Synergistic Activity of Cisplatin and Anise Extract Nanoparticles to Inhibit the Tumor of Mammary Cancer Cells in Mice

Original Article

Nael Mohammed Sarheed[1], Hamid K. AL-tameemi[2] and Ameer Ali Imarah[3]

[1]*College of Medicine, Al-Muthanna University, Al-Muthanna-Iraq.*

[2]*Medical Laboratory Techniques, Bilad AlRafidain University College, Diyala-Iraq.*

[3]*Biology Department, Faculty of Science, University of Kufa. Iraq*

ABSTRACT

Introduction: Cancer is a major health problem worldwide, and it is one of the leading causes of death. Preventing tumor development is a major challenge due to the similarity in cellular mechanisms between cancerous and normal cells; however, when using biological substances have high efficiency against cancerous cells and low toxicity against normal cells associated with a good prognosis. Therefore, the current study is designed to study the role of Anise extract (AE) and Anise extract-Chitosan nanoparticles in the synergistic effect with cisplatin in treatment of tumor-induced in female mice.

Materials and Methods: Five groups of female mice, 6 mice for each group were included to achieve the goal of the present study. These study groups were control, tumor-induced, Cisplatin treated, Cisplatin – Anise extract treated, and Cisplatin-Anise extract- Chitosan nanoparticles treated groups. The tumor size and some biomarkers (IL-33, LRG-1, and CRP) were considered as indicators for tumor development and response to the treatment protocol.

Results: The present study found that the tumor size in the tumor-induced group reach to 33.1 mm after 30 days of inducement, while the tumor size in Cisplatin treated group was significantly smaller than the tumor-induced group. The tumor size in Cisplatin- Anise extract group was smaller than Cisplatin treated group. Also, the effect of cisplatin against a tumor increased when combining anise extract with Chitosan nanoparticles. Due to biomarkers showed IL-33, LRG-1, and CRP levels were higher in tumor group than control and other study groups.

Conclusion: The current study concluded that the anise extract has high effectiveness in reinforcement Cisplatin action against tumor development and at the same time increased their effect by nanoparticles.

Received: 31 October 2023, **Accepted:** 21 January 2024

Key Words: Anise extract, biomarkers, cispaltin, nanoparticles, tumor.

Corresponding Author: Hamid K. AL-tameemi, PhD, Medical Laboratory Techniques, Bilad AlRafidain University College, Diyala-Iraq, **Tel.**: 07721475497, **E-mail:** hamid.altameme@yahoo.com, dr.hamed@bauc14.edu.iq

ISSN: 1110-0559, Vol. 48, No. 1

INTRODUCTION

Cisplatin is an effective and valuable platinum-based chemotherapy treatment used to treat many malignant diseases related to types of cancers in various body tissues[1,2]. As cisplatin works in cancer tumor cells to form a cross-link with DNA, which end up stopping the multiplication of cancer cells and weakening their other activities[3].

On the other hand, studies indicated an increase in the effectiveness of cisplatin by herbal materials in inhibiting the proliferation of cancer cells, which was attributed to an increase in the effectiveness of the antioxidants GSH and GST that work to inhibit cisplatin resistance by the cell, as the decrease in toxicity associated with an increase in cisplatin resistance to cellular aggregates containing sulfhydryls as GSH and regulate the enzymatic activity of GST to be more effective by synergizing with this substance[4]. This was observed when using some herbs that increased the effect and effectiveness of cisplatin against cancer cells, where ancient scientists used in traditional medicine natural compounds derived from medicinal plants called (phytochemicals), are biologically effective compounds such as flavones, alkaloids, polyphenols terpens, glycosids and carotenoids, which brought the attention of researchers in the field of pharmaceutical industries to treat several diseases, including cancerous tumors[5].

AN3, or called ANM3 (Ahmed Nahi Mohammed), is one of the types of malignant tumors (metastasis) of mammary gland cells that were discovered in 2008 by Dr. Ahmad Majeed Al-Shammari and his colleagues[6] and named after them later, where the sample was taken from tumor epithelium of aged female mice with an immunosuppressed system to be implanted subcutaneous in young female mice with suppressed immunity also by injection of Cyclosporine A and Methyl prednisolone sodium at 24 hours before implantation, It is worth noting that the number of cells in every 0.1 ml of AN3 contains approximately 10×10^6 viable cells[7]. These cellular samples of tumor tissue were later used as cell lines that could be implanted in mice to generate tumors for research

DOI: 10.21608/ejh.2024.242091.1963

purposes, including experiments on effectiveness of anti-tumor drugs[6].

According to the above, the aim of this study was to investigate the synergistic effectiveness of cisplatin and anise extract in reducing the size of cancerous tumors in experimental animals, with the use of nanoloading technology to increase the efficiency of the plant extract.

MATERIAL AND METHODS

Preparing of extract and nanoparticles

The seeds of the anise plant available in Iraqi markets were cleaned and washed with DW and exposed to air to dry by sun light. Used the electric mixer for the grounding of aniseeds to powder, then the extraction process was carried out by soxhlet through using a proportion 1:5 from the plant/solvent according to method for Abu-Rumman[8], then loading of extract on chitosan nanoparticles by an ionic gelation method according to[9,10]. Where dissolve 200 mg of extract in 50 ml of chitosan solution (4 mg / ml) by slow distillation to obtain a ratio 1:1 under stirring for 30 minutes at 900rpm in a hot plate stirrer. Then adding 10 ml of STPP (0.25%) with 5:1 ratio of solution and stirring to allow the chitosan particles to adsorb anise extract on their surface. After that the solution exposure to sonication for 3 minutes, and filtered with filter paper.The solution is kept at 4 °C.

Characterization of Nanoparticles

According to[12], Particle size analysis were examined, Zeta potential analysis were tested according to[12], and FTIR (Fourier Transform Infrared Analysis) were tested according to[11,13].

The experiment (In vivo)

The experimental animals

30 young BALB/C albino strain of female laboratory mice with weights ranging (10-16 g) and 6-8 weeks of life were used in the experiment. The animals were placed in the animal house of the iraqi Center for Cancer and Medical Genetics Research (ICCMGR)/Al-Mustansiri University, with environmental conditions that included moderate temperature, a 12-hour dark and 12 hours light cycle.

The animals were treated with the approval of the ethics committee at the center, where they were kept in meshed plastic cages containing sawdust; the pellets were fed (mix from corn, wheat and milk) and drinking with tap water throughout the experiment. The animals were left to adapt for 14 days before starting the experiment.

