PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (4) 815-821 April 2023

Advancements in Layered Double Hydroxide-Based Chemotherapeutic Nanosystems for Cancer Treatment

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ABSTRACT: Globally, Cancer therapy is still a major issue. With the latest developments in nanotechnology, layered double hydroxide (LDH)-based nanosystems are receiving special attention due to their pH dependent biodegradability, superior biocompatibility, easy surface modification, anion exchange capacity and high chemical stability, giving rise to great potential for cancer therapy. By intercalating inorganic, organic, or bio molecules into their lamellar lattice, new hybrid materials with dual or multi-functional features, including anticancer capabilities, can be developed from layered double hydroxide (LDH). Despite the fact that outstanding research has been published, few review papers address these essential and promising discoveries to stimulate the ongoing development of LDH-based nanosystems in the field of cancer therapy. This paper therefore study focuses on the most recent advancements in LDH-based chemotherapeutic nanosystems for cancer treatment. The information utilized in this review was gathered from studies that have previously been published and were retrieved from several Journal outlets. These reports dealt with the usage of layered doubled hydroxide-based chemotherapeutic nanosystems for the treatment of cancer. The research demonstrates that layered double hydroxides may be utilized to develop single or composite nanosystems that distribute therapeutic components precisely without causing cumulative damage for the field of nanomedicine.

DOI: **https://dx.doi.org/10.4314/jasem.v27i4.24**

Cite this paper as: OMONMHENLE, S. I; IFIJEN, I. H. (2023). Advancements in Layered Double Hydroxide-Based Chemotherapeutic Nanosystems for Cancer Treatment. *J. Appl. Sci. Environ. Manage.* 27 (4) 815-821

Dates: Received: 07 February 2023; Revised: 18 March 2023; Accepted: 28 March 2023
Published: 31 March 2023

Keywords: layered double hydroxide; nanosystems; cancer therapy; drug resistance

Cures for cancer have evolved thanks to the emergence of alternative treatments like customized or targeted therapy (Maliki *et al.*, 2022; Ifijen *et al.*, 2022), but they still have a lot of drawbacks. Due to its great selectivity, one of the most promising treatments, phototherapy (Ifijen *et al*., 2023a; Ifijen *et al.*, 2023b), can be used to treat even deeply seated cancers, such as liver tumors, with relative ease. The two main therapeutic approaches used in phototherapy are photothermal therapy (PTT) (Zhong *et al*., 2021) and photodynamic therapy (PDT) (Perni *et al*., 2021), which uses light to create therapeutic reactive oxygen species (ROS) (Algorri *et al*., 2021). These treatment approaches are frequently used to increase the total efficacy of phototherapy thanks to recent developments in nanotechnology. The term photochemotherapy (PCT), which refers to the use of phototherapies in conjunction with chemotherapy, has also been used in recent studies. However, there are a few drawbacks to phototherapy, particularly in terms of phototherapeutic drugs (Rejinold *et al*., 2022). Near-infrared (NIR) dyes or ROS producers typically become unstable owing to deterioration. Through encapsulating technologies, such as polymers, liposomes, or micelles, researchers have tried to increase the stability of phototherapeutic drugs (Kumari *et al*., 2019; Xu *et al*., 2018). Several nanotechnology-based phototherapy instruments are

