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Charcoal Nanoparticles as a Delivery System for Doxorubicin and Sorafenib in Treatment of Hepatocellular Carcinoma

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Abstract

Background: Hepatocellular carcinoma (HCC) is the most common type of liver cancer and one of the leading causes of cancer-related death worldwide. Advanced HCC displays strong resistance to chemotherapy, and traditional chemotherapy drugs do not achieve satisfactory therapeutic efficacy. The delivery of therapeutic compounds to the target site is a major challenge in the treatment of many diseases. **Objective:** This study aims to evaluate activated charcoal nanoparticles as a drug delivery system for anticancer agents (Sorafenib and Doxorubicin) in Hepatocellular Cancer Stem Cells. **Method:** The percent efficiency of entrapment (% EE) of the doxorubicin and sorafenib entrapped onto the activated charcoal was obtained by determining the free doxorubicin and sorafenib concentration in the supernatant-prepared solutions. Then the characterizations of nanoparticles were formed by determination of the particle size distribution, zeta potential, and polydispersity index (PDI). The anticancer activity of activated Charcoal, Doxorubicin-ACNP, sorafenib-ACNP, free doxorubicin, and free sorafenib solutions was measured based on cell viability percentage in HepG2 cell lines (ATCC-CCL 75). *In vitro* RBC's toxicity of Doxorubicin/sorafenib loaded charcoal was estimated by hemolysis percentage. **Results:** The synthesized Doxorubicin-ACNP and Sorafenib-ACNP were evaluated and their physiochemical properties were also examined. Essentially, the percent Efficiency of Entrapment (EE %) was found to be 87.5% and 82.66% for Doxorubicin-ACNP and Sorafenib-ACNP, respectively. The loading capacity was 34.78% and 24.31% for Doxorubicin-ACNP and Sorafenib-ACNP. Using the Dynamic Light scattering [DLS] for the determination of the hydrodynamic size and surface zeta potential, a

narrow sample size distribution was obtained of (18, 68, and 190 nm for charcoal, 105, 255, and 712 nm for doxorubicin, and 91, 295, and 955 nm for sorafenib), respectively. A surface charge of −13.2, −15.6 and −17 was obtained for charcoal, doxorubicin/charcoal, and sorafenib/charcoal nanoparticles. The cytotoxic activity of Doxorubicin-ACNP and Sorafenib-ACNP was evaluated *in-vitro* against HepG2 cell lines and it was observed that Drug loaded ACNP improved anticancer activity when compared to Doxorubicin or Sorafenib alone. Moreover, testing the toxicity potential of DOX-ACNP and Sorafenib-ACNP showed a significant reduction in the hemolysis of red blood cells when compared to Doxorubicin and Sorafenib alone. **Conclusion:** In conclusion, it is notable to state that this study is regarded as the first to investigate the use of Activated charcoal for the loading of Doxorubicin and Sorafenib for further use in the arena of hepatocellular carcinoma. Doxorubicin-ACNP and Sorafenib-ACNP showed noteworthy anticancer activity along with a reduced potential of RBCs hemolysis rendering it as an efficacious carrier with a low toxicity potential.

Keywords

Activated Charcoal Nanoparticles (ACNP), Drug Delivery System, Sorafenib and Doxorubicin, Hepatocellular Cancer Stem Cells

1. Introduction

Liver cancer is the sixth most common cancer in the world. The 5-year survival rate of patients with hepatocellular carcinoma (HCC) is only 18%, making it the second most fatal tumor after pancreatic cancer [1] [2]. HCC is the most common type of liver cancer and occurs in Sub-Saharan Africa [3]. In the United States, age-adjusted incidence rates of the disease have tripled from 1992 to 2010 [4]. Recent studies in the US have shown a decrease in the incidence rates of hepatocellular carcinoma in younger and middle-aged adults [5], due to the availability of a wide range of treatment options, including hepatectomy, image-guided transcatheter tumor therapy, liver transplantation, transcatheter arterial chemoembolization (TACE), radiotherapy, chemotherapy, and combination therapy [6] [7].

Several antineoplastic agents that can halt the progression of tumors through direct or indirect mechanisms have been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) [8]. These drugs fall into the following categories based on how they work: alkylating agents, antimetabolites, mitotic inhibitors, topoisomerase inhibitors, and antitumor antibiotics, in addition to a more diverse group of agents with various or unknown anticancer activities [9] [10] [11].