Tumor inducing

The tumor was induced in experimental animals in ICCMGR/Al-Mustansiriya University by induce of AN3 mammary cell carcinoma. Where withdrawn a quantity of previously generated tissue tumor from implanting of AN3 sample in female mice that were nursed for more than 30 days, bringing the tumor diameter to 3-4 cm to be sources for these cells. AN3 cells are withdrawn from the tumor by a syringe (10ml, gauge 18) after sterilization with iodine, where a sample ranging from 6-8 ml of tumor fragment in each infected animal that appear as semi-liquid was obtained according to the size of the tumor

Immediately, the samples for a group of animals are placed in a sterile container under ultraviolet rays and near a fire in the hood, then the sample were washed with a neutralized phosphate buffer PBS (contain streptomycin and penicillin) to get rid of the suspended material, including blood and pus. The cellular precipitate is taken each time and washed again 4-5 times. The tumor cells are withdrawn with a syringe (1ml) and injected directly into mice ready for injection. A dose of 0.2-0.4 ml of AN3 sample is injected subcutaneously of each animal in the posterior region of the back after being sterilized with iodine or 70% ethyl alcohol.

The animals were followed under the same conditions of the experiment, and it was noticed that the tumor was grown in the same area or close to it within 12-15 days from the injection time, where the tumor size reached 0.8-1.2 cm. After animal reach this size of tumor, start treatment with prepared materials. It is worth noting that some of the animals did not grow tumors, others are died and may be tumor as metastasis[7].

Preparation of drug concentrations and doses

The required concentrations were prepared for the purpose of this study according to the following:

1. According to[14,15], determine the dose of Cisplatin at 6mg/kg/week, was used by (KOCAK FARMA) company.
2. According to[16], determine the dose of Anise extract was 200 mg/kg /day orally.
3. According to[17], determine the dose of chitosan nanoparticles loaded by anise extract (CNP-AE) was 40 mg/kg/day by orally.

The required doses were calculated according to the weight of the animal, then the volumes were mixed in a 1:1 ratio from therapeutic substances and PBS (pH = 7.4) for regulating of pH. The animals were weighted weekly, and appropriate doses of therapeutic substances were given daily for 4 weeks.

Distribution of experimental animals

30 of female (BALnB/C albino strain) laboratory mice used. Divided of animals to 5 groups (each group 6 mice). The mice were dosed orally from treatments by gavage to the stomach directly for a period of 4 weeks, according to the following groups:

1. Control group: 6 uninfected mice were administrated orally 0.2 ml/day of normal saline solution (0.9%).
2. tumor group: 6 infected mice were administrated orally 0.2 ml/day from normal saline solution.

3. Cisplatin group: 6 infected mice were injected 6 mg/kg/week intraperitonially from cisplatin.
4. Cisplatin + AE group: 6 infected mice were injected 6 mg/kg/week from cisplatin intraperitonially, and administrated 200 mg/kg/day of anise extract orally.
5. Cisplatin + CNP-AE group: 6 infected mice, were injected 6 mg/kg/week intraperitonial from cisplatin, and administrated 40 mg/kg/day from CNP-AE by oral.

After four weeks, the tumor size of each group of animals was measured by electronic vernier caliper, then the animals were sacrificed by anesthesia with chloroform, and blood samples were taken from the heart directly by syringe 5 ml. Serum was obtained in an amount of approximately 0.4-0.5 ml by centrifugation and kept in the freezer for later use.

Measuring of biomarkers and CRP

Biomarkers (IL-33 and LRG-1) were measured in the serum according to the procedure prepared in (Sunlong Biotech Company,China), and C-reactive protein (CRP) index was measured according to the Company (Bioassay Technology Laboratory Company) by ELISA.

Statistical analysis

The experimental data were analyzed for statistical significance by one-way analysis of variance and post hoc comparison using the SPSS version 25. All data were reported as mean ± SD and statistical significance was accepted at $P < 0.05$[18].

RESULTS

Characterization of nanoparticles

The results of the material characterization examination showed that the particle size of anise extract was 129.5 nm, while the particle size after loading on chitosan nanoparticles reached 138.4 nm (Figure 1).

While Zeta potential found that the particles of anise extract were negatively charged (-29.5mV), while the charge appeared positive after being borne on the nanoparticles (+48.06Mv) (Figure 2).

Concerning the FTI-R, the functional groups of anise extract and chitosan nanoparticles were diagnosed, which appeared together for the same materials after their loading, which proves that the loading process was successful (Figure 3).

Tumor development

To confirm and link the relationship between the treatment protocols and tumor development among study groups, tumor size was documented in each study groups. Due to tumors-induced protocol, after 10 days of tumor cells injection in back of mouse, the size of tumor will reach to about 5 mm, and after 30 days, its reach to approximately 31-36 mm. The purpose of this study to show possible the role of Anise extract, and Chitosan nanoparticles in increase the activity of Cisplatin in prevent or delayed increase in tumor size (tumor development) and in finally, given enough time to tumor management protocols.

With first time of tumor cells injection in all study groups except control group, the treatment protocol was started. And after 30 days, the size of tumor were recorded as shown in (Figure 4 A-E)

The tumor size rate in tumor-induced treated group was reached 33.01 mm while in Cisplatin, Cisplatin-Anise extract , and Cisplatin- Anise extract- Chitosan nanoparticles treated groups were 26.935, 16.685, and 15.17 mm, respectively as shown in (Figure 4 B-E). Due to current results, there were significant differences in tumor size among study groups (*P value*<0.001). The size of tumor in Cisplatin treated group was significantly lower than tumor-induced group, where in tumor - inducing group were 33.01 + 1.457mm, while was 26.94 + 0.007071 mm in cisplatin group, to refer to the dampening effect of tumor by Cisplatin. Also, the size of tumor appear to progressive decrease in other groups, copmare to Cisplatin- Anise extract, where the tumor size of Cisplatin treated group, Cisplatin- Anise extract, and Cisplatin-Anise extract- Chitosan nanoparticles treated groups were 26.94+0.007071, 16.69+0.4455, and 15.17+0.2970 mm respectively, while there was no significant difference in tumor size between Cisplatin- Anise extract , and Cisplatin- Anise extract- Chitosan nanoparticles treated group, although there is a decrease in the size of the tumor, were appeared 16.69+0.4455 mm and 15.17+0.2970 respectively (*P value* 0.544), as shown in (Figure 5). These results confirm the role of anise extract and the use of chitosan nanoparticles in showing a more effective synergestic role than the use of cisplatin alone.

Biomarkers

To explain the possible mechanism which responsible on delayed tumor development and to study the relationship among the study groups in response to, different treatment protocols, three parameters were taken, was IL-33, LRG-1 and CRP.

