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Melittin and/or nanogold displayed an *in vivo* cytotoxic effect on colorectal carcinoma (CRC) in CRC-bearing mice: molecular and histopathological approach.

Tarek Khamis[1*], Hossam Nagi A. Ibrahim[2*], Alaa Abdel-Alim[3*], Shafika Elsayed[4], Lamiaa L.M. Ebraheim[4], Nesma I. El-Naseery[4], Adel Abdelkhalek[5], Saydat S. Abdelhameed[2]

[1]Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig university, Zagazig 44511, Egypt
[2]Department of Biochemistry, Faculty of Veterinary Medicine, Zagazig university, Zagazig 44511, Egypt.
[3]Department of Cytology and Histology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt
[4]Department of Cytology and Histology, Faculty of Veterinary Medicine, Badr University in Cairo (BUC), Badr City, Cairo 11865, Egypt
[5]Department of Food Hygiene, Safety and Technology, Faculty of Veterinary Medicine, Badr University in Cairo, Badr City 11829, Egypt.

Email: T.khamis@vet.zu.edu.eg

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Abstract: One of the most commonly therapeutic implications of nano gold is treatment of small cancers especially during the early twentieth century, also melittin has been approved via many clinical trials as a valid therapeutic approach in several types of cancers. The present study was designed to investigate the possible therapeutic potential of nano gold and/or melittin. *In vivo* studies were performed on fifty colorectal carcinoma bearing mice which were allocated into five equal groups 10 rats of each, mice bearing colorectal carcinoma group, mice bearing colorectal carcinoma treated with melittin group, mice bearing colorectal carcinoma treated with nanogold group, mice bearing colorectal carcinoma treated with cisplatin group, mice bearing colorectal carcinoma treated with mixture of melittin and nanogold group. The gene expression analysis for P13K, PTEN, AKT, P53, mTOR, mir-21, histopathological and immunohistochemical analysis for cytokeratin were done. The result showed that PI3K, mTOR, and mir-21 levels upregulate in mice bearing colorectal carcinoma group while their levels downregulate in the other groups respectively. Otherwise TP53 and PTEN levels were downregulated in mice bearing colorectal carcinoma group while upregulated in the other groups respectively. So, these results showed that combining melittin with nano gold achieved a therapeutic potency like cisplatin with minimal hepatic and renal adverse effects followed by melittin and nano gold both In vivo and In vitro. It can be concluded that combination of nano gold with melittin can be used as a safe alternative for cisplatin in dealing with colorectal carcinoma.

Keywords: Nanogold, Mellitin, Cisplatin, Human colorectal carcinoma, gene expression

Introduction

Colorectal cancer (CRC) is third in terms of recognition (6.1%) and second in terms of mortality (9.2%). It is estimated that by the year 2035, the total number of deaths from rectal and colon cancer will increase by 60% and 71.5%, respectively [**Douaiher J. et al 2017**]. These figures may differ from country to country depending on the degree of economic development. Therefore, the disease is widely recognized as a marker of the country's socioeconomic development [**Bray F. et al, 2018**]. The increase in morbidity is also influenced by lifestyle, body fatness and dietary patterns [**Arnold M. et al, 2017**]. There is convincing evidence that physical activity has a protective effect. The risk of developing the disease is increased by more frequent red and processed meat and alcohol drinks (**Bray F. et al, 2018)**, **(WCRF/AICR, 2016)**. The progress of civilization and economic development, apart from improving socioeconomic conditions, also causes a change in dietary patterns, referred to as the Westernization of the lifestyle. This means higher consumption of animal fats, processed meats, refined grains or sweets, a low supply of dietary fibers, fruits, and vegetables, and low physical activity. The occurrence of overweight or obesity is often the result of such a lifestyle [**Murphy N. et al, 2019**]. Overweight and obesity are associated with an increased risk of many civilization diseases. Visceral obesity has been reported to adversely affect the prognosis of CRC in men [**Silva A. et al, 2019**]. About a quarter of a contributor to genetic predisposition. The development time of CRC usually lasts from several to several years; therefore, it is very important to diagnose it early in developing the disease. Based on follow-up examinations and nutrition prevention based on a balanced diet, secondary prevention is also important [**Zaytseva Y. 2021**]. In 2020, CRC was the most diagnosed cancer (out of 36 cancers) among men in 18 of the 186 countries worldwide and women in 6 of the 185 countries [**Sung H. et al, 2020**]. However, in 2018, CRC was the most diagnosed among men in 10 of 185 countries, and no country had CRC as the most diagnosed cancer among women [**Bray F. et al, 2018**]. So, the CRC incidence rate has increased to about 10 from 5% in the last two years in men. Women were predominant in 3.24% of countries. CRC is more common among men than women and more than four times more common in high-income countries than in low-income countries. The deaths were also about 2.5 times higher in high-income nations than in low-income nations. For many years, cancer patients have been treated with surgery and chemotherapy as the initial lines of defense against the disease. However, individuals with metastatic disease have historically had a poor prognosis for CRC. Primary and adjuvant therapy advancements have improved CRC survival time. In usual cases, surgery is required to remove the tumor [**Kelland L.2007**] altogether. Nearly a quarter of CRC cases are diagnosed at the advanced stage, and 20% of the remaining cases acquire metachronous metastases; therefore, curative surgical control alone is often challenging, resulting in tumor-related mortality [**Persons D.L.,2000**]. Notably, chemotherapy or radiotherapy may be used before or after surgery to help shrink or stabilize the tumor. Current chemotherapy comprises single-agent therapy (primarily fluoropyrimidine (5-FU)) and multiple-agent regimens, including oxaliplatin (OX), irinotecan (IRI), and capecitabine (CAP or XELODA or XEL). The combined therapy regimens FOLFOX (5-FU + OX), FOXFIRI (5-FU + IRI), XELOX or CAPOX (CAP + OX), and CAPIRI (CAP + OX) remain the mainstream approaches in first-line treatment. Patients with poor performance or low risk of deterioration are recommended single-agent therapy. Choosing additive agents appears to be similar in efficacy, with only side effects varying [**Qing Jiao et al, 2014**]. However, it has several irreversible drawbacks, such as systemic toxicity, unsatisfactory response rates, variable innate and acquired resistance, and low tumor-specific selectivity. As a result, a large amount of money has been invested in developing innovative ways to refine or even replace standard CRC chemotherapy. Cisplatin (CAS No. 15663-27-1, MF-$Cl_2H_6N_2Pt$; NCF-119875), cisplatinum, also called *cis*-diamminedichloroplatinum(II), is a metallic (platinum) coordination compound with a square planar geometry. It is a white or deep yellow to yellow-orange crystalline powder at room temperature. It is slightly soluble in water and soluble in dimethylprimanide and *N,N*-dimethylformamide. Cisplatin is stable under normal temperatures and pressures, but may transform slowly over time to the *trans*-isomer (**Akron 2009**). Cisplatin has a molecular weight of 301.1 gm/mol, a density of 3.74 g/cm^3, a melting point of 270° C, a log K_{ow} of -2.19 and a water solubility of 2.53 g/L at 25° C (**HSDB 2009**). Cisplatin enters the cells by both passive diffusion through the plasma membrane and by active transport

