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İnsan Akciğer Hücre Hatlarında Noskapinin Antianjiogenik ve Apopitotik Etkileri

Apoptotic and Antiangiogenic Effects of Noscapine in Human Lung Cancer (A549) Cells

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Öz

Giriş ve Amaç: Günümüzün en önemli sağlık sorunlarından biri olan akciğer kanseri hem kadınlarda hem de erkeklerde en sık ölüme neden olan kanser türüdür.
İnsan alveolar karsinom hücrelerinden kaynaklanan A549 akciğer hücreleri, tip II alveolar hücre fenotipiyle eşleşmesine rağmen, insan primer alveolar epitel hücrelerinin sahip olduğu birçok özelliğe de sahiptir.
Noskapin, haşhaş somniferum (afyon) içindeki bileşenlerden oluşur. İlk olarak 1817'de Papaver somniferum'dan (afyon) izole edilmiştir. Morfinden sonra afyon bitkisinde (toplam bileşimin %10'una kadar) bulunan en bol opioidlerden oluşur. Narcotine, Nectodon, Nospen, Anarcotine ve (arkaik) Opiane olarak da bilinir ve S, R stereokimyasına sahip bir (-) izomerdir (fitalid-karbonda S stereokimyası ve izokinolin-karbonda R). Noskapin yapısal ve kimyasal olarak morfin, kodein, tebain, papaverin ve narsein gibi diğer afyon alkaloidlerinden farklıdır. Bu çalışmanın amacı, insan akciğer kanseri (A549) hücrelerinde noskapin'in apoptotik ve antianjiogenik etkilerinin araştırılmasıdır.
Gereç ve Yöntemler: Çalışmada 10 ppm, 15 ppm, 20 ppm, 25 ppm, 40 ppm, 50 ppm, 60 ppm, 65 ppm, 70 ppm, 75 ppm, 80 ppm, 90 ppm ve 100 ppm konsantrasyonları kullanılmıştır.
Bulgular: Canlılık değerlerinin çoğalma deneyi oranı. Düştüğü gözlendi ve LD50 değeri 40 ppm olarak belirlendi. Kullanılan istatistiksel analiz: Analizlerin istatistiksel analizi SPSS Statistics 20,0 programı kullanılarak yapıldı. Bulgular: VEGF değerleri 20 ppm, 40 ppm ve 80 ppm noskapin konsantrasyonlarında kontrol grubuna göre azaldı. 80 ppm'de PARP değerlerinde önemli bir düşüş oldu.
Sonuç: Bulgular sonucunda; Noskapin konsantrasyonlarının 10 ppm, 20 ppm olduğu düşünülmektedir. 40 ppm, VEGF seviyelerini düşürerek anjiyogenezi azaltabilir ve metastazı önleyebilir. PARP seviyeleri tüm noskapin konsantrasyonlarında azaldı, ancak en önemli fark 80 ppm'de görüldü.

Anahtar Kelimeler: A549, Noskapin, PARP, Papaver Somniferum, VEGF

Abstract

Objective: Lung cancer, one of the most important health problems today, is the most common type of cancer that causes death in both women and men.

Although A549 lung cells originating from human alveolar carcinoma cells match the type II alveolar cell phenotype, it also has many characteristics that human primary alveolar epithelial cells have.

Noscapine consists of the components in the poppy somniferum (opium). It was first isolated from Papaver somniferum (opium) in 1817. It consists of the most abundant opioids found in the opium plant (up to 10% of the total composition) after morphine. It is also known as Narcotine, Nectodon, Nospen, Anarcotine and (archaic) Opiane, and S is an (-) isomer with R stereochemistry (S stereochemistry in phthalid-carbon and R in isoquinoline-carbon). Noscapine is structurally and chemically different from other opium alkaloids such as morphine, codeine, thebaine, papaverine and narcein.The aim of the study the ınvestıgatıon of the apoptotıc and antıangıogenıc effects of noscapine ın human lung cancer (A549) cells.