The results shown the level of IL-33 and LRG-1 ware difference among study groups (Figures 6,7). IL-33 and LRG-1 levels ware significantly lower ($P > 0.001$) in control group by compared with tumor-induced group, were 25.43 + 3.460 mm and 48.25+8.190 mm for IL-33, and 16.67+3.812 mm and 43.02+5.731 mm for LRG-1 respectively (*p value*< 0.001). While there is significant increase of tumor-induced group compare with Cisplatin treated group, Cisplatin –Anise extract treated, and Cisplatin- Anise extract - Chitosan nanoparticles treated groups, where showen respectively 48.25+ 8.190 mm, 36.61+1.919mm, 34.01+5.113 ,and 29.31+6.757 mm for IL-33 as shown in figure 6. And 43.02+5.731, 31.32+7.638, 24.13+4.607, and 19.53+3.174 mm for LRG-1 in the same groups as shown in figure 7. So, in Cisplatin treated group,

the levels of biomarkers higher than the other groups. Also the results showen no significant decrease for this markers in Cisplatin –Anise extract - Chitosan nanoparticles treated group compare to Cisplatin-Anise extract treated groups, as shown in (Figures 6,7). and this reflect a role of Anise extract and Chitosan nanoparticles to increase the activity of Cisplatin in decrease the level of the two biomarkers and decrease the tumor.

(Figure 8) shown level of CRP among the study groups. CRP in control group was significantly lower (*p vale* <0.001) than Tumor induced group. These groups were 9.067+0.9750 mm and 13.84+1.369 mm respectively. While the level of CRP was progessive inhibition for each of Cisplatin treated, Cisplatin –Anise extract treated, and Cisplatin- Anise extract- Chitosan nanoparticles treated groups compare to the tumor induced group.

The results of these three parameters reflect the vital role of Anise extract and especially when combined with Chitosan nanoparticles in increase activity of Cisplatin in treatment the tumor.

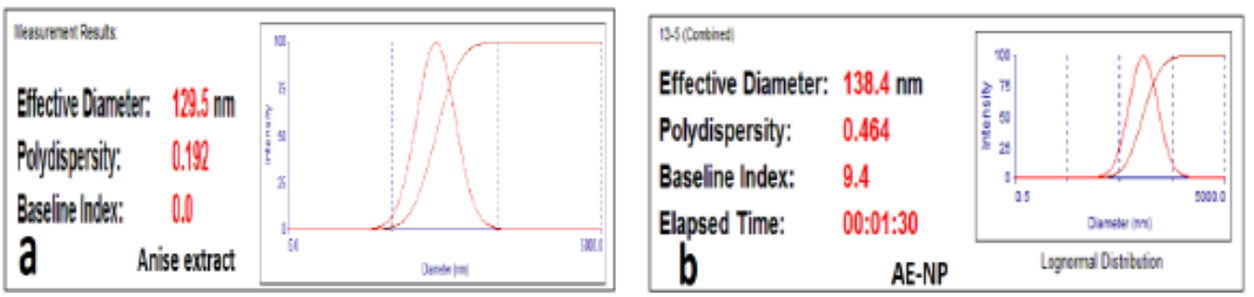

Fig. 1: The particle size of anise extract before and after loading on chitosan nanoparticles

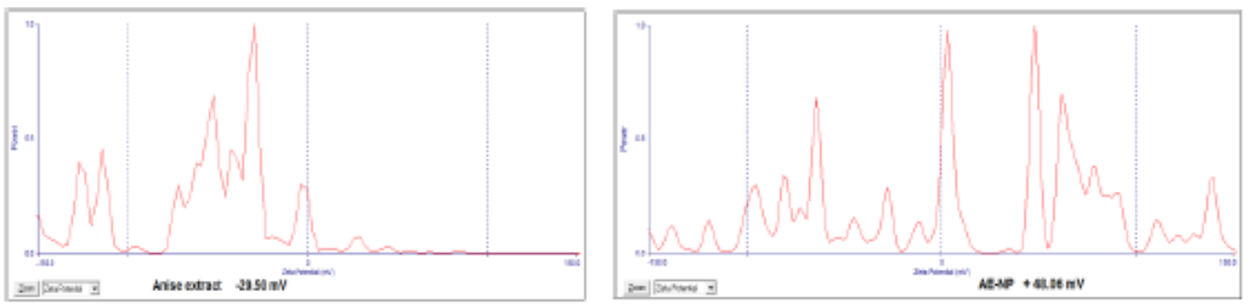

Fig. 2: Zeta potential of anise extract befor and after loading on chitosan nanoparticles

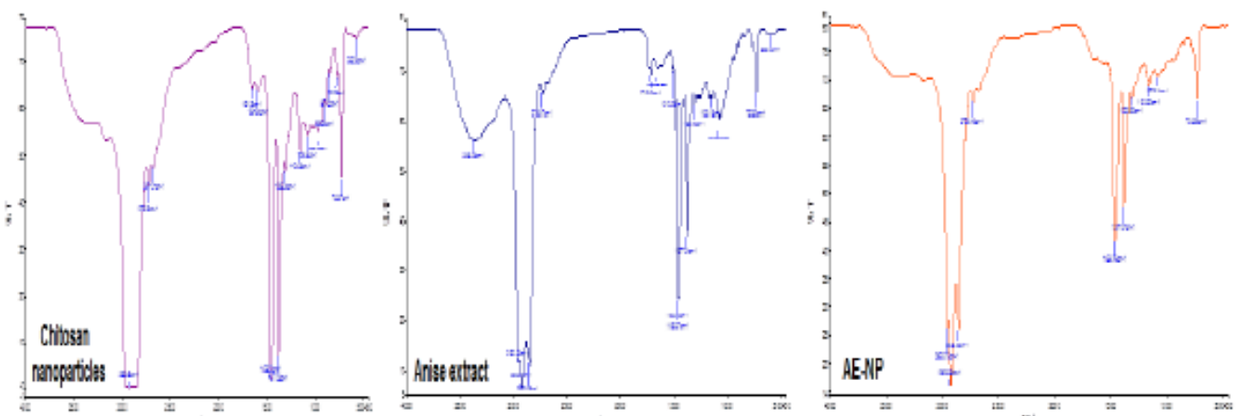

Fig. 3: FTI-R of chitosan nanoparticles , anise extract befor and after loading on chitosan nanoparticles

Fig. 4 (A-E): shown the results of tumor size after 30 days of treatment protocols. Figure A shown control group which act as negative control without any indicated on tumor found. Figure B1 and B2 shown size of tumor on back of mouse in tumor-inducing treated group. FigureC1 and C2 shown size of tumor on back of mouse in Cisplatin treated group. Figure D1 and D2 shown size of tumor on back of mouse in Cisplatin–Anise extract treated group. Figures E1 and E2 shown tumor size of back of mouse in Cisplatin- Anise extract- Chitosan nanoparticles treated groups.

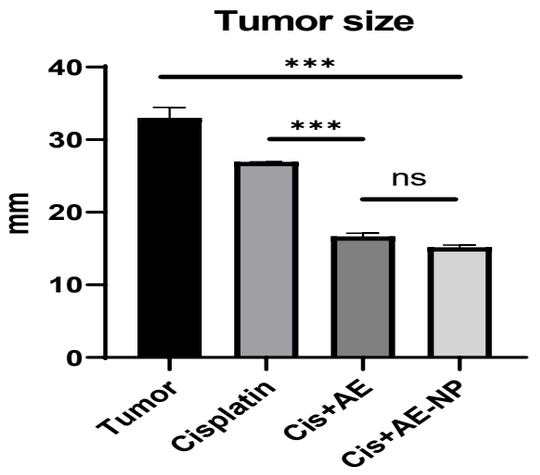

Fig. 5: shown a tumor size among a study groups.