mediated by several membrane transporters [**Spreckelmeyer S., 2014** , **Hall M.D., 2008**]. Once inside the cell, the two labile chloride leaving groups of cisplatin are replaced by water resulting in activation of the drug [**Qi L.et al 2019**]. The aquated form of cisplatin forms covalent bonds with the N7 position of guanine and adenine resulting in DNA crosslinks between two purine bases. Most crosslinks are formed on the same strand of DNA, such as GpG 1,2-intrastrand crosslinks, ApG 1,2-intrastrand crosslinks and, to a lesser extent, GpXpG 1,3-intrastrand crosslinks. Crosslinks between two guanine bases on the opposite strands of DNA (DNA ICLs) are less abundant accounting for 1-2% of cisplatin-induced lesions [**Kelland L.2007**]. In response to platinum DNA damage, cells activate various signal transduction pathways which either induce cell cycle arrest and therefore facilitate DNA repair or trigger apoptosis and hence cell death [**Wang D., Lippard S.J.(2005)**]. Central in the DNA damage response is the tumor suppressor protein p53, which is phosphorylated and activated upon treatment with cisplatin [**Persons D.L et al 2000**]. Moreover, a significant problem among patients with ovarian cancer, treated with cisplatin, is the development of numerous resistance mechanisms during therapy, including changes in the processes of cellular drug import and export, changes in the DNA damage repair mechanisms, as well as numerous changes in the processes of apoptosis and autophagy. So the therapeutic effect of cisplatin remains elusive Thus searching for a novel therapeutic regime is recommended. Although nanoparticles (NPs) have promising applications in medicine. The immune system is an important protective system to defend organisms from non-self-matters. NPs interact with the immune system and modulate its function, leading to immunosuppression or immunostimulants. These modulating effects may bring benefits or dangers. Compositions, sizes, and surface chemistry, and so forth, affect these immunomodulations. Here we give an overview of the relationship between the physicochemical properties of NPs, which are candidates to be applied in medicine, and their immunomodulation properties. GNPs with different surface modifications showed different immunogenicity in organisms. The immunogenicity of GNPs coated with C-terminal 19 kDa fragment of merozoite surface protein 1 (MSP-1_{19}) was an important vaccine candidate. In this study, GNPs showed poor immunogenicity in mice but enhanced antibody response when formulated with alum [**Parween et al,2011**]. However, GNPs coated with monosaccharide or disaccharides could initiate the immune response by activating the macrophages. Some studies indicated that high concentrations of PEG coated on GNPs could induce antibody production and trigger immune responses. High doses of injected PEG-coated GNPs were cleared through these mechanisms [**Simpson et al, 2010**, **Agrawal et al,2011**].

GNPs can also induce inflammatory responses *in vivo*. Well-dispersed PEG-coated GNPs (13 nm) can be recognized by host defense mechanism and induce acute inflammation and apoptosis in the liver [**Cho et al, 2009**]. If inflamed tissues are exposed, stronger immune responses may be induced . When exposed to sensitized mice, 40 nm GNPs could lead to a threefold increase in airway hyper reactivity and increase the number of neutrophils and macrophages [**Hussain et al, 2011**]. Although the results achieved from many studies are very encouraging, several critical issues deserve to be taken seriously into account. One important problem is that the toxicity of AuNPs requires to be addressed properly. Despite chemical inertness, gold is, after all, a noble metal and has inherently chemical toxicity to a certain degree. Introducing functional moieties such as stabilizing materials and biocompatible materials seems to reduce toxicity of AuNP core to some extent, but it is worth noting that several surface modifications may also cause unwanted side effects. Except for the nanoparticle core, some toxicity may be attributed to these modifying materials. Further research should be required to quantify the trade-off between treatment diagnosis benefit and toxicity after functional modifications. **(Yue et al, 2012)**

It is equally important to consider whether and how the functional substances affect biodistribution and consequent side effects. Similarly, if we want to avoid the plague of immunogenicity in vivo, surface modification technology requires further optimization. Another possible effect on biosafety is the biodistribution of AuNPs because AuNPs can accumulate in the spleen liver, and other sites, hence showing toxic effects in these organs. There is a need for further knowledge to understand the biodistribution profile of AuNPs. So the therapeutic effect of nanogold remains elusive Thus searching a novel therapeutic regimes is recommended .(**Fallarini et al , 2013)**

