Polyaniline – (Au, Ag and TiO) Nanocomposite as autophagy activator

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Abstract

The fast development of nanotechnology and the widespread use of nanoparticles has resulted in an increase in the amount of nanoparticles entering the environment. However, there has been little research on their influence on human and environmental health. In this study, Polyaniline (Ag, Au and TiO_2) nanocomposites were prepared using the polymerization method and conformed using UV-visible spectroscopy, SEM, and XRD. The anti-proliferative activity of Polyaniline (Ag, Au and TiO_2) against AMJ-13 cell lines were studied. This study suggests that Polyaniline (Ag, Au and TiO_2) Enhancing Autophagy as a therapeutic for inflammation prevention and treatment has the potential to be a powerful cancer therapy.

Keywords: polyaniline, polymerization, Nanocomposite, AMJ-13 and Autophagy.

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Introduction

Because of their unique physicochemical characteristics, such as special ultrastructure, dispersity, and effective cellular uptake properties, nanoparticles have been widely used in industry and biomedical fields for drug delivery, anti-pathogen, nanocomposite material, and diagnostic imaging applications in recent decades. However, concerns have been raised about nanotoxicity as a result of the widespread use of nanoparticles. [Li J, Fan C and Pei H, 2013], [Linko V, Ora A and Kostiainen MA, 2015]Scientists have discovered a number of pathways that contribute to nanoparticle-induced toxicity, including apoptosis, necrosis, oxidative stress, and autophagy, among other things. Autophagy, among other mechanisms, has lately been recognized as a key cell dying strategy in the context of different nanoparticle-induced toxicities. [Fu PP, Xia Q and Hwang HM, 2014], [Liu Y, Liang and J, Wang , 2016]Despite the fact that the role of Autophagy in nanoparticle-polymer toxicity was still unclear, the general process of Autophagy summarized the different functions of Autophagy in various nanoparticle-polymer in vitro models and thoroughly analyzed the physicochemical and biochemical (cellular and molecular) mechanisms of Autophagy during nanoparticle-induced toxicity through the listing and summarizing representative examples, the role of Autophagy in polymer toxicity was still unclear. Dispersity, size, charge, and surface chemistry are some of the most important physicochemical processes [Deter RL and De Duve C,1967], [Glick D, Barth S and Macleod KF, 2010], [Mizushima N, 2007]. Due to the fact that autophagy is a double-edged sword in cancer [P. Jiang ,

2015], [F. Joris, 2018], and that it plays many and even competing roles in the disease, it is well recognized that cancer cells use autophagy to resist anticancer agent-induced apoptosis [Z.J. Zhou, 2019], [Z.J. Zhou, 2017].As a consequence, the suppression of Autophagy has been proposed as a technique to make cancer cells more sensitive to chemotherapeutic drugs. In nanomedicine, one of the most potential uses of PANI-Np is cancer treatment, particularly in the next generation of cancer therapies. It has been established in our research that the efficacy of these nanoparticles may be proved by the application of a novel therapy that combines light and conductive nanomaterials, known as photothermal therapy [Bongiovanni, Molina, Rivarola, Kogan and Barbero, 2014]. In addition, there is a potential application of these nanoparticles to dissolve protein aggregates of neurodegenerative diseases [Borm,P.J.A.,2002], [Stejskal, J. andSapurina, I., 2005]. This type of polymer nano conjugated synthesis is increased and, hence, could be a potential candidate for medical applications [Stejskal, Hajná, Kašpárková, Humpolíček, Zhigunov and Trchová, 2014], [Mohamad, GadEl and Sawsan , 2013]. In this paper,It was possible to manufacture PANI-Np in the presence of polymeric stabilizers using dispersion polymerization. Because of their biocompatibility, nanoparticles were chosen to provide improved stability in aqueous conditions and to prevent precipitation of the nanoparticles. Specifically, the nanoparticles exist as an aqueous dispersion that has been stabilized by hydrophilic polymers. Because of this, we investigated the Autophagy of these dispersions.