currently available, however they each have their own drawbacks, such as low phototherapeutic stability and toxicity. Inorganic clay nanoparticles (NPs) may be employed in PCT applications, according to recent findings. Clay NPs, also known as sheet silicates or phyllosilicates, are clay minerals that constitute a component of inorganic layered nanomaterials (Guggenheim & Martin, 1995). As bleeding inhibitors and as healing agents, they have been employed (Rejinold *et al*., 2022). Additionally, they are applied topically as dermatological and anti-inflammatory protectors and used in pharmaceutical preparations as disintegrants, diluents, binders, emulsifying agents, thickening agents, anticaking agents, flavor reservoirs, and the delivery modifiers of active agents (Carretero & Pozo, 2010). They are also used in pharmaceuticals as active ingredients in oral antacids, gastrointestinal protectors, and anti-diuretics (Carretero, & Pozo, 2009). Two-dimensional clay nanosheets in particular are a common class of nanomaterials with distinctive features, including a high surface-area-to-mass ratio and peculiar physical attributes resulting from their two-dimensional morphological feature and ultrathin thickness (Tan & Zhang, 2015). The utilization of these qualities as functional materials in the treatment of cancer is encouraged. Layered double hydroxide (LDH), commonly known as anionic clay, belongs to the family of two-dimensional nanosheets and has garnered a lot of interest. The general chemical formula for LDH is $[M_1^{II}{}_{-X}M_X^{III}(OH)_2]^{X+}(A^{n-})_{x/n}.mH_2O]$, where M^{II} represents divalent metal; M^{III} represents trivalent metals; x is the mole ratio of $M^{III}/(M^{II} + M^{III})$, and An^- is a non-framework charge compensating inorganic or organic anion (Omonmhenle & Shannon, 2019a). Tetravalent cations as well as monovalent cations may be present in LDH depending on certain circumstances (Omonmhenle & Shannon, 2019b). Due to the even distribution of metal ions and the simple exchangeability of intercalated anions, LDH demonstrates a variety of unique benefits and application potentials (Okolo *et al*., 2015). The fields of physics, energy evolution, and environmental science have all made extensive use of it (Omonmhenle & Shannon, 2016).

Due to their excellent biocompatibility, pH dependent biodegradability, anion exchange capacity, ease of surface modification, and high chemical stability, LDH-based nanosystems have generated interest in the biomedical disciplines during the past ten years (Wen *et al*., 2021). They are excellent candidates for the treatment of several diseases, including cancer, due to these qualities. Although many great studies have been reported, there aren't many review articles that discuss these significant and encouraging developments to support the continued advancement of LDH-based nanosystems in the field of cancer therapy. This review primarily focuses on the most recent developments in LDH-based chemotherapeutic nanosystems for cancer therapy.

Circumventing Resistance to Drugs: The fact that tumor cells commonly become resistant to anticancer medications during clinical treatment is unquestionably a major downside of chemotherapy (Devalapally *et al*., 2008; Bukowski *et al*., 2020). Therefore, it is crucial to create a therapeutically powerful anticancer agent without side effects in order to achieve successful chemotherapy. Various inorganic nanovectors have developed in this regard for creating sophisticated DDSs for chemotherapy (Komiski *et al*., 2020). Due to its controlled drug-release behavior, improved cellular absorption mechanism, and high drug efficacy, 2D-LDHs have been identified as one of the most promising inorganic delivery vehicles (Choi *et al*., 2008). In addition to treating common cancers including breast cancer, acute leukemia, and osteogenic sarcoma, methotrexate (MTX) also treats uncommon malignancies such head and neck tumors by preventing the folate cycle in the cell (Moscow, 1998; Komiski *et al*., 2020). The reduced folate carrier (RFC) (Sirotnak, 1987) and the glycosylphosphatidylinositol-anchored folate receptor (Antony, 1992) are the two primary locations on the cell membrane that allow folic acid (FA) and MTX medicament molecules to enter mammalian cells, respectively. When the MTX molecules entered the cell, they were used as a building block for the enzyme folylpolyglutamyl synthetase (FPGS), which then converted them into MTX polyglutamates (Jolivet & Schilsky, 1981). This action helped to inactivate the enzyme dihydrofolate reductase (DHFR), which is necessary for the folate cycle. Next, the MTX molecules were screened for intracellular pathways where the synthesis However, due to the well-documented multidrug resistance, the ineffectiveness of MTX has been frequently observed for patients with specific tumors (Gottesman, 2020). In broad terms, MTX resistance is associated with impaired MTX transfer caused by RFC defects (Jansen *et al*., 1998), reduced activity of MTX polyglutamation triggered by low FPGS expression, and/or increased levels of DHFR caused by gene amplification or DHFR mutations causing reduced MTX affinity (Schimke, 1984; Yamamoto *et al*., 2016).