Melittin inhibited the viability of CRC cell lines and induced apoptosis in SW480 cells by regulating apoptosis-related proteins. Melittin triggered endoplasmic reticulum (ER) stress and caused an imbalance in calcium homeostasis in SW480 cells. An absence of melittin triggered ER stress via the calcium chelating agent BAPTA/AM, and the IP3R inhibitor 2-aminoethoxydiphenyl borate (2-APB) impaired melittin-induced apoptosis in SW480 cells. Melittin treatment suppressed tumour growth but did not affect the body weight of SW480 tumour-bearing mice. Unlike cisplatin and 5-fluorouracil, melittin treatment did not change the biochemical and haematological parameters of the tumour-bearing mice. Finally, in these mice, melittin treatment induced ER stress, which was then blocked by BAPTA/AM, whilst 2-APB impaired the growth inhibitory effect of melittin. So from our study we concluded that treatment with the combination of both melittin and nanogold have a great therapeutic effect on colorectal carcinoma bearing mice (**Yan Luo et al, 2023)**

2. Material and methods

2.1 Chemicals and media

RPMI1640 (Roswell Park Memorial Institute) medium with L-glutamine (Cambrex, Belgium), HAuCl4, and Trypan Blue (Sigma, USA). Biochemical kits purchased from the local market, colorectal carcinoma cell line HCT 116 purchased by the National Cancer Institute (NCI), Cairo University, Egypt, and was maintained by weekly intra-peritoneal transplantation of 2.5 x 10^6 /ml cells in male Swiss albino mice. On the other hand, Cisplatin was obtained from (Meylan, USA) and Melittin was purchased from (Sigma Aldrich, USA)

2.1 Experimental animals and design:

Fifty male white mice weighing 25-45 gm were used in this study. The rats were housed in the research building, faculty of Veterinary Medicine, Zagazig University. They were housed in clean stainless-steel cages, drinking water and food were supplied ad libitum, and all animals were kept under the same constant environmental and nutritional conditions throughout the experiment. The animals were left 10 days before starting the experiment for acclimatization.

The experiment was done according to the general rules of the National Institutes of Health (NIH) for the Care and Use of Laboratory Animals in scientific investigations and affirmed by the Ethics of Animal Use in Research Committee (EAURC), Zagazig University, Egypt.

Fifty male albino mice were allocated into 5 equal groups, G1: colorectal carcinoma bearing mice, G2: colorectal carcinoma bearing mice treated with cisplatin, G3: colorectal carcinoma bearing mice treated with nanogold, G4: colorectal carcinoma bearing mice treated with melittin, and G5: colorectal carcinoma bearing mice treated with a combination of nanogold and melittin.

2.3 Gold Nano Particles (AuNP) preparation

AuNP prepared according to **Rajesh et al., (2012)**. Briefly, 200 ml of 1 mM HAuCl4 was boiled and stirred under the reflux condition for 30 min. 20 ml of 38.8 mM aqueous tri-sodium citrate was added directly into the boiled solution. The color of the solution changed from pale yellow to deep red within 7–10 min after the addition. Further, the reaction continued for an additional ~20 min after which 20 mg of polyvinyl pyrrolidone (PVP, M. W=40,000) in 30 ml of water was added to the above solution and stirred for the next 45 min. The solution cooled at room temperature.

2.4 Real-Time RT-PCR

Total RNA was extracted from the different tissues using the TRIzol™ reagent kit in accordance with the manufacturer's instructions (Invitrogen, Thermofisher Scientific, Waltham, MA, USA). As was previously reported, 500 ng of total RNA was used for transcription, leading to the production of mRNA (Abd El-Hakim et al., 2021; El-Shetry et al., 2021). In this case, miRNA transcription was performed using TaqMan™ Small RNA Assays (Thermofisher Scientific, Waltham, MA, USA) on 10 ng of RNA according to the manufacturer's guidelines. Primers specific to miRNAs, stem loops, and the universal reverse primer were all designed with the help of http://genomics.dote.hu:8080/mirnadesigntool (viewed on 10 September 2020) assay design software (Czimmerer et al., 2013). Sangon Biotech (Beijing, China) kindly supplied a list of the primers used in this investigation. For real-time PCR, we used the Maxima SYBR Green/Rox qPCR 2X Master Mix from

Thermofisher Scientific in Waltham, MA, USA. Each gene's relative expression was calculated using the $2^{-\Delta\Delta Ct}$ technique, with mRNA and miRNA normalized to housekeeping β-actin and U6, respectively (Livak and Schmittgen, 2001).

3.5 Histopathological Examination and immunohistochemical analysis.

Fixation and tissue processing: The formalin preserved spleen, liver and bone marrow tissues were processed in an automated tissue processor. The processing consisted of an initial 2 steps fixation and dehydration. Fixation comprising tissue immersion in 10% buffered formalin for 48 hours, followed by removal of fixative in distilled water for 30 minutes. Dehydration was then carried out by running the tissues through a graded series of alcohol (70%, 90%, and 100%). The tissue was initially exposed to 70% alcohol for 120 minutes followed by 90% alcohol for 90 minutes and then two cycles of absolute alcohol, each for one hour. Dehydration was then followed by clearing the samples in several changes of xylene. It consisted of tissue immersion for an hour in a mixture comprising 50% alcohol and 50% xylen. followed by pure xylene for one and a half hour. Samples were then impregnated with molten paraffin wax, then embedded and blocked out. Slices of 5 µm thick deparaffinized pancreatic tissue were incubated with 3% H_2O_2 for 30 min, after which they were treated for 1 h at 37 °C with anti-cytokeratin antibody (Affinity Bioscience Inc., China, 1:200) according to the manufacturer's instructions. The slices were then rinsed twice with PBS before being treated with the secondary antibody for 20 minutes. Following the avidin-biotin-peroxidase immunohistochemistry procedure established by Hsu et. al. **(Hsu et al., 1981)**, the slices were washed in PBS and stained with diaminobenzidine (DAB).