2. experimental and material

2.1. material

AMJ-13 cells are Iraqi from patient breast cancer cell lines; the cells were kindly provided by the Iraqi Centre for Cancer and Medical Genetic Research (ICCMGR), Al-Mustansiriyah University, Baghdad, Iraq. RPMI-1640, trypsin-EDTA, dimethyl sulfoxide (DMSO), fetal bovine serum, ovalbumin, 3-(4,5-dimethylthiazal-z-yl)-2,5-diphenylterazolium (MTT), from Escherichia coli O111:B4 (Cat. No. L2630, Sigma- Aldrich, MO, USA). Silver nanoparticles from (Hongwu international group), gold nanoparticles lab synthesis, titanium dioxide nanoparticles from (Hongwu international group), aniline from (Alpha chemika), ammonium peroxidisulphate ($(NH_4)_2S_2O_8$) from (central drug house) and hydrochloric acid HCl from (Alpha chemika).

2.2. experimental work

2.2.1. Synthesis of Polyaniline

The chemical oxidative polymerization technique was used to create polyaniline by oxidative polymerization of aniline in the presence of hydrochloric acid as a catalyst and ammonium peroxidisulphate as an oxidant in the presence of hydrochloric acid. The synthesis consisted of 5 ml 1M HCl and 1.5 ml aniline, which were combined in a 55ml flask connected with an electromagnetic stirrer and allowed to sit overnight. Then, in a burst of energy, 6gm of (NH4)2S2O8 (ammonium peroxidisulphate) in 20 ml of 1M HCl was abruptly added to the previously prepared solution. The polymerization temperature of 0° C was maintained for 2 hours to ensure that the polymerization process was completed successfully. The precipitate that was produced was then filtered. The product was washed with 1M HCl, followed by distilled water, in this order, until the wash solution became colorless. After that, it was re-filtered and completely washed with distilled water, yielding the emeraldine salt(ES) form of polyaniline as a result of the process. It was necessary to dry the solution for 24 hours at 60° C in a vacuum oven in order to get the emeraldine base (EB) form of PANI. As a

result, a powder of insulating polyaniline (EB) polymer was obtained [Zhang, Wan , Wei and Synth.2005] at the end of the process.

2.2.2. preparation of Polyaniline (Ag, Au and TiO_2) nanocomposite

Anline powder slowly was diluted in an ultrasonic device and then stirred in a magnetic stirrer. Polyaniline protected Ag, Au, and TiO_2were synthesized by interfacial polymerization method, wherein different ratios of Ag, Au and TiO_2 (0.05, 0.1) dispersed in aniline resin.

2.2.3. Maintenance of cell cultures

AMJ13 cells were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 g/mL streptomycin for 24 hours. A trypsin-EDTA solution was used to passage the cells, and the cells were reseeded at 80 percent confluence twice a week and incubated at 37 °C[Al-Ziaydi, Hamzah, Al-Shammari, Kadhim, and Jabir, 2020], [Majid S. Yasmin, Ghassan, Nahi , Usama , Yaser, 2021].

2.2.4. Flow cytometry assay for Autophagy marker LC3

By incubating 1 g/mL of a rat anti-mouse CD16/CD32 antibody at 4° C for 30 minutes, nonspecific Fc-mediated binding of antibodies to Fc receptors was inhibited and performed. The level of LC3 is determined by utilizing a flow cytometry test. The findings were examined using a flow cytometer of the FACS Caliber type (BD). [Majid, Yasmin, Ghassan, Nahi, Usama, Yaser, Mona and Dina,2021], [Jihad, Noori, Jabir, Albukhaty, AlMalki, Alyamani, 2021].