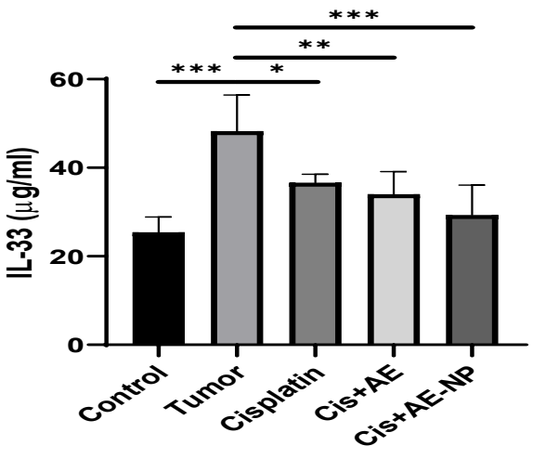

Fig. 6: Shown IL-33 level among a study groups.

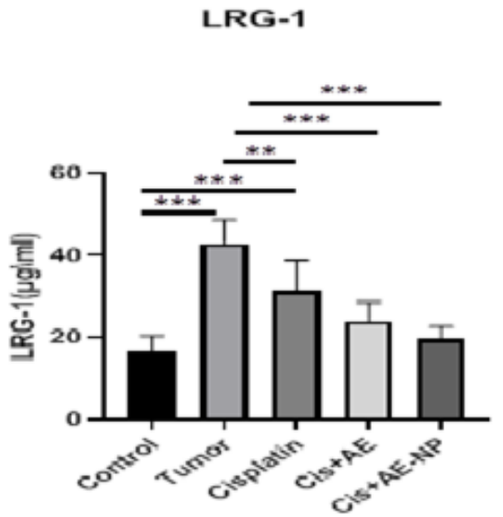

Fig. 7: Shown LRG-1 level among a study groups.

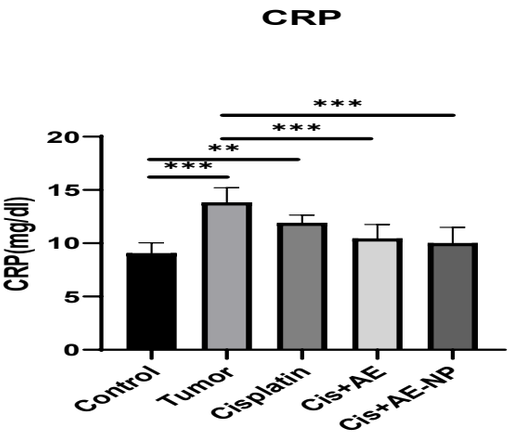

Fig. 8: Shown CRP level among a study groups.

DISCUSSION

The treatment of cancer can be challenging and often involves a combination of surgery, chemotherapy, radiation therapy, and targeted therapies. The earlier cancer is detected, the better the chances of successful treatment and survival. Regular cancer screenings and adopting a healthy lifestyle can also help reduce the risk of developing cancer. However, the Medical plant extracts have been used in traditional medicine for centuries, and some of these extracts have been found to have potential anti-cancer properties. Several plant extracts have been studied for their potential use in breast cancer treatment, and one of these extracts is anise extract[19,20].

Recently, the plant extracts combine with nanoparticles to enhance cancer treatment by improving drug delivery and targeting cancer cells more effectively. A study published in the Journal of Controlled Release investigated the use of nanoparticles for the targeted delivery of a chemotherapy drug, doxorubicin, to breast cancer cells. The researchers developed a nanoparticle system that could release doxorubicin specifically in response to the acidic environment found in tumors. They study found that the nanoparticle system increased the effectiveness of doxorubicin against breast cancer cells and reduced toxicity to healthy cells[21,22].

Depending on information mentioned above, the current study designed to study the role of Anise extract (AE) and Anise extract- Chitosan nanoparticles in synergestic effect with the cisplatin in treatment for the tumor induced in female mouse. The tumor size and some biomarkers (IL-33, LRG-3, and CRP) were considered as indicator for tumor development. The present study found that a tumor size in tumor-induced group reach to 33.1 mm after 30 days of induce. The tumor size in Cisplatin treated group was significantly smaller than tumor-induced group. This finding agree with a study achieved by Dasari et al., Who define the Cisplatin as a chemotherapy drug that is commonly used to treat various types of cancer[21,23].

Also the current study in line with another study found that Cisplatin act as drug for tumor treatment and it works by damaging the DNA of cancer cells by create an cross linkage between the two strand, preventing them from dividing and growing[24]. Due to the large toxic effects associated with the great role of cisplatin in the treatment of cancer patients, the current studies tended to use efficient natural plant antioxidants to prevent or reduce the resulting inflammation and increase the effectiveness of this treatment to reduce the size of the resulting tumors[25,26,27]. In addition, anise has been considered in recent years as a new (novel) source of chemical compounds that prevent or mitigate cancer through its antiproliferative and antiapoptic properties[28]. So, the tumor size in Cisplatin treated group was larger than in both Cisplatin- Anise extract, and Cisplatin- Anise extract- Chitosan nanoparticles treated groups. This results in line with a study achieved by Abotaleb and colleagues[29] found that anise extract has a wide range of biological and pharmacological activities including antioxidant, antibacterial, anticancer, antiulcer, antidiabetic, antifibrotic, antiviral, anti-inflammatory, analgesic, cardiac, antiaging, hepatoprotective, neurological, and nephroprotective efficacy.This may be due to the possession of many aromatic compounds, including anethole, eugenol and estragol which gave him many characteristics, the most important of which is an antioxidant and aniproliferation of the cancer cell[30].

These results consistent with many previous studies, a study achieved in 2014, found that anise extract inhibited the growth of breast cancer cells, suggesting that it may have potential as an anti-cancer agent[31]. In 2013, the researchers investigated in the effect of anise extract on colon cancer cells. They found that anise extract induced apoptosis (cell death) in these cancer cells, and suggesting that it may be a promising treatment for colon cancer[32]. Olaywi, (2020)[33] investigated the effect of anise extract on the growth of lung cancer cells in vivo, they found that anise extract inhibited the growth of these cancer cells and induced cell death. They suggested that anise extract might have potential as an anti-cancer agent for lung cancer. In some situations, the tumor do not response to treatment with cisplatin these cases called “resistance to cisplatin”. Jafarzadeh et al.,(2021)[34] found the combined use of cisplatin and Fisetin increases the induction of apoptosis in cisplatin-resistant ovarian cancer cells (A2780); therefore, the combined use of cisplatin and some substances can be considered a promising strategy in the treatment of ovarian cancer.