3.6 Statistical analysis:

The data were analyzed using the statistical package program SPSS version 23 Software for Windows. The results were expressed as mean ± standard error of studied groups and using the analysis of variance test (one-way ANOVA. If the p-value is less than 0.05, we reject the null hypothesis that there is no difference between the means and conclude that a significant difference does exist. Means with the same letter are not significantly different from each other. **(Corp IBM, 2021)**

3. Results

3.1 Effect of cisplatin, Nanogold, and melittin on the relative expression of PI3K in colorectal carcinoma-bearing mice:

The demonstrated study showed that The PI3K pathway extends beyond directly regulating cancer cell proliferation and survival. In B-cell malignancies, targeting PI3K purges the tumor cells from their protective microenvironment. The expression of PI3K was dramatically downregulated in mice bearing human colorectal treated with cisplatin, nanogold, bee venom, and a combination of nanogold, and **melittin** respectively than the control group (Fig. 1).

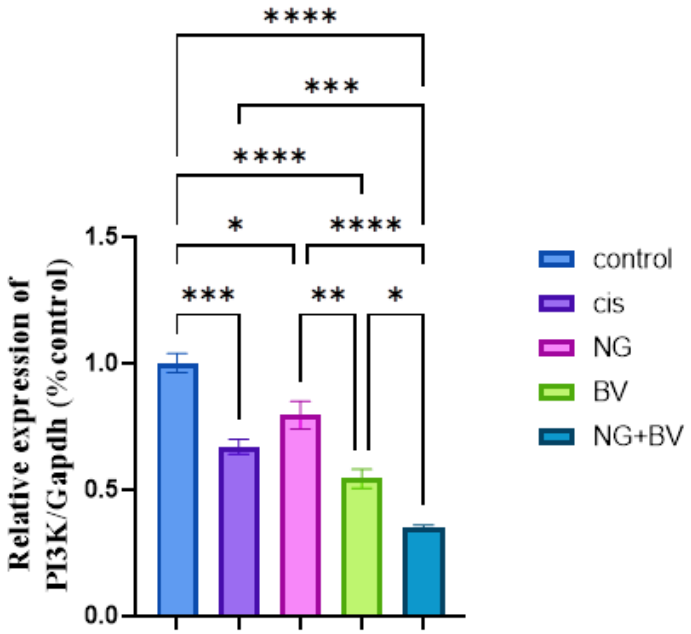

Fig. 1 Effect of Intraperitoneal (I/P) administration of cisplatin, Nanogold, and/or Bee venom on the relative expression of PI3K in colorectal carcinoma-bearing mice. values are the mean of 6 mice per group ± S.E.M. *P<0.05 , **P<0.01 , ***P<0.001 and ****P<0.0001

3.2 Effect of cisplatin, Nanogold, and melittin on the relative expression of AKT in colorectal carcinoma-bearing mice:

Also, this study examined that AKT over-expression initiated the development of colon cancer as shown in (Fig 2). The results illustrated that the expression level of AKT significantly decreased in mice bearing colorectal carcinoma group otherwise increased in groups treated with Cisplatin, nanogold, **melittin,** and a mixture of nanogold with **melittin (Fig 2).**

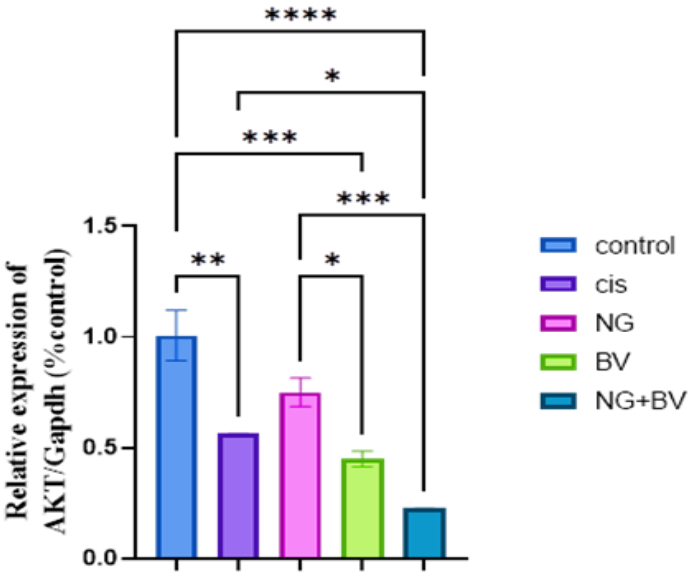

Fig. 2 Effect of Intraperitoneal (I/P) administration of cisplatin, Nanogold, and/or Bee venom on the relative expression of AKT in colorectal carcinoma-bearing mice. values are the mean of 6 mice per group ± S.E.M. *P<0.05, **P<0.01 , ***P<0.001 and ****P<0.0001

3.3 Effect of cisplatin, Nanogold, and melittin on the relative expression of PTEN in colorectal carcinoma-bearing mice:

The presented study proved that Cisplatin, nanogold, **melittin,** and a mixture of nanogold with **melittin** administration in colorectal carcinoma-bearing mice **significantly upregulated the expression of PTEN respectively than the colorectal carcinoma-bearing mice in order (Fig 3).**

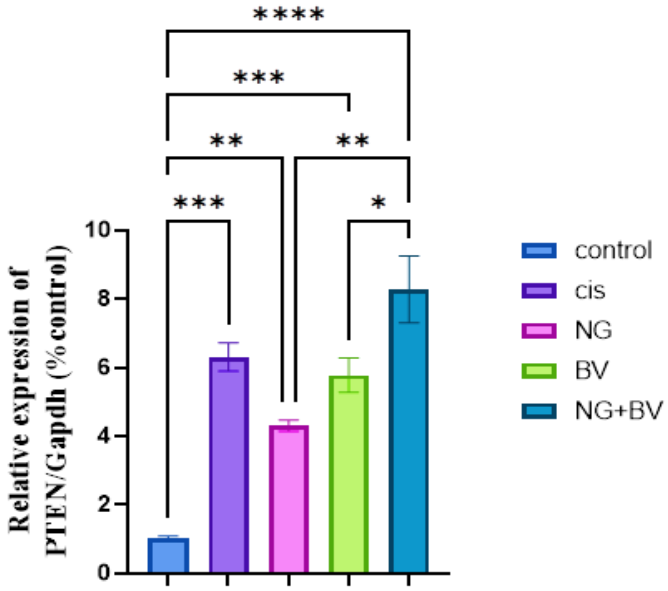

Fig. 3 Effect of Intraperitoneal (I/P) administration of cisplatin, Nanogold, and/or Bee venom on the relative expression of PTEN in colorectal carcinoma-bearing mice. values are the mean of 6 mice per group ± S.E.M. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001.