2.2.5. characterization of Ag, Au and TiO_2 nanoparticles

In order to validate the presence of nanoparticlesAg, Au and TiO2, a spectroscopic study (Hitachi U-2910 Spectrophotometer, Hitachi Ltd., Tokyo, Japan) was carried out on the NPs, with the NPs being continuously scanned at 280–760 nm. Scanning electron microscopy was used to characterize the NPs' morphological characteristics (SEM, AA-7000; Shimadzu, Tokyo, Japan). With CuK radiation, an XRD-6000 X-ray diffractometer (Shimadzu, Tokyo, Japan) was used to confirm that the nanoparticles were crystalline. A voltage of 40 kV and a current of 30 mA were applied to the nanoparticles, and the results were analyzed.

3. Results and discussion

3.1. characterization of prepared PANI- Ag, Au and TiO2 nanoparticles

The formation of silver nanoparticles AgNPs during the interfacial polymerization was monitored by UV–visible spectral studies using a different $_2$ratio of AgNPs (0.05 and 0.1) to aniline dimer [Yan, Han and Tay,2007], [Bedre, Basavaraja, Salwe, Shivakumar, Arunkumar and Venkataraman 2009]. A shoulder peak that appeared at 304 nm for 0.05 PANI-AgNPs is due to the formation of silver nanoparticles during the polymerization reaction of the aniline dimer. On the other hand, a broad peak is seen at 622 nm due to molecular exciton for polyaniline. A slight shift in peak positions was noted in the case of 0.1 PANI–AgNPs nanocomposite system when compared with the UV–visible spectrum of 0.05 PANI-AgNPs [Oliveira, Castro, Canestraro, Zanchet,Ugarte, Roman and Zarbin 2006]Fig. 1A while, in cases0.05 and 0.1 PANI-AuNPs The UV–vis spectrum 315 and 601 nm are the two absorption bands in Fig. 1B. The benzenoid ring – transition is responsible for the absorption peak at 315 nm, whereas the polaron/bipolaron transition is responsible for the absorption peak at 610 nm. A peak absorption of 770 nm is seen when the polyaniline concentration is high enough to span the whole 500 to 900 nm range [Wang , Liu, Han, Sun, Huang Y and Yang G 2005].Fig. 1C shows peaks at 305 and 603 nm for PANI-TiO_2NPs. Meanwhile, the findings of FESEM

imaging showed the production of nanoparticles with diameters ranging from 54 to 150 nm for pure pani, 40 to 78 nm for PANI-AgNPs, 60 to 90 nm for PANI-AuNPs, and 50 to 70 nm for PANI-TiO2NPs. The doping of Ag, Au, TiO_2strongly affects the PANI's morphology. With the increase of NPs contents, the composites show a transformation in morphology from typical fibrous PANI to particles Fig2. The XRD pattern obviously demonstrated that the semi-crystalline nature of nanostructure PANI thin film is shown by the X-ray diffraction curve in Fig. 3Atwo peaks located at 2θ = 20º, and 25º correspond to (020), and (002) lattice planes [Bhagwat, Sawant and Mahajan, 2016]. The preferred peak along (002) direction comes from the periodicity of polymer plains perpendicular to the polymer chain, while the (020) direction along the chain of the polymer [Pouget, Józefowicz, Epstein, Tang, MacDiarmid, 1991]. These results agree with the PANInanostructure prepared by polymerization techniques [Bhagwat, Sawant and Mahajan, 2016]. In PANI–AgNPs The diffraction peaks at 2θ = (27.8˚), (32.2˚), (46.6˚) (55.05°), (58°), (68°) and (77.5˚) to the (101), (111), (211), (220), (221), (031) and (311) diffraction planes, respectively.Meanwhile,In PANI–AuNPs, The diffraction peaks at 2θ = (12.07˚), (38.4˚), (44.6˚) (64.8°), and (77.9˚) to the (001), (020), (200), and (200) and for PANI-TiO_2NPs The diffraction peaks at 2θ = (11.84˚), (25.4˚), (37.9˚) (48.11°), (54.56°) and (62.32°) to the (011), (101), (004), (200), (211), and (204).