The previous studies were agree with the current study which shown the effect of Cisplatin was enhancement when combine with anise extract[21]. The progress in the field of nano-applications for cancer treatments had a significant impact and showed a synergistic action for many compounds, including platinum compounds, in which a significant improvement appeared in the solubility of non-hydrophobic substances and their enhancement in cells by increasing the permeability of the cell interior[35]. accordingly showed the current study found the effect of cisplatin against a tumor increase when combine Chitosan nanoparticle and these results consistent with a study[36] have shown that nanoparticles can prolong the lifespan of therapeutic agents. This has been demonstrated through the use of sulfonatocalix4arene to carry cisplatin, which increased its lifespan by 3-2 times compared to the drug alone. In addition, the toxicity associated with cisplatin was reduced by increasing its resistance to cellular groups containing the antioxidant glutathione (GSH) and regulating the enzymatic activity of GST. The nanoparticles also exhibit a high affinity for caveolae, which are formed in the plasma membrane during cellular uptake[37].

The therapeutic delivery of tumors by the activated targeting mechanism depends on the size of the particles, and it is mentioned that the tumor tissue has characteristics that differ from the normal tissue, including the large size of the vascular system, its distribution, its high density, its heterogeneous distribution, and its high permeability[38] Which leads to an abundance of vessels compared to a weak fibrous drainage system for large particles, which provides therapeutic materials with high molecular weights in the tumor tissue. This condition is known as the enhanced permeability and retention (EPR)effect. Which allows to increase the concentration of therapeutic substances in the tumor vessels through the infiltration of nanoparticles with less than 150-200 nm from normal blood vessels to the swollen tissue[39] In addition to what he mentioned[40] that the size of the nanoparticles has the ability to circulate in the blood in the form of vacuoles for long periods, which causes a reduction in the mononuclear phagocyte system (MPS) as a result of saturating the blood with high doses of active compounds loaded on nanomaterials, which sometimes may cause pathological effects, the destruction of phagocytic cells and the nullification of their functional role, according to the safety of the used nanomaterials and the therapeutic materials loaded on them. It is also indicated that the binding of nanomaterials to plasma proteins and the action of complement in the blood may remove particles in vivo, depending on their size[41,42].

The present study included some biomarkers, IL-33, LRG-3, and CRP. IL-33,LRG-3 ,and CRP level were higher in Cisplatin treated group than control group and other study groups. This reveals the toxic effect of Cisplatin and this toxicity decrease with anise extract alone or with Chitosan nanoparticles. This finding agree with Zhao and his colleagues (2018)[43] found IL-33, also known as an "alarm" cytokine, is considered an internal signal that is released from damaged cells as a nuclear-associated cytokine after exposure to tissue injury or shock. The expression of this cytokine is stimulated during cell necrosis or tissue damage, indicating its role in activating the immune response to act as an alarm after damage to epithelial and endothelial cells during stress, shock, viral infection, and toxic damage in various tissues, including liver toxicity[44]. Epithelial and endothelial cell nuclei in organs such as the liver, lungs, and kidneys have

the ability to express IL-33 protein, and it has also been expressed in endothelial veins and small and large blood vessels in several tissues as a nuclear protein, especially in blood vessels for the cancer patients[45]. Another study that investigated the role of IL-33 in cancer is Zhao et al. (2018). The study found that IL-33 can promote the growth and metastasis of lung cancer cells by regulating multiple oncogenic pathways.

IL-33 has been shown to play a role in tumor development and progression, as it can promote inflammation and angiogenesis in the tumor microenvironment. In some cases, IL-33 has also been found to have anti-tumor effects, possibly through activation of anti-tumor immune responses. IL-33, also known as interleukin-33, is a cytokine that is involved in various biological processes such as immune response and inflammation. It is produced by a variety of cells including epithelial cells, fibroblasts, and endothelial cells. One study that investigated the role of IL-33 in the immune response is "IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation" by L. P. Pichery et al. (2012)[46]. Either the concentrations of IL-33 and LRG-1 in Cisplatin- Anise extract, and Cisplatin- Anise extract-Chitosan nanoparticles treated groups were return to same level as control group i.e there was no significant difference, and this refer to the role of Anise extract and Chitosan nanoparticles was vital to induce the Cisplatin action to reduce the level of LRG-1 and in tumor treatment. And these results in line with a study achieved by Y. Yanagisawa and his colleagues. (2018)[47] found that high serum LRG-1 levels were associated with worse survival outcomes in patients with pancreatic cancer. Also Li and his colleagues (2020)[48] investigated the role of LRG-1 in gastric cancer. The study found that overexpression of LRG-1 inhibited cell invasion and survival in gastric cancer cells and suggested that LRG-1 may function as a tumor suppressor in gastric cancer.

CRP (C-reactive protein) is a marker of inflammation and has been implicated in cancer development and progression. And the results in the current study agree with a prospective cohort and Mendelian randomization analysis achieved by Zhu and his colleagues (2022)[49], found that CRP was a potential biomarker to assess risks of overall cancer and 12 site-specific cancers. The increase in this indicator in the current study may be attributed to the use of cisplatin, which causes toxicity to the tubular epithelial cells, vasoconstriction in the renal capillary vessels, and inflammatory effects that appear in most areas of the kidney due to free radicals resulting from the harmful effects of using this treatment, the most important of which are fibrosis and dysfunction. Proinflammatory of proximal tubule cells[50]. As that the activation of leucocytes (white cells) and cytokines of the proximal tubule cells leads to the emergence and prolonged period of inflammation, where the level of IL-1, IL-6, and TNF-alpha secretion is increased by increasing the activity of the NF-KB pathway to end with the development of the renal wound[51,52].

The current results also showed a decrease in the inflammatory marker (CRP) after using anise extract with cisplatin, as some studies indicated the extract and oil of anise fruits played a major role in modifying the biomarkers affected by oxidation, such as the ability to reduce ferric acid and lipid peroxidation, which was attributed to the main role of its contents that act as antioxidants[53]. The essential oil showed high efficacy in the antioxidant capacity relative to the substance anethole, which represents 82.3% of the content of the oil itself, and it was also attributed to the presence of phenols, D-limonene, astracol and other compounds that have beneficial effects in this field, or it may be due to its action of synergistic compounds. Therefore, it was indicated for its independent use as a protective agent against many degenerative processes such as tumors[54]. Also, this plant has a role in providing the manganese element to the enzyme Superoxide Dismutase (SOD), one of the most important antioxidants with an intracellular role to reduce oxidative stress resulting from free radicals, therefore, it is considered protective of many diseases such as cancer, heart and kidney diseases[55-57].