3.4 Effect of cisplatin, Nanogold, and melittin on the relative expression of mTOR in colorectal carcinoma-bearing mice:

The result of the current study illustrated that mTOR was implicated in the direct regulation of cancer cell proliferation and survival. Since, the expression level of mTOR was significantly ($p < 0.01$) downregulated with the intraperitoneal administration of cisplatin, nano-gold, melittin, and a combination of nano-gold with melittin in colorectal carcinoma-bearing mice respectively than the control group (Fig. 4).

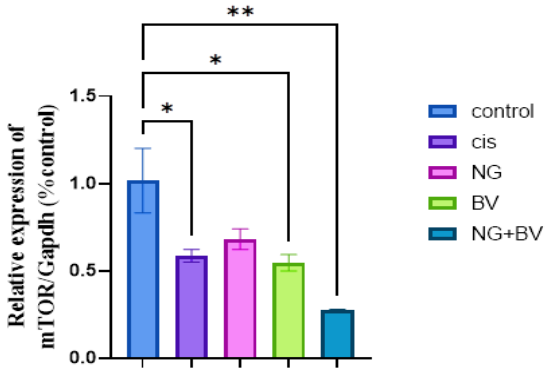

Fig. 4 Effect of Intraperitoneal (I/P) administration of cisplatin, Nanogold, and/or Bee venom on the relative expression of mTOR in colorectal carcinoma-bearing mice. values are the mean of 6 mice per group ± S.E.M. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001.

3.5 Effect of cisplatin, Nanogold, and melittin on the relative expression of P53 in colorectal carcinoma-bearing mice:

The results of the current investigation revealed that the administration of cisplatin, nanogold, and/or melittin in colorectal carcinoma-bearing mice induced a significant upregulation ($p < 0.001$) in the expression level of the TP53 than the control group with an outperforming result for the combination than the other treated groups (Fig 5).

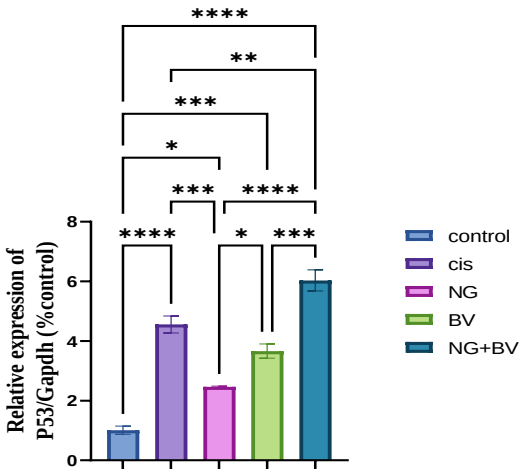

Fig. 5 Effect of Intraperitoneal (I/P) administration of cisplatin, Nanogold, and/or Bee venom on the relative expression of P53 in colorectal carcinoma-bearing mice. values are the mean of 6 mice per group ± S.E.M. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001.

3.6 Effect of cisplatin, Nanogold, and melittin on the relative expression of mir-21 in colorectal carcinoma-bearing mice:

The results of the current investigation revealed that the administration of cisplatin, nanogold, and/or melittin in colorectal carcinoma-bearing mice induced a significant downregulation ($p < 0.001$) in the expression level of the mir-21 than the control group with an outperforming result for the combination than the other treated groups (Fig 6).

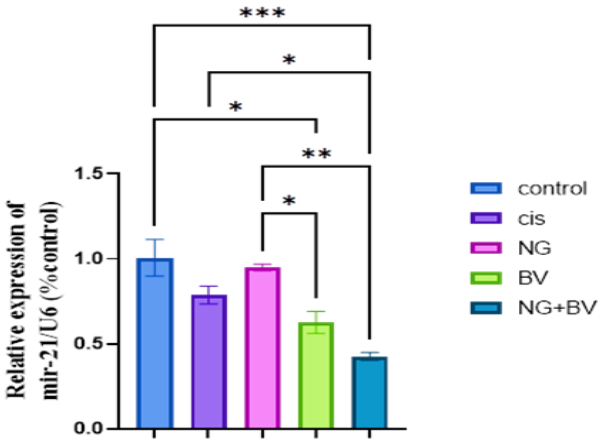

Fig. 6 Effect of Intraperitoneal (I/P) administration of cisplatin, Nanogold, and/or Bee venom on the relative expression of mir-21 in colorectal carcinoma-bearing mice. values are the mean of 6 mice per group ± S.E.M. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001.

3.7 Effect of cisplatin, Nanogold, and melittin on the immunohistochemical expression of cytokeratin as a cancer biomarker in colorectal carcinoma-bearing mice:

Immune-stained sections from different organs of colorectal carcinoma-bearing mice revealed a strong positive brownish cytoplasmic staining reaction in the viable peritoneal tumor mass and the metastatically deposited tumor cells involving the brain tissue, liver, and spleen. Other organs were free from any tumoral deposits. (Fig.7).

Investigated immune-stained tissues of the cisplatin-treated group demonstrated partial therapy effect of the cytotoxic routinely used chemotherapeutic agent as some of the intraperitoneal tumor mass cells appeared viable with moderate brownish cytoplasmic staining reaction. Metastatic malignant deposits of the used Colo-rectal cell line were seen involving some of the pulmonary blood vessels. Other examined organs appeared free from any malignant deposits (Fig.8).