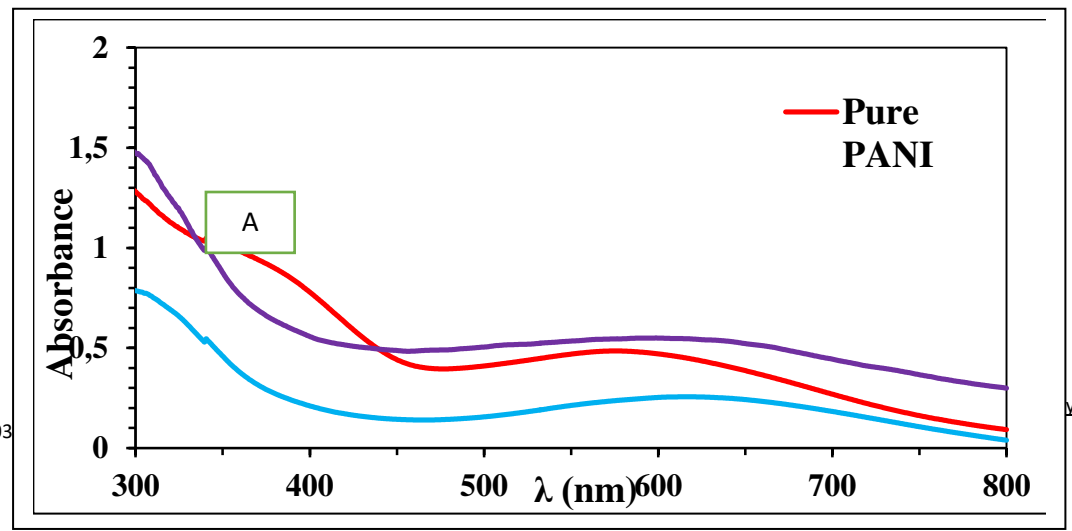
Ag, Au and TiO_2 nanoparticles increase Autophagy

We hypothesized that the prepared PANI- Ag, Au and TiO_2We would augment Autophagy following 1 µg/mL of a rat anti-mouse CD16/CD32 antibody of AMJ-13 cells. with or without PANI- Ag, Au and TiO2 (5 µgmL-1) treatment. The ability of the prepared PANI-Ag, Au and $TiO_2$to increase Autophagy was measured using flow cytometry, as shown in Fig.4. The results showed that the prepared PANI- Ag, Au and TiO_2increased autophagosome formation and LC3 protein levels. Autophagy is well-known for its ability to minimize the production of reactive oxygen species (ROS), which perform critical activities associated to the mediation of the intrinsic apoptosis pathway. These findings revealed that PANI-Ag, Au, and TiO_2 were effective in inducing autophagy.

4. conclusion

In this study, PANI and PANI- Ag, Au and TiO_2 prepared by polymerization method were then investigated as autophagy activators for AMJ-13 cancer breast cell lines. We found that PANI- Ag, Au and TiO_2 nanocomposite exhibited potent anti-proliferative on the AMJ-13 cell line. The results confirmed that PANI- Ag, Au and TiO_2, different forms of tumors and the promotion of immune system activities are possible applications for this compound.