CONCLUSION

It can be concluded from the current study that anise extract has a synergistic effect with cisplatin in reducing tumor size in the animal body. In addition to the effective role of nanoloading in increasing the effectiveness of this plant extract, which confirms the possibility of using this therapeutic method in different types of tumors, especially in the mammary glands. However, the limitations in current study were the results do not confirmed by histopathology and IHC examination due to limitations associated with time and funds. Therefore, we recommended to researchers to confirming the current results.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Coffetti, G., Moraschi, M., Facchetti, G., & Rimoldi, I. (2023). The challenging treatment of cisplatin-resistant tumors: State of the art and future perspectives. Molecules, 28(8), 3407. https://doi.org/10.3390/molecules28083407

2. Zoń, A., & Bednarek, I. (2023). Cisplatin in Ovarian Cancer Treatment—Known Limitations in Therapy Force New Solutions. International Journal of Molecular Sciences, 24(8), 7585. DOI 10.3390/ijms24087585

3. Bhat, A., Verma, S., Chander, G., Jamwal, R. S., Sharma, B., Bhat, A., ... & Shah, R. (2023). Cisplatin-based combination therapy for cancer. Journal of Cancer Research and Therapeutics. DOI: 10.4103/jcrt.jcrt_792_22.

4. Dai, X., Zhou, X., Liao, C., Yao, Y., Yu, Y., & Zhang, S. (2019). A nanodrug to combat cisplatin-resistance by protecting cisplatin with p-sulfonatocalix [4] arene and regulating glutathione S-transferases with loaded 5-fluorouracil. Chemical Communications, 55(50), 7199-7202.doi.org/10.1039/C9CC03012C

5. Strickland, L. R., Pal, H. C., Elmets, C. A., & Afaq, F. (2015). Targeting drivers of melanoma with synthetic small molecules and phytochemicals. Cancer letters, 359(1), 20-35. doi.org/10.1016/j.canlet.2015.01.016.

6. AL-SHAMMARY, A. M., AL-MUDHAFR, M. A., E. D. CHALAP AL- GRAWI3 , Z. A. AL-HILI1 & N. YASEEN1 newcastle disease virus suppresses angiogenesis in mammary adenocarcinoma models

7. Al-Shamery, M. J., Alshami , M. A., Emran, M. A. and Almukhtar, A. (2015). Establishment and characterization of a receptor-negative, hormone-nonresponsive breast cancer cell line from an Iraqi patient Breast Cancer Targets and Therapy, 7, 223-2302 DOI:10.2147/BCTT.S74509

8. Wang, Z., Luo, T., Cao, A., Sun, J., Jia, L., & Sheng, R. (2018). Morphology-Variable Aggregates Prepared from Cholesterol-Containing Amphiphilic Glycopolymers: Their Protein Recognition/Adsorption and Drug Delivery Applications. Nanomaterials, 8(3), 136. doi.org/10.3390/nano8030136.

9. Ferji, K., Venturini, P., Cleymand, F., Chassenieux, C., & Six, J. L. (2018). In situ glyco-nanostructure formulation via photo-polymerization induced self-assembly. Polymer Chemistry, 9(21), 2868-2872. doi.org/10.1039/C8PY00346G.

10. 0- Abouelhag, H. A., Sivakumar, S. M., Bagul, U. S., Eltyep, E. M., & Safhi, M. M. (2017). Preparation and physical characterization of cisplatin chitosan nanoparticles by zeta nano sizer “prime step for formulation and development”. Int. J. Pharm. Sci. Res, 8(10), 1-14. 10.13040/IJPSR.0975-8232.8(10).4245-49

11. Jabar, J. G. and Karam, F. F. (2020) Preparation characterization and application of Chitosan nanoparticles as drug carrier. J. Phys.: Conf. Ser. 1664 012071. doi:10.1088/1742-6596/1664/1/012071

12. Banerjee, P., Satapathy, M., Mukhopahayay, A., & Das, P. (2014). Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. Bioresources and Bioprocessing, 1, 1-10. DOI:10.1186/s40643-014-0003-y

13. Rafique, M., Sadaf, I. , M. and Tahir, M. B. (2017).A review on green synthesis of silver nanoparticles and their applications A review on green synthesis of silver nanoparticles and their applications. An international journal.V45. Issue. Pages 1272-1291. https://doi.org/10.1080/21691401.2016.1241792

14. Zhou, X., Ling, K., Liu, M., Zhang, X., Ding, J., Dong, Y., ... & Zhang, J. (2019). Targeted delivery of cisplatin-derived nanoprecursors via a biomimetic yeast microcapsule for tumor therapy by the oral route. Theranostics, 9(22), 6568.doi:10.7150/thno.35353.

15. A. A. Imarah. Protective effect of Eruca Sativa leaves oil extract against induced renal failure in rats according to certain physiological and histopathological criteria. Master thesis. Biology dep. Faculty of Science. University of Kufa,(2017). DOI:10.1016/S2221-1691(12)60490-0

16. Aprotosoaie, A. C., Costache, I. I., & Miron, A. (2016). Anethole and its role in chronic diseases. Drug discovery from mother nature, 247-267. DOI: 10.1007/978-3-319-41342-6_11

17. Jabir, M. S., Hussien, A. A., Sulaiman, G. M., Yaseen, N. Y., Dewir, Y. H., Alwahibi, M. S., ... & Rizwana, H. (2021). Green synthesis of silver nanoparticles from Eriobotrya japonica extract: a promising approach against cancer cells proliferation, inflammation, allergic disorders and phagocytosis induction. Artificial cells, nanomedicine, and biotechnology, 49(1), 48-60. doi.org/10.1080/21691401.2020.1867152

18. Rahman, A., Golam Muktadir, M. (2021). SPSS: An Imperative Quantitative Data Analysis Tool for Social Science Research International Journal of Research and Innovation in Social Science (IJRISS) |Volume V, Issue X, October 2021|ISSN 2454-6186 DOI:10.47772/IJRISS.2021.51012

19. A. Manjamalai, & V.M. Berlin Grace (2013). Anticancer activity of anethole in MCF-7 breast cancer cells. Food and chemical toxicology, 62, 810-818. doi: 10.1016/j.fct.2013.10.039.