On the basis of the MTT assay, the effects of intact MTX or the MTX-LDH nanohybrid on the inhibition of tumor cell growth were investigated in MTX sensitive HOS cells and MTX resistant HOS cells, respectively (Yu *et al*., 2023). When cells were treated

with intact MTX, the proliferation of the sensitive cells was considerably more inhibited than that of the resistant cells, demonstrating that drug resistance resulted in decreased treatment efficacy (Choi & Choy, 2021). However, the MTX-LDH nanohybrid suppressed cell growth in sensitive and resistant cells in a manner that was very similar, demonstrating that MTX-LDH was an effective hybrid medication even in DHFR-overexpressing cells like HOS. As a result, it was determined that the LDH delivery vehicle effectively smuggled MTX through clathrin-mediated endocytosis rather than the folate receptor and RFC, leading to increased intercellular uptake of MTX and the overriding of drug resistance (Neradil *et al*., 2015).

LDH-Based Chemotherapeutic Nanosystems: Chemotherapy, a popular cancer treatment method, involves giving chemical treatments to human body systems. Nevertheless, chemotherapy is frequently constrained by the low solubility and instability of many anticancer medications as well as the nonspecific targeting of cancer cells, which results in considerable side effects (Yang *et al*., 2018). To address these concerns, efficient drug delivery systems (DDSs) based on different nanomaterials have been used to improve stability and load efficiency as well as trigger the controlled release of therapeutic pharmaceuticals at targeted tumour tissues (Wen *et al*., 2019). Due to their varied chemical composition, excellent biocompatibility, high anion exchange capacity, and high drug loading capacity, LDH and its nanocomposite stand out among these as promising candidates for using chemotherapy to treat cancer using a variety of anti-cancer drugs, such as methotrexate (MTX), doxorubicin (DOX), 5-fluorouracil (5-FU), dacarbazine (DAC), etc. The protonation of -OH groups surrounding metal ions enables LDH to release intracellular medicines in response to acidic tumour microenvironments, and positively charged LDH is simple to internalize into a negatively charged cell membrane (Dugosz *et al*., 2023). Since the MgAl-LDH intercalated with MTX drug was applied to chemotherapy in vitro cell-line experiment (Choy *et al*., 2004), a variety of MTX-LDH nanohybrids have been examined on drug contents, controlled release profile, toxicity, and improvement of colloidal stability as delivery methods. Mei *et al*. (2018) used 2D monolayered double hydroxide (MLDH) nanosheets with a loading capacity of up to 3.6 mg mg-1 (w/w) to localize the anticancer drug doxorubicin (DOX). According to structural characterizations and theoretical calculations, the DOX molecule is consistently structured and orientated at the surface of the MLDHs with a binding energy of 15.90 eV, suggesting significant electrostatic interaction. For MLDH nanosheets, DLS, AFM, and HRTEM verified a diameter of 50 nm and a thickness of 0.8 nm (Figure 1).

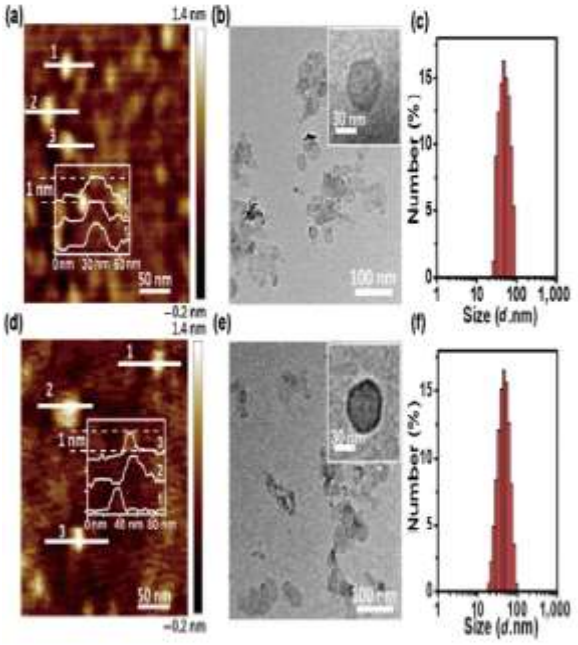

Fig 1: AFM images, HRTEM images, and particle size distribution of (a)–(c) DOX/MLDHs and (d)–(f) FA-DOX/MLDHs (Mei *et al*. (2018)