Materials and Methods: In the study, concentrations of 10 ppm, 15 ppm, 20 ppm, 25 ppm, 40 ppm, 50 ppm, 60 ppm, 65 ppm, 70 ppm, 75 ppm, 80 ppm, 90 ppm and 100 ppm were used in the proliferation experiment proportion of vitality values. It was observed that it decreased and the LD50 value was determined as 40 ppm. Statistical analysis used: Statistical analysis of the analyzes was performed using SPSS Statistics 20,0 program.

Results: VEGF values decreased at 20 ppm, 40 ppm and 80 ppm noscapine concentrations compared to the control group. There was a significant decrease in PARP values at 80 ppm.

Conclusions: As a result of the findings; It is thought that concentrations of noscapine 10 ppm, 20 ppm and 40 ppm can reduce angiogenesis and prevent metastasis by lowering VEGF levels. PARP levels decreased at all noscapine concentrations but the most significant difference was seen at 80 ppm noscapine concentration.

Keywords: A549, Noscapine, PARP, Papaver Somniferum, VEGF

1.Introduction

Turkey as well as in the whole world which is characterized by uncontrolled cell division and proliferation and located among the most common causes of cancer death; It is basically a genome disease that occurs as a result of changes in DNA, and then manifests itself with proteomics and metabolic changes as well as changes in the genome structure [1].

Genetic susceptibility, mutations, changes in hormones and changes in the immune system are the main factors that lead to cancer, but some external factors such as smoking and alcohol use, unbalanced diet, viral and bacterial infections, chemicals, radiation and air pollution can also play an effective role in cancer development [2].

It is important to diagnose and diagnose cancer at early stages in controlling cancer by decreasing the mortality rates due to cancer and increasing the survival time of patients. The methods used in cancer treatment are generally; Chemotherapy, radiotherapy, immune therapy, hormone therapy, targeted therapy and finally, surgical intervention [3].

Although one or a combination of these methods is preferred in the treatment, some undesirable side effects are observed in patients from time to time. There is a need for new treatment approaches that are more selective and effective that target only the relevant cancer cells, with minimizing toxic side effects. For this reason, in recent years, under the name of alternative medicine, there has been a tendency towards herbal treatments with natural and / or secondary metabolites containing anti-cancer properties. By determining effective cancer treatment strategies for target cancer cells, in order to diagnose cancer in the early stages, the changes in the protein mechanism as a result of the metastasis of the related cancer should be determined precisely and protein biomarkers should be determined [4]. Lung cancer is a type of tumor that occurs as a result of uncontrolled and rapid division of different cell groups in the lung tissue and is categorized in different histopathological subclasses according to the lung tissue group in which it develops. In general, as in other cancer groups, lung cancer is a multifactorial disease and a serious health problem that occurs as a result of the accumulation of genetic and epigenetic changes [5].

A549 cells D.J. It was isolated from lung carcinoma tissue of a 58-year-old male from Central Asia in 1972 by Giard et al. [6]. These isolated cells were cultured for 1000 generations and it was concluded that these cells were immortal [7]. There are two types of alveolar cells. Type I covers 96% of the inner surface of the alveoli and it is difficult to produce in vitro. Type II, on the other hand, takes up less surface and has many functions. In addition, type II cells are the progenitor cells of type I cells. Although A549 lung cells originating from human alveolar carcinoma cells match the type II alveolar cell phenotype, they also have many characteristics of human primary alveolar epithelial cells [8,9]. Therefore, it has widespread use as a model not only in lung cancer models but also in in vitro studies of human alveolar epithelial cells [10,11,7].

While lung cancer constitutes 13.4% of all cancers, lung cancer is responsible for 28.4% of all cancer deaths. While 5-year survival is 52% in non-pulmonary cancers, it is 14% in lung cancer, and mortality has increased even more despite increasing treatment methods [12].