Immune-stained tissue sections of Nano Gold treated group demonstrated characteristic remedy effect of the used technologically prepared nano-gold material as most parts of the intraperitoneal tumor mass cells appeared markedly degenerated and or necrotic or apoptotic with only a few viable cells showing a brownish cytoplasmic staining reaction. All other examined organs appeared free from any malignant deposits (Fig.9).

Investigation of immune stained tissue sections of the Bee Venom treated group demonstrated mild to moderate remedy effect of the used bee venom compound as most parts of the intraperitoneal tumor mass cells appeared viable and strongly reacted with pan-cytokeratin specific marker (brownish cytoplasmic staining reaction). Intravascular malignant cell permeation of some peritoneal blood vessels was seen. Moreover, the metastatic deposit was recorded in some of the pulmonary blood vessels. Other examined organs appeared free from any malignant deposits (Fig.10).

Examined sections from different organs of the Nanogold and Bee Venom treated group. pointed out marked synergistic cytotoxic response of the used compounds against the malignant cells of the intraperitoneal tumoral mass and some of the occasionally seen metastatic cells. Such effect was represented by complete shrinkage, necrosis, and apoptosis of the tumoral mass with total and or partial disappearance, a part of very few viable cells that appeared mildly to moderately react to the specific pan-cytokeratin marker. Nearly the same remedy effect was clear in hepato-portal metastatic malignant cells. Other examined organs were normal and free from any metastatic deposits. (Fig.11).

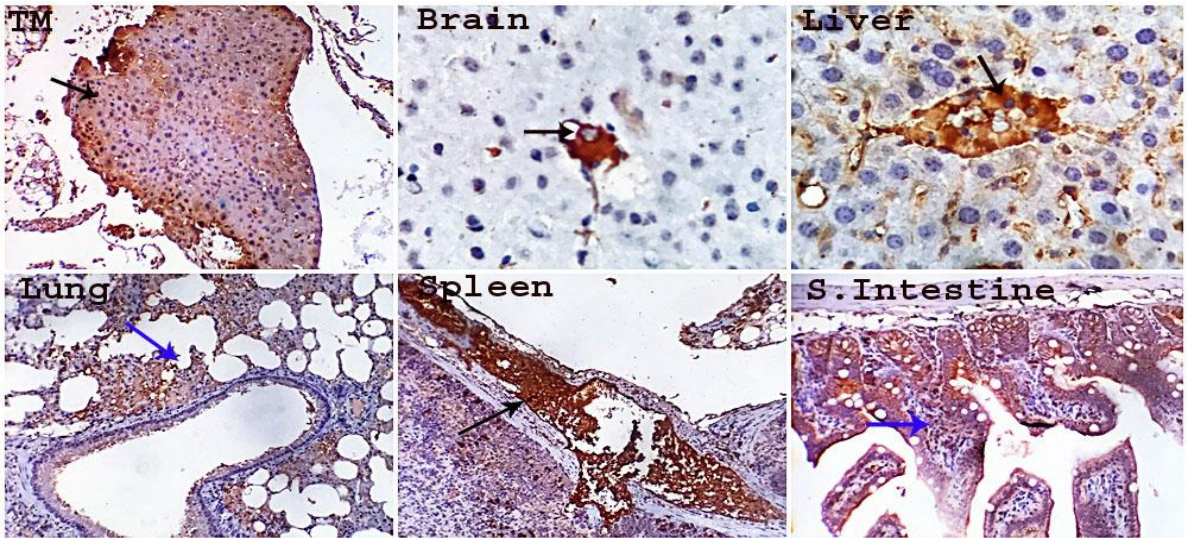

Fig.7 . Photomicrograph from different organs of (Carcinoma -Cell line , control positive group) immune-stained with pan-cytokeratin ,CK, showing strong positive brownish cytoplasmic staining reaction in the viable peritoneal tumor mass and in the metastatically deposited tumor cells involving the brain tissue, liver and spleen (black arrows). Other organs were free from any tumoral deposits(blue arrows)

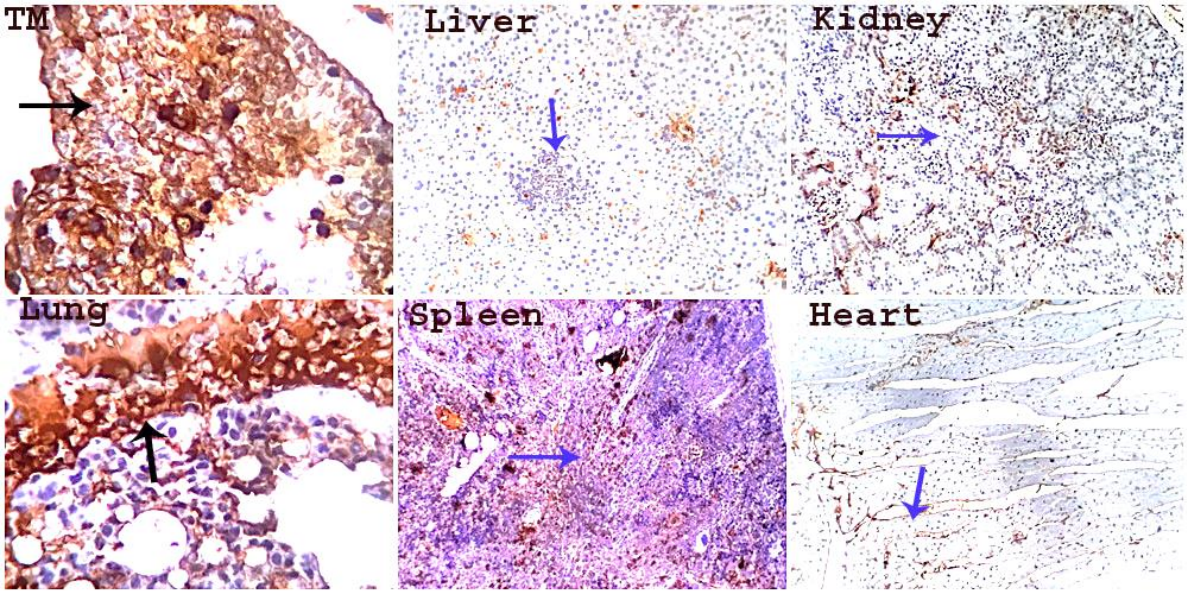

Fig.8 Photomicrograph from different organs of (Carcinoma Cell-Line -Cisplatin treated group) immune-stained with pan-cytokeratin,CK, showing some of the intraperitoneal tumor mass cells appears viable with moderate brownish cytoplasmic staining reaction(black arrow) . Metastatic malignant deposits of the used Colo-rectal cell line are seen involving some of the pulmonary blood vessels(black arrow) . Other examined organs appears free from any malignant deposits(blue arrows). X100, 400.