The anticancer drug doxorubicin (DOX) was isolated from Streptomyces paucities var. caesious. It is a water-soluble, photosensitive anticancer drug that is used as a potent chemotherapeutic agent to treat a variety of cancers, including

breast, ovarian, leukaemia, and lung. DOX induces cell death through multiple intracellular targets: histone eviction, DNA adduct formation, topoisomerase II inhibition, ceramide overproduction, reactive oxygen species generation, and Ca^{2+} and iron homeostasis regulation [12], however, its use can result in serious adverse side effects including irreversible cardiotoxicity. Consequently, many studies have focused on the development of DOX delivery carriers to improve their efficacy and safety [13].

Sorafenib (SOR) is an oral kinase inhibitor that inhibits tumor cell proliferation and angiogenesis and induces cancer cell apoptosis [2] [14]. It also improves the survival rates of patients with advanced liver cancer. However, due to its poor solubility, fast metabolism, and low bioavailability, clinical applications of sorafenib have been substantially restricted [15]. Currently, conventional antitumor drugs lack selectivity for tumor tissues, and the main obstacles to chemotherapy are multidrug resistance (MDR) and drug toxicity [2] [16].

In recent years, various studies have been conducted on the use of nanoparticles to improve drug targeting and therapeutic efficacy in HCC [17]. Among solid tumors, HCC is considered a typical drug-resistant tumor, and strategies designed to overcome MDR are urgently needed [18]. Uncontrolled phase 2 study comprising 137 patients with advanced hepatocellular carcinoma and Child-Pugh class A or B status suggested that sorafenib might have a positive therapeutic effect when used alone; after all, sorafenib treatment produced a median overall survival of 9.2 months and a median time to progression of 5.5 months [19].

A conventional drug application is characterized by limited effectiveness, poor biodistribution, and a lack of selectivity. These limitations and drawbacks can be overcome by controlling drug delivery. Through the use of controlled drug delivery systems (DDS), the drug is transported to the site of action, minimizing its impact on vital tissues and unwanted side effects [20]. DDS also protects the drug from rapid degradation or clearance and increases drug concentration in target tissues, requiring lower dosages of medication [21].

Numerous studies on nano-drug delivery systems have demonstrated that nanocarriers can enhance the effects of anti-cancer drugs. For example, carbon nanotubes, one type of nanocarrier, have been shown to enhance the effects of paclitaxel, gene, and cisplatin, small molecules, to kill cancer cells. The mechanism by which nanocarriers worked was because they could carry drugs into the cells through endocytosis or something similar [22]. L. Sun *et al.* in 2013 found that the therapeutic effects of ACNPs utilized as anti-cancer medication carriers on experimental malignancies [23]. Develop stabilizing strategies for amorphous drugs with drug loading into porous materials showed great potential interactions between the carrier and the adsorbed drug, as well as the carriers' small pore size, which limits the crystallization of the drug and assists in improving the absorption of the drugs [24] [25] [26]. Activated charcoal has the potential to be used as a microcarrier [27].

Several novel sorafenib and doxorubicin Nanocarriers have been created by

researchers to combat drug resistance in HCC [2] [28] [29] [30]. Typically, nanoparticles (NPs) used in medication delivery applications have a size between 5 and 200 nm [31]. SOR and DOX-loaded nanoparticles actively target tumor tissues and have a high release efficiency and bioavailability [2] [32]. An elevated absolute zeta potential demonstrates a high surface charge density, which boosts cancer cell death and enhances SOR and DOX-NPs' therapeutic efficacy [2] [33]. Moreover, NPs efficiently lower the therapeutic dose and frequency of administration by regulating drug release. Chemotherapy medication cytotoxicity and degradation rate are decreased by NPs. Furthermore, a lot of drug-loaded nanoparticles are delivered to tumor tissues *in vivo* by magnetic fields, and acidic tumor microenvironments can initiate drug release [34]. SOR and DOX-NPs efficiently treat cancer by overcoming the physiological and physical obstacles that prevent conventional medications from working. As a result, nanotechnology holds the ability to overcome MDR and change the way cancer cells respond to cancer medications [35].

The purpose of this study is to examine the use of activated carbon (AC) as a drug carrier for amorphous drug delivery. Nanotechnology can improve disease diagnosis and treatment specificity by addressing challenges like biodistribution and intracellular trafficking and enhancing disease diagnosis through cell-specific targeting and molecular transport.