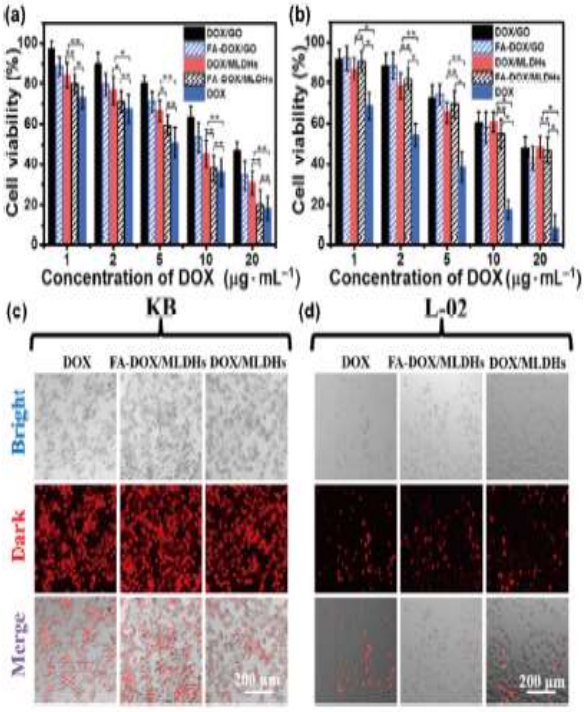

Fig 2: MTT assay of viabilities after incubation with free DOX, DOX/MLDHs, FA-DOX/MLDHs, DOX/GO, and FA-DOX/GO with various concentrations for 24 h: (a) KB cells and (b) L-02 cells. Fluorescence images of (c) KB cells and (d) L-02 cells stained with PI after incubated with DOX, DOX/MLDHs, and FA-DOX/MLDHs (equivalent DOX: 10 μg·mL^{-1}. *p < 0.05, **p < 0.01) (Mei *et al*., 2018)

The FA-DOX/MLDH combination, as produced, demonstrated superb fluorescence imaging, selective anticancer performance, and relatively moderate cytotoxicity to normal cells after further folic acid (FA) incorporation. The FA-DOX/MLDH combination also demonstrated excellent biocompatibility, high storage stability, and targeting potential. Based on in vitro studies with cancer cells, DOX-FA/MLDHs exhibit targeted cellular absorption and superior anticancer behavior with the aid of the targeting agent folic acid (FA) (Figure 2). This composite substance additionally demonstrates a selective release toward cancer cells and strong biocompatibility with normal cells, which would ensure its useful applications in cancer therapy. The development, physicochemical characteristics, and anticancer potency of drug-incorporated LDH nanohybrids were shown by Kim *et al*. (2014). To incorporate pharmacological molecules into LDHs while maintaining the topochemical features of original LDHs, they employed the process of reconstruction technique. Through reconstructive techniques, drug compounds as MTX, 5-FU, and their combination were successfully added to LDH while maintaining the lateral size of LDH nanoparticles. In cell culture conditions with 10% FBS, all of the nanohybrids were shown to exhibit positive zeta potential, indicating a facilitated contact with cancer cells. Furthermore, it has been demonstrated that proteins in normal conditions lower the level of aggregates in nanohybrids, potentially making them easier for cancer cells to recognize. HeLa human cervical cancer cells were used in an anticancer efficacy experiment, and the results revealed that the nanohybrids had better drug efficacy than the free drug alone (Figure 3). Comparing the combination administration of MTX + 5-FU and ML + FL, it was noteworthy that the MFL nanohybrid that included MTX and 5-FU together demonstrated the highest anticancer activity. The combined inclusion of a medication cocktail into LDHs, according to these studies, could improve the combination anticancer therapy. The engineering of magnesium aluminum layered double hydroxides utilizing coprecipitation and hydrothermal treatment at 100 °C for 6 hours was the focus of a study conducted by Abdelgalil *et al*. (2023). The chemotherapeutic medication pemetrexed (PMX) was then intercalated through anion exchange at an alkaline pH within the brucite layers of LDH. The LDH2-PMX was then coated with sericin protein using a desolvation process, and the resulting NPs were stabilized by chemical crosslinking with glutaraldehyde. Finally, the carboxylic groups of the sericin protein were chemically linked to the amino groups of the APTES-ZnO QDs. The created nanocarriers are designed to be administered intravenously in order to treat and detect breast cancer.

