بجرعة 4 مجم لكل واحد كيلو جرام عن طريق الفم للفئران التى سبق حقنها ببنزوالفابيرين بجرعة 100 مجم لكل واحد كيلو جرام مرتين الاولى فى الاسبوع الاول والثانية بعد الاسبوع 20 من بداية التجربة وذلك لإحداث سرطان الرئة تجريبيا. اكد ت النتائج ان البنزوالفابيرين ادى الى احداث الاصابة بسرطان الرئة فى الفئران عن طريق التشريح الهستوباثولوجى والى نقص ملحوظ فى سوبر اكسيد ديسميوتاز و الجلوتاثيون المختزل والكتاليز وزيادة ملحوظة فى كلا من النيتريك اكسيد والليبيد بيروكسيداز وبروتين 53 والكاسباس 3 و 9 .وقد اظهرت النتائج ان العلاج بالنانو ادى الى نقص فى الليبيد بيروكسيداز النيتريك اكسيد وزياد مضادات للاكسدة وزيادة وبروتين 53 والكاسباس 3 و9 ثم نعد يل نسبهم الى القيم الطبيعية او بالقرب منها.

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