

AG-NPS WITH HUMAN SERUM ALBUMIN COATED PHYTO THERAPEUTIC AGENT CAPPED ACT AS A DRUGS DELIVERY AGAINST BREAST CANCER

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ABSTRACT

Due to its ability to produce perfect release of drugs and biodistribution, drug delivery systems based on nanotechnology have recently attracted the interest of researchers in the field of nanomedicine. Due to their remarkable biological features and the availability of numerous different materials used to build drug delivery vehicles for effective cancer treatment, serum albumin-based nano vehicles have been extensively developed and explored. Human serum albumin is one of them and is a remarkably effective and promising carrier for an anti-cancer pharmaceutical. Long half-life, frequent recycling, and specific accumulation are the benefits of HSA. HSA has many binding pockets where various ligands, such as fatty acids and ions, can bind, providing pharmaceuticals with a variety of non-covalent binding sites. In addition, HSA has increased drug half-lives, decreased renal drug clearance, and increased drug accumulation in specific cancer tissues. HSA is an ideal chance to deliver widely used drugs in a brand-new way. *Wrightia tinctoria* leaves were used to create the plant alkaloid substance known as brucine. Silver nitrate was combined with brucine solution to synthesis silver nanoparticles. UV-vis Spectrophotometer, SEM, and FTIR were used to characterise nanoparticles. This review examined the bioconjugation of HSA with Phyto therapeutic agent-capped Ag-NPs. The ex-vivo approach was used to execute and evaluate human serum albumin as a drug delivery vehicle against the MCF-7 breast cancer cell line. Phyto therapeutic agent capped Ag-NPs coated with HSA may be used as a workable drug delivery system in the treatment of cancer, according to the results.

Keywords: Human serum albumin, Michigan Cancer Foundation-7, Drug delivery, AgNPs.

1. INTRODUCTION

The majority of the drugs that are currently available on the market and used to treat a variety of human disorders are composed of small chemical components. However, these small-molecule drugs typically have negative side effects, such as rapid breakdown, short circulation times, rapid renal clearance, non-specific distribution, and dangerous accumulation in certain organs or tissues (Wang et al., 2020). Serum albumin, a naturally occurring ligand carrier that is highly concentrated and circulates for a long time in the blood, has shown amazing potential as a delivery method for anti-cancer medications. Drugs that are generally swiftly eliminated from circulation can have their half-lives extended by albumin, and more importantly, albumin can promote the accumulation of these drugs inside tumors (Hoogenboezem & Duvall, 2018). Serum albumin is a well-behaved carrier material that is used by one class of efficient drug delivery systems to encapsulate or conjugate therapeutic chemicals for administration of medications that are directed at tumors (Elzoghby et al., 2012). Human serum albumin, which is the most frequent protein in plasma, maintains plasma colloidal osmotic pressure and carries endogenous chemicals. Albumin protein was chosen as the drug delivery payload primarily because to its superior biocompatibility, biodegradability, and non-immunogenicity (An & Zhang, 2017). The efficient therapeutic advantages of cancer therapy supported by nanomaterials that have emerged in recent decades have captivated researcher attention and enthusiasm for creating fresh uses of nanotechnology to cure various types of cancer (Barreto et al., 2011, Nazir et al., 2014). Due to AgNPs inherent anticancer impact, which is widely employed in in-vitro and ex-vivo investigations employing a variety of cancer cell models, several recent scientific studies have sought to utilise AgNPs in combination with anticancer medications (Morais et al., 2020). A breakthrough in the treatment of metastatic cancer is the result of AgNPs extraordinary anticancer capability. We also assessed the most recent research on AgNPs cellular-level toxicological activity, which depicts it as a potent anticancer agent with obvious therapeutic efficacy (Jabeen et al., 2021). Analytical techniques like FT-IR, SEM analysis, and UV-visible spectrophotometer are utilised to characterise the chemical composition and shape of the synthesised AgNPs (Revathy et al., 2022). HSA conjugates have the capacity to control medication release and localise pharmaceuticals at particular places. As a result, HSA is seen to be a good choice for the administration of medications for the treatment of cancer (Taheri et al., 2011). Because AgNPs have no negative effects on serum proteins, their use in the transportation of conjugated drug molecules is advised. Since HSA is found in the circulatory system, using AgNPs in a range of biomedical applications may be possible (Hazarika et al., 2020). One of the most urgent areas of joint research in materials science and biology is the

