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Assessment of the toxicity of Nanokaolinite on colorectal cancer cells

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Abstract

One of the leading causes of death worldwide is cancer, particularly colorectal cancer, which ranks third among cancer-related deaths according to recent studies. In light of this reality, the importance of finding a treatment that avoids the pitfalls of previous treatments increases. These treatments may be beneficial in treating colorectal cancer, but they also harm the entire body. Thanks to the promising idea of using nanotechnology to develop an efficient treatment, there are many options to work on. Despite its rocky nature, kaolin possesses properties that could transform treatments if exploited optimally. Many researches illustrated the promising role of kaolinite in many medical applications. This research aims to obtain pure kaolinite in nano scale form, to make sure of the validity of kaolinite itself chemically using Fourier Transform Infrared **(**FTIR) and analysis technique X-ray Fluorescence Spectroscopy (XRF), then, to test its potential toxicity in eliminating colorectal cancer cells using cell viability 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay (MTT assay).

1. Introduction

Colorectal cancer (CRC) represents as one of the leading causes of morbidity and mortality of cancer cases worldwide. In 2020, it was estimated that there were over 1.9 million new cases and nearly 1 million deaths globally [1,2]. Geographical distribution in terms of high- or low-income areas is considered one of the determinants of incidence rates [3]. Major risk factors for CRC include lifestyle changes, dietary habits, diets high in red or processed meats, low fiber intake, alcohol consumption, tobacco use, obesity and physical inactivity [4], age (most common in elderly people) [3,5], gender differences [6], family history of CRC, genetic syndromes and personal history of inflammatory bowel disease [5,7-8]. Cryotherapy could be saved as a treatment option [9], and also radiotherapy, to be used in order to eliminate CRC [10]. Conventional treatment for CRC is stage-dependent. Early-stage cancers are typically treated with surgical resection, which can be curative [11].

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Adjuvant chemotherapy (e.g., FOLFOX, FOLFIRI) is often administered in stage III or high-risk stage II disease to reduce recurrence risk. For metastatic CRC (mCRC), systemic chemotherapy remains the cornerstone of treatment, often in combination with targeted agents such as Bevacizumab as anti-VEGF, and Cetuximab or panitumumab as anti-EGFR [11]. One of the major challenges in CRC treatment is the non-specificity of conventional chemotherapeutic agents, which attack both healthy and cancerous tissues, leading to significant side effects. Moreover, many drugs have poor solubility or rapid degradation in vivo, limiting their effectiveness [11]. Nanotechnology offers a promising alternative by enabling targeted drug delivery to tumor tissues, controlled and sustained drug release, combination therapies in a single nano-carrier, and enhanced imaging and diagnostic capabilities [12-14]. Among the nano materials being explored, nanokaolinite has shown considerable potential due to its natural abundance, low cost, biocompatibility, and modifiability [15]. This sets the stage for exploring the use of kaolinite-based nanomaterials as next-generation vehicles for CRC therapy. Among various nanomaterials, clay minerals, particularly kaolinite, have gained attention for their unique physicochemical properties, biocompatibility, and ease of functionalization

Kaolinite is a naturally occurring clay mineral that is composed of aluminosilicate with a layered structure and a chemical formula $Al_2Si_2O_5(OH)_4$ [16]. Kaolinite is characterized by its high surface area and cation-exchange capacity, with a 1:1 dioctahedral phyllosilicate structure. These properties position it as an attractive candidate for the applications of drug delivery. Kaolinite's biocompatibility and low toxicity profile further support its potential in biomedical applications [17]. These properties have made kaolinite a valuable material in various fields, including ceramics, paper coating, cosmetics, drug delivery, and more recently, biomedical applications like cancer treatment [18,19]. Kaolinite can provide a controlled and sustained release as it adsorbs and intercalate chemotherapeutic drugs, due to its lamellar structure and surface charge [20]. For instance, methoxy-modified kaolinite has been shown to increase the interlayer spacing, enhancing drug loading ability and facilitating controlled release [19,21]. Kaolinite also has a contribution in targeted therapy. Kaolinite also achieved the pH-responsive delivery. Kaolinite can be engineered for selective drug release drugs selectively in the acidic tumor microenvironment, minimizing systemic side effects. For example, doxorubicin-loaded kaolinite exhibits increased drug release at pH 5.5, which is typical of tumor tissues, compared to neutral pH conditions [19]. Besides, kaolinite has approved to contribute in photothermal and photodynamic therapy. Magnetic or fluorescent kaolinite composites support simultaneous imaging (Magnetic Resonance Imaging (MRI), fluorescence) and therapy ushering in the era of theranostics. For instance, Mn_3O_4-modified kaolinite composites have been used for drug delivery that depends on magnetic resonance imaging (MRI)-guided. This allows a real-time monitoring of treatment efficacy [19].

