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NOVEL THERAPEUTIC REGIMENS FOR URETHANE-INDUCED EARLY LUNG CANCER IN RATS: II CISPLATIN NANOPARTICLES COMBINED WITH CURCUMIN NANOPARTICLS ADJUVANT

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Materials & Methods: *A total of seventy two male wistar rats were included in the study. Early lung cancer was induced by urethane in the rats except for the control group. Rats were divided randomly into 7 groups (10 animals each) as follows: I-Control, II-cancer, untreated, III-cancer + free cisplatin alone IV- cancer + cisplatin nanoparticles alone V- cancer + free cisplatin + free curcumin VI-cancer + cisplatin nanoparticles + curcumin nanoparticles, VII-cancer + cisplatin nanoparticles + free celecoxcib.* ***Results:*** *Treatment of lung cancer rats with either free or nanoparticles of cisplatin alone showed a significant suppression of the plasma levels of the CYFRA21-1 tumor marker, as well as the tissue expressions of the pro-inflammatory, proliferative & antiapoptotic parameters as compared to those of group II cancer rats. Moreover , the free form of curcumin or its nanoparticles or free celecoxcib adjuvants supplementation combined with cisplatin nanoplarticles led to a further decrease of all parameters as compared to those treated with cisplatin alone, that was more evident in the group treated with cisplatin nanoparticles combined with curcumin nanoparticles adjuvant.* ***Conclusion:*** *The results of the present study emphasized the synergestic effect of cisplatin nanoparticles combined with either forms of curcumin or free celecoxib adjuvants as promising efficient new nanotherapy against lung cancer. To the best of our knowledge, it is the first study that established the urethane induced experimental lung cancer model in Upper Egypt, along with its treatment trial with cisplatin & curcumin nanoparticles adjuvant therapy*

Keywords: *Lung cancer; cisplatin; curcumin; nanoparticles*

INTRODUCTION

Lung cancer accounts for the first incidence of all malignancies in males & its mortality rate accounts for the second of all cancers.[1] The 5 year-survival with pulmonary tumors is 23% which is poor compared with other types of cancer. In 2023, the new cases and deaths from NSCLC and SCLC lung cancer in the United States are estimated to be 238, 34 and 127,070 thousands respectively. It varies markedly for patients diagnosed at local stage (61%), or distant stage (7%).[2]

Chemical induced carcinogenesis in mouse models represents an important approach aimed at the explanation of the various mechanisms relating genotype and ecological features in tumor progress, comprising pulmonary tumors. The induction of pulmonary tumors in rats with urethane led to a respected model of Kras-driven pulmonary tumors.[3] The activation of the oncogene

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Kristen rat sarcoma virus (k-ras) is an early event in non-small cell lung cancer occurrence which imitates those identified in human NSCLC.[4]

Numerous tumor screening methods now exist in experimental practice. Examples comprise low-dose computed tomography (LDCT) for prediction of pulmonary tumors.[5]

While some researches have revealed that CEA, CA-125, and CYFRA 21-1 are predictive elements for stage III-IV non-small cell lung cancer, yet, they did not explore the hepatic metastasis of pulmonary tumors.[6]

Tumor progress and its response to treatment are controlled by inflammation. Long-lasting inflammation assists cancer development and drug resistance, while induction of acute inflammatory responses regularly motivates the progress of dendritic cells (DCs) and antigen appearance, leading to anti-tumor immune response.[7]

The cancer inflammatory microenvironment encourages the appearance of a diversity of pro-inflammatory cytokines & stimulates angiogenesis & cancer development.[8]

Nuclear factor-kappa B (NF-κB) is a marker of inflammation, host immune response, cell adhesion, growth signs, cell proliferation, cell differentiation, and apoptosis resistance. NF-κB pathways are actively responsible for lung carcinoma. Moreover it shows a vital role in infective illnesses as COVID-19.[9] Moreover, several antiapoptotic genes including bcl_2 are induced by the NFkB pathway that promotes cell survival & tumor development.[10]

The protein kinase B (PKB or AKT) is a serine / threonine kinase acts as a downstream effector of the PI3K. AKT controls numerous cellular procedures such as cell proliferation and existence, metabolism, cancer development, and metastasis.[11]

Cisplatin (cp-diamine dichloroplatinum II), the frequently employed antineoplastic agent, is the key drug used in the management of various tumors including pulmonary tumors.[12]

Owing to the adverse effects, treatment relapse or toxicity associated with cisplatin, more recent drug trials have focused on natural sources that have minimal side effects.

Curcumin, being a nontoxic, highly antioxidant & antiinflammatory, has been confirmed to keep good therapeutic & pharmacologic effects against lung cancer.[13] It has been offered to raise the treatment efficacy & oral bioavailability of other therapeutics.[14]

The clinical advantage of the selective cyclooxygenase-2 inhibitor, celecoxib, sharing with antitumor treatment in progressive non-small-cell lung cancer (NSCLC) is still undistinguishable. A meta-analysis was achieved to report the effectiveness and safety of celecoxib in sick people with progressive NSCLC. Celecoxib don't assist survival of patients with progressive NSCLC but enhanced the objective response rate (ORR) of primary stage management.[15]

With the beginning of nanotechnology, it was probable to design cisplatin or curcumin – encapsulated polymer nanoparticles with a small bulky size, enhanced & improved bioavailability.[16]

The anti-tumor properties of curcumin itself or associated with cisplatin, disrupted the cell proliferation cycle, transcription and growth factors, inflammatory cytokines, protein kinases, and oncogenic fragments.

Combination of diverse therapeutic factors similar to curcumin or celecoxcib using platinum medications as cisplatin for cancer treatment has been shown to potentiate the susceptibility of the resistant tumor cells to the effect of chemotherapy.[17,18]

The aim of this research was to evaluate cisplatin nanoparticles with curcumin nanoparticles adjuvant as new nanotherapy for urethane induced initial pulmonary tumor in rats that may lead to a better management of the disease.

MATERIALS AND METHODS

Ethics approval

This research project was approved by the Institutional Review Board of the Faculty of Medicine, Assiut University, Assiut, Egypt (IRB no.17400006).

Substances

Urethane (ethylcarbamate) was bought from Sigma / Aldrich, USA; Trading Dynamic company, Egypt

Cisplatin (CP-diamine dichloroplatinum II) was bought from Sigma, United States of America; Chemocare corporation, Egypt.

Curcumin (1,7bis (4 hydroxy-3methoxy phenol)-1,6-heptadiene-3,5 dione) was bought from Sigma/Aldrich, USA; Trading Dynamic corporation, Egypt)

Celebrex (celecoxib) was purchased from PFizer pharmaceuticals, Egypt

Total substances used were of analytical grade purity.

Preparation of Cisplatin/PLGA nanoparticles

This was described in our previous paper E. Radwan et al.[19,21]

Preparation of curcumin/PLGA nanoparticles and its TEM photograph

It was described in a supplement for the paper.