20. Al-Tameemi, H. K., Al-Husseini, R. M., Al-Mudhafer, R. H., Abid, H. A., Al-Gazali, H. R., Abdullah, D. A., & Albaldawy, M. T. (2023). Molecular and immunohistochemically study of APC exon 16 and their possible role in colorectal carcinoma development, 1-7. Heliyon. http://dx.doi.org/10.2139/ssrn.4534678

21. Sarheed, N. M. and Jaffat. H. S. (2022). Protective effect of anise extract loaded by chitosan nanoparticles in mice treated with cisplatin. AIP Conference Proceedings 2450, 020026,. https://doi.org/10.1063/5.0094569

22. Wang, C., Xu, H., Liang, C., Liu, Y., Li, Z., Yang, G., ... & Liu, Z. (2013). Iron oxide@ polypyrrole nanoparticles as a multifunctional drug carrier for remotely controlled cancer therapy with synergistic antitumor effect. ACS nano, 7(8), 6782-6795. doi: 10.1016/j.jconrel.2017.05.189)

23. Dasari, S., Njiki, S., Mbemi, A., Yedjou, C. G., & Tchounwou, P. B. (2022). Pharmacological effects of cisplatin combination with natural products in cancer chemotherapy. International Journal of Molecular Sciences, 23(3), 1532. doi.org/10.3390/ijms23031532

24. De Giorgi, U., Casadei, C., Bronte, G., Altini, M., & Martinelli, G. (2020). High-dose chemotherapy in a patient with coronavirus disease (COVID-19). European Journal of Cancer, 136, 130-131. doi.org/10.1016/j.ejca.2020.06.026.
25. Crona, D. J., Faso, A., Nishijima, T. F., McGraw, K. A., Galsky, M. D., & Milowsky, M. I. (2017). A systematic review of strategies to prevent cisplatin-induced nephrotoxicity. The oncologist, 22(5), 609-619. doi.org/10.1634/theoncologist.2016-0319
26. Abdel-Daim, M. M., Abushouk, A. I., Donia, T., Alarifi, S., Alkahtani, S., Aleya, L., & Bungau, S. G. (2019). The nephroprotective effects of allicin and ascorbic acid against cisplatin-induced toxicity in rats. Environmental Science and Pollution Research, 26, 13502-13509. doi.org/10.1007/s11356-019-04780-4
27. Hayati, F., Hossainzadeh, M., Shayanpour, S., Abedi-Gheshlaghi, Z., & Mousavi, S. S. B. (2016). Prevention of cisplatin nephrotoxicity. Journal of nephropharmacology, 5(1), 57. doi: 10.1007/s00280-008-0711-0.
28. Bekara, A., Hamadouche, N. A., Kahloula, K., Harouat, S., Tabbas, D., & Aoues, A. E. K. (2015). Effect of Pimpinella anisum L (Aniseed) aqueous extract against lead (Pb) neurotoxicity: neurobehavioral study. International Journal of Neuroscience and Behavioral Science, 3(3), 32-40. DOI: 10.13189/ijnbs.2015.030302.
29. Abotaleb, M., Liskova, A., Kubatka, P., & Büsselberg, D. (2020). Therapeutic potential of plant phenolic acids in the treatment of cancer. Biomolecules, 10(2), 221. doi.org/10.3390/biom10020221
30. Sarheed, N. M. and Jaffat, H. S. (2022). Detection of chemical compounds and its antioxidant activity of aniseeds extract. AIP Conference Proceedings 2398, 040049. https://doi.org/10.1063/5.0093811.
31. Zhao, S., Baik, O. D., Choi, Y. J., & Kim, S. M. (2014). Pretreatments for the efficient extraction of bioactive compounds from plant-based biomaterials. Critical reviews in food science and nutrition, 54(10), 1283-1297. https://doi.org/10.1080/10408398.2011.632698.
32. Kadan, S., Rayan, M., & Rayan, A. (2013). Anticancer activity of anise (Pimpinella anisum L.) seed extract. The Open Nutraceuticals Journal, 6(1). DOI: 10.2174/1876396001306010001.
33. Olaywi, H. S(2015). Analyses of Anise and Ginger as Antitumor Compounds for cell line A549, HeLa and BT549 (PhD dissertation, Tennessee State Uni). Digitalscholarship.tnstate.edu/dissertations/AAI10003947.
34. Jafarzadeh, S., Baharara, J., & Tehranipour, M. (2021). Apoptosis Induction with Combined Use of Cisplatin and Fisetin in Cisplatin-Resistant Ovarian Cancer Cells (A2780). Avicenna Journal of Medical Biotechnology, 13(4), 176. https://doi.org/10.18502/ajmb.v13i4.7202
35. J. Shi, P. W. Kantoff, R. Wooster and O. C. Farokhzad, Cancer nanomedicine: progress, challenges and opportunities. Nat. Rev. Cancer, 2016, 17, 20. doi:10.1038/nrc.2016.108.
36. J. Zhou, G. Yu and F. Huang, Chem. Soc. Rev. 46, (2017)7021–7053. DOI: 10.1039/C6CS00898D
37. J. Voigt, J. Christensen and V. P. Shastri, Proc. Natl. Acad. Sci. U. S. A., 2014, 111, 2942. doi: 10.1073/pnas.1322356111.
38. Aw-Yong, P. Y., Gan, P. H., Sasmita, A. O., Mak, S. T., & Ling, A. P. (2018). Nanoparticles as carriers of phytochemicals: Recent applications against lung cancer. Int. J. Res. Biomed. Biotechnol, 7, 1-11. https://doi.org/10.1080/17425247.2022.2041599
39. Maeda, H. (2015). Toward a full understanding of the EPR effect in primary and metastatic tumors as well as issues related to its heterogeneity. Advanced drug delivery reviews, 91, 3-6. doi.org/10.1016/j.addr.2015.01.002
40. Caracciolo, G. (2018). Clinically approved liposomal nanomedicines: lessons learned from the biomolecular corona. Nanoscale, 10(9), 4167-4172. doi.org/10.1039/C7NR07450F.
41. Tefas, L. R., Sylvester, B., Tomuta, I., Sesarman, A., Licarete, E., Banciu, M., & Porfire, A. (2017). Development of antiproliferative long-circulating liposomes co-encapsulating doxorubicin and curcumin, through the use of a quality-by-design approach. Drug design, development and therapy, 1605-1621. doi.org/10.2147/DDDT.S129008
42. Li, M., Shi, F., Fei, X., Wu, S., Wu, D., Pan, M., ... & Dou, J. (2017). PEGylated long-circulating liposomes deliver homoharringtonine to suppress multiple myeloma cancer stem cells. Experimental Biology and Medicine, 242(9), 996-1004. doi.org/10.1177/1535370216685
43. Zhao, J. et al. IL-33 promotes the growth and metastasis of human lung cancer cells via regulation of multiple oncogenic pathways." Oncotarget 9.19: (2018)15719-15736. doi: 10.1002/mc.22491.
44. Pecaric-Petkovic, T., Didichenko, S. A., Kaempfer, S., Spiegl, N., & Dahinden, C. A. (2009). Human basophils and eosinophils are the direct target leukocytes of the novel IL-1 family member IL-33. Blood, The Journal of the American Society of Hematology, 113(7), 1526-1534. DOI: 10.1182/blood-2008-05-157818.
45. N. S.Grotenboer, ME. Ketelaar, GH. Koppelman, MC. Nawijn. Decoding asthma: Translating genetic variation in IL33 and IL1RL1 into disease pathophysiology. J Allergy Clin Immunol. 2013;131:856-865. doi.org/10.1182/blood-2008-05-157818