3.2. PANI-



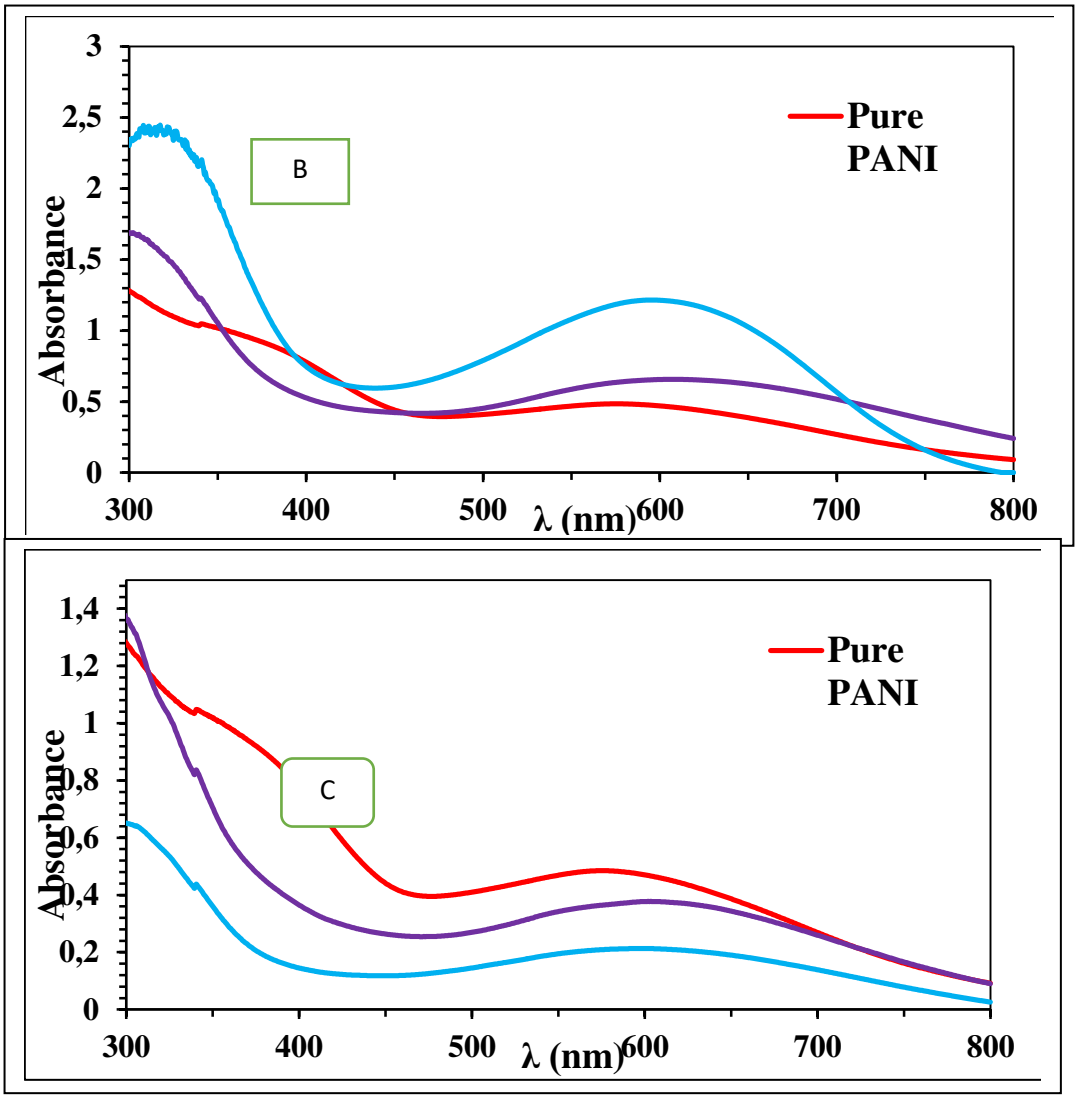


Fig.3. UV-Vis spectrum A. PANI–AgNPs, B. PANI–AuNPs and C. PANI–TiO_2NPs.