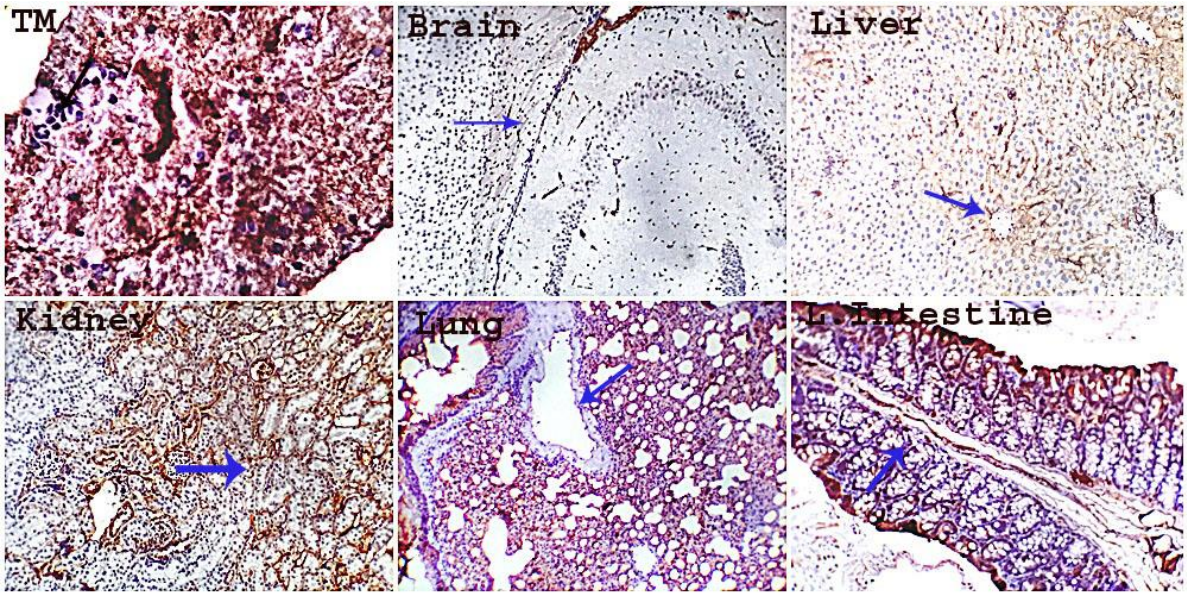

Fig.9 Photomicrograph from different organs of (Carcinoma Cell-Line -Nano-gold treated group) immune-stained with pan-cytokeratin,CK, showing characteristic remedy effect of nano-gold material as most parts of the intraperitoneal tumor mass cells appear markedly degenerated and or necrotic or apoptotic with only a few viable cells showing brownish cytoplasmic staining reaction(black arrow). All other examined organs appeared free from any malignant deposits(blue arrows). X 100 , 200 , 400.

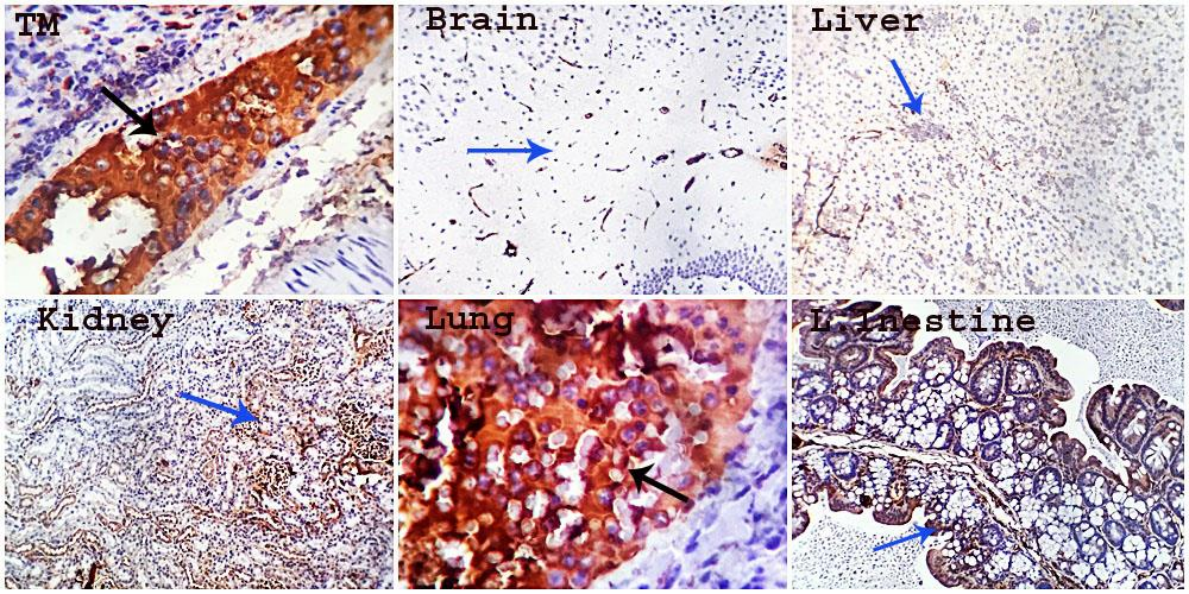

Fig.10 Photomicrograph from different organs of (Carcinoma Cell-Line -Bee venom treated rats) immune-stained with pan-cytokeratin ,CK, showing mild to moderate remedy effect as intravascular malignant cells permeation of the some peritoneal blood vessels is seen (black arrow). Metastatic deposit appears in some the pulmonary blood vessels(black arrow) . Other examined organs appears free from any malignant deposits.(blue arrows).X 100, 200, 400.