Noscapine closed formula is C22H23NO and its molecular weight is 413.43 g / mol. Its melting point is

176 oC. Noscapine contains 63.91% C, 5.61% H, 3.39% N and 27.09% O atoms. Since it was thought to be narcotic in the past, the name of noscapine, which was called narcotine, was changed when the antitussive (cough suppressant) property was discovered. The cough-suppressing properties of codeine are the same. It is used as a medicine in medicine because of its antitussive properties [13,15].

Joshi's team found that noscapine, an opium-derived alkaloid, is a potent anti-tumor agent. This discovery was so surprising and exciting because noscapine has been used medically as an antitussive in humans and experimental animals, with little side effects and no addictive burden. In addition, its water solubility and feasibility for oral solubility is a valuable advantage over many other drugs for cancer therapy. As an anti-tumor agent, it induces apoptosis in various cell lines and stops metaphase in cell division [16].

Although noscapine has chemical moieties similar to that of colchicine and podophyllotoxin, the binding assay and the effects of noscapine on colchicine and tubulin binding time indicate that noscapine and colchicine bind to different sites on tubulin. Superficially it shares similar chemical groups with noscapine, colchicine and podophyllotoxin; however, the stereo structure of noscapine, colchicine, and podophyllotoxin is different. Therefore, they concluded that noscapine could create other contacts on the tubulin surface [16]. A similar group reported that the anti-mitotic activity is specific to noscapine because closely related compounds do not inhibit the growth of a lymphoma cell line. In addition, noscapine has been shown to be effective in reducing lymphoma growth and increasing the survival of tumor-bearing mice when administered in drinking water.

It is noteworthy that at tumor suppressing doses, noscapine has little or no toxicity to the kidney, liver, heart, bone marrow, spleen or small intestine [17]. Moreover, oral noscapine did not inhibit primary immune responses that are critically dependent on proliferation of lymphoid cells. Therefore, these results indicate that noscapine has the potential to be an effective chemotherapeutic agent for the treatment of human cancer [18]. Microtube binding drugs such as paclitaxel, docetaxel, and vinca alkaloids are currently in clinical use in cancer chemotherapy. Unfortunately, toxicity and low aqueous solubility have limited the applicability of these drugs in cancer chemotherapy.

Moreover, their use has been hampered by the development of drug resistance contributed by multifactorial mechanisms such as Pglycoprotein overexpression [19], altered expression of tubule isotypes [20], and the presence of tubulin mutations [21]. Therefore, the development and / or discovery of microtubule based compounds such as noscapine [22] is demanded. Noscapine has been shown to effectively inhibit proliferation and induce apoptosis in both paclitaxel sensitive and paclitaxel resistant human ovarian carcinoma cells [23].

As stated in other studies in this context, noscapine effectively inhibits the progression of various types of cancer both in vitro and in vivo without any significant side effects. These types of cancer are lymphoma cancer [17], breast cancer [24], melanoma cancer [25], ovarian cancer [23], colon cancer [26], lung cancer [27].

Based on this information, we aimed to investigate the effects of various Noscapine concentrations on cell proliferation, VEGF and PARP levels in our study.

2. Materials and Methods

2.1. Sterilization Before Cell Culture

All plastic materials used in cell culture and ready-made sterile media were obtained from commercial companies. Analyzes of the relevant parameters were made using the methods described below in lysate and medium samples prepared from cells obtained after incubations.

2.1. Cell Culture

The main content of the medium used in the culture of A549 cells is RPMI 1640, but it contains 10% fetal bovine serum (FBS) and 1% penicillin / streptomycin. Cell culture was made by incubating in sterile conditions in T25 and T75 flasks containing medium prepared in a sterile condition in a carbon dioxide incubator with 5% CO2 and 37 oC temperature conditions.

Passaging processes were carried out when the cells being propagated reached a density covering approximately 85% of the culture flask. Cells were used in experiments when they reached a sufficient number.

2.2. CCK 8 Cell Viability Test

For CCK 8 cell viability test, the cells were seeded in a 96-well plate at 5x105 cells / ml in RPMI medium containing 10% FBS and 1% penicillin / streptomycin, and incubated in a 5% CO2 and 37oC incubator. After 24 hours, the wells were divided into the following groups and applications were made as 10 µl (Table 1). Three repetitions were done from each group.