So, in this research, we aim to testify the ability of nano to affect the ability of colorectal cancer cells to proliferate, thus its ability to continue as a cancerous threat by hindering its main characteristics, uncontrollable growth and proliferation.

2. Materials and Methods

A high-quality raw kaolin sample with industrial and commercial value was chosen from West Central Sinai, Egypt. It is collected from Abu Zenima deposit, ant it is given the code K3 (Awad et al., 2017). Prior to Kaolin purification, raw kaolin is prepared by unifying its particles sizes. To obtain a smaller particle size, the sample was grinded, and then sieved to get the finest particle sizes in a range below 125nm using 0.125mm pores sieve. Then, it was washed using deionized water and 70% ethanol relatively, and then dried on 70°C for 30 min. To get red oh hematite, a mechanical stirring at a 100°C as a constant temp was done for a water slurry of kaolin.

2.1. Purification of Kaolin

A 500 ml of 0.5 M oxalic acid solution were added to the flask containing the slurry and the temperature was set to the desired value. All leaching tests were made at atmospheric pressure. A mixture of kaolinite in dist.H_2O with Sodium Carbonate Na_2CO_3, and sodium lauryl sulphate $NaC1_2H_{25}SO_4$ were stirred using the mechanical stirrer, then the mix was let to separate into two phases, liquid phase representing suspension and the mixture containing the wet purified kaolinite. Suspension was centrifuged to get colloid kaolinite, and then was dried on 70°C.

2.2. Preparation of nanokaolinite

Purified nanokaolinite is heated in oven in a heating cycle up to 400 for 40 °C min as the temperature was rose by 10°C/min, and then to cool by the same criteria. The resulted nano sheets were washed using dist.H2O by sonicator for 10 min to make sure of size reduction process, and then by magnetic stirrer for 15 min on a slow velocity, then samples were centrifuged and dried at 70 °C over night, and then were kept at refrigerator to be analyzed and stored.

2.3. Characterization of nanokaolinite

2.3.1. Fourier Transform Infrared (FTIR)

FT-IR spectroscopy is used to visualize the variability and orientations of hydroxyl groups based on the structural disorder specifically on the external basal plane surfaces of the pure kaolinite [16]. This is conducted using a PerkinElmer Spectrum IR Spectrometer with Spectrum IR Version 10.6.2 (Thermo Scientific, Waltham, MA, USA) FT-IR spectrometer. The transmittance peaks were recorded in the 450–4000 cm^{-1} frequency range.

2.3.2. X-ray Fluorescence Spectroscopy (XRF)

X-ray fluorescence (XRF) technique is employed to analyze the mineralogical and chemical constituents of the purified kaolinite powder as well as the raw kaolin [16]. This is conducted using Niton™ XL2 XRF Analyzer.

2.4. Biological Toxicity of nanokaolinite.

2.4.1. Cell viability assay (MTT Assay)

The cytotoxic potential of kaolinite against the Caco-2 colorectal cancer cell line was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric assay, which evaluates cell viability by measuring the metabolic reduction of the yellow tetrazolium salt (MTT) to insoluble purple formazan crystals by NAD(P)H-dependent oxidoreductase enzymes in viable cells. The intensity of the resulting color, is quantified by absorbance at 570 nm, is directly proportional to the number of active cells [22]. Caco-2 colorectal cancer cell line is seeded by 1×10^5 cells/ml into 96-well plates, incubated for 24 hours at 37°C to allow the formation of a confluent monolayer. After washing, test samples were prepared in RPMI medium containing 2% serum and added to designated wells, while control wells received only maintenance medium. The morphological indicators of cytotoxicity, including cell roundness, shrinkage, granulation, and monolayer disruption were observed after the application of treatment. Then, 20 µl of 5 mg/ml, in Phosphate-Buffered Saline (PBS) (BIO BASIC CANADA INC), MTT solution was added to each well, then plates were shaken for 5 minutes at 150 rpm, then incubated for 4 hours at 37°C in a 5% CO_2. After incubation, the medium was removed, and the resulting formazan crystals were solubilized in 200 µl of dimethyl sulfoxide (DMSO). Absorbance was measured at 560 nm with background subtraction at 620 nm. The viability of treated Caco-2 cells was calculated relatively to the untreated cells.