development of new functional and safe NPs. However, the underlying cause-effect correlations are still poorly understood, making a deeper knowledge of the NP-protein interactions necessary (Goy-López et al., 2012). The activities of the cell lines were examined after bioconjugation. Breast cancer is a prevalent malignancy that accounts for 23% of all cancer cases and 14% of cancer deaths. Michigan Cancer Foundation-7 (MCF-7) cells are used in this investigation (Rao et al., 2020). Since the mammary epithelium of breast cancer patients differs from that of the MCF-7 cell line in several ways, the human breast cancer cell line Michigan Cancer Foundation-7 (MCF-7) is commonly used in experimental studies. The use of this cell line as a research tool in the study of cancer is therefore widespread (Holliday et al., 2011). In light of these data, we examined human serum albumin potential for use as a targeted drug delivery system for the Michigan Cancer Foundation-7 cell line employing Phyto therapeutic agent-capped Ag-NPs.

2. ALBUMIN AND ITS FUNCTIONAL STRUCTURE

Human serum albumin (HSA) is a single chain of 609 amino acids and a molecular weight of 66.5 kDa. It contains the active albumin (585 amino acids) as well as signal peptides (1–18) and pro-peptides (19–24) (Larsen et al., 2016). The primary structure of HSA consists of one N-terminus, one C-terminus and three homologous domains such as domain I, II, and III. Each of the domains has two helical subdomains (IA and IB, IIA and IIB, IIIA and IIIB) respectively. Each of these subdomains consists of 4 to 6 α -helices (Sand et al., 2015a). Other blood proteins are glycosylated, while HSA is not. Moreover, because of its negatively charged surface and ability to diffuse in and out of blood arteries, HSA has a half-life of around 19 days in blood and is highly stable (Bern et al., 2015; Foss et al., 2016). HSA and BSA share 76% identity in the sequence (Huang et al., 2004) whereas serum albumins from different origins have an average sequence identity of over 62% (Majorek et al., 2012). HSA is a globular, heart-shaped protein contains repeating series of six helical subdomains (He et al., 1992; Sugio et al., 1999). HSA displays its excellent capacity to carry various ligands mainly due to its hydrophobic packets, providing potential applications in drug delivery. In the 3D structure of an albumin, there are two key regions—Sudlow site I and Sudlow site II—that serve as the protein's primary drug-binding sites (Sand et al., 2015).

3. TARGETED THERAPY

The creation of novel therapy approaches that specifically target tumors with certain molecular abnormalities has advanced extraordinarily over the past few decades. These cutting-edge medicines, also called targeted therapies. The secret to increasing overall survival while reducing the unfavorable side effects of cancer treatment is targeted therapy. Comparing patients who got matching targeted therapy to those who did not, the former demonstrated noticeably better overall survival (OS) and progression-free survival (PFS). But because each patient has a different genetic profile, each responds to targeted therapy differently (Zhou et al., 2022). However, the majority of cancer patients were not informed that they were eligible for targeted therapy when they were diagnosed, which could have improved their overall prognoses.

Small molecules and macromolecules (such as monoclonal antibodies, polypeptides, antibody-drug conjugates, and nucleic acids) are broadly two groups into which targeted medications can be divided. Small molecule targeted therapies have advantages over macromolecule medications in various areas, such as the pharmacokinetic (PK) characteristics, prices, patient compliance, and drug storage and transportation (Zhong et al., 2021). These medications have a wide range of targets, including kinases, proteins that regulate epigenetic processes, enzymes that repair DNA damage, and proteasomes. Small-molecule focused anti-cancer medications undoubtedly yet face several difficulties, including low response rates and drug resistance. Targeted therapeutic medicines have effective anticancer effects with fewer side effects than traditional chemotherapy. However, a significant disadvantage of molecular targeted therapy is the development of drug resistance. Several methods have been tried to increase therapeutic efficacy by overcoming this resistance. Here, we provide a summary of current understanding of a number of targeted therapeutic drugs, including classification, mechanisms of action, instances of targeted therapy employed in clinical settings, and prospects for future research (Min et al., 2022).