After the applications were made, it was left to incubate for 48 hours. At the end of 48 hours, 10 µl of CCK 8 solution was added on them. It was left to incubate for 2 hours. At the end of the incubation period, 10 seconds of agitation was made and measurement was made with a plate reader at 450 nm wavelength. (Kit Lot Number: AB1714A2) (Noscapine CAS Number: 912607)

2.3. PARP Analysis

Cells in 75 cm² flasks were used for PARP analysis. Cells were removed with the help of trypsin. Then it was centrifuged, the supernatant was discarded and the pellet obtained was suspended by the addition of medium.

24-well cell culture plates were used for the experiment. A549 cell was inoculated into the wells at a density of 5x10 °. The plate was kept in the incubator for 24 hours and at the end of 24, the cells taken from the incubator were treated with various concentrations of Noscapine.

Table 1. Working Groups

Groups	Applications made
Group 1	RPMI with 10% FBS and 1% penicillin / streptomycin
Group 2	30 µM Cis Platin
Group 3	10 ppm Noscapine
Group 4	15 ppm Noscapine
Group 5	20 ppm Noscapine
Group 6	25 ppm Noscapine
Group 7	30 ppm Noscapine
Group 8	40 ppm Noscapine
Group 9	50 ppm Noscapine
Group 10	60 ppm Noscapine
Group 11	65 ppm Noscapine
Group 12	70 ppm Noscapine
Group 13	75 ppm Noscapine
Group 14	80 ppm Noscapine
Group 15	90 ppm Noscapine
Group 16	100 ppm Noscapine

It was placed in a 37ºC incubator for 48 hours. After 48 hours, cells were removed from the incubator. Media was removed from the plated cells and treated with approximately 700µl PBS. PBS was drawn from the treated cells. 200µl trypsin was added to the cells and placed in a 37ºC incubator and the cells were allowed to rise. The effect of trypsin was removed by adding 450µl medium to the cells separated from the surface. Cells were transferred to the ependorph for PARP with a disposable pipette.

The cells in the epondorf were centrifuged at 2100 rpm for 20 minutes at 15ºC. After centrifugation, the supernatant was discarded. Medium was added into the eppendorfs and mixed gently, then plated onto a 96-well plate loaded with single-use antibody in the kit. Reagents, samples and standards were prepared. Prepared samples and standards were seeded into a 96-plate plate and allowed to incubate at 37 ° C for 60 minutes. After incubation, the wells were washed five times with washing solution, Chromogen solution A and B were added, and incubated at 37 ° C for 10 minutes. Stop solution was then added and the measurement was carried out. (Human PARP ELISA Kit Lot No: 201911)

2.4. VEGF Analysis

Cells in 75 cm² flasks were used for VEGF analysis. Cells were removed with the help of trypsin. Then it was centrifuged, the supernatant was discarded and the pellet obtained was suspended by the addition of medium. 24-well cell culture plates were used for the experiment. A549 cell was seeded in the wells at a density of 5x10 °. The plate was kept in the incubator for 24 hours and at the end of 24, 33the cells taken from the incubator were treated with various concentrations of Noscapine. It was placed in a 37ºC incubator for 48 hours. After 48 hours, cells were removed from the incubator. Media was removed from the plated cells and treated with approximately 700µl PBS. PBS was drawn from the treated cells. 200µl trypsin was added to the cells and placed in a 37ºC incubator and the cells were allowed to rise. The effect of trypsin was removed by adding 450µl medium to the cells separated from the surface. Cells were transferred to the ependorph for VEGF analysis with a disposable pipette.