3. Results

3.1. Fourier Transform Infrared (FTIR)

The results in figure (1) represent FTIR spectroscopy peak positions (in cm^{-1}) and their corresponding transmittance (%T). 3685.54cm^{-1} (81.71%T) and 3619.70cm^{-1} (85.32%T), these are typical of stretching vibrations of surface hydroxyl (–OH) groups. Si–O stretching (1115, 1004, and 787–750cm^{-1}), and Al–OH/Si–O bending (911cm^{-1}).

3.2. X-ray Fluorescence Spectroscopy (XRF)

The results of XRF chemical analysis of nanokaolinite, which are illustrated in table (1), revealed a change between raw and purified kaolinite regarding the Wt% of major oxides.

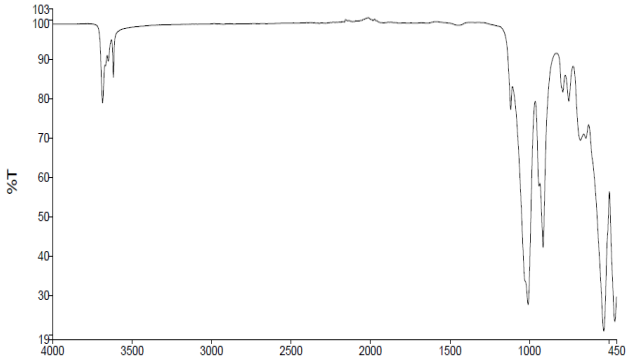

Figure 1: FTIR results of nanokaolinite.

Table1: Chemical composition of the raw and purified kaolinite samples determined by XRF analysis.

Major Oxides (Wt %)	Raw Kao In Literature	Pure Kao in Literature	Raw Kao K3	Pure Kao K3
SiO_2	46.98	47.04	63.27	46.62
Al_2O_3	35.28	36.01	31.89	34.32
TiO_2	1.2	1.3	6.49	12.57
Fe_2O_3	0.54	0.52	٢,٩	2.13
MnO	<0.01	--	--	--
MgO	0.18	0.14	--	--
CaO	0.04	0.04	--	--
Na_2O	0.26	0.46	--	--
K_2O	0.44	0.49	--	--
P_2O_5	0.05	0.06	--	--
SO_3	0.01	0.06	0.002	--

3.3. Cell viability assay (MTT)

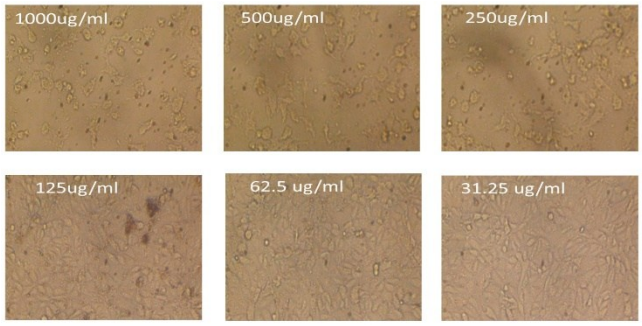

Figure 2: Effect of different concentrations of nanokaolinite on

Caco-2 cells.

The findings revealed that when colorectal adenocarcinoma cells of Caco-2 cell line were treated with 31.25, 62.5, 125, 250, 500 and 1000 µg/ml of nanokaolinite. The 100% cell proliferation was significantly reduced in a dose- dependent manner to 99.9%, 79.6%, 31.8%, 7.7%, 6.7 %, and 5.6% respectively as in figure (2), to get 100.82µg/ml as the IC50 value as indicated in figure (3).

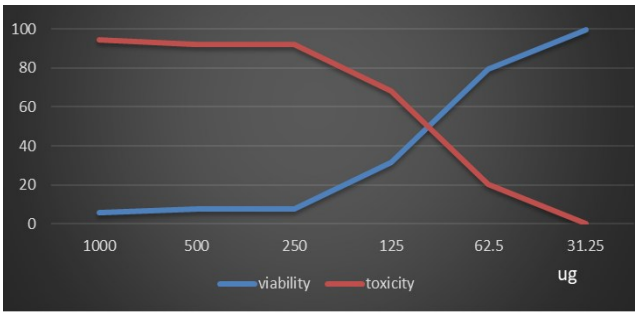

Figure 3: Effect of different concentrations of on Caco-2 cells, and the IC50 value.