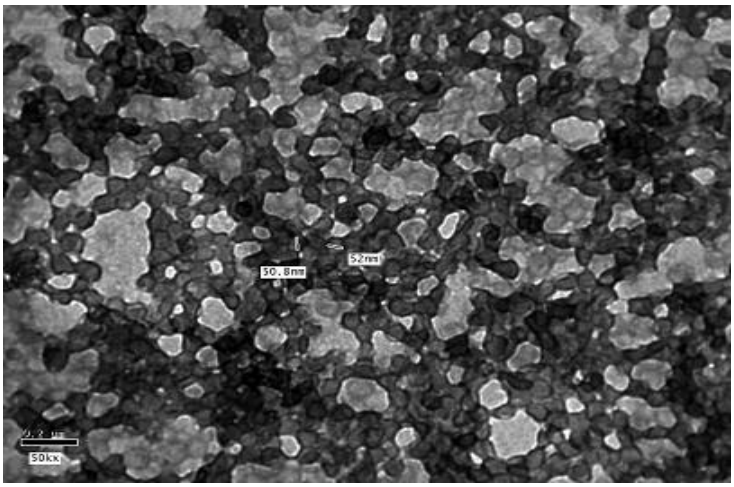


Fig. 1: Transmission electron microscope (TEM) of free PLGA.

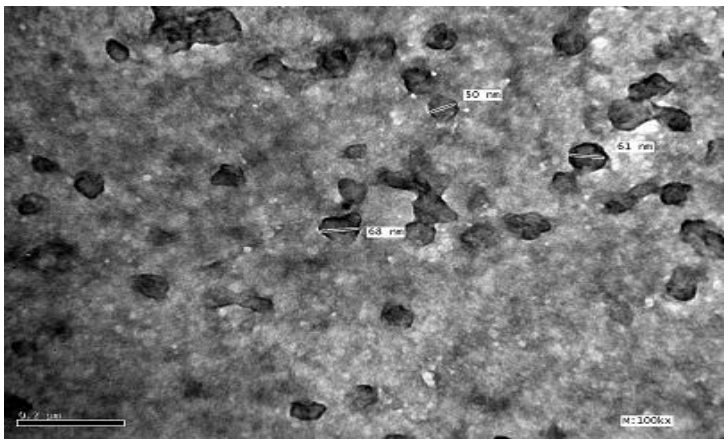


Fig. 2: TEM of Cisplatin loaded PLGA.

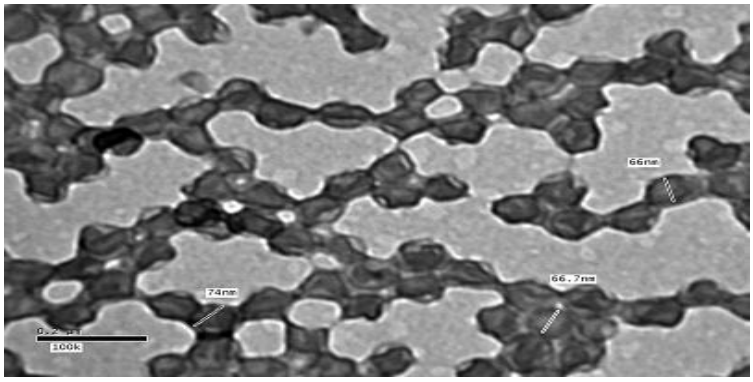


Fig. 3: TEM of Curcumin loaded PLGA.

From the **Fig.s**, it is clear that, the middle particle size of pure PLGA is in the range 50:52 nm and increased by loading of drug onto the polymer surface, where the particle mass got to 74 nm in case of curcumin loaded PLGA and reached to 68 nm in case of cisplatin loaded PLGA.

Animals

This study is an extension of the previous work [21]. It was performed on an overall of 72 adult wistar albino rats with an average weight of 110-120 g (10 weeks). They were bought from animal household, Assiut Animal Experimental unit, Medicine's College, Assiut University. Rats were conserved for 2 weeks in metal cages in animal house in a 12 hour light/dark round at a continuous temprature of 25°C & 50 -60% moisture. Entirely rats were allowed free access to regular food & water ad lipitum in the whole test duration. Rats were observed carefully for their overall health throughout the research time & were treated in agreement with the strict supervisory guidelines of the National Institution of health (USA). Totally events concerning rats were accepted by the Institution of Animal Care Group, Faculty of Medicine, Assiut University.

Control Group

- **(Group I)**: After their acclimatization for 2 weeks, completely healthy 10 control rats were chosen haphazardly; left un-treated & maintained on usual care & represented the normal negative control group.

The remaining 62 rats were taken to induce pulmonary tumors model.

Induction of primary lung tumors

Fig. (4) represents the protocol of the study design. Rats were administered 4 intra-peritoneal (I.P.) injections of urethane of 0.375 g/kg/throughout twelve weeks duration with an interval of three weeks (between each injection)[22] .Three weeks next to the 4th dosage of urethane injection, two rats were killed & pulmonary tumors were proven histo-pathologically in them.

N.B. It is noteworthy to mention that another two extra control groups (10 rats each) were prepared as follows:

10 control rats, got acetonitrile alone (the vehicle of nanoparticles polymer) in a dosage of 5 mg/kg/week for 3 weeks by oral gavage.

10 control rats, got cisplatin nanoparticls alone in a dosage of 5 mg/kg/week for 3 weeks by oral gavage.

Then, they were omitted from the study after they proved histopathologically & biochemically to have non-significant changes from the healthy control group.

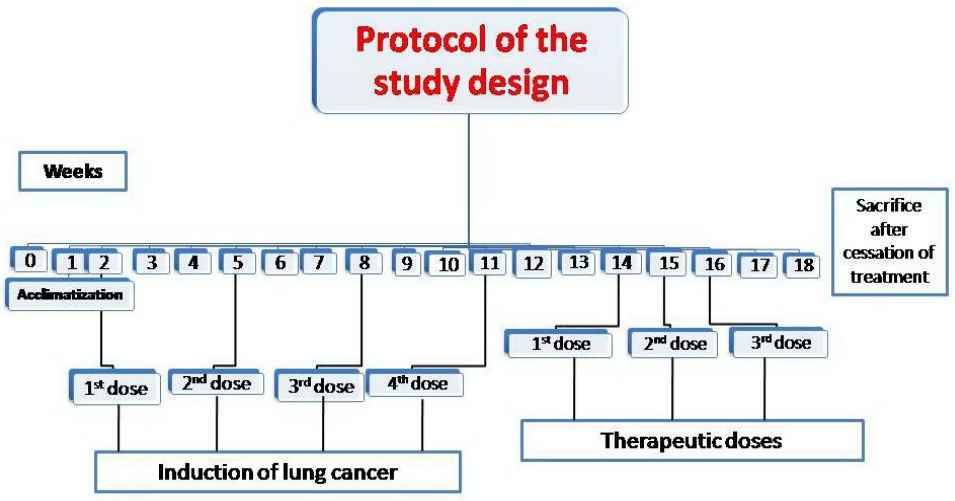


Fig. 4: The protocol of the study design.