The cells in the epondorf were centrifuged at 2100 rpm for 20 minutes at 15ºC. After centrifugation, the supernatant was discarded. Medium was added into the eppendorfs and mixed gently, then plated onto a 96-well plate loaded with single-use antibody in the kit. Reagents, samples and standards were prepared. Prepared samples and standards were seeded into a 96-plate plate and allowed to incubate at 37 ° C for 60 minutes. After incubation, the wells were washed five times with washing solution, Chromogen solution A and B were added, and incubated at 37 ° C for 10 minutes. Stop solution was then added and the measurement was carried out. (Human VEGF ELISA Kit Lot No: 201911)

2.5. Statiscital Analysis

The data were analyzed using SPSS-18 computer program and the results were given as mean ± standard deviation (SD). After the homogeneity of the groups was tested, ANOVA- Duncan test were used in one-way to find differences between groups. Differences were considered statistically significant at $P<0.05$.

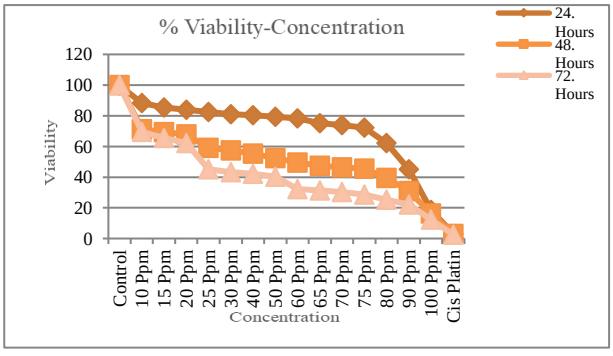
3. Results and Discussion

3.1. Proliferation Experiment Results

Dose ranges for A549 lung cancer cell line; 5, 10, 20, 40, 75, 100 Ppm. Labyrinth dose (LD50) was determined as

40 ppm since it caused the death of 50% of cancer cells by using the CCK-8 kit at the end of 24, 48 and 72. hours, and the viability rate of cancer cells gave better results at the 48th hour (Table 2). In other experiments, this time and dose were taken as basis.

Table 2. Viability vs Concentration Chart



3.2.PARP and VEGF Analysis Findings

PARP and VEGF analyzes were performed on A549 cell line treated with Noscapin at concentrations of 20 ppm, 40 ppm and 80 ppm. The results obtained are given in Table 3.

Table 3. PARP and VEGF Findings

	VEGF (ng/L)	**PARP (ng/L)**
Control	0,0615±0,03[a]	0,0596 ± 0,004[a]
Noscapine 20	0,0515±0,01[b]	0,0423 ±0,002[a,b]
Noscapine 40	0,0434±0,02[c]	0,0421±0,006[a,b]
Noscapine 80	0,0431±0,01[c]	0,0425±0,002[b]
Cisplatin	0,0697±0,01[a]	0,0858±0,004[c]

4. Conclusion

Noscapine is one of the ingredients in Papaver somniferum (opium). It was first isolated from Papaver somniferum (opium) in 1817. It is one of the most abundant opioids found in the opium plant (up to 10% of the total composition) after morphine.

It was used as an antitussive agent, but later Ye et al discovered its anti-neoplastic properties in 1998. It works as a tubulin inhibitor by stoichiometrically binding with the tubule, causing a change in its compliance. He has been continuously working on the development of plant-derived anticancer agents such as noscapine for the last 20 years.

Anticancer activity and synthesis of potent analogues of 9-12 noscapine, a plant-derived anticancer agent, have been reported. Structure-activity analysis showed that the isoquinoline ring in noscapine (Nos) can be made without affecting the proton binding to tubulin at the C-9 position [28,29]. It is used as a medicine in medicine because of its antitussive properties [13-15]. Based on this information, this study was conducted to investigate the apoptotic and anticarcinogenic effects of different concentrations of noscapine in the A549 human lung cancer cell line.

MCF-7, MCF-10F, MDA-MD-231 cell lines were used in the study of the apoptotic effect of noscapine on breast cancer cell lines by Edwin and Gloria. MTT (3-(4,5-dimethylthiazol-2) -2,5-diphenyltetrazolium bromide) method was used to determine the cell viability of these cell lines. The IC-50 value for MCF-10F, MCF-7 and MDA-MB-231 cells at the end of 48 hours was determined as 53,30,20 μM, respectively. It has been shown to induce a concentration-dependent cytotoxicity in MCF-10F, mcf-7 and MDA-MB-231 cells [30].