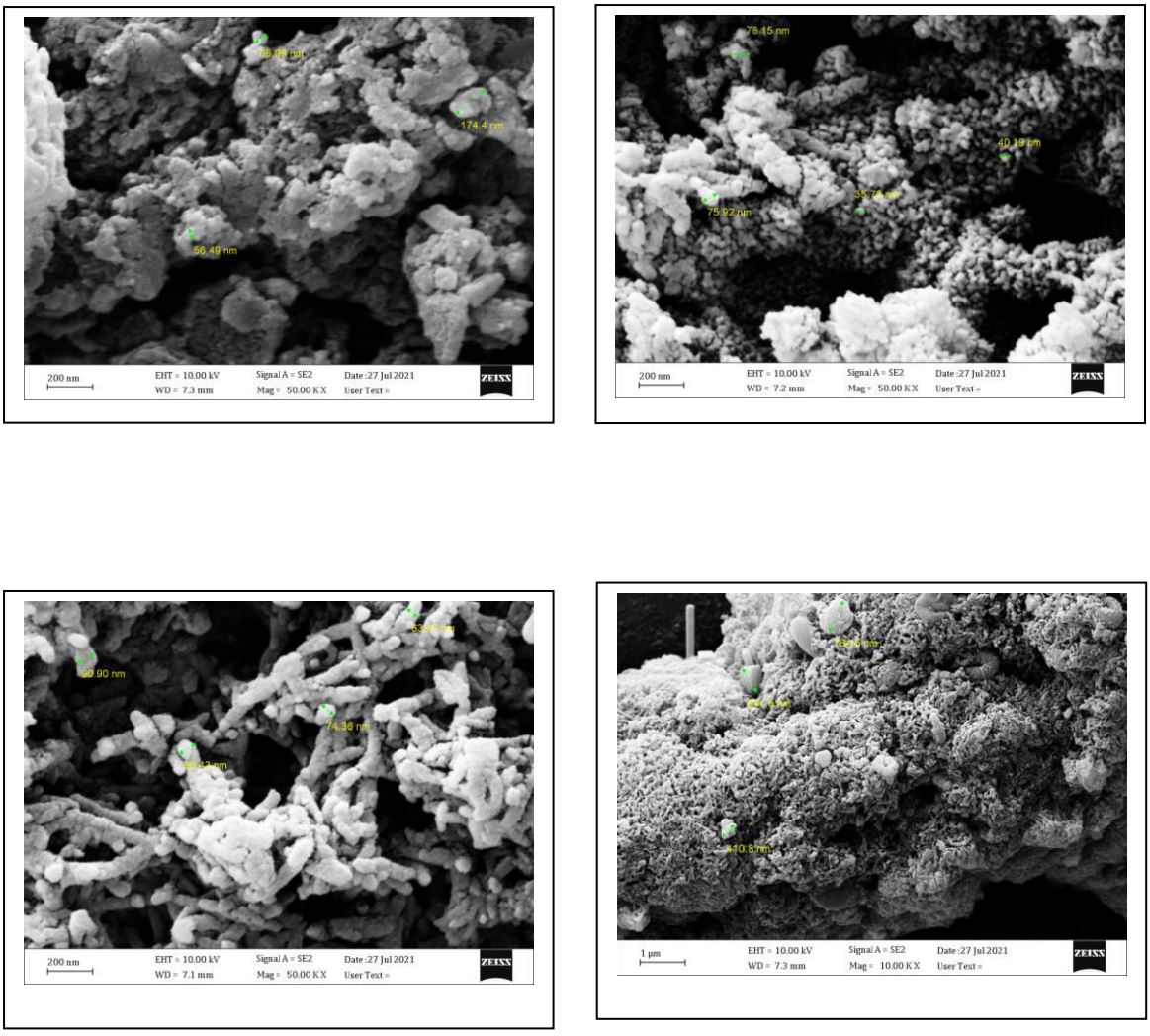


Fig. 3 FESEM images A. pure PANI, B. PANI–AgNPs, C. PANI–AuNPs and D. PANI–TiO_2NPs.