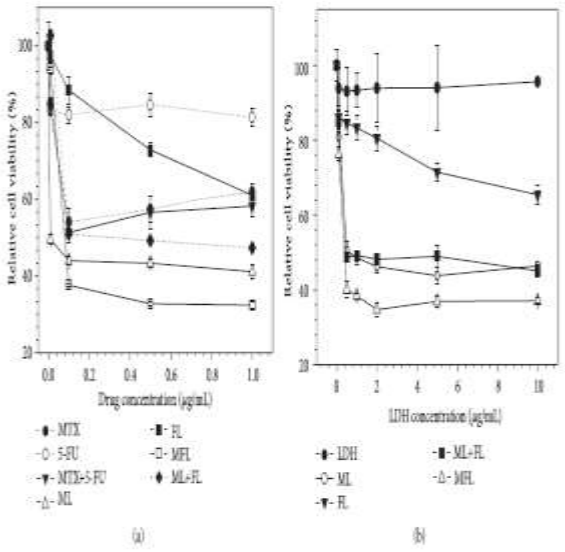

Fig 3: Anticancer efficacy of drug/LDH nanohybrids based on (a) drug concentration and (b) LDH concentration in HeLa cells (Kim *et al*., 2014).

The synthesized NPs had an appropriate particle size of 201.9 nm, a narrow PDI of 0.294 ± 0.02, and a high PMX intercalation efficiency of 90.70 ± 0.65%. They were negatively charged ((−21.1 ± 0.51 mV). PMX, LDH, sericin, QD, APTES-ZnO QDs/Seri@LDH2-PMX (GTA) and LDH-PMX, were all thoroughly physiochemically characterized, structurally identified, and subjected to optical, crystallinity, thermal, and morphological investigations. Utilizing FT-IR, UV-visible, photoluminescence (PL), XRD, DSC, ICP, and HRTEM, the spectral analysis was conducted and thoroughly examined. Additionally, the amide link between sericin and ZnO QDs was created through the application of bioconjugation chemical processes. The hydrophilic PMX clearly displayed a sustained release mode. With an IC50 of 1.34 ± 0.03 and 0.31 ± 0.007 μg/mL at 24 and 72 hours, respectively, an in vitro cytotoxicity research on MDA-MB-231 breast cancer cells confirmed our improved formula's potential to significantly increase anticancer activity compared to free PMX. Its hemocompatibility and in vitro serum stability were both satisfactory. Furthermore, as shown by the cellular uptake research, the produced NPs were effectively incorporated into MDA-MB-231 cells. Additionally, the study demonstrated through thorough XRD, particle size, and probable outcomes that the guest anion's (PMX) ability to intercalate inside the LDH layers increased with guest anion concentration. The proper selection of a crosslinker in a formulation containing an inorganic bioimager, however, is crucial as it may affect the

photoluminescence capabilities; in our investigation, GTA crosslinker was more appropriate. Finally, since PMX was effectively absorbed by cancer cells and then retained, as opposed to the free drug, it can be said that PMX's anticancer activity was significantly increased. The improved colloidal stability of LDH due to sericin may also account for its effective cellular absorption. Additionally, it offers an additional sustained PMX release profile from F6 that is expected to prevent PMX from entering the bloodstream while facilitating medication release at the tumor site (drug release was approximately 99.23% after 30 hours and 99.3% after 11 hours at pH 7.4 and 4.8, respectively). Additionally, the incorporation of APTES-ZnO QDs with the surface of NPs would allow for tumor imaging for theranostic applications and could contribute to overall anticancer activity via a variety of pathways.

Choi *et al*. (2016) developed hydrodynamic layered double hydroxide-methotrexate (LDH-MTX) hybrid nanoparticles with an average particle diameter of 100 nm as an efficacious DDS for chemotherapy in another investigation. The biodistribution studies revealed that the LDH drug delivery vehicle had a clear targeting impact, as mice injected with LDH-MTX had a 3.5-fold more substantial tumor-to-liver ratio and a five times greater tumor-to-blood ratio of MTX at 30 minutes postinjection compared to those administered with free MTX. The profiles of in vivo inflammatory response and body weight change show that incorporating MTX into a nontoxic clay carrier (LDH) result in a new nanohybrid DDS. As a result of its low in vitro and in vivo toxicities and strong targeting impact, the present nanohybrid colloid with an average particle size of 100 nm can be offered as a viable injectable chemotherapy.