The residual 60 cancer rats were separated into further 6 groups as follows:

- **Group II:** 10 rats with pulmonary tumors were left without management & characterize the tumor positive control group. They were normally maintained till the end of the research.
- **Group III:** 10 rats with pulmonary tumors, injected I.P. with free cisplatin of 5 mg/kg/ week for 3 weeks duration.[23]
- **Group IV:** 10 rats with pulmonary tumors, supplemented with cisplatin nanoparticles of 5 mg/kg/week for three weeks by oral gavage.
- **Group V**: 10 rats with pulmonary cancer, received I.P. free cisplatin (in the same previous dose) plus free curcumin in a dose of 200 mg/kg/twice a week for 3 weeks.[24]
- **Group VI:** 10 rats with pulmonary tumors, supplemented with cisplatin nanoparticles (in the similar earlier amount) plus curcumin nanoparticles in a dosage of 200 mg/kg/twice a week for 3 weeks orally.
- **Group VII:** 10 rats with pulmonary tumors, supplemented with cisplatin nanoparticles (in the similar earlier amount) plus free celecoxcib in a dosage of 5 mg/kg/week for 3 weeks orally. The smallest oral dosage of celecoxcib was elected to decrease the probability of undesirable actions that might be related to the higher doses in different organs including the lung.[25,26]

Sampling

After 2 weeks from the end of the treatment intervention period, blood specimen of each rat in each group was drawn (from the retro –orbital vein) on EDTA coated tubes & the plasma were separated by centrifugation & kept subdivided at -20^{o}C for subsequent assessments. Then, rats were killed by cervical dislocation. The entire upper & medium portions of right lung of every rat were separated, washed with cold phosphate buffered saline, blotted and marked then saved at -80^{o}C in liquid nitrogen prior to RNA extraction.

Histopathology

The inferior part of right lung was dissected from every rat directly after they were died, pulmonary samples were fixed in 10% neutral buffered formalin, dehydrated in ordered alcohol sequences, cleared with methyl benzoate & embedded in paraffin wax. Five microns thick sections were cut. Sections were marked with hematoxylin & eosin (H & E).

Biochemical analysis

Real-Time Quantitative (PCR) and Enzyme-Linked Immunosorbent Assay (ELIZA)

Real-time quantitative PCR

Frozen lung tissue specimens were homogenized using a rotary homogenizer. Total RNA was extracted using Pure Link RNA minikit (Catalog No. 12183020, Ambion-Life technologies) following the manufacturer's instruction. The RNA purity and concentration was determined using a Biotek Nanodrop system. cDNA was synthesized using high capacity reverse transcription kit (Catalog No. 4368814, Applied biosystems) and subjected to quantitative polymerase chain reaction (qPCR) that was performed in a Step One Plus Real-time PCR system (Applied biosystems) using Maxima SYBR Green qPCR Mastermix (Catalog No. K0251, Thermo Fischer Scientific). A two-step reaction protocol was used with an initial denaturation of 1 min at 95°C, followed by 40 cycles of 95°C for 15 s, then 60°C for 1 min. The primers used are represented in **table .** β actin was utilized to normalize expression data. Results were expressed as fold change by the $2^{-\Delta\Delta CT}$ method.

Enzyme-linked immunosorbent assay and kits

Multi drug resistance protein-1 was measured by a Rat Multi drug resistance protein1 (ABCB1) ELISA, while Cytokeratin 19 protein was measured by a Rat Cytokeratin 19 (CK-19/KRT 19) ELISA kit. Both kits were purchased from SinogeneClon Biotech Co., China. and used according to the manufacturer's instructions (Catalogs no. SG-21162 and SG-20500, respectively).

Table : Primers used in qPCR experiments.

β-actin	**F: (5′-TGTTGTCCCTGTATGCCTCT-3′**
	R: (5′-TAATGTCACGCACGATTTCC-3′)
BCL2	**F: (5′-ATCGCTCTGTGGATGACTGAGTAC-3′)**
	R: (5′-AGAGACAGCCAGGAGAAATCAAAC-3′)
NF-κB	**F: (5′-ACAACCCCTTCCAAGTTCCCT-3′)**
	R: (5′-TGTGGGCGACTT CATCCT-3′)
AKT	**F: (5′-CCGCTATTATGCCATGAAGAT-3′)**
	R: (5′-TGTGGGCGACTT CATCCT-3′)

Statistical analysis

It was done by Graph pad prism program, v5. Data are expressed as the mean values of estimated parameters ± SEM. One way analysis of variance (ANOVA) was used for comparing groups, tailed by Tukey's multi comparison test. Values of P<0.05 were considered significant.

RESULTS AND DISCUSSION

Results

I- Cytokeratin -19 tumor marker (CYFRA21-1)

Acquired resistance to the cytotoxic anticancer agents has been observed. The drug resistance was evaluated in this study by MDR-1.

Urethane therapy caused a significant rise in the mean plasma CYFRA 21-1 in the tumor group as compared to that of controls (P< 0.001) & other groups (P<0.05), **table (1) & Fig. (5)**. The cytostatic free cisplatin treatment to the cancer group (III) led to a significant rise in CYFRA 21-1 plasma level as compared to other cancer treated groups. Supplementation of cisplatin nanoparticles either alone or associated with curcumin nanoparticles or celecoxcib adjuvant therapy (groups V to VII) showed normalization of levels when compared to the control group, with a non-significant reduction in comparison to the free cisplatin group. The lowest value was exhibited by the VI group that was treated with both cisplatin & curcumin nanoparticles.

Table 1 : The levels of inflammatory, proliferative,antiapoptotic, tumor & drug resistance markers in different study groups of a rat model of lung cancer.

	Controls	Cancer group	Cancer + cisplatin group	Cancer + N- cisplatin group	Cancer + N-Cisplatin + Curcumin group	Cancer + N-Cisplatin + N curcumin group	Cancer + N-Cisplatin+ Celecoxib group	P value
NFkB	1.07	27.96	11.02	5.34	1.15	0.53	1.57	<0.0001
	0.24	4.04	0.73	0.63	0.40	0.34	0.75	
Akt	1.97	8.36	4.26	3.69	0.66	0.20	1.03	< 0.0001
	0.15	1.24	0.41	0.54	0.10	0.08	0.21	
BCL2	1.18	11.48	5.62	2.85	0.82	0.46	0.80	< 0.0001
	0.43	1.26	0.47	0.25	0.10	0.06	0.27	
CK19	259.40	673.80	514.20	325.00	349.40	290.00	303.80	< 0.0001
	20.75	49.73	19.38	28.61	22.29	21.23	39.95	
MDR-1	1082.00	1344.00	1541.00	1164.00	1195.00	965.60	1113.00	< 0.002
	31.04	154.50	134.20	76.03	38.30	94.01	54.08	

Data are presented as mean ± SEM, ANOVA was used for comparison between groups followed by Turkey's multiple comparisontest. P-value is considered significant when < 0.05.

N-cisplatin: cisplatin nanoparticles, N-curcumin: curcumin nanoparticles, NFkB: Nuclear factor kappa B, AKt: protein kinase B, Bcl_2 :B- cell lymphoma -2, CK19: Cytokeratin-19, MDR-1: multidrug resistance-1.