In another study, Mahavir et al. They investigated the anticancer activity of Noscapine, an opioid alkaloid, in combination with Cisplatin in non-small cell lung cancer. Cell viability testing in each treatment group was determined by the crystal violet dye assay. Cells were applied various dilutions of Cis with or without Nos at 10-30 and 30-50 μM, respectively, against H460 and A549 cells. Noscapin was found to inhibit the proliferation of H460 and A549 cells in a dose-dependent manner with IC50 values of 34.7 ± 2.5 μM and 61.25 ± 5.6 μM, respectively [31].

In another study; In human colon cancer cells, the SW480 cell line was used and the MTT method was used to determine cell viability. Six groups (control, noscapine, negative control (NC), siCDH17, NC + noscapine and siCDH17 + noscapine) were used for cell viability testing. They found that after treatment with 10 μg / mL noscapine, cell proliferation in siCDH17 + noscapine was significantly reduced compared to the noscapine group [32].

rezou et al. Studied the Synergistic Anticancer Effect of Paclitaxel and Noscapin on Human Prostate Cancer Cell Lines. In this study, the effect of noscapine (10-100) applied in varying doses to LNCaP and PC-3 prostate cancer cell lines on the viability of cells at the end of 48th hour was investigated by MTT method.

As a result, they showed that single and combination therapies of both paclitaxel and noscapine significantly decreased the viability of both LNCaP and PC-3 cells compared to control cells, depending on the dose and time [33].

In this study, in order to determine the IC50 (inhibition) value, cell proliferation with CCK8 cell viability test is 10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm, 40 ppm, 50 ppm, 60 ppm, 65 ppm, 70 ppm, 75. It was observed that by administering noscapine at doses of ppm, 80 ppm, 90 ppm, 100 ppm and at 24, 48, and 72 hours, the A549 lung cancer cell line showed a proportional decrease in cell proliferation depending on concentration and time. However, as a result of the very high antiproliferative effect obtained in the 72th hour data, our study was conducted over the 48-hour incubation period, taking other studies as reference.

Poly (ADP-Ribose) Polymerase (PARP) is a family of enzymes that perform multiple cellular processes in addition to DNA repair. PARP1 has been best characterized and is one of the two DNA damage-activated PARPs. Poly (ADPRibose) Polymerase (PARP, MA: 116 kDa) is one of the most abundant proteins in the nucleus. It catalyzes the polymerization of ADP-ribose from NAD + molecules in target cellular proteins by attaching to linear or branched polymers. PARP plays many roles in many molecules and cellular processes. DNA damage detection and repair, chromatin modifications, transcription and cellular death pathways. These processes are; It is very critical in physiological and pathological consequences such as genome repair, carcinogenesis, aging, inflammation, and neuron functions. PARP, one of the first identified substrates of caspase; In particular, it is involved in apoptosis and necrosis. In the process of apoptosis, caspase 7 and caspase 3 cleave PARP between Asp214 and Gly215 and cleaves it into p85 and p25 fragments.

PARP cleavage separates the DBD from the catalytic domain and renders enzymes inactive. This process destroys PARP activation in response to DNA fragmentation during apoptosis, and prevents futile efforts for DNA repair and ATP consumption required in necrotic cell death. Hence, PARP cleavage helps the cell enter the apoptotic pathway and is considered the hallmark of apoptosis [34].

Kocak C. et al. Noscapine and docetaxel in their research on the cytotoxic, anti-proliferative and apoptotic effects of Noscapine on human estrogen receptor positive (MCF-7) and negative (MDA-MB-231) breast cancer cell lines They found that it resulted in a greater decrease in the percentage of -231 cells and a greater increase in the percentage of BAX positive MDA-MB-231 cells. They found that tamoxifen caused a greater decrease in the percentage of Bcl-2-positive and an increase in the percentage of BAX-pos-itive MCF-7 cells. Thus, noscapine and docetaxel were found to have a greater apoptotic effect on MDA-MB-231 cells than MCF-7 cells, while tamoxifen was found to have a greater apoptotic effect on MCF-7 cells [35].