4. MECHANISM OF HSA SERVES AS A DRUG DELIVERY VEHICLE

The primary role of albumin is derived from its contribution to the transport and oncotic pressure of plasma colloid. Albumin contains hormones, enzymes, drugs, and poisons in addition to stabilizing blood volume in circulation. In addition to maintaining the integrity of the capillary membrane, other physiological processes include antioxidant characteristics, free radical scavenging, and others (Zunszain et al., 2003). Many endogenous and external targets exist for this medication. Additionally, human albumin binds and transports a wide range of hydrophobic molecules, including endogenous (such as cholesterol, fatty acids, bilirubin, and thyroxine) and exogenous (such as drugs and toxins) substances, transition metal ions, and gas (nitric oxide NO), with implications for their solubilization, transport, metabolism, and detoxification (Neumann et al., 2010). Human serum albumin used as a potential drug

delivery vehicle based on its properties like long half-life and accumulation in cancer tissues. It has a characteristic such as ease-of-diffusion towards epithelia, Basically the tumor containing blood vessels are not well-organized, immature and leaky. Through leaky tumor blood vessels albumin can carry anti-cancer agents to tumor sites (Hoogenboezem et al., 2018; Sand et al., 2015). There are many strategies for HSA to deliver drugs, including covalent linking drugs to the albumin are with chemical techniques, fusing peptide or protein using recombinant technology, non-covalently binding drugs to albumin and drug carrying albumin nano particle (An et al., 2017). With notable benefits such abundance in blood, high concentration in tumor areas, superior binding efficacy with a variety of ligands, and an extension of circulatory half-life, human serum albumin (HSA) has proven its adaptable and functional capabilities (Larsen et al., 2016; Sand et al., 2015b; Toh et al., 2020). They forecast that HSA via non-covalently binding, covalently binding, and genetic fusion techniques could generally function as a good drug delivery vehicle. In fact, a number of HSA-based or HSA-binding medications, including Albiglutide, Semaglutide, Abraxane, and Levemir, have been developed and successfully used in clinical settings (Hoogenboezem et al., 2018b).

5. MATERIALS AND METHODS

Silver Nitrate

Nanoparticles (NPs) are used for drug encapsulation and delivery because they are more biocompatible than conventional treatments (Wang et al., 2008). Over the years, it has been emphasized that NP size reduction is crucial for increasing their bioavailability and suitability for therapeutic applications in conditions like cancer (Kim et al., 2007). Silver nanoparticles (AgNPs) have a great deal of potential for treating cancer because they selectively disrupt the mitochondrial respiratory chain, which produces ROS and prevents ATP synthesis, both of which result in DNA damage (AshaRani et al., 2009; Morones et al., 2005). The use of plants for the synthesis of AgNPs is justified since it is not only straightforward, quicker, and easier, but also because the synthesized particles were more reliable, stable, and affordable than those produced by other traditional methods (Mohanpuria et al., 2008). With these evidences, here we investigated the green synthesis of AgNPs using aqueous extract obtained from leaf of *Wrightia tinctoria* and its cytotoxicity against ex-vivo MCF-7 cell line.

MCF-7 cell line

Glucocorticoid, progesterone, and estrogen receptors are present in the human breast cancer cell line MCF-7. It was created in 1970 by Dr. Soule of the Michigan Cancer Foundation in Detroit, Michigan, from the pleural effusion of a 69-year-old White woman who had metastatic breast cancer (adenocarcinoma). MCF-7 cells are beneficial for in vitro breast investigations because they retained a number of desirable traits unique to mammary epithelium, such as processing estrogen via estrogen receptors (ER) in the cell cytoplasm to produce estradiol. It is the first breast cancer cell line to respond to hormones (Electroporation-Based Therapies for Cancer, 2014). Our work has revealed that silver nitrate has a high cytotoxic potential at low concentrations on MCF-7 human breast carcinoma cells and the apoptosis proportion of cells was increased by treatment of silver nitrate in MCF-7 human breast carcinoma cells depolarizing mitochondrial membrane potential. These findings demonstrate that silver nitrate could be used as a pharmaceutical agent against breast cancers (Kaplan et al., 2020).