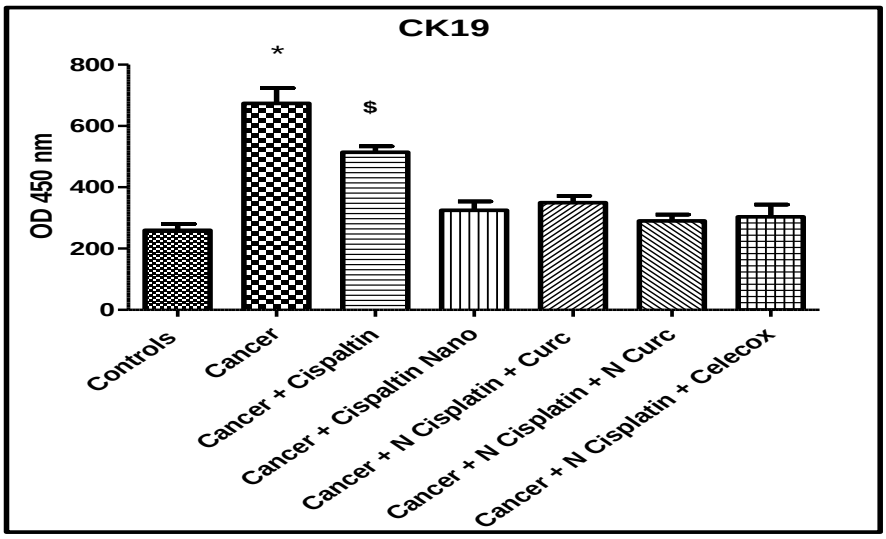


Fig. 5 : Levels of cytokeratin 19 in different study groups of a rat lung cancer model.

Data are presented as mean ± SEM, ANOVA was used for comparison between groups followed by Turkey's multiple comparisontest. P-value is considered significant when < 0.05.

*compared to other groups

$ Related to other tumor treated groups except free cisplatin treated cancer group

N-cisplatin: cisplatin nanoparticles, N-curcumin: curcumin nanoparticles, Celecox: Celecoxib free form, NFkB: Nuclear factor kappa B, AKt: protein kinase B, Bcl_2 :B- cell lymphoma -2, CK19: Cytokeratin-19, MDR-1: multidrug resistance-1.

II-The pro inflammatory biomarker (NFkB)

Treating rats with urethane initiated a significant rise in NFkB mean expression levels in group II as compared to their levels in controls & other groups (P< 0.001 each), **table (1) & Fig. (6).**

Administration of free cisplatin caused a significant decrease in their levels as compared to group II with a significant increase in comparison to other cancer treated groups (P< 0.01 each).

- The cancer groups treated with cisplatin nanoparticles plus either curcumin or free celecoxcib showed normalization of NFkB levels.
- Supplementation of tumor rats with cisplatin nanoparticles in addition to curcumin nanoparticles adjuvant, demonstrated a significant reduction in the NFkB expression levels in relation to the free cisplatin treated group (P< 0.001) which was the lowest value amongst all groups.

III-The proliferative (AKt) & antiapoptotic (bcl2) markers

The mean relative expression of AKT & bcl_2 levels were significantly increased in tumor group (II) as related to the other groups (P< 0.01 each), **table (1) & Fig.s (7, 8).**

Significant down regulation of their levels were observed in all treated groups (P< 0.05) but there was a slight activation in groups III & IV. Combination of free curcumin or its nanoparticles with cisplatin nanoparticles for cancer treatment (group V & VI), down regulates AKT & bcl_2 (P < 0 .05 & P< 0.001 correspondingly) as related to those of free cisplatin treated group.

Cancer rats supplemented with both cisplatin & curcumin nanoparticles (group VI) was found to exhibit the lowest AKT & bcl_2 expression levels that was significantly dissimilar (P<0.05) from the group supplemented with cisplatin nanoparticles alone.

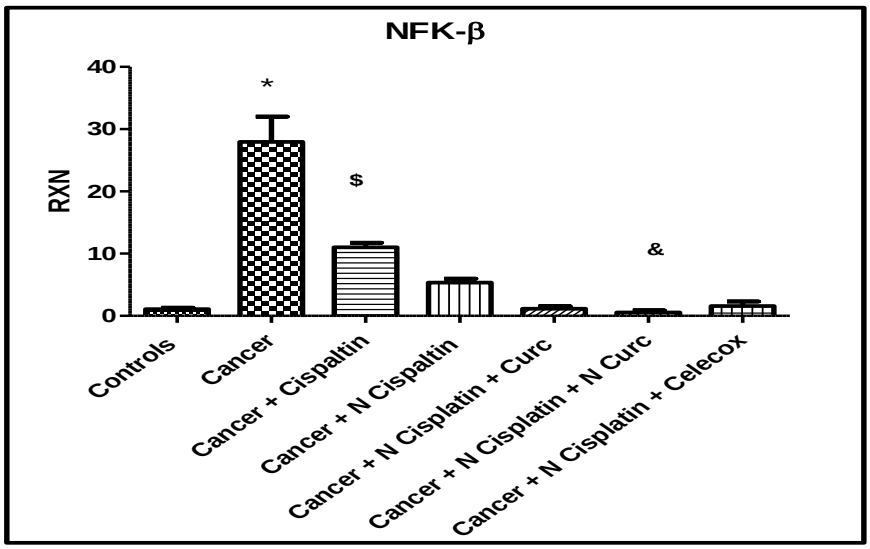


Fig. 6 : Expression of the pro inflammatory marker (NFkB) in different study groups of a rat model of lung cancer.

Data are presented as mean ± SEM, ANOVA was used for comparison between groups followed by Turkey's multiple comparisontest. P-value is considered significant when < 0.05.

*related to other groups

$ compared to other tumor treated groups

& compared to free cisplatin treated cancer group

NFK-B: nuclear factor kappa-B, N-cisplatin: cisplatin nanoparticles, Curc: Curcumin free form, N-curc: curcumin nanoparticles, Celecox: Celecoxib free form.

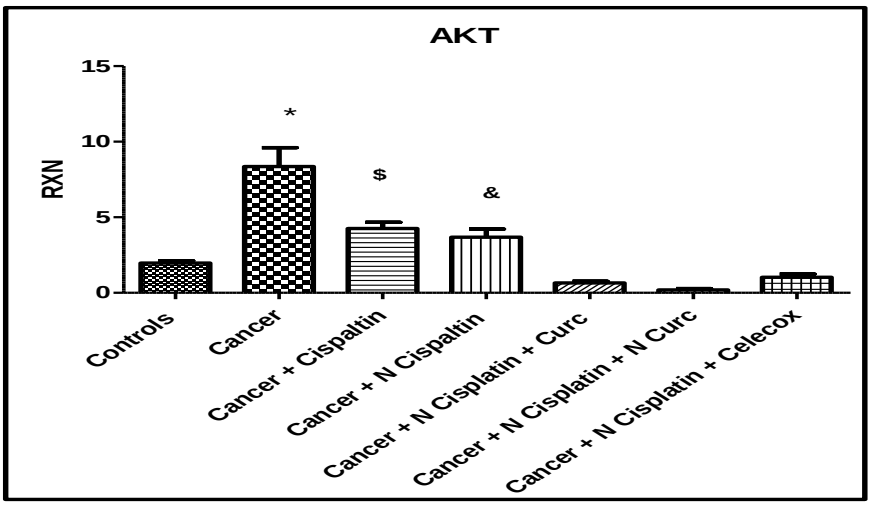


Fig. 7: Expression of the proliferative marker (AKT) in different study groups of a rat model of lung cancer.