4. Discussion

Colorectal cancer (CRC) represents as one of the leading causes of morbidity and mortality of cancer cases worldwide. Also, Caco-2 colorectal cancer cell line represents a partially chemotherapy resistant cancer cells depending on the combination or the form of treatment delivery as the treatment efficacy may need a carrier to achieve its mission [23]. Researchers have enhanced kaolinite therapeutic potential by developing nanokaolinite to offer improved drug loading, pH-responsive release, and synergistic anticancer effects [17].

In FTIR, the stretching vibrations of surface hydroxyl (–OH) groups likely from structural or adsorbed water. Such peaks are common in minerals like kaolinite or other clays. Si–O stretching and Al–OH/Si–O bending, all confirm this is a layered silicate, such as kaolinite. The findings of FTIR are supported with XRF results showed that the ratio or Wt% of SiO_2 and Al_2O_3, with presence of iron and L.O.I in each sample [24].

The MTT experiment findings describes the cytotoxic impact of nanokaolinite. The results were so promising regarding the toxicity of nanokaolinite toward colorectal cancer cells represented in Caco-2 cell line. The control untreated sample is 100% viability, while there is a great decrease in cell viability with different concentrations of pure kaolin recording IC50 values (as 50% of cells are died) at 100.82µg/ml. These findings reveal the strong effect of kaolinite on the main key element that specialize cancer cells, which is up normal proliferation [17]. In front of such results, besides knowing the kaolinite's biocompatibility and low toxicity profile [18,19], these evidences demonstrate that nanokaolinite can be great candidate in the treatment and controlling cancers [25], specifically colon cancer.

Conclusion

According to the discussed findings, the clear toxicity of nanokaolinite toward colorectal cancer cells is illustrated, which supports the further research on the promising ability of nanokaolinite toward colorectal cancer cells, not only as drug carriers as previously mentioned in previous researches, but also as a drug itself. Further research is needed to understand the mechanism of the effect of nanokaolinite on colorectal cancer cells on the molecular level.

A statement of no conflict of interest

Authors have no conflict.

References

[1] Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229-263. doi:10.3322/caac.21834

[2] Xin X, Tang Y, Lu M, et al. Prognostic value of diffusion-weighted imaging to cytoreductive surgery with or without hyperthermic intraperitoneal chemotherapy for patients with gastric cancer and peritoneal metastases. BMC Cancer. 2025; 25:616. doi:10.1186/s12885-025-14008z

[3] Eng C, Yoshino T, Ruíz-García E, et al. Colorectal cancer. Lancet. 2024;404(10449):294–310. doi:10.1016/S0140-6736(24)00360-X

[4] Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Prz Gastroenterol. 2019;14(2):89-103. doi:10.5114/pg.2018.81072

[5] Sawicki T, Ruszkowska M, Danielewicz A, Niedźwiedzka E, Arłukowicz T, Przybyłowicz KE. A Review of Colorectal Cancer in Terms of Epidemiology, Risk Factors, Development, Symptoms and Diagnosis. Cancers (Basel). 2021;13(9):2025. Published 2021 Apr 22. doi:10.3390/cancers13092025

[6] Schmuck R, Gerken M, Teegen EM, et al. Gender comparison of clinical, histopathological, therapeutic and outcome factors in 185,967 colon cancer patients. Langenbecks Arch Surg. 2020;405(1):71-80. doi:10.1007/s00423-019-01850-6

[7] Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 2017;66(4):683-691. doi:10.1136/gutjnl-2015-310912

[8] Hnatyszyn A, Hryhorowicz S, Kaczmarek-Ryś M, et al. Colorectal carcinoma in the course of inflammatory bowel diseases. Hered Cancer Clin Pract. 2019; 17:18. Published 2019 Jul 12. doi:10.1186/s13053-019-0118-4

[9] Jiang X, Ji Z, Lei X, Liu C, Yuan F. Suitable T stage for cryosurgery to spare the anus in patients with low rectal cancer. Cryobiology. 2023; 111:121-125. doi: 10.1016/j.cryobiol.2023.05.004