2. Materials and Method

2.1. Preparation of ACNP

For the preparation of samples of drugs, Doxorubicin HCl (2 mg/mL) solution was purchased from Ebewe Pharma, Australia, and Sorafenib was kindly obtained from MEDISELLER (New Delhi, India). Activated charcoal was supplied by Advent Chembio Pvt. Ltd., India (Ltd. Co., India) and dissolved in ethanol (2 mg/ml). Then, the drugs (doxorubicin or sorafenib, 2 mg/ml) were added to the activated charcoal ethanol solution (drug: charcoal ratio, 1:3). The solution was mixed by a magnetic stirrer for 24 hours at room temperature with magnetic stirring at 800 rpm, then subjected to centrifugation at 15,000 rpm for 10 minutes, then left to dry in an oven overnight at a temperature of 40˚C. The samples were weighed and kept for further studies [36]. All other reagents were of analytical grade, and ultra-purified water with a resistivity of 18 μS/cm was obtained from an ultrapure water system (Millipore Milli-Q system; Milford, MA, USA) and used in all aqueous preparations.

2.2. Efficiency of Entrapment

The percent efficiency of entrapment (% EE) of the doxorubicin and sorafenib entrapped or adsorbed onto the charcoal was obtained from the determination of free doxorubicin and sorafenib concentration in the supernatant recovered after particle centrifugation (18,000 rpm, 15 mins) by absorbance measurement at λmax = 485 and 264 nm, respectively. These doxorubicin and sorafenib quan-

tities were determined using a multiskan sky spectrophotometer (Thermo Scientific, Germany). The supernatant recovered from unloaded charcoal particles (without doxorubicin or sorafenib) was used as a blank. Doxorubicin and sorafenib entrapment efficiency (%) was the percentage of entrapped cecropin B to the total amount of doxorubicin and sorafenib added. The % EE was calculated using Equation (1):

$$\text{Efficiency of entrapment} = (\text{drug B0} - \text{drug Bf})/\text{drug B0} \quad (1)$$

where; drug B0: is the initial amount of doxorubicin and sorafenib added for encapsulation

drug Bf: is the amount of non-entrapped doxorubicin and sorafenib in the supernatant after centrifugation of the particles, respectively [37].

Also, the loading capacity of doxorubicin and sorafenib onto the chitosan particles was determined according to the Equation (2):

$$\text{Loading capacity} = (\text{drug B0} - \text{drug Bf})/\text{NPs wt} \times 100 \quad (2)$$

where NPs wt is the weight of the recovered particles [38].

2.3. Characterization of the Doxorubicin/Sorafenib-Loaded Charcoal Particles

Malvern ZetaSizer Nano ZS (Malvern Instruments Ltd. Malvern, Worcestershire, UK) was used to characterize nanoparticles. 2 mg of each particle was suspended in 2 ml of double distilled water; sonicated for 10 min to ensure uniform dispersion. The particle size analysis was carried out in triplicates at 25˚C, an angle of 90˚ for the photomultiplier, and a wavelength of 633 nm. The surface charge (zeta potential) of the nanoparticles was determined from electrophoretic mobility. The zeta potential measurements were performed in triplicates using the 100 µl aqueous dip cell by Zeta Sizer, Nano ZS (Malvern Instruments Ltd., Malvern, Worcestershire, UK), the samples were diluted 1:10 with double distilled water before measuring [39].

2.4. Anti-Cancer Assay

Cell lines and culture conditions

HepG2 cell lines (ATCC-CCL 75) were obtained from the department of cell culture (Vacsera, Egypt) and were grown in RPMI-1640 media supplemented with 10% heat-inactivated Phosphate buffered saline (FBS) and 100 IU/ml penicillin and 100 IU/ml streptomycin. Cultures were maintained in a humidified atmosphere with 5% CO_2 at 37˚C. The cells were seeded in 96-well culture plates at a density of 5×10^4 cells/ml and incubated for 24 hours at 37˚C to reach 70% confluency. Cells were treated with 3.85 - 250 $\mu g \cdot ml^{-1}$ of each test substance. Activated Charcoal, Dox-charcoal particles, sorafenib-charcoal particles, free doxorubicin, and free sorafenib solutions were prepared using PBS and diluted using culture media. After 24 hours, the medium containing all the samples was removed and the cells were washed with PBS. Then, 50 µl of 0.5% crystal violet

staining solution was added to each well and incubated for 20 min at room temperature on a bench rocker with a frequency of 20 oscillations per minute. The plates were washed four times in a stream of tap water and inverted on filter paper to remove any residual fluid. The plates were air-dried for at least 2 hours at room temperature, then 200 µl of methanol were added to each well and incubated for 20 min at room temperature on a bench checker with a frequency of 20 oscillations per minute. The optical density of each well was measured at 570 nm (OD570) with a plate reader.