Data are presented as mean ± SEM, ANOVA was used for comparison between groups followed by Turkey's multiple comparisontest. P-value is considered significant when < 0.05.

*related to other groups.

$ compared to other tumor treated groups except free cisplatin treated cancer group

& compared to free and nanoparticls of curcumin combined with cisplatin nanoparticles treated cancer group.

AKT: protein kinase B, N-cisplatin: cisplatin nanoparticles, Curc: Curcumin free form, N-curc: curcumin nanoparticles, Celecox: Celecoxib free form.

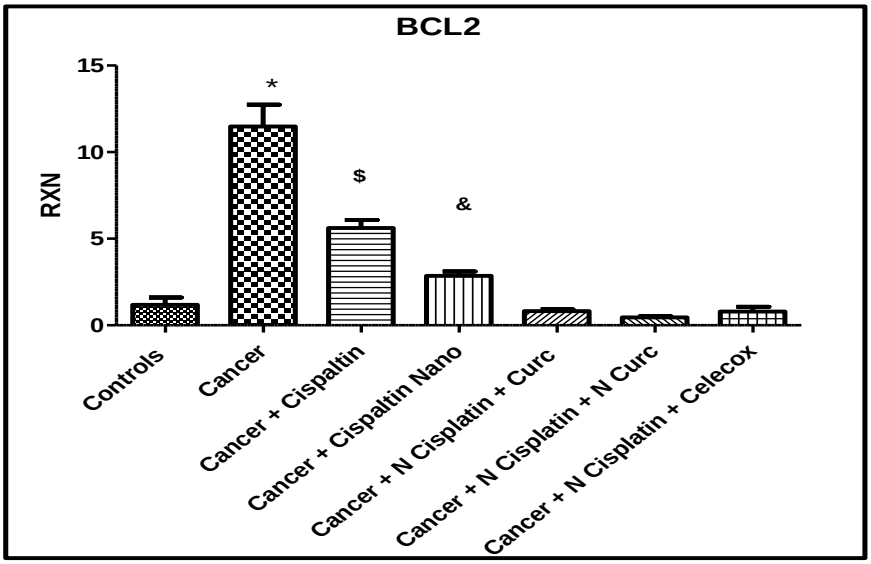


Fig. 8 : Expression of the antiapoptotic marker (bcl_2) in different study groups of a rat model of lung cancer.

Data are presented as mean ± SEM, ANOVA was used for comparison between groups followed by Turkey's multiple comparisontest. P-value is considered significant when < 0.05.

*related to other groups.

$ compared to other tumor treated groups except free cisplatin treated cancer group.

& compared to free curcumin,curcumin nanoparticls and celecoxcib combined with cisplatin nanoparticls treated cancer groups.

Bcl_2: B-cell lymphoma -2, N-cisplatin: cisplatin nanoparticles, Curc: Curcumin free form, N-curc: curcumin nanoparticles, Celecox: Celecoxib free form.

IV-The multidrug resistance -1 indicator (MDR-1)

Urethane treatment lead to a non-significant rise in the plasma levels of MDR-1 in group II in relation to that of controls, **table (1) & Fig. (9).**

Free cisplatin treatment to cancer rats, produced a significant rise ($P< 0.05$) in the mean plasma MDR-1 levels (in group III) when compared to their equivalent levels of controls & groups VI & VII. Non-significant differences were observed in the levels of other treated groups as compared to those of controls. The group treated with both cisplatin & curcumin nanoparticles exhibited the lowest level in comparison to other treated groups.

Histopathological results (Fig. 10)

Fig. 10A demonstrates the standard histological structure of the lung of a healthy rat. Histological variations related to urethane treatment are showed in **Fig.s10B, C, D, E** where adenocarcinoma , small cell carcinoma & adenocarcinoma of mixed type were detected. **Fig. 10F** indicates the necrosis & apoptosis in tumor rats treated with free cisplatin . **Fig.s 10G**&H show raised apoptosis, necrosis & hemorrhage in tumor rats treated with either cisplatin nanoparticles or free cisplatin with curcumin. **Fig. 10I** shows multiple foci of necrosis & apoptosis in tumor rats supplemented with combinations of cisplatin and curcumin nanoparticles.**Fig.10J** shows adenomatous hyperplasia with lymphocytes infiltration& hemorrhage in cancer rats treated with cisplatin nanoparticles & celecoxcib.

Lung cancer is the principal reason of tumor death & one of the higher mortality rate malignancies in population life of the universe.[13]

Despite advances in its systemic standard treatment, its late symptoms & poor prognosis necessitate the intensive research for novel effective therapeutic intervention.

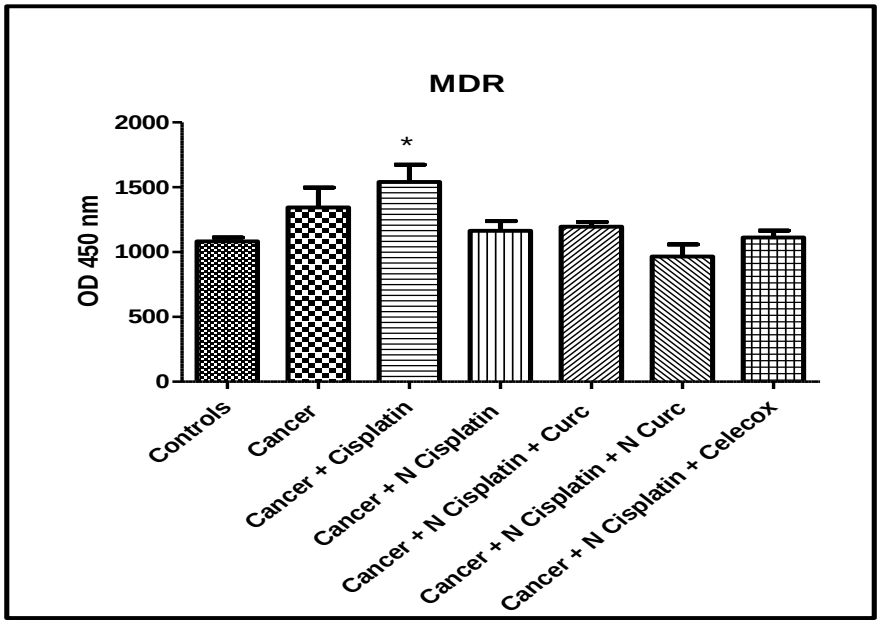


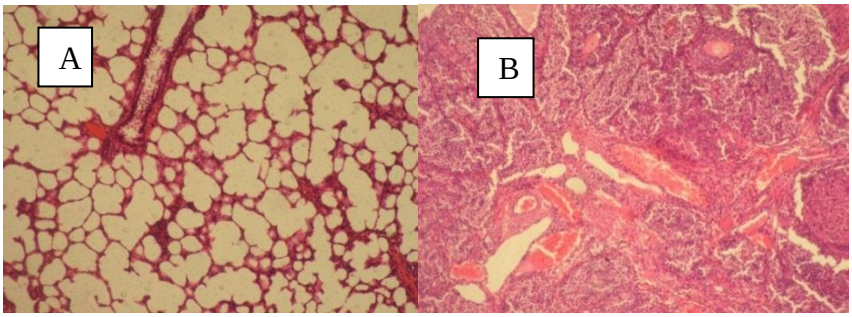
Fig. 9: Levels of multidrug resistance (MDR-1) in different study groups of a rat model of lung cancer.