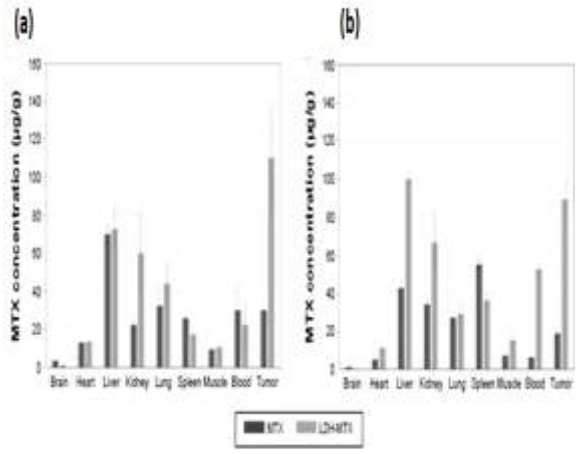

Fig 4: Biodistribution studies of MTX in each tissue of C33Aorthotopic tumor-bearing mice treated with MTX (dark gray) and LDH-MTX (gray) for (a) 30 minutes and (b) 60 minutes after administration (Choi *et al*., 2016).

Doxorubicin (DOX) is a powerful anti-cancer medication, and efforts have been made to build nanostructures for its distribution to tumor cells. The nanoparticles increase the cytotoxicity of DOX against tumor cells, reducing the negative effects on normal cells. The safety profile of nanostructures is an essential problem, and recently, green synthesis of nanoparticles has received an abundance of attention for the development of biocompatible carriers. Kiani *et al*. (2022) developed layered double hydroxide (LDH) nanomaterials for doxorubicin (DOX) administration. Cu-Al LDH nanoparticles were generated by mixing $Cu(NO_3)_2.3H2O$ and $Al(NO_3)_3.9H2O$, subsequently autoclaving at 110 degrees Celsius. DOX was loaded onto nanoscale structures after green modification of LDH nanoparticles using Plantago ovata (PO). The FTIR, XRD, and FESEM techniques were used to characterize LDH nanoparticles, verifying their correct production and drug loading. The drug release investigation indicated pH-sensitive release of DOX from LDH nanoparticles, with the largest release of anti-tumor drug occurring at pH 5.5 and the lowest drug release occurring at pH 4.5, possibly due to the deleterious impact of low and extremely acidic pH on nanoparticle structure. The MTT experiment exhibited great biocompatibility of PO modified Cu-Al LDH nanoparticles; thus, PO-modified LDH nanoparticles demonstrated partial and low toxicity towards PC12 and HEK-293 cells, while decreasing viability of HT-29 and MCF-7 tumor cells. The reduction in viability of HT-29 and MCF-7 cells was smaller in PO-modified LDH nanoparticles compared to non-modified LDH nanocarriers, which should be investigated further in future investigations. CLSM results demonstrated that LDH nanoparticles delivered DOX to the cytoplasm and nucleus of MCF-7 and HEK-293 cells. Likewise, histological investigation of kidney tissue revealed no cell damage, normal cell and tubule architecture, and no blood vessel obstruction, indicating the great biocompatibility of PO-modified LDH nanoparticles. The antibacterial test revealed that Cu-Al LDH nanostructures exhibit cytotoxicity against Gram-positive and -negative bacteria, and they can be used to treat bacterial illnesses in future investigations.

Conclusion: This review has demonstrated that LDH and its nanocomposites have a great deal of potential as adaptable nanosystems for various cancer treatments without cumulative harm. The stability and characteristics of LDH-based nanocomposites, which combine the advantages of LDH with one or more other nanomaterials in a single nanosystem, have greatly improved. The influence of the surface characteristics and physicochemical properties of

LDH and its nanocomposites must be understood in order to develop safe LDH formulations.

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