Synthesis of AgNPs

Briefly, 3ml of ethanol were diluted in 1g of powdered leaf extract (brucine) before being combined with 17ml of distilled water. This subsequent research made use of this plant aqueous extract solution. Then, 100ml of distilled water was used to dissolve 0.022g of silver nitrate (Song et al., 2009). To reduce Ag⁺ ions, individually add 5ml of produced leaf extract aqueous solution to 95ml of 1mM aqueous silver nitrate solution. By conducting the reaction in a water bath at 95°C for 20 minutes, the impact of temperature on the rate of synthesis and the size/shape of the produced AgNPs was examined. As a result, the solution was filtered by centrifuging it repeatedly for 30 minutes at 3500 rpm while it remained at room temperature, then resuspending the pellet in distilled water to get rid of any undesired biological molecules. The centrifugation and resuspension in sterile deionized water procedures were done three times to guarantee greater separation of free entities from metal nanoparticles. We can eliminate any uncoordinated biological molecules by doing this (Sukirtha et al., 2012).

Preparation of bioconjugates

Using a Superdex 75 column that had been pre-equilibrated with 0.01 M phosphate before to usage, HSA was purified using liquid chromatography. 1 mL of an HSA stock solution with a concentration 10 times more than a predetermined volume of Ag NP solution (10^{12} NPs/mL) was added in order to completely cover the surface of a given volume of HSA coated Ag-NP solution. The protein concentration was determined by spectrophotometric analysis using a molar absorption value of $35\,219\text{ M}^{-1}\text{cm}^{-1}$ at 280 nm (Pace et al., 1995). Protein-NP bioconjugates were incubated for varied amounts of time (between 0 and 48 h) with moderate stirring in order to monitor the growth

of protein adsorption on the NP surfaces. After incubation, the bioconjugate samples were centrifuged for 20 mins at speeds ranging from 8000 to 16000 rpm (the larger the sample, the slower the speed). The sample was then resuspended in a protein-free water solution and centrifuged once more to remove any excess protein molecules that were either loosely or unbound to the NP surfaces (Capule et al., 2012; Casals et al., 2011). Then, the bioconjugates were determined by Dynamic Light Scattering method.

6. BIOMEDICAL APPLICATION

One of the biggest causes of death and illness in the globe is cancer. Radiation therapy, chemotherapy, and surgery are currently the most widely used cancer treatments (Urruticoechea et al., 2010). However, their drawbacks, such as renal toxicity, liver toxicity, or decreased drug availability at the target location, outweigh their advantages (Lomis et al., 2016). A target-specific and biocompatible drug delivery method, such as human serum albumin, can be used to solve these issues (Abbasi et al., 2012; Jeong et al., 2016; Satya Prakash, 2010). The properties of HSA-NPs include biocompatibility, biodegradability and non-immunogenicity (Satya Prakash, 2010). By enhancing the increased permeability and retention (EPR) effect rather than administering free medicines, this has improved tumour targeting (Maeda et al., 2000).

7. CONCLUSION

Human serum albumin (HSA) has demonstrated its adaptability and functionality with major advantages including abundance in blood, high concentration in tumor areas, superior binding efficacy with a range of ligands, and an extension of circulatory half-life. They predicted that HSA may generally serve as an effective drug delivery vehicle by non-covalently binding, covalently binding, and genetic fusion approaches. Albiglutide, Semaglutide, Abraxane, and Levemir are only a few of the HSA-based or HSA-binding medicines that have been created and utilized successfully in clinical settings. To conclude, the present study documented the first ever synthesis, characterization and cytotoxicity of biosynthesized AgNPs from *Wrightia tinctoria* against ex-vivo MCF-7 cell line with human serum albumin. Collectively, our data suggests that AgNPs possess superior cytotoxic activity compared to the *Wrightia tinctoria* aqueous extract. With little uncovered mechanism in the current study, there is a wide scope for detailed investigation in the future for the application of AgNPs in cancer therapy. Human serum albumin as a drug delivery vehicle against MCF-7 breast cancer cell line was performed and analysed in ex-vivo method. The results suggest that Phyto therapeutic agent capped Ag-NPs coated with HSA can be employed as a practical drug delivery system in cancer treatment.