Data are presented as mean ± SEM, ANOVA was used for comparison between groups followed by Turkey's multiple comparisontest. P-value is considered significant when < 0.05.

*related to controls, curcumin nanoparticls and celecoxcib combined with cisplatin nanoparticls treated cancer groups

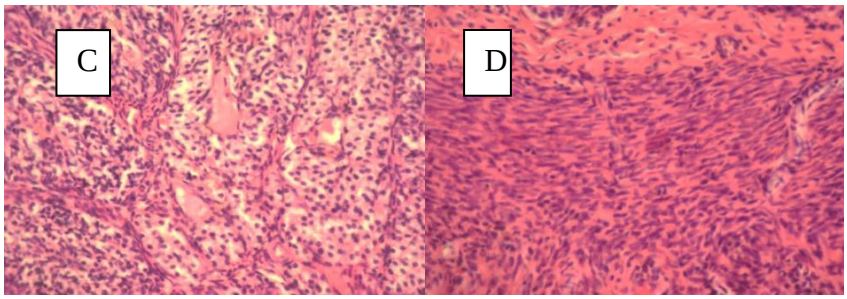
MDR-1: multidrug resistance-1, N-cisplatin: cisplatin nanoparticles, Curc: Curcumin free form, N-curc: curcumin nanoparticles, Celecox: Celecoxib free form

The Photomicrographs of lung showing the following:

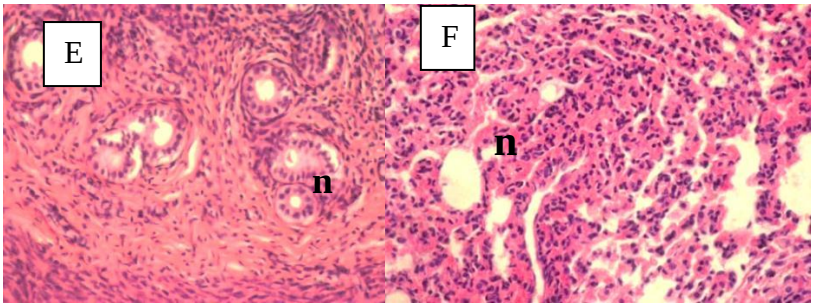


(A) Healthy histological structure from control rat.

In urethane treated rats, the lung presented variant patterns of cancer development (B) Lung adenocarcinoma.

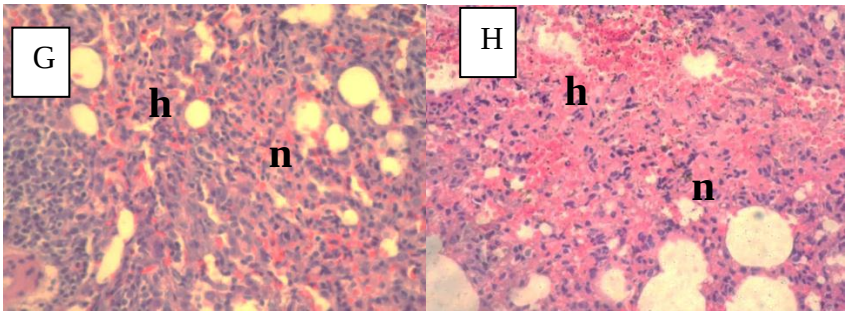


(C) bronchoalveolar growth of adenocarcinoma , (D) small cell carcinoma.

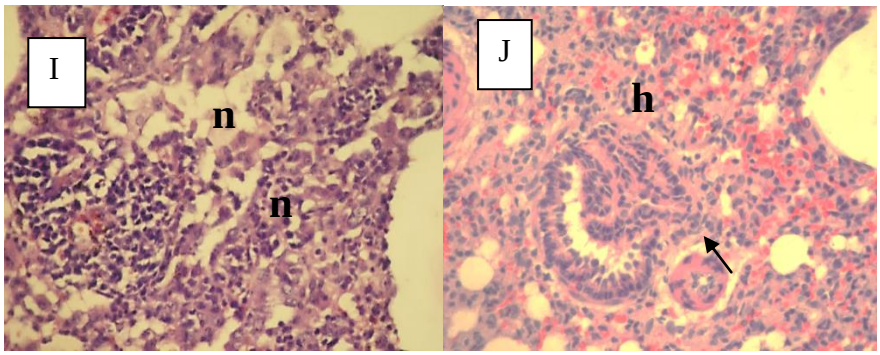


(E) adenocarcinoma of mixed type.

In the additional treated rats groups, (F) the cancer cells go through necrosis (n) or apoptosis in urethane co-treated with cisplatin rats.



(G) tumor necrosis(n) & hemorrhage(h) were observed in rats co-treated with cisplatin nanoparticles, (H) the tumor development showing severe necrosis(n) & hemorrhage(h) in urethane co-treated with cisplatin and curcumin rats.



multiple foci of necrosis or apoptosis of tumor cells (n) in lung of rat co-treated with combination of cisplatin and curcumin nanoparticles, (J) bronchial adenomatous hyperplasia with minimal infiltration of lymphocytes (arrow) and hemorrhage(h) in rats treated with urethane in association with the mixture of cisplatin nanoparticles and celecoxcib (COX 2 inhibitor).

Fig. 10: The histopathological photomicrographs of the lung

The present study established a reliable primary pulmonary tumor model in wister rats by urethane which is distinctly linked to human lung cancer. A murine tumor cell line LA-4 was formed through urethane treatment to an A/He rat and established typical appearance of pulmonary adenoma cells that were translated into the human genome and is well-matched as a model for human squamous cell pulmonary tumor.[22] The authors found that the powerful inflammatory state & activated epithelial NFkB promotes the urethane induced lung carcinogenesis.

The induction of pulmonary cancer in rats with urethane is appreciated as a model of Kras-driven pulmonary tumor. However, inborn rat strains illustrate liability to pulmonary cancer creation, which is particularly similar to that identified in human lung cancer.[32]

The early suspicious lung masses & their response to therapy were detected by cytokeratin -19 tumor marker (CYFRA 21-1).

Their mean plasma levels showed significant increase in group II rats. Moreover, free cisplatin treatment to group III caused a significant rise in CYFRA 21-1 levels as compared to other treated groups that reflects the poor response to the free cisplatin.