In a different study, Kiechle FL et al. Showed that programmed cell death (apoptosis), necrosis, autophagy, and mitotic catastrophe differ from other cell deaths by increasing caspase-3 levels among 4 types of cell death [36-38].

Mahavir C. et al. Investigated the anticancer activity of noscapine in combination with Cisplatin. TUNEL method was used to define the 3p-hydroxyl end of fragmented DNA, which is one of the biochemical markers of apoptosis. It has been shown that apoptosis is induced in H460 and A549 cells following treatment with combinations of Cis, Nos or Nos. Nos + Cis treatment caused apoptosis in treated H460 cells after 72 hours compared to Cis and Nos, respectively. Similarly, combination therapy against A549 cells was found to induce apoptosis in Cis and Nos, respectively [39].

Xia T. et al., In their study of noscapine-induced apoptosis in human colon cancer cells, detected the effects of apoptosis in SW480 cells by AnnexinV-FITC / PI flow cytometry. As shown compared to the control group, the percentage of apoptotic cells treated with the noscapine group and transfected with the siCDH17 group increased significantly. They also found that SW480 cells significantly increased apoptosis in the siCDH17 + noscapine group compared to the noscapine group [32].

This study in which the apoptotic and anticarcinogenic activity of Noscapine in A549 cell lines were associated with PARP is the first in the literature. PARP levels were found to be lower than the control group at all noscapine concentrations. The most significant difference was seen at 80 ppm Noscapine concentration. The decrease in the PARP level suggests that the cell goes to apoptotic death.

Angiogenic factors, the extracellular matrix and many growth factors released from cells surrounding the matrix, cytokines and their receptors play a major role in angiogenesis [40,41]. Among the angiogenic factors bFGF, tumor growth factor- β (TGF – β), angiopoetins, PDGF and VEGF. The most important angiogenic molecules are VEGF and VEGF receptors. VEGF, an endothelial specific mitogen, is an angiogenic factor that plays a role in endothelial cell growth and increases vascular permeability. Activation of VEGF receptors phosphorylates intracellular signal transduction proteins, allowing the proliferation, migration and differentiation of endothelial cells [42,43].

MMP, a family of endopeptidases, facilitates the invasion of malignant cells and endothelial cells into the connective tissue and vessel wall during the angiogenesis process. MMPs break down the basement membrane, ECM and contribute to the release of proangiogenic factors associated with ECM [44-46].

VEGF is a survival factor for endothelial cells, in vivo and in vitro [47,48]. The VEGF family is important because of its angiogenic and cell survival characteristics [49]. Neoplastic cells secrete VEGF, provide oxygen and nutrients with the formation of new vessels and also facilitate metastasis. VEGF is a survival factor, protecting tumor cells from stress situations such as hypoxia, chemotherapy, radiotherapy.

In the light of the information given above, this study is a first in the literature, since no angiogenic factor studies have been found associated with noscapine, one of the important alkaloids, in A549 cell lines. As a result of these findings; VEGF levels decreased at all concentrations of noscapine. Concentrations of 40 ppm Noscapine and 80 ppm Noscapin are more effective in reducing VEGF levels. The decrease in VEGF levels suggests that noscapine may prevent metastasis by lowering angiogenesis.

As a result; Various concentrations of noscapine have been shown to reduce cell proliferation. It is thought that all concentrations of noscapine (20 ppm, 40 ppm and 80 ppm) will reduce angiogenesis and prevent metastasis by lowering VEGF levels. It was observed that the PARP level was lower than the control group. The decrease seen in PARP values suggests that Noscapine leads the cell to apoptotic death.

5. Acknowledgements and Disclosures

This work was carried out in collaboration between all authors. Author FKC and İİ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FKC and AK designed the study and managed the analyses of the study. All authors read and approved the final manuscript. There are no ethical issues after the publication of this manuscript.

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