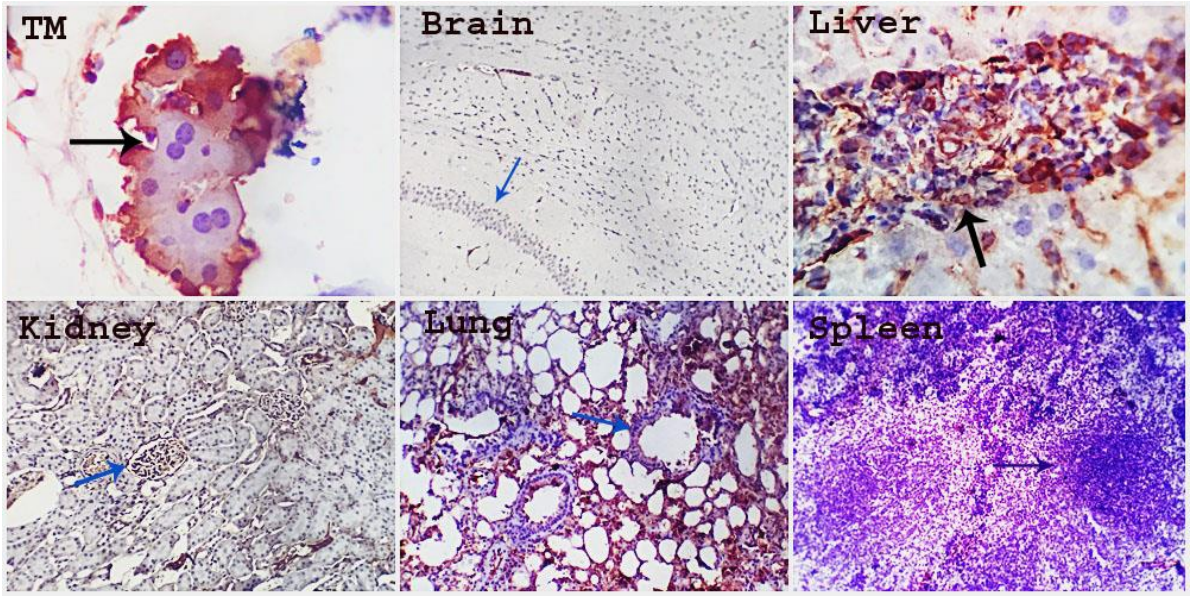

Fig.11. Photomicrograph from different organs of (Carcinoma Cell-Line -Nano gold and bee venom treated rats) immune- stained with pan-cytokeratin, CK, showing complete shrinkage , necrosis and apoptosis of the tumoral mass with total and or partial disappearance, a part of very few viable cells which appears mildly to moderately reacted to the specific pan-cyto- keratin marker(black arrow). The same remedy effect is clear in hepato-portal metastatic malignant cells(black arrow) . Other examined organs appears normal and free from any metastatic deposits(blue arrows).X 100 , 200 , 400.

4. DISCUSSION

Colorectal cancer begins when healthy cells in the lining of the colon or rectum change and grow out of control, forming a mass called a tumor. A tumor can be cancerous or benign. Cisplatin has demonstrated efficacy against various types of cancers such as germ-cell tumors, sarcomas, carcinomas as well as lymphomas. **(Camillo Porta et al, 2014)**

Nanogold is used in clinical trials to treat cancer, especially small cancers in the head area. The nanogold can be injected into the body. Once in the bloodstream, the nanogold particles will stick to the cancerous cells. Next, the nanogold is bombarded with a near-infrared laser, killing the cancerous cells and leaving the healthy cells unharmed. The activities that follow will give an introduction to lasers, nanogold, applications of nanogold, and how nanotechnology is useful for drug delivery. Recently, physicians have been using bee venom for treating patients who suffer from chronic or autoimmune diseases. Both clinical trials and lab testing confirmed that a bee venom is an excellent form of biotherapy **(Fei Ren et al, 2019).**

The PI3K, AKT, and mTOR pathways extend beyond the direct regulation of cancer cell proliferation and survival. The level of those genes up-regulates in cell line fold change in mice bearing human colorectal carcinoma while down-regulate in those taking nanogold, **melittin** and in the treated group with the combination mixture of nanogold and **melittin** as well as cisplatin-treated group Our findings were similar to those of previous studies **(Camillo Porta et al, 2014)**

P53 and PTEN prevented the development of tumors (Tumor suppressor) and our data demonstrated that there was a downregulation in the level of P53 and PTEN in mice bearing colorectal carcinoma group otherwise upregulation in groups treated with nanogold, **melittin** and in the group treated with a mixture of nanogold and **melittin** Our findings were similar to those of previous studies **(Francesca Molinari and Milo franttini 2013)** and **(Xiao-Lan li et al, 2015)**. **Tong liu et al, (2021)** investigated the role of MiR-21 in cell proliferation is the modulating expression of multiple cancer target genes was agreed with our findings as MiR-21 level was upregulated in mice bearing colorectal carcinoma groups while downregulated in groups treated with nanogold, **melittin,** and in the group treated with combination mixture of nanogold and **melittin** as well as the cisplatin-treated group.

5. conclusion

Based on the previous findings we could speculate that nanogold and/or melittin represent a valid therapeutic tool for colorectal carcinoma via modulating the expression of the mir-21 – PI3K / AKT / mTOR / PTEN – TP53 signaling pathway.

Conflicts of Interest: The authors declare no conflict of interest.

Author contribution: all the listed authors contributed equally.

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