[10] Chiang CH, Chao TY, Huang MY. Adaptive radiotherapy of locally advanced sigmoid colon cancer with intra fractional motion using the MRIdian system: A case report. Oncol Lett. 2023;26(5):487. doi:10.3892/ol.2023.14074
[11] Fadlallah H, El Masri J, Fakhereddine H, et al. Colorectal cancer: Recent advances in management and treatment. World J Clin Oncol. 2024;15(9):1136-1156. doi:10.5306/wjco. v15.i9.1136
[12] Wang B, Xue Y, Zhai W. Integration of tumor microenvironment in patient-derived organoid models help define precision medicine of renal cell carcinoma. Front Immunol. 2022; 13:902060. doi:10.3389/fimmu.2022.902060
[13] Zhang D, Wang L, Tian L, Chen W, El-kott A, Negm S, Eltantawy W, Alshaharni M. Bio-inspired supported of gold nanoparticles over surface of kaolin towards human ovarian cancer management in vitro and regulating the inflammatory action of adenomyosis in mice in vivo through MAPK signaling route. J Sci Adv Mater Devices. 2024. doi: 10.1016/j.jsamd.2024.100714
[14] Kordkatouli M, Mahmood Janlou MA, Sateei A, Mousavi MMH, Dulskas A. Recent progress in nanoparticle-driven drug delivery strategies for cancer therapy: Focus on colorectal cancer. Zahedan J Res Med Sci. 2024;27(1): e158109. doi:10.5812/zjrms-158109
[15] Cheng H, Liao J, Ma Y, Sarwar MT, Yang H. Advances in targeted therapy for tumor with nanocarriers: A review. Mater Today Bio. 2025; 31:101583. doi: 10.1016/j.mtbio.2025.101583
[16] Awad ME, López-Galindo A, Medarević D, Milenković M, Ibrić S, El-Rahmany MM, Viseras Iborra C. Enhanced antimicrobial activity and physicochemical stability of rapid pyro-fabricated silver-kaolinite nanocomposite. Int J Pharm. 2021; 598:120372. doi: 10.1016/j.ijpharm.2021.120372
[17] Persano F, Leporatti S. Nano-Clays for Cancer Therapy: State-of-the Art and Future Perspectives. J Pers Med. 2022;12(10):1736. Published 2022 Oct 19. doi:10.3390/jpm12101736
[18] Awad ME, Farrag AM, El-Bindary AA, El-Bindary MA, Kiwaan HA. Photocatalytic degradation of Rhodamine B dye using low-cost pyrofabricated titanium dioxide quantum dots–kaolinite nanocomposite. Appl Organomet Chem. 2023;37(7): e7113. doi:10.1002/aoc.7113
[19] Wu Q, Liao J, Yang H. Recent Advances in Kaolinite Nanoclay as Drug Carrier for Bioapplications: A Review. Adv Sci (Weinh). 2023;10(25): e2300672. doi:10.1002/advs.202300672
[20] Zhang Y, Huang P, Long M, et al. Intercalated kaolinite as an emerging platform for cancer therapy. Sci China Chem. 2019;62(1):58–61. doi:10.1007/s11426-018-9364-8
[21] Tan D, Yuan P, Annabi-Bergaya F, Dong F, He H, Liu D. High-capacity loading of 5-fluorouracil on the methoxy-modified kaolinite. Appl Clay Sci. 2014; 100:60–65. doi: 10.1016/j.clay.2014.02.022
[22] Ghasemi M, Turnbull T, Sebastian S, Kempson I. The MTT assay: Utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. Int J Mol Sci. 2021;22(23):12827. doi:10.3390/ijms222312827
[23] Haffez H, Sanad HH, Ebrahim H, Hassan ZA. Synergistic effects of abietic acid combined with doxorubicin on apoptosis induction in a human colorectal cancer cell line. Sci Rep. 2025;15(1):16102. doi:10.1038/s41598-025-99616-2
[24] Senoussi H, Osman H, Courtois C, Bourahli MEH. Mineralogical and chemical characterization of DD3 kaolin from the east of Algeria. Bol Soc Esp Ceram Vidrio. 2016;55(3):121–126. doi: 10.1016/j.bsecv.2015.12.001
[25] Vital N, Cardoso M, Kranendonk M, Silva MJ, Louro H. Evaluation of the cyto- and genotoxicity of two types of cellulose nanomaterials using human intestinal cells and in vitro digestion simulation. Arch Toxicol. 2024;99(2):575–596. doi:10.1007/s00204-024-03911-2