Cytokeratin -19 is the soluble protein constituent of the intermediate filament proteins in epithelial cells. It is released in blood as a result of malignant cells necrosis.[2]

Supplementation of cisplatin nanoparticles either alone or in combination with curcumin in either forms or celecoxib adjuvant therapies showed normalization of CYFRA 21-1 levels where the lowest value was possessed by the VI group that was treated with both cisplatin & curcumin nanoparticles.

This illustrated the respectable response of tumor cells to cisplatin nanoparticles either alone or with adjuvant therapies of curcumin or celecoxib. Serum cancer indicators can be added as harmonizing tests that predict tumors, its development and management checking. [28]

In the present research, urethane treatment caused a significant increase of the mean comparative expressions of the proinflammatory marker (NFkB) in group II. This is in line with the earlier results of Stathopoulos et al.[22] who reported an initial NFkB stimulation with an associated inflammatory state in mouse lungs after urethane treatment.NFkB signaling & crosstalk are found in numerous stages of tumor growth comprising a stimulus, long-lasting inflammation, fibrosis, formation of precancerous nich & conversion of the healthy cell to a tumor one.[29]

NFkB can promote cancer progression via induction of several antiapoptotic & cell cycle regulatory genes controlling epithelial to mesenchymal transition (EMT).[30]

The present research demonstrated a noteworthy downregulation of NFkB expression levels in rats who received free cisplatin. Furthermore, tumor groups supplemented with cisplatin nanoparticles in addition to either free curcumin or celecoxcib showed normalization of NFkB expression levels.

In addition, supplementation of cancer rats with cisplatin nanoparticles coadministrated with curcumin nanoforms showed a significant downregulation of NFkB expression levels that was the lowest value amongst other groups.

This was confirmed histopathologically by the multiple foci of increased apoptosis in rats supplemented with cisplatin & curcumin nanoparticles adjuvant therapy.

Cisplatin is a cytostatic & cytotoxic chemotherapeutic agent emerged as a front line option for several forms of tumor treatment, comprising pulmonary tumors.[12] Its mechanism of action leads to inhibition of protein synthesis in dividing cancer cells.[31,32]

Curcumin (4 hydroxy-3 methoxy phenol)-1, 6 heptatadiene-3,5dione) is a yellow phenolic spice. The Curcuma genus has an extended history of therapeutic presentations, collected from about 120 species. Amongst the Curcuma classes, Curcuma longa L. (Curcuma; Turmeric) is the extensively documented; a cultivated plant, full-grown in a warm weather, in several areas of the universe[33,34]. Its poor aquous solubility, rapid degradation & short half-life limit its efficiency as a talented healing therapy in tumor treatment. [13] To be more effective, it must be supplemented in a high concentration but patients showed low-response to its bulky treatment.[35]

Several reports indicated that curcumin can augment the anticancer effects of cisplatin, by blocking tumor initiation, promotion & invasion, inhibiting the proinflammatory cytokines & inflammatory cell signals through NFkB down regulation & its target genes (e.g. cyclin D_1 Bcl_2) in human cancer both in vivo & in vitro.[36,18,33,24] The usage of nanoparticles can beat the hydrophobic environment of curcumin, besides its steadiness success and cellular bio-availability in vitro and in vivo[37-39]. Numerous strategies for nanocurcumin construction have been established, by its individual set of features and exclusive characteristics, where most of them are still in the conceptual stage[37-39]

Cox_{-2} signaling pathway has a pivotal character in the pathogenesis of pulmonary tumors. About 70% of adenocarcinoma in NSCLC has been found with increased cox_2 expression. Previous studies have indicated that non-small cell lung cancer (NSCLC) cell lines with increased COX-2 expression were characterized by high expression of CD44 and their aggressive nature was expressively negotiated due to the effect of exact CD44

down regulators. Researches on colorectal and pulmonary tumors also demonstrated that COX-2 upregulation raises tumor invasiveness through a CD44-pathway.[40]

Consequently, inhibition of cox_2 is anticipated to be an approach to stabilize the tumor suppressor P53 functionality[41]

Growing evidence has emphasized the highly selective cox_2 inhibitor celecoxcib as a hopeful medicinal approach in a diversity of malignancies comprising lung tumors.[52,53] Rich epidemiological and preclinical/medical researches established that celecoxib, an exact COX-2 inhibitor, was correlated to the destruction of tumor cell multiplication and reduction in tumor events.[42] In addition, celecoxib is a non-steroidal anti-inflammatory drug that has shown promise in prevention of cancer and has been used as adjunct to surgery to reduce the member of adenomatous colorectal polyps in patients with familial adenomatous polyposis.

The PI_3k/AKT/mTOR pathway is related to the cell proliferation & cancer.[43]

The data of the present research demonstrated a noteworthy upregulation in both the multiplying AKT marker & anti-apoptotic bcl_2 expression values in urethane treated tumor rats, that were significantly decreased on treatment with either free cisplatin or its nanoparticles or those treated with both curcumin forms or celecoxcib adjuvant. Interestingly, this downregulation was the highest in the group treated with both cisplatin & curcumin nanoparticles in comparison with other treated groups. These findings indicate the synergistic effect between curcumin & cisplatin nanoparticles treatment that clue to the return of apoptotic role & stopping the proliferation of tumor cells in rat pulmonary tissues.

AKT activation induces cell survival in numerous types of cancer cells.[44] A residual activation of AKT was demonstrated in rats of the current study that were treated with either free or nanoform of cisplatin. The expression of JAK2 and p-STAT3 pathway was reduced by cisplatin and doxorubicin[45,46] which participate in inducing AKT gene expression.

It has been reported that curcumin can inhibit cancer development by inducing apoptosis[47] & downregulating bcl_2expression. The result of both complexes of doxorubicin and curcumin together lead to caspase-9 and Bax up regulation plus the down regulation of Bcl-2 expression,[48] several cell signaling paths & their cross talks[49].

Combination of curcumin either forms with cisplatin nanoparticles for cancer treatment in the present study, significantly downregulates AKT & in turn inhibits proliferation of cancer cells. This may be attributed to the induction of G2/M transition phase cell cycle arrest through the modulation of AKT & p38 MAP kinase & the downstream effector P53 gene.[33] Similarly, Pugazhenthi et al.[50] reported that, curcumin has a cytoprotective effect on mouse pancreatic B-cells as mediated through PI3K/AKT signaling downregulation. Moreover, Johnson et al.[51] results suggested that curcumin is a good inhibitor of PI_3K/AKT/mTOR signaling through the modulation of their expression & their phosphorylation in some cancer cell lines.

Furthermore, free celecoxcib has been reported to exert its effects through AKT signaling suppression. It promotes antiproliferative effects on colon cancer cells & reduces cancer development in vivo.[52] Earlier researches have revealed that celecoxcib can induce lung cancer apoptosis via inhibiting bcl_2 gene & maintaining the cells in G_1 phase.[53] It can enhance the activity of the standard chemotherapeutic agents by its effective treatment of resistant tumors with overexpression of bcl_2.[53]

Again, our results confirmed the fact that cisplatin nanoparticles combined with either curcumin or celecoxcib adjuvant therapy were able to exert a more pronounced effect on cancer cells as compared to the cisplatin free drug alone.

The former studies suggested that, the combined treatment of curcumin & cisplatin in NSCLC cell lines, can cause cell cycle arrest, which in turn, leads to the start of apoptosis besides the proteosomal degradation of bcl_2 that mediates cisplatin resistance & increased the P53 expression.[54,55] These drugs combination can decrease tumor mass & cancer progression.[56]

The data of the current study are in agreement with the results of previous investigators: Tomeh et al.[18] & Chakraborty et al.[13], who found that curcumin augmented the cell proliferation inhibition, enhanced the

cytotoxic activity of cisplatin & corroborated the apoptotic action of cisplatin-resistant pulmonary tumor cells.

Cisplatin & curcumin nanoparticles, carried on the PLGA & designed in a size of 50-100 nm, as in the current study, go in line with other previous investigators who found that this particle size appears promising chemopreventive therapeutic compounds against enzymatic deprivation, extended circulating period, improved solubility, sustained release, and stability, selective absorption,passive targeting into deep lung, reduced toxicity & improved efficacy as compared to the free drug[57-60]

The histopathological examination of the current research are in harmony with the biochemical outcomes, which confirmed apoptosis in rats-cancer cells co-treated with cisplatin-either froms. It was evident that apoptosis was severely increased in rats treated with curcumin adjuvant therapy with a return to the precancerous phase of NSCLC (adenomatous hyperplasia) in rats supplemented with cisplatin nanoparticles associated with free celecoxcib.

Tumor cell resistance to apoptosis appears frequently with treatment failure.[61] Cell cycle arrest in tumor cells with different p53 values induced by neoplastic therapy can be tailed by poly-ploidization and reprogramming which cause variations in the appearance of genes involved in meiosis control.[62] In the current research, drug resistance was evaluated by MDR-1 & the data revealed a noteworthy rise in the mean plasma values in rats treated with free cisplatin alone.

Cisplatin, when used as NSCLC therapy often provide a resistant pulmonary tumor cells that possess epithelial mesenchymal transition (EMT) phenotype [55]. Enhancement of drug efflux out of cells by ATP connecting cassette protein transporters lead to a restricted influx of the drug to the cell, activation of cell survival pathways e.g. DNA-repair or detoxification & blockade of apoptosis[56,59].

Normalization of MDR-1 levels in the present study was observed in the other treated groups, where the lowest value was exhibited by the group treated with both cisplatin & curcumin nanoparticles adjuvant that reflects efficient therapeutic intervention.

These results support the findings of Panda et al.[63] who suggested the usage of adjuvants combined to chemotherapy to decrease the undesirable properties of drug resistance & rise the active targeting to tumor cells.

Thus, the utility of drugs with diverse mechanisms against several signaling pathways implicated in lung cancer could provide a more effective & a better response for the selected anticancer drug[64,32].

In conclusion

The results of the existing study proposed the use of cisplatin nanoparticles combined with either curcumin nanoparticles or celecoxcib adjuvants as effective promising synergistic new therapeutic intervention for NSCLC induced by urethane in rats.

Additional researches with greater sample size & extended period are required to affirm our outcomes.

To the best of our knowledge, it is the first study that established the urethane-induced experimental lung cancer model in Upper Egypt along with its treatment trial.

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أنظمة علاجية جديدة لسرطان الرئة المبكر الناجم عن اليوريثان في الفئران: II جسيمات سيسبلاتين نانوية مقترنة بجسيمات نانوية من الكركمين

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الخلاصة:

يمثل سرطان الرئة الإصابة الأولى ومعدل الوفيات الثاني لجميع الأورام الخبيثة لدى الذكور. مظاهره المتأخرة تمنع اكتشافه في مرحلة مبكرة. ولذلك، يجب تصميم طرق علاجية جديدة لتحسين معدل الوفيات.

المقدمة:

أجريت هذه الدراسة لفحص جسيمات الكركمين النانوية المساعدة لجسيمات السيسبلاتين النانوية كعلاج نانو جديد لسرطان الرئة المبكر الناجم عن اليوريثان في الفئران والذي قد يؤدي إلى سيطرة أفضل على المرض.

المواد والطرق:

تم تضمين ما مجموعه اثنين وسبعين من فئران ويستار الذكور في الدراسة. تم إستحداث سرطان الرئة المبكر بواسطة اليوريثان في الفئران باستثناء المجموعة الضابطة. تم تقسيم الفئران عشوائياً إلى ٧ مجموعات (١٠ حيوانات لكل مجموعة) على النحو التالي:

I-Control، II-السرطان، غير المعالج، III-السرطان + سيسبلاتين حر وحده IV- السرطان + جسيمات سيسبلاتين النانوية وحدها V- السرطان + سيسبلاتين حر + الكركمين الحر VI- السرطان + جسيمات سيسبلاتين النانوية + جسيمات الكركمين النانوية، VII-السرطان + جسيمات سيسبلاتين النانوية + سيليكوكسيب الحر.

تم تقييم تأثير العلاج باليوريثان وأدوية السرطان على أنشطة الالتهاب والتكاثر وموت الخلايا المبرمج بواسطة تعبيرات NFkB و AkTوbcl2، على التوالي في أنسجة الرئة. تم تقييم مقاومة الأدوية بواسطة MDR-1 وتم اكتشاف أورام الرئة المبكرة بواسطة مستويات البلازما .CYFRA21-1 كما تم إجراء التقييم النسيجي.

النتائج:

أظهر علاج فئران سرطان الرئة إما بالجسيمات الحرة أو النانوية من سيسبلاتين وحده قمعًا كبيرًا لمستويات البلازما لمؤشر الورمCYFRA21-1 ، بالإضافة إلى تعبيرات الأنسجة للمؤشرات المؤيدة للالتهابات والتكاثرية والمضادة لموت الخلايا مقارنة بتلك الخاصة بـ المجموعة الثانية من الفئران السرطانية. علاوة على ذلك، أدى الشكل الحر للكركمين أو جسيماته النانوية أو مكملات السيليكوكسيب المساعدة الحرة مع جسيمات السيسبلاتين النانوية إلى انخفاض إضافي في جميع المؤشرات مقارنة بتلك المعالجة بالسيسبلاتين وحده، وكان ذلك أكثر وضوحًا في المجموعة التي عولجت بجسيمات السيسبلاتين النانوية جنبًا إلى جنب مع جسيمات الكركمين النانوية المساعدة.

الخاتمة:

- أكدت نتائج الدراسة الحالية على التأثير التحفيزي لجسيمات السيسبلاتين النانوية مع أي من المواد المساعدة من أشكال الكركمين أو السيليكوكسيب الحر كعلاج نانوي جديد فعال واعد ضد سرطان الرئة.
- هناك حاجة إلى مزيد من الدراسات ذات حجم أكبر للعينات ومدة علاجية أطول لتأكيد النتائج المتحصل عليها.
- إن أفضل ما نعرفه أن هذه الدراسة هي الأولى التي أنشأت نموذجًا تجريبيًا لسرطان الرئة الناجم عن مادة اليوريثان في صعيد مصر، إلى جانب تجربة العلاج بالسيسبلاتين وجسيمات الكركمين النانوية المساعدة.