$$\text{Cell viability \%} = (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}/\text{Abs}_{\text{mc}} - \text{Abs}_{\text{blank}}) \times 100$$

where $\text{Abs}_{\text{sample}}$ is the absorbance of the sample, $\text{Abs}_{\text{blank}}$ is the absorbance of the blank and Abs_{mc} is the absorbance of the control medium [40].

2.5. *In Vitro* RBC's Toxicity of Doxorubicin/Sorafenib Loaded Charcoal

The blood was collected from a healthy human volunteer (without use of Non-Steroidal Anti-Inflammatory Drugs) for 2 weeks. The blood tubes were centrifuged at 3000 rpm for 10 min, then plasma was poured, and RBCs were washed three times with an equal volume of normal saline. The volume of the blood was measured and re-formed as a 10% v/v suspension with normal saline. Different concentrations of charcoal, Dox-charcoal, sorafenib-charcoal particles, free doxorubicin, and free sorafenib were prepared as follows: 125, 62.5, 31.25, and 15.56, 7.78, and 3.89 µg/ml. A volume of 250 µl of each sample was added to 250 µl of erythrocyte suspension (1 ml (about 0.03 oz) of packed cells in 10 ml of PBS).

The PBS solution and 10% v/v solution of Triton X-100 were used for negative and positive controls, respectively. After 4 hrs of incubation at 37˚C, the samples were centrifuged for 10 min at 2000 rpm, the supernatant was collected, and hemolysis was determined from the 540 nm optical density of haemoglobin released into the supernatant using a multiskan sky spectrophotometer (Thermo Scientific, Germany).

The percentage of hemolysis was estimated by assuming the hemolysis produced in the control was 100%. The results are expressed as percent hemolysis and were calculated according to the equation:

$$\text{Hemolysis \%} = (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{neg}}/\text{Abs}_{\text{pos}}) \times 100\%$$

where $\text{Abs}_{\text{sample}}$ is the absorbance of the sample, Abs_{neg} is the absorbance of the negative control, and Abs_{pos} is the absorbance of the positive control [41].

3. Results

3.1. Efficiency of Entrapment

Using the percent Efficiency of Entrapment (% EE) formula, the entrapment efficiency of the drug/charcoal nanoparticles was determined to be 87.5% and 82.66% for doxorubicin and sorafenib, respectively. In accordance with Loading

Capacity formula [38], the loading capacity was found to be 34.78% and 24.31% for doxorubicin and sorafenib, respectively.

3.2. Characterization of the Doxorubicin/Sorafenib Loaded Charcoal Particles

Upon synthesis and drying, the drug/charcoal nanoparticle dispersions were characterized by dynamic light scattering (DLS) for the determination of hydrodynamic size and the surface zeta potential. The resultant charcoal and drug/charcoal nanoparticles showed a narrow size distribution with hydrodynamic size (18, 68, and 190 nm for charcoal, 105, 255, and 712 nm for doxorubicin, and 91, 295, and 955 nm for sorafenib), respectively (**Figures 1(a)-(c)**).

Moreover, a surface charge of −13.2, −15.6 and −17 was obtained for charcoal, doxorubicin/charcoal and sorafenib/charcoal nanoparticles, respectively (**Figures 1(d)-(f)**). The surface charge of the nanoparticles reflects the stability, dispersability and *in-vivo* activity. [42] Essentially, the surface charge reflects the storage stability of the nano-carriers. Zeta-potential values greater than +30 mV and lower than −30 mV are indicative of stable conditions, whereas values between −30 mV and +30 mV indicate unstable conditions that cause aggregation and coagulation of nanoparticles. [43]