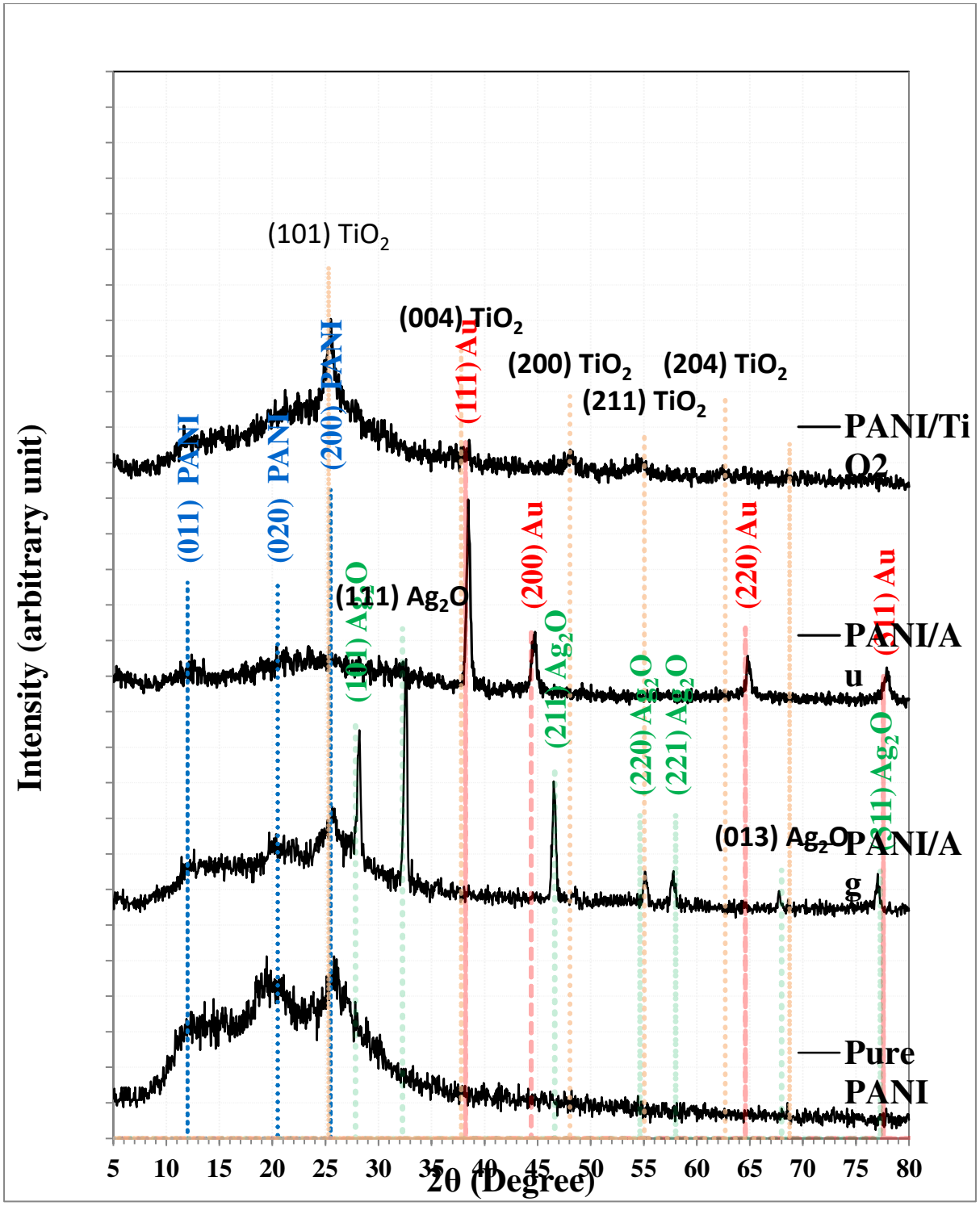


Fig. 4. XRD pattern of A. pure PANI, B. PANI–AgNPs, C. PANI–AuNPs and D. PANI–TiO_2NPs.