46. Kurowska-Stolarska, M., Stolarski, B., Kewin, P., Murphy, G., Corrigan, C. J., Ying, S., ... & Liew, F. Y. (2009). IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. The Journal of Immunology, 183(10), 6469-6477. doi.org/10.4049/jimmunol.0901575

47. Takenaka, et al. Serum Leucine-Rich α2 Glycoprotein: A Novel Biomarker for Transmural Inflammation in Crohn's Disease The American Journal of Gastroenterology 118(6):p 1028-1035, June 2023. DOI:10.14309/ajg.0000000000002127

48. Li, D., Xing, Y., Tian, T., Guo, Y., & Qian, J. (2020). Overexpression of LRRC59 is associated with poor prognosis and promotes cell proliferation and invasion in lung adenocarcinoma. OncoTargets and therapy, 6453-6463. doi.org/10.2147/OTT.S245336

49. Zhu, M., Ma, Z., Zhang, X., Hang, D., Yin, R., Feng, J., ... & Shen, H. (2022). C-reactive protein and cancer risk: a pan-cancer study of prospective cohort and Mendelian randomization analysis. BMC medicine, 20(1), 1-13. https://doi.org/10.1186/s12916-022-02506-x

50. Gonzalez-Vitale, J. C., Hayes, D. M., Cvitkovic, E., & Sternberg, S. S. (1977). The renal pathology in clinical trials of cis-platinum (II) diamminedichloride. Cancer, 39(4), 1362-1371. doi.org/10.1002/1097-0142(197704)39:4<1362::AID-CNCR2820390403>3.0.CO;2-N.

51. Hall, A. M., & Schuh, C. D. (2016). Mitochondria as therapeutic targets in acute kidney injury. Current opinion in nephrology and hypertension, 25(4), 355-362. doi.org/10.1097/MNH.0000000000000228

52. Perše, M., & Večerić-Haler, Ž. (2018). Cisplatin-induced rodent model of kidney injury: characteristics and challenges. BioMed research international, 2018. doi.org/10.1155/2018/1462802

53. Asadollahpoor, A., Abdollahi, M., & Rahimi, R. (2017). Pimpinella anisum L. fruit: Chemical composition and effect on rat model of nonalcoholic fatty liver disease. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences, 22. Doi:10.4103/1735-1995.202147.

54. Destro, B. G. I., Jorge, R. M. M., & Mathias, A. L. (2020). Maximization of essential oil antioxidant capacity via star anise hydrodistillation. Brazilian Journal of Chemical Engineering, 36, 1679-1688. doi.org/10.1590/0104-6632.20190364s20190099 .

55. Holley, A. K., Bakthavatchalu, V., Velez-Roman, J. M., & St. Clair, D. K. (2011). Manganese superoxide dismutase: guardian of the powerhouse. International journal of molecular sciences, 12(10), 7114-7162. doi.org/10.3390/ijms12107114.

56. AL-Tameme, H. K., AL-Husseini, R. M., & AL-Mudhafer, R. H. (2023, March). Molecular study of PIK3CA exon 20 and their role in PIK3CA expression in patients having colorectal carcinoma. In AIP Conference Proceedings (Vol. 2475, No. 1). AIP Publishing. doi.org/10.1063/5.0102766.

57. AL-Tameme, H. K., AL-Husseini, R. M., & AL-Mudhafer, R. H. (2023, March). Molecular study of TP53 exon 5 and their role in P53 expression in patients with colorectal carcinoma. In AIP Conference Proceedings (Vol. 2475, No. 1). AIP Publishing. doi.org/10.1063/5.0102765

الملخص العربى

التأثير التآزري للسيسبلاتين وجسيمات مستخلص اليانسون النانوية لتثبيط ورم خلايا سرطان الثدي في الفئران

نائل محمد سرهيد[١]، حامد خليل علي التميمي[٢]، أمير علي شاكر[٣]

[١]كلية الطب، جامعة المثنى، المثنى - العراق.

[٢]تقنيات المختبرات الطبية، كلية بلاد الرافدين الجامعة، ديالى - العراق.

[٣]قسم الأحياء، كلية العلوم، جامعة الكوفة، العراق

المقدمة: يُعد السرطان من أبرز التحديات الصحية عالميًا، حيث يمثل أحد الأسباب الرئيسية للوفاة. يكمن التحدي في علاج الأورام في التشابه بين الخلايا السرطانية والخلايا الطبيعية، مما يستلزم استخدام مواد علاجية فعالة ضد الخلايا السرطانية مع تقليل السُمية للخلايا السليمة. يُعتبر الجمع بين العلاجات الكيميائية والمستخلصات النباتية ذات الفعالية العالية والسمّية المنخفضة خيارًا واعدًا. لذا، تهدف هذه الدراسة إلى تقييم التأثير التآزري لمستخلص اليانسون (AE) وجسيماته النانوية المحملة على الكيتوزان مع السيسبلاتين في علاج الأورام المستحثة في إناث الفئران.

المواد والطرق: شملت الدراسة خمس مجموعات من الفئران الإناث (٦ فئران لكل مجموعة)، حيث تم تقسيمها إلى: مجموعة ضابطة، مجموعة مصابة بالورم، مجموعة معالجة بالسيسبلاتين، مجموعة معالجة بالسيسبلاتين ومستخلص اليانسون، ومجموعة معالجة بالسيسبلاتين ومستخلص اليانسون المحمل على جسيمات الكيتوزان النانوية. تم قياس حجم الورم وتحليل بعض المؤشرات الحيوية (IL-٣٣، LRG-١، وCRP) كمؤشرات على تطور الورم والاستجابة للعلاج.

النتائج: أظهرت الدراسة أن حجم الورم في المجموعة المصابة بلغ ٣٣,١ ملم بعد ٣٠ يومًا من التحفيز، بينما كان حجم الورم أصغر بشكل ملحوظ في مجموعة السيسبلاتين مقارنةً بالمجموعة المصابة. كما انخفض حجم الورم بشكل أكبر عند الجمع بين السيسبلاتين ومستخلص اليانسون، وكانت أعلى نسبة تثبيط للورم عند استخدام مستخلص اليانسون المحمل على جسيمات الكيتوزان النانوية. بالإضافة إلى ذلك، أظهرت النتائج ارتفاع مستويات IL-٣٣، LRG-١، و CRP في المجموعة المصابة مقارنةً بالمجموعات الأخرى، مما يشير إلى استجابة التهابية متزايدة مرتبطة بتطور الورم.

الاستنتاج: تشير هذه الدراسة إلى أن مستخلص اليانسون يتمتع بفعالية عالية في تعزيز تأثير السيسبلاتين ضد نمو الورم، كما أن تحميله على جسيمات الكيتوزان النانوية يزيد من فعاليته المضادة للسرطان، مما يجعله خيارًا واعدًا للعلاج التآزري للأورام.