8. REFERENCE

- [1] Abbasi, S., Paul, A., Shao, W., & Prakash, S. (2012). Cationic Albumin Nanoparticles for Enhanced Drug Delivery to Treat Breast Cancer: Preparation and In Vitro Assessment. *Journal of Drug Delivery*, 2012, 1–8. <https://doi.org/10.1155/2012/686108>
- [2] An, F.-F., & Zhang, X.-H. (2017). Strategies for Preparing Albumin-based Nanoparticles for Multifunctional Bioimaging and Drug Delivery. *Theranostics*, 7(15), 3667–3689. <https://doi.org/10.7150/thno.19365>
- [3] AshaRani, P. V., Low Kah Mun, G., Hande, M. P., & Valiyaveetil, S. (2009). Cytotoxicity and Genotoxicity of Silver Nanoparticles in Human Cells. *ACS Nano*, 3(2), 279–290. <https://doi.org/10.1021/nn800596w>
- [4] Barreto, J. A., O'Malley, W., Kubeil, M., Graham, B., Stephan, H., & Spiccia, L. (2011). Nanomaterials: Applications in Cancer Imaging and Therapy. *Advanced Materials*, 23(12), H18–H40. <https://doi.org/10.1002/adma.201100140>
- [5] Bern, M., Sand, K. M. K., Nilsen, J., Sandlie, I., & Andersen, J. T. (2015). The role of albumin receptors in regulation of albumin homeostasis: Implications for drug delivery. *Journal of Controlled Release*, 211, 144–162. <https://doi.org/10.1016/j.jconrel.2015.06.006>
- [6] Capule, C. C., & Yang, J. (2012). Enzyme-Linked Immunosorbent Assay-Based Method to Quantify the Association of Small Molecules with Aggregated Amyloid Peptides. *Analytical Chemistry*, 84(3), 1786–1791. <https://doi.org/10.1021/ac2030859>
- [7] Casals, E., Pfaller, T., Duschl, A., Oostingh, G. J., & Puentes, V. F. (2011). Hardening of the Nanoparticle-Protein Corona in Metal (Au, Ag) and Oxide (Fe₃O₄, CoO, and CeO₂) Nanoparticles. *Small*, 7(24), 3479–3486. <https://doi.org/10.1002/sml.201101511>
- [8] Electroporation-Based Therapies for Cancer. (2014). Elsevier. <https://doi.org/10.1016/C2013-0-18150-0>
- [9] Elzoghby, A. O., Samy, W. M., & Elgindy, N. A. (2012). Albumin-based nanoparticles as potential controlled release drug delivery systems. *Journal of Controlled Release*, 157(2), 168–182. <https://doi.org/10.1016/j.jconrel.2011.07.031>

- [10] Foss, S., Grevys, A., Sand, K. M. K., Bern, M., Blundell, P., Michaelsen, T. E., Pleass, R. J., Sandlie, I., & Andersen, J. T. (2016). Enhanced FcRn-dependent transepithelial delivery of IgG by Fc-engineering and polymerization. *Journal of Controlled Release*, 223, 42–52. <https://doi.org/10.1016/j.jconrel.2015.12.033>
- [11] Goy-López, S., Juárez, J., Alatorre-Meda, M., Casals, E., Puentes, V. F., Taboada, P., & Mosquera, V. (2012). Physicochemical Characteristics of Protein–NP Bioconjugates: The Role of Particle Curvature and Solution Conditions on Human Serum Albumin Conformation and Fibrillogenesis Inhibition. *Langmuir*, 28(24), 9113–9126. <https://doi.org/10.1021/la300402w>
- [12] Hazarika, Z., & Jha, A. N. (2020). Computational Analysis of the Silver Nanoparticle–Human Serum Albumin Complex. *ACS Omega*, 5(1), 170–178. <https://doi.org/10.1021/acsomega.9b02340>
- [13] He, X. M., & Carter, D. C. (1992). Atomic structure and chemistry of human serum albumin. *Nature*, 358(6383), 209–215. <https://doi.org/10.1038/358209a0>
- [14] Holliday, D. L., & Speirs, V. (2011). Choosing the right cell line for breast cancer research. *Breast Cancer Research*, 13(4), 215. <https://doi.org/10.1186/bcr2889>
- [15] Hoogenboezem, E. N., & Duvall, C. L. (2018a). Harnessing albumin as a carrier for cancer therapies. *Advanced Drug Delivery Reviews*, 130, 73–89. <https://doi.org/10.1016/j.addr.2018.07.011>
- [16] Huang, B. X., Kim, H.-Y., & Dass, C. (2004). Probing three-dimensional structure of bovine serum albumin by chemical cross-linking and mass spectrometry. *Journal of the American Society for Mass Spectrometry*, 15(8), 1237–1247. <https://doi.org/10.1016/j.jasms.2004.05.004>
- [17] Jabeen, S., Qureshi, R., Munazir, M., Maqsood, M., Munir, M., Shah, S. S. H., & Rahim, B. Z. (2021). Application of green synthesized silver nanoparticles in cancer treatment—a critical review. *Materials Research Express*, 8(9), 092001. <https://doi.org/10.1088/2053-1591/ac1de3>
- [18] Jeong, K., Kang, C. S., Kim, Y., Lee, Y.-D., Kwon, I. C., & Kim, S. (2016). Development of highly efficient nanocarrier-mediated delivery approaches for cancer therapy. *Cancer Letters*, 374(1), 31–43. <https://doi.org/10.1016/j.canlet.2016.01.050>
- [19] Kaplan, A., & Mehtap Kutlu, H. (2020). Investigation of Silver Nitrate on Cytotoxicity and Apoptosis in MCF7 Human Breast Carcinoma Cells. *Asian Pacific Journal of Cancer Biology*, 5(2), 49–56. <https://doi.org/10.31557/apjcb.2020.5.2.49-56>
- [20] Kim, J. S., Kuk, E., Yu, K. N., Kim, J.-H., Park, S. J., Lee, H. J., Kim, S. H., Park, Y. K., Park, Y. H., Hwang, C.-Y., Kim, Y.-K., Lee, Y.-S., Jeong, D. H., & Cho, M.-H. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*, 3(1), 95–101. <https://doi.org/10.1016/j.nano.2006.12.001>
- [21] Larsen, M. T., Kuhlmann, M., Hvam, M. L., & Howard, K. A. (2016). Albumin-based drug delivery: harnessing nature to cure disease. *Molecular and Cellular Therapies*, 4(1), 3. <https://doi.org/10.1186/s40591-016-0048-8>
- [22] Lomis, N., Westfall, S., Farahdel, L., Malhotra, M., Shum-Tim, D., & Prakash, S. (2016). Human Serum Albumin Nanoparticles for Use in Cancer Drug Delivery: Process Optimization and In Vitro Characterization. *Nanomaterials*, 6(6), 116. <https://doi.org/10.3390/nano6060116>
- [23] Maeda, H., Wu, J., Sawa, T., Matsumura, Y., & Hori, K. (2000). Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *Journal of Controlled Release*, 65(1–2), 271–284. [https://doi.org/10.1016/S0168-3659\(99\)00248-5](https://doi.org/10.1016/S0168-3659(99)00248-5)
- [24] Majorek, K. A., Porebski, P. J., Dayal, A., Zimmerman, M. D., Jablonska, K., Stewart, A. J., Chruszcz, M., & Minor, W. (2012). Structural and immunologic characterization of bovine, horse, and rabbit serum albumins. *Molecular Immunology*, 52(3–4), 174–182. <https://doi.org/10.1016/j.molimm.2012.05.011>
- [25] Mohanpuria, P., Rana, N. K., & Yadav, S. K. (2008). Biosynthesis of nanoparticles: technological concepts and future applications. *Journal of Nanoparticle Research*, 10(3), 507–517. <https://doi.org/10.1007/s11051-007-9275-x>
- [26] Morais, M., Teixeira, A. L., Dias, F., Machado, V., Medeiros, R., & Prior, J. A. V. (2020). Cytotoxic Effect of Silver Nanoparticles Synthesized by Green Methods in Cancer. *Journal of Medicinal Chemistry*, 63(23), 14308–14335. <https://doi.org/10.1021/acs.jmedchem.0c01055>
- [27] Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramírez, J. T., & Yacaman, M. J. (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology*, 16(10), 2346–2353. <https://doi.org/10.1088/0957-4484/16/10/059>
- [28] Nazir, S., Hussain, T., Ayub, A., Rashid, U., & MacRobert, A. J. (2014). Nanomaterials in combating cancer: Therapeutic applications and developments. *Nanomedicine: Nanotechnology, Biology and Medicine*, 10(1), 19–34. <https://doi.org/10.1016/j.nano.2013.07.001>

- [29] Neumann, E., Frei, E., Funk, D., Becker, M. D., Schrenk, H.-H., Müller-Ladner, U., & Fiehn, C. (2010). Native albumin for targeted drug delivery. *Expert Opinion on Drug Delivery*, 7(8), 915–925. <https://doi.org/10.1517/17425247.2010.498474>
- [30] Pace, C. N., Vajdos, F., Fee, L., Grimsley, G., & Gray, T. (1995). How to measure and predict the molar absorption coefficient of a protein. *Protein Science*, 4(11), 2411–2423. <https://doi.org/10.1002/pro.5560041120>
- [31] Rao, P. B., & Deeba, F. (2020). Expressions of biomarkers in MCF7 Breast and Colon Cancer Cell Lines. *Journal of Drug Delivery and Therapeutics*, 10(2), 107–114. <https://doi.org/10.22270/jddt.v9i4-s.3993>
- [32] Revathy, R., Joseph, J., Augustine, C., Sajini, T., & Mathew, B. (2022). Synthesis and catalytic applications of silver nanoparticles: a sustainable chemical approach using indigenous reducing and capping agents from *Hyptis capitata*. *Environmental Science: Advances*, 1(4), 491–505. <https://doi.org/10.1039/D2VA00044J>
- [33] Sand, K. M. K., Bern, M., Nilsen, J., Noordzij, H. T., Sandlie, I., & Andersen, J. T. (2015a). Unraveling the Interaction between FcRn and Albumin: Opportunities for Design of Albumin-Based Therapeutics. *Frontiers in Immunology*, 5. <https://doi.org/10.3389/fimmu.2014.00682>
- [34] Satya Prakash, S. (2010). Human serum albumin nanoparticles as an efficient noscapine drug delivery system for potential use in breast cancer: preparation and in vitro analysis. *International Journal of Nanomedicine*, 525. <https://doi.org/10.2147/IJN.S10443>
- [35] Song, J. Y., Jang, H.-K., & Kim, B. S. (2009). Biological synthesis of gold nanoparticles using *Magnolia kobus* and *Diopyros kaki* leaf extracts. *Process Biochemistry*, 44(10), 1133–1138. <https://doi.org/10.1016/j.procbio.2009.06.005>
- [36] Sugio, S., Kashima, A., Mochizuki, S., Noda, M., & Kobayashi, K. (1999). Crystal structure of human serum albumin at 2.5 Å resolution. *Protein Engineering, Design and Selection*, 12(6), 439–446. <https://doi.org/10.1093/protein/12.6.439>
- [37] Sukirtha, R., Priyanka, K. M., Antony, J. J., Kamalakkannan, S., Thangam, R., Gunasekaran, P., Krishnan, M., & Achiraman, S. (2012). Cytotoxic effect of Green synthesized silver nanoparticles using *Melia azedarach* against in vitro HeLa cell lines and lymphoma mice model. *Process Biochemistry*, 47(2), 273–279. <https://doi.org/10.1016/j.procbio.2011.11.003>
- [38] Taheri, A., Atyabi, F., Salman Nouri, F., Ahadi, F., Derakhshan, M. A., Amini, M., Ghahremani, M. H., Ostad, S. N., Mansoori, P., & Dinarvand, R. (2011). Nanoparticles of Conjugated Methotrexate-Human Serum Albumin: Preparation and Cytotoxicity Evaluations. *Journal of Nanomaterials*, 2011, 1–7. <https://doi.org/10.1155/2011/768201>
- [39] Toh, W. H., Louber, J., Mahmoud, I. S., Chia, J., Bass, G. T., Dower, S. K., Verhagen, A. M., & Gleeson, P. A. (2020). FcRn mediates fast recycling of endocytosed albumin and IgG from early macropinosomes in primary macrophages. *Journal of Cell Science*, 133(5). <https://doi.org/10.1242/jcs.235416>
- [40] Urruticoechea, A., Alemany, R., Balart, J., Villanueva, A., Vinals, F., & Capella, G. (2010). Recent Advances in Cancer Therapy: An Overview. *Current Pharmaceutical Design*, 16(1), 3–10. <https://doi.org/10.2174/138161210789941847>
- [41] Wang, S., Liu, S., Zhang, Y., He, J., Coy, D. H., & Sun, L. (n.d.). Human Serum Albumin (HSA) and Its Applications as a Drug Delivery Vehicle. <https://doi.org/10.36648/1791-809X.14.2.698>
- [42] Wang, X., Yang, L., Chen, Z., & Shin, D. M. (2008). Application of Nanotechnology in Cancer Therapy and Imaging. *CA: A Cancer Journal for Clinicians*, 58(2), 97–110. <https://doi.org/10.3322/CA.2007.0003>
- [43] Zunszain, P. A., Ghuman, J., Komatsu, T., Tsuchida, E., & Curry, S. (2003). *BMC Structural Biology*, 3(1), 6. <https://doi.org/10.1186/1472-6807-3-6>