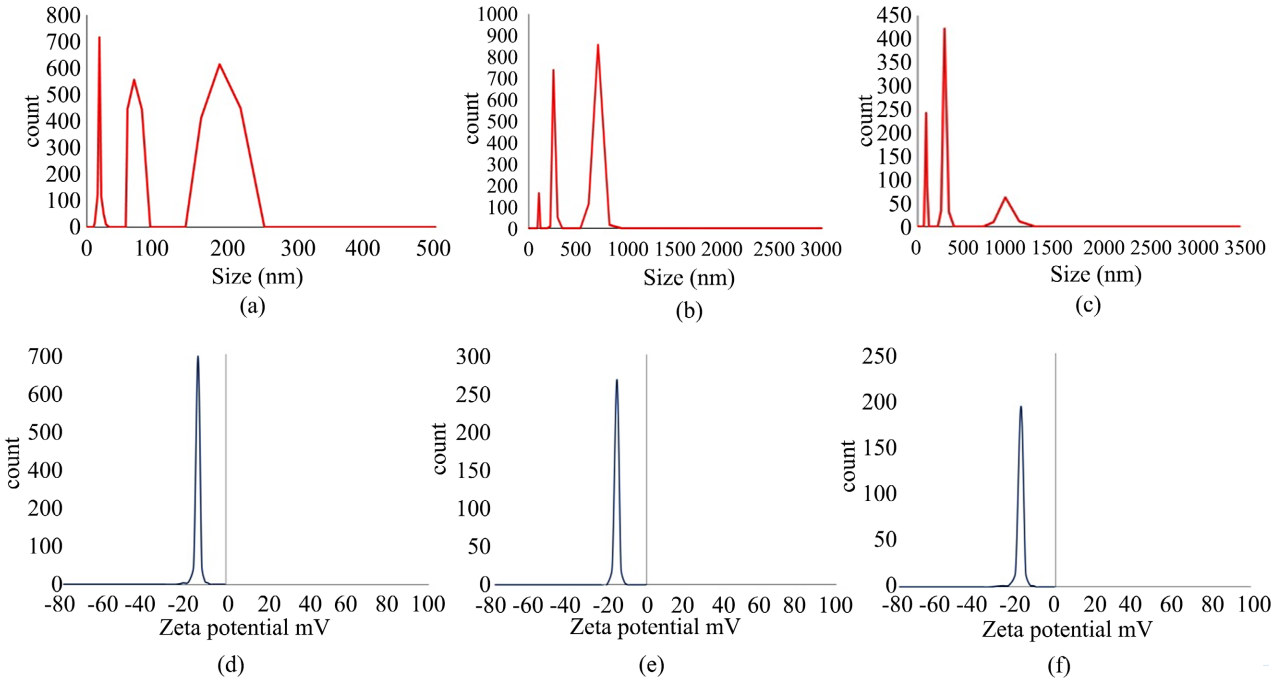

Figure 1. (a)-(c): The particle size of (a) charcoal, (b) doxorubicin/charcoal NP and (c) sorafenib/charcoal NP. (d)-(f): Zeta Potential of (d) charcoal, (e) doxorubicin/charcoal NP and (f) sorafenib /charcoal NP.

3.3. Anti-Cancer Assay and Cytotoxic Activity

The anti-cancer activity of charcoal, doxorubicin/charcoal NPs, sorafenib/charcoal NPs, free doxorubicin, and sorafenib was evaluated *in vitro* against HepG2

hepatocellular carcinoma cell lines. After 24 hr of exposure, IC50 values were determined from a graph of cell viability measured over a range of concentrations between 3.85 and 250 μg/ml. For this data, a line graph was plotted between concentrations (X-axis) versus % cell viability (Y-axis) using GraphPad Prism 8, and then an intersection was drawn at 50% inhibition on the Y-axis and then correlated to the concentration value on the X-axis (Figure 2).

From this data, it is clear that charcoal, doxorubicin/charcoal, sorafenib/charcoal NPs, free doxorubicin, and sorafenib showed a different range of significant anti-cancer activity varying from 3.85 to 250 μg/ml. It was also noticed that the loading of anticancer drugs, doxorubicin and sorafenib, onto charcoal NPs increased their anti-cancer activity against HepG2 hepatocellular carcinoma cell lines. (Table 1 and Figure 2)