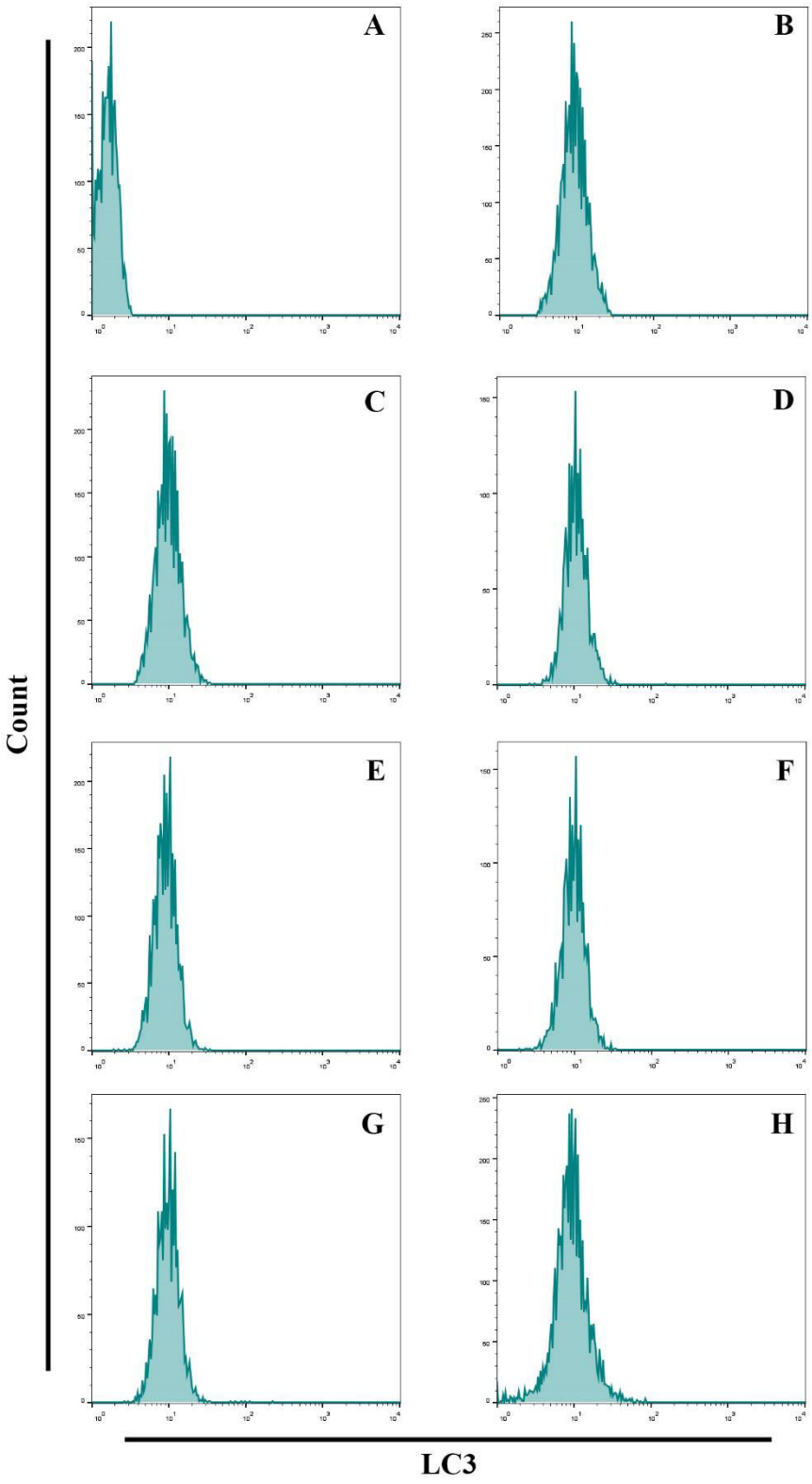


Fig .4 Prepared NPs induced Autophagy in AMJ3 cells. A, control untreated cells. B, AMJ3 cells treated with PANI pure at concentration 10 µg/ml. C, AMJ3 cells treated with PANI + Ag nanoparticles (1%) at concentration 10 µg/ml. D, AMJ3 cells treated with PANI + Ag nanoparticles (0.1%) at concentration 10 µg/ml. E, AMJ3 cells treated with PANI+ TiO nanoparticles (1%) at concentration 10 µg/ml. F, AMJ3 cells treated with PANI+ TiO nanoparticles (0.1%) at concentration 10 µg/ml. G, AMJ3 cells treated with PANI + Au

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nanoparticles (1%) at concentration 10 µg/ml.

AMJ3 cells treated with PANI+ Au nanoparticles (0.1%) at concentration 10 µg/ml. Data are represented as mean ± SD.

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