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Original Article

Formulation, Characterization, and Cytotoxic Effect of PVA Incorporated Iron Oxide Nanoparticles of Gramine Using HCT-116 Cell Line *in vitro*

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ABSTRACT

Background and Objectives: Globally, Colorectal cancer is the major cause of death. The current work emphasizes the synthesis of Gramine-loaded polyvinyl alcohol (PVA) coated Iron Oxide nanoparticles (NPs) and the evaluation of the antiproliferative and anticancerous activity in the HCT 116 cell line. **Materials and Methods:** The physiochemical, structural, and morphological characteristics of synthesized Gramine loaded PVA coated Iron Oxide NPs were assessed by UV-Visible spectrophotometer, Fourier Transform-Infrared Spectroscopy (FT-IR), X-ray Diffraction (XRD), and scanning electron microscopy (SEM). The cytotoxic activity against HCT 116 (Colon cancer) cell line was assessed using a tetrazolium-based colorimetric assay (MTT), trypan blue exclusion assay, phase contrast microscopy, Nitric Oxide (NO), Lipid Peroxidation (LPO), and Reactive Oxygen Species (ROS) estimation. **Results:** The UV spectrum revealed the synthesis of Iron Oxide NPs at the peak of 266.25 nm. The FT-IR results confirmed that Gramine was successfully conjugated and PVA was efficaciously coated to Iron Oxide NPs. The XRD data revealed that the synthesized Gramine loaded PVA coated Iron Oxide NPs are crystallite in nature. The micrograph of SEM revealed the presence of tetragonal shaped Iron Oxide NPs. In the MTT assay, the proliferation of HCT 116 cells was inhibited in a dose-dependent manner and the IC_{50} was fixed at 25 µg/mL which showed 52.68% of inhibition. In the trypan blue exclusion assay, the percentage of viable cells gradually decreased and the deformation of cells was seen in phase contrast microscopic analysis. In treated groups, the oxidative stress indicators such as NO, LPO, and ROS levels were substantially higher compared to the control. **Conclusion:** The synthesized Gramine loaded PVA coated Iron Oxide NPs exhibited outstanding anticancer activity against HCT 116 cell line.

Keywords: Colorectal cancer cell line, Gramine, Polyvinyl alcohol, Iron oxide nanoparticles, Drug delivery.

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INTRODUCTION

Colon cancer is a widespread condition impacting people all over the world. It is considered as an end result of many years of continued neoplasia in the colon. According to the statistics, it ranks as the third most common factor in cancer-related mortality, behind breast cancer and lung cancer.[1] Adjuvant radiotherapy and chemotherapy with a cytotoxic drug like 5-fluorouracil, oxaliplatin, and cisplatin created drug resistance and resulted in several side effects.[2–4] These challenges encouraged several researchers to investigate strategies, which can provide alternative treatment options to be effective, affordable, and highly compatible. Metal oxide NPs have shown promise in numerous applications, such as gene and medication delivery, cell imaging, and diagnostics.[5–8] Iron oxide Nanoparticles (NPs) are biocompatible and approved for therapeutic application by FDA and are contrast enhancement agents in cell imaging.[9–11] As magnetic nanoparticles have the propensity to aggregate, they are frequently coated using surfactants or polymers to reduce aggregation, especially in the bloodstream to minimize toxicity because of their biocompatibility and biodegradability.[12,13] It is important to note that, the hydrophilic coasting provided in the nanoparticle allows it to stabilize in the body fluids. In addition, it also helps in the bioavailability with quick clearance with the reticuloendothelial system.[14]

The application of medicinal plants and their bioactive compound is an alternative method for treating colon cancer. The major phytocomponents such as alkaloids, phenolics, volatile oils, saponins, flavonoids, quinines, and terpenoids possess a potent cytotoxicity with fewer adverse complications. Due to poor solubility, low bioavailability, ineffective targeting, and commercial

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scaling-up issues, bioactive compounds are conjugated to nanoparticles.[15] In the current study, Gramine delivery using PVA coated Iron Oxide NPs were investigated. Gramine is an indole alkaloid that exhibits potential curative properties including anti-oxidant, anti-proliferatory, anti-inflammatory, antiviral, and antiangiogenic effects but its effect on colon cancer is poorly understood.[16–18] Being a potent anticancer agent, target delivery can provide better treatment without any side effects. Thus, this study investigated the potential of Gramine loaded PVA coated Iron Oxide NPs on its anticancer activity using HCT 116 cell line.

MATERIALS AND METHODS

Materials

Iron (II) chloride ($FeCl_2$), Ferric chloride ($FeCl_3$), Polyvinyl alcohol, and Gramine were purchased from Sigma-Aldrich, St. Louis, MO, USA. DMEM medium, FBS, and Antibiotics were purchased from Gibco, Grand Island, NY, USA. MTT was purchased from Invitrogen, USA. Trypan blue solution, 0.4%, was purchased from Thermo Fisher Scientific, USA. All other reagents used were of analytical grade.

Synthesis of Gramine Loaded PVA-Coated Iron Oxide NPs

Preparation of Iron Oxide NPs

Iron Oxide NPs were synthesized by co-precipitation method.[19] 240 mL $FeCl_2.4H_2O$ (0.6 M) and $FeCl_3.6H_2O$ (1.08 M) were prepared in distilled water and stirred at 600 rpm for 25 min at 60°C. Simultaneously, 400 mL of NaOH (1.6 M) solution was prepared by stirring at 800 rpm for 25 min at 60°C followed by which already prepared solution of $FeCl_2.4H_2O$-$FeCl_3.6H_2O$ was added dropwise resulting in the formation of black precipitates at pH-11. The precipitates were washed to remove residues and the pH was lowered to 7.2 and subjected to drying at 40°C.

Coating of PVA to Iron Oxide NPs

The coating of PVA to synthesized Iron Oxide NPs was performed as mentioned by Nadeem *et al.*[19] with slight modifications. 1 g of synthesized Iron Oxide NPs was mixed with 3 g of PVA and make up to 100 mL using distilled H_2O. The solution was vigorously stirred at 80°C. Following this, the suspension was stirred for an additional 24 hr at room temperature. PVA-coated Iron Oxide NPs were finally dried at 35°C.

Loading of Gramine to PVA Coated Iron Oxide NPs

Gramine was loaded to PVA-coated Iron Oxide NPs by a method mentioned by Nadeem *et al.*[19] with minor modifications. In 100 mL of distilled H_2O, 10 mg of gramine was dissolved and mixed for 10-15 min. 50 mg of synthesized PVA coated Iron Oxide NPs was mixed with Gramine solution and stirred at 300 rpm at 25°C for 22-24 hr. The final precipitates were dried at 50°C.

Characterization of Gramine Loaded PVA Coated Iron Oxide NPs

The absorbance spectrum of Gramine loaded PVA coated Iron Oxide NPs was analysed using a UV-vis-spectrophotometer (Shimadzu, Tokyo, Japan) in the range of 200-800 nm. The functional groups responsible for capping and effective stabilization of the Gramine loaded PVA coated Iron Oxide NPs were determined by FT-IR in the range of 500-3500 cm^{-1}. The FT-IR spectrometer (Alpha, Bruker, Germany) was utilized in order to observe the spectra. The crystallite nature of Iron Oxide NPs was determined using Rigaku Miniflex 600 X-ray diffractometer using a high-power Cu K source at 40 kV/15 mA. The SEM (EVO MA18) was used to investigate the morphological structure.

Cell Line

The colon cancer cells originated from human (HCT116) was purchased from ATCC, USA. The cells were cultured and maintained in a DMEM supplemented with 10% FBS. All cells were incubated at 37°C in 5% CO_2, 95% air, and the media was replaced by fresh media every 2-3 days.

MTT Assay

MTT assay has been employed for the determination of the cell death capacity of the samples compared to the control. The methodology was followed as earlier described with slight modification.[20] Confluent HCT 116 cells were trypsinized and then plated into 96 well treated culture plates at a density of 10000 cells per well. The final volume of each well was kept at 200 µL. The culture plate was then incubated for 24 hr in the CO_2 incubator for attachment of cells, then different concentrations of Gramine loaded PVA coated Iron Oxide NPs were treated in triplicates and kept in the incubator for further effects. The doses used for this experiments was from 0.1 till 100 µg/mL. At the end of experiment, 15 µL of MTT solution prepared (5 mg/mL in PBS) was added to each well and incubated again for 4 hr. Later the media that remained in the cells were removed without disturbing the formazan crystals. To each well, 100 µL of DMSO was added and the crystals were dissolved to form the purple colour. The intensity of the colour (optical density; OD) was then measured at 490 and 630 nm. The percentage inhibition of cells was determined by,

$$\text{Inhibition } (\%) = (1 - \{(\text{OD Treated})_{490} - (\text{OD Treated})_{630} / (\text{OD Control})_{490} - (\text{OD Control})_{630}\} \times 100)$$

Trypan Blue Exclusion Assay

The viability of cells was evaluated using the trypan blue assay after treatment with drug.[21] In 96-well plates, HCT 116 cells were seeded and left for attachment for 24 hr. The cells were treated with Gramine loaded PVA coated Iron Oxide NPs (12.5, 25, and 50 µg /mL) for 24 hr. After treatment, cells were rinsed with PBS,

and trypsinized for 2 min at 37°C in an incubator and cells were pelleted with growth media. The live cells were stained with 4% trypan blue and counted manually by a haemocytometer.

Cell Morphology Analysis

Confluent HCT 116 cells were placed into 24 well plates for overnight. Different concentrations of Gramine loaded PVA coated Iron Oxide NPs were added to it and incubated for 24 hr. The changes in morphological characteristics of the cells were analyzed by a phase contrast microscope (Olympus, Tokyo, Japan).[22]

Nitric Oxide Estimation

Using Griess reagent, the total NO level was estimated from the sample as per the method described earlier with slight modifications.[23] Briefly, 50 µL of the culture supernatant was added to equivalent amount of Griess A reagent (1% sulphanilamide), and set aside for 10 min for reaction at room temperature and dark. Then to each well, 50 µL of Griess B reagent (0.1% NED) solution was added and again incubated for another 10 min at dark and room temperature. At the end of the reaction, the colour formed (purple) was subjected to spectrophotometric measurement at a wavelength of 520 nm and 550 nm.

Lipid Peroxidation Determination

The assay has been carried out to determine the lipid peroxidation followed by the method reported by Ohkawa *et al.* with minor modifications.[24] Firstly the cells from both treated and control groups were harvested by centrifugation. To each samples, ice-cold KCl buffer were added and homogenised. The resultant homogenate (100 µL) were added with 200 µL of 8.1% SDS, 1.5 mL of 20% acetic acid, 1.5 mL of 0.8% TBARS and 700 µL of distilled water. This mixture was then heat to boil for 1 hr. The resultant solution was measured at 532 nm and compared with the standard curve.

Reactive Oxygen Species Analysis

The reactive oxygen species was determined by the method reported by Noori *et al.*[25] The wells were seeded with $1x10^5$ cells/ml and incubated for 24 hr. Following this, cells are treated with various concentrations of Gramine loaded PVA coated Iron Oxide NPs and incubated for 24 hr. After washing with 1X PBS, 100 µL of 0.1% NBT solution was added and incubated for 1 hr and washed thrice using 70% methanol. The dried wells was treated with 12 M potassium hydroxide (120 µL) and DMSO (120 µL). The OD was measured at 630 nm.

Statistical Analysis

All experiments were triplicated and GraphPad Prism version 5.1 was used to perform a One-way analysis of variance on the data. The comparative analysis between the control and the treated groups was performed using Tukey's multiple comparison test. Data are shown as Mean ± SD. $p<0.05$ is considered as significant.

RESULTS

Characterization of Gramine loaded PVA Coated Iron Oxide NPs

The observed spectrum at 266.25 nm in UV-vis. Spectrophotometer confirmed the synthesis of Iron Oxide NPs shown in Figure S1. FT-IR analysis is shown in Figures S2 and S3. The stretching vibrations at 3232 cm^{-1} denote the occurrence of -NH and -OH groups. The band at 2946 cm^{-1} ensures the occurrence of sp^3 bonding in the $-CH_3$ group. The peak at 1639 cm^{-1} corresponds to H-O-H stretching vibrations. The peaks at 1143 cm^{-1} are attributed to C-O bending. The band at 572 cm^{-1} evidenced that the presence of a characteristic band belonging to Fe-O confirms the synthesized NPs predominantly composed of Fe_3O_4. The peaks between 500 cm^{-1} to 800 cm^{-1} correspond to Iron Oxide bonding. The XRD pattern in Figure S4 revealed that synthesized Gramine-loaded PVA-coated Iron Oxide NPs were observed to be crystalline in nature. The lattice planes (311), (400), (422), (511), and (440) confirm the tetragonal structure. The sharper peaks correspond precisely to the cubic spinel phase of Fe_3O_4, which is closer to the American Society for Testing and Materials, and the particles are confirmed as γ-Fe_2O_3 based on the XRD analysis. The microstructures of Gramine loaded PVA coated Iron Oxide NPs were studied using SEM. The tetragonal-shaped NPs with rough surfaces were observed. The SEM micrograph was represented in Figure (1).

Cytotoxicity Assay

The HCT 116 cell line was treated with different concentrations of Gramine-loaded PVA-coated Iron Oxide NPs to evaluate its anti-cancer potential. The MTT results obtained was depicted in Figure 2. The dosage range tested was 0.1–100 µg/mL. The graph showed there was significant inhibition in the proliferation of the HCT 116 cell line in a dose-dependent manner. The 52.68% of inhibition was observed in 25 µg/mL dosage, which is fixed as MIC of Gramine-loaded PVA-coated Iron Oxide NPs against HCT 116. Increased dosage such as 50 µg/mL, showed high inhibition percentage of 98.44. From the results of MTT, it was confirmed that the Gramine-loaded PVA-coated Iron Oxide NPs have remarkable anti-cancer potential.

Trypan Blue Exclusion Assay

The cell viability was assessed for Gramine loaded PVA coated Iron Oxide NPs towards HCT 116 cell line. From the cytotoxicity study, the IC_{50} value was fixed as 25 µg/mL. The dosage fixed for viability assay was ½ MIC, MIC, and 2 MIC such as 12.5, 25, and 50 µg/mL. The results were represented in Figure 3. The figure showed 98.18% of viable cells in the untreated group, whereas the treated groups showed a gradual decrease in the viability

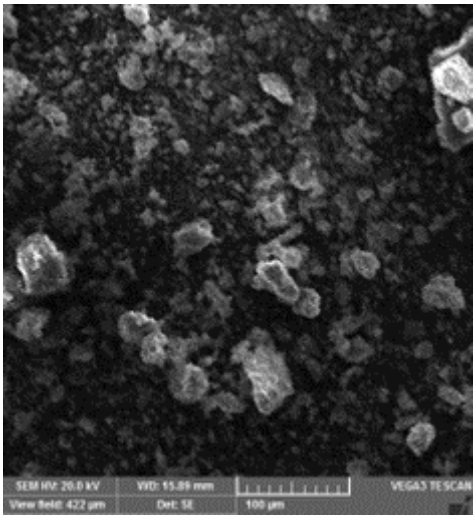

Figure 1: SEM images of Gramine-loaded PVA coated Iron Oxide NPs. The tetragonal shaped Iron Oxide NPs at 100 µm have been depicted in the figure.

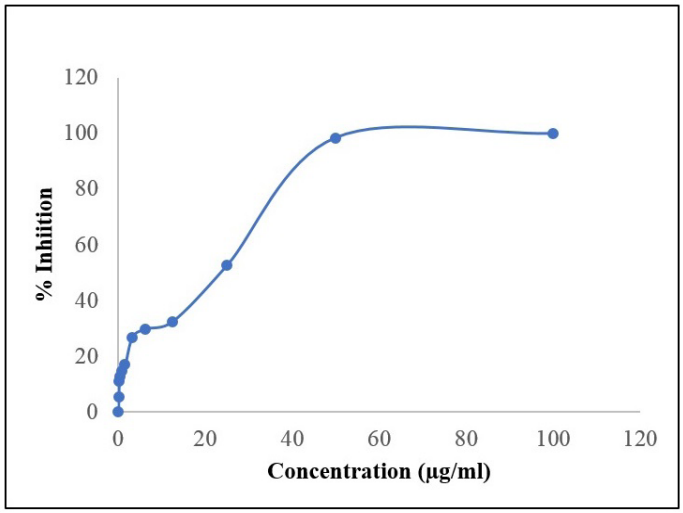

Figure 2: Cytotoxicity analysis. Cytotoxicity potency of Gramine loaded PVA coated Iron Oxide NPs against HCT 116 cell line was depicted in this figure. The graph showed the gradual increase in inhibition percentage in a dose-dependent manner. The 52.68 % of inhibition was obtained in 25 µg/mL concentration, which was fixed as an IC_{50} value.

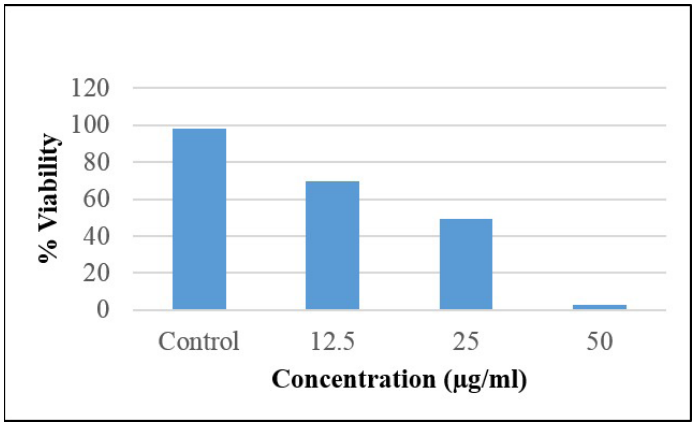

Figure 3: Trypan blue exclusion assay. The figure showed the results obtained from the trypan blue exclusion assay. The decreased % viability was observed with an increase in dose concentration.

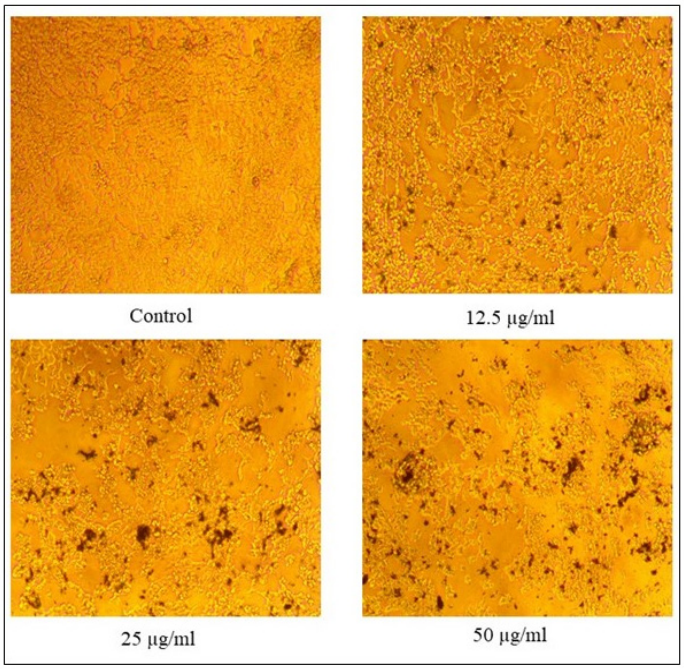

Figure 4: Morphometric analysis. The images showed the morphology of Gramine loaded PVA coated Iron Oxide NPs treated HCT 116 cell line at different dosages. In the control, the cells were observed with fine morphology whereas in the treated group the condensed and dead cells were observed in a dose-dependent manner.

percentage as 69.43, 49.20, and 2.58 for 12.5, 25, and 50 µg/mL, respectively. The viability results are analogues to the cytotoxicity result.

Phase Contrast Images

The morphometric analysis was performed under a phase contrast microscope to evaluate the morphological deformation of the cell line treated with the Gramine-loaded PVA-coated Iron Oxide NPs at different concentrations (Figure 4). The control image showed a large number of healthy cells with a distinct morphology. The cells in the treated groups exhibited cell deformations which includes disintegration, cell shrinkage, cell blubbing, and cell debris from the deformed cells. This observation indicated that the cells in treated groups were typically in the path of apoptosis. The degree of deformation of cells was significant with the dosage concentration.

Nitric Oxide Assay

The NO was estimated by the Griess reagent method. Different concentrations such as 12.5, 25, and 50 µg/mL were employed to evaluate the status of NO production in the HCT 116 cell line upon being treated with Gramine loaded PVA coated Iron Oxide NPs. The results were shown in Figure 5. The control group showed 1.20 ± 0.05 µM of NO production whereas the

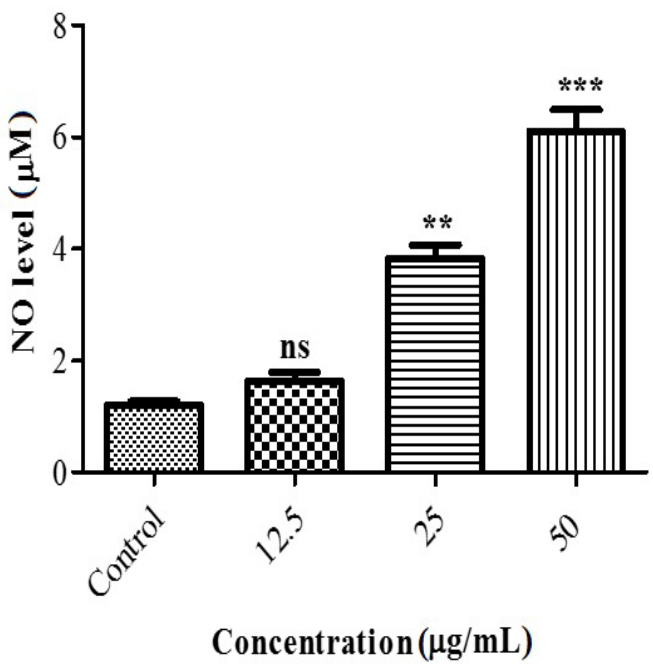

Figure 5: Nitric oxide assay. The graph showed the Nitric oxide level of HCT 116 treated with different concentrations of Gramine loaded PVA coated Iron Oxide NPs such as 12.5, 25, and 50 µg/ml. The NO level was increased in a dose-dependent manner compared to the control group. Data are represented as Mean ± SD. (p<0.001).