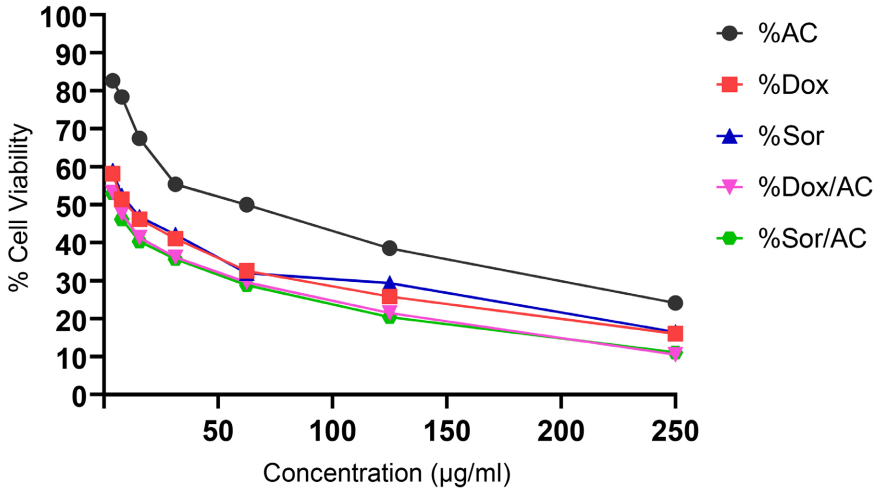

Figure 2. The cytotoxic effect of charcoal, doxorubicin/charcoal, sorafenib/charcoal NPs, free doxorubicin, and sorafenib on the $HepG_2$ cell line.

Table 1. The cytotoxic effect of charcoal, doxorubicin/charcoal, sorafenib/charcoal NPs, free doxorubicin, and sorafenib on the HepG2 cell line.

Concentration (μg/ml)	% Cell viability				
	AC	Dox	Sor	Dox/AC	Sor/AC
250	24.12	15.99	16.43	10.49	11.11
125	38.48	25.77	29.33	21.44	20.39
62.5	49.97	32.56	31.99	29.55	28.77
31.25	55,34	41.04	42.13	36.14	35.66
15.56	67.44	46.18	46.77	41.33	40.27
7.78	78.32	51.39	52.44	47.66	46.09
3.85	82.59	58.14	59.02	53.176	52.99

3.4. *In Vitro* RBC's Toxicity of Doxorubicin/Sorafenib Loaded Charcoal NPs

All the charcoal NPs showed a slight effect on membrane stabilization, whereas doxorubicin and sorafenib/charcoal NPs moderately affected RBCs. (Table 2 and Figure 3) It was observed that the loading of anticancer drugs onto charcoal NPs significantly decreased the hemolysis of red blood cells. The percent of hemolysis was maintained around 6% - 18% even at a maximum concentration of 250 µg/ml for charcoal, 12% - 45% for doxorubicin/charcoal, and sorafenib/charcoal, whereas a high hemolytic effect of 35% - 98% was observed in the case of both free anticancer drugs, doxorubicin and sorafenib.

4. Discussion

Given the poor prognosis and high mortality rates associated with hepatocellular

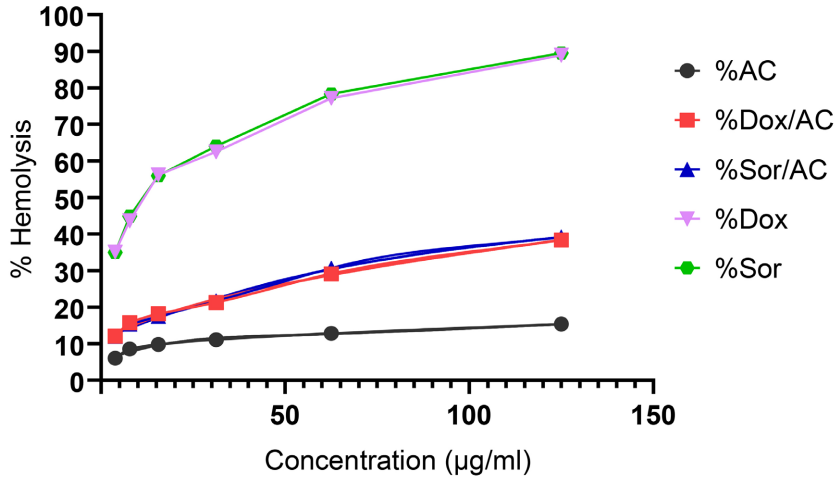

Figure 3. The hemolytic effect of charcoal, doxorubicin/charcoal, sorafenib/ charcoal NPs, free doxorubicin and sorafenib on the RBC's.

Table 2. The hemolytic effect of charcoal, doxorubicin/charcoal, sorafenib/charcoal NPs, free doxorubicin, and sorafenib on the RBC's.

Concentration (µg/ml)	% Hemolysis				
	AC	Dox/AC	Sor/AC	Dox	Sor
250	18.03	45.02	44.89	98.05	97.86
125	15.34	38.33	39.14	88.92	89.52
62.5	12.82	29.13	30.65	77.15	78.33
31.25	11.11	21.25	21.66	62.47	64.02
15.56	9.79	18.16	17.44	56.13	55.92
7.78	8.54	15.72	15.33	43.65	44.86
3.85	6.01	12.05	11.95	35.05	34.94
IC50 (µg/ml)	-	>250	>250	13.01	12.82

carcinoma, novel and effective strategies are urgently required for treatment. Essentially, advances in the field of nanotechnology represent a promising arena for tumor-targeted delivery of chemotherapeutics. Huge strides of progression have been made in the fight against hepatocellular carcinoma, with newer Nano-agents being devised as delivery systems and vehicles for chemotherapeutic drugs. This study investigates the use of activated charcoal as an efficient drug delivery system for better penetration of doxorubicin and sorafenib into hepatic cancer cells.

Following the synthesis of doxorubicin and sorafenib-loaded charcoal NPs, the percent efficiency of entrapment (% EE) was determined to be 87.5% and 82.66% for doxorubicin and sorafenib, respectively. The entrapment efficiency is an essential metric that indicates the nanoparticles' physicochemical properties and ability to act as a delivery system. Both doxorubicin and sorafenib achieved high entrapment efficiency indicative of the effectiveness of charcoal as a delivery system. In a study conducted to evaluate the use of Poly (lactic-*co*-glycolic) Acid (PLGA) and poly (ethylene glycol) PEG-PLGA carriers to entrap doxorubicin and sorafenib together as chemotherapeutic agents [44], the entrapment efficiency of doxorubicin and sorafenib with PLGA and PEG-PLGA carriers was lower than the percentages in our study ranging as (52%, 69% and 74% for doxorubicin) and (55%, 67% and 88% for sorafenib), respectively. This gives an insight into the advantages conferred by the utilization of charcoal as a nanocarrier when compared to PLGA and PEG-PLGA carriers for doxorubicin and sorafenib delivery in hepatocellular carcinoma. Conversely, another interpretation of the low entrapment efficiency obtained in the previous study using PLGA and PEG-PLGA carriers would be attributed to the fact that both doxorubicin and sorafenib were co-loaded into a single carrier, which is different from our study that focused on entrapping a single chemotherapeutic agent with charcoal.

Moreover, in the present study, the loading capacity was found to be 34.78% and 24.31% for doxorubicin and sorafenib, respectively. In a study conducted to evaluate the use of DOX and SOR loaded on ZIF-67 as chemotherapeutic agents in hepatocellular carcinoma, the loading efficiency obtained was as high as 59.7% and 60.2% with doxorubicin and sorafenib, respectively [45]. Additionally, this study found that modifications of the nanocarriers led to better stabilization of the nanocomposite and improved retaining of the drug within the nanocarrier. On the other hand, Babos *et al.* [46] noted that the loading efficiency of doxorubicin and sorafenib on PHB and PEGylated PHB carriers was much lower than the ones obtained in this study (2.6% and 8.4% for doxorubicin and sorafenib with PHB) and (2.6% and 7.7% for doxorubicin and sorafenib with PEGylated PHB), respectively.

The characterization of doxorubicin and sorafenib-loaded charcoal nanoparticles was conducted using dynamic light scattering (DLS) for the determination of hydrodynamic size and the surface zeta potential. The nanoparticle size plays a defining role in determining the efficiency of EPR-based tumoritropic accumulation [47]. In this study, the resultant charcoal and drug/charcoal nanoparti-

cles revealed a narrow size distribution with hydrodynamic size (18, 68, and 190 nm for charcoal, 105, 255, and 712 nm for doxorubicin, and 91, 295, and 955 nm for sorafenib). In a similar study evaluating the synthesis and cytotoxic activity of 5-fluorouracil (5FU) and Sorafenib 32 (SF)-loaded in chitosan nanoparticles, the size of SF/5FU-CS and SF/5FU-CS-FA nanoparticles obtained was about 78 ± 14 nm and 142 ± 25 nm [48]. In a study set to evaluate the potential impacts of particle size of Doxorubicin (DOX) loaded in lipid/glycocholic acid mixed micelles (LGs), particle sizes at around 10 nm and 100 nm were both observed [49]. Nonetheless, it is important to note that nanoparticles smaller than 10 nm are quickly cleared by renal filtration while nanoparticles bigger than 300 nm are quickly identified and eliminated by the reticuloendothelial system (RES) and removed from the blood circulation [44]. Multiple studies from the literature have suggested that nanoparticles with diameters ranging from 50 to 150 nm are considered to be the optimal size for EPR-mediated tumor targeting [50] [51].

Furthermore, a surface charge of −13.2, −15.6, and −17 was obtained for charcoal, doxorubicin/charcoal, and sorafenib/ charcoal nanoparticles, respectively. It is well indicated in the literature that a neutral or slightly negative surface charge of nanoparticles is the best for overcoming the retaining of nanoparticles in vascular endothelial luminal or damage by the reticuloendothelial system in the body [52]. Moreover, the morphology of the synthesized charcoal nano-carriers was not analysed in this study using techniques like TEM, SEM, and XRD, which are commonly used for visualizing nano-carriers. This is because multiple sources in the literature devise the use of Dynamic Light Scattering (DLS) and zeta sizer as the standard characterization techniques for nanocarriers used in drug delivery systems (DDS). [53] [54] However, the use of TEM, SEM and XRD may provide an enhanced overview of the crystallographic structure and the morphology of the charcoal-based nanocarriers.

The cytotoxic activity of charcoal, doxorubicin/charcoal NPs, sorafenib/charcoal NPs, free doxorubicin, and sorafenib was evaluated *in vitro* against HepG2 hepatocellular carcinoma cell lines and significant anti-cancer activities varying from 3.85 to 250 μg/ml were notable. These results are compliant with the literature that shows that the nanoparticles of doxorubicin and sorafenib have been extensively explored to improve the therapeutic efficacy of HCC [2] [12]. Malarvizhi GL *et al.* revealed that sorafenib released from the nano-shell inhibited aberrant oncogenic signaling involved in tumor cell proliferation, whereas doxorubicin from the nano-core evoked DNA intercalation thereby killing > 75% of cancer cells. While in this study involving higher concentrations, >90% of cancer cells were killed [30].

In vitro, determination of hemolytic properties is a common and important method for the preliminary evaluation of cytotoxicity of anticancer drugs. Therefore, in this study the determination of the hemolysis effect was considered and found to be maintained around 6% - 18% even at a maximum concentration of 250 μg/ml for charcoal, 12% - 45% for doxorubicin/charcoal, and soraf-

enib/charcoal, whereas a high hemolytic effect of 35% - 98% was observed in the case of both free anticancer drugs, doxorubicin and sorafenib which indicated the high safety profile of using the ACNPs as a carrier for the anticancer agents (doxorubicin and sorafenib) and this result was found to be compliant with the literature as the hemolysis was found to vary by 2 - 7 folds based on the concentrations of the agents used [41].

5. Conclusion

To our knowledge, this is the first study demonstrating the efficacy of activated charcoal as a nanocarrier for chemotherapeutic agents. This study reveals the potential of activated charcoal as an efficient drug delivery system for anticancer agents and investigates its therapeutic effects on the HepG2 human cell line. In essence, doxorubicin and sorafenib-loaded charcoal nanocarriers were found to be more effective in targeting hepatocellular carcinoma cells in comparison to free charcoal, doxorubicin, or sorafenib. In conclusion, ACNP could strengthen the chemical and physical efficacy of doxorubicin or sorafenib by improving the drug penetration to the microenvironment of the cancer, indicative of ACNP's ability to serve as a good nano-carrier for anti-cancer drugs to target cells. ACNP has great potential for therapeutic applications in anti-tumor chemotherapy. Following their notable *in-vitro* activity, further research investigating the use of ACNP *in vivo* would provide a better overview of the potential of drug-loaded charcoal nanoparticles.

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Author Contribution

Aishah AL-Qurashi Abdullah: Project administration, writing the first draft of the manuscript; Toga Khalid Mohamed Gader: Supervision, writing & editing the final version of the manuscript; Marvit Osman Widdatallah Omer: Co-supervision, writing & editing the final version of the manuscript; Abdullah E. Gouda: Conceptualization of methodology, Project administration; Samah Mamdouh: Conceptualization of methodology, Project administration; Mohamed A. Shemis: Conceptualization of methodology, Project administration.

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Conflicts of Interest

The authors have no conflicts of interest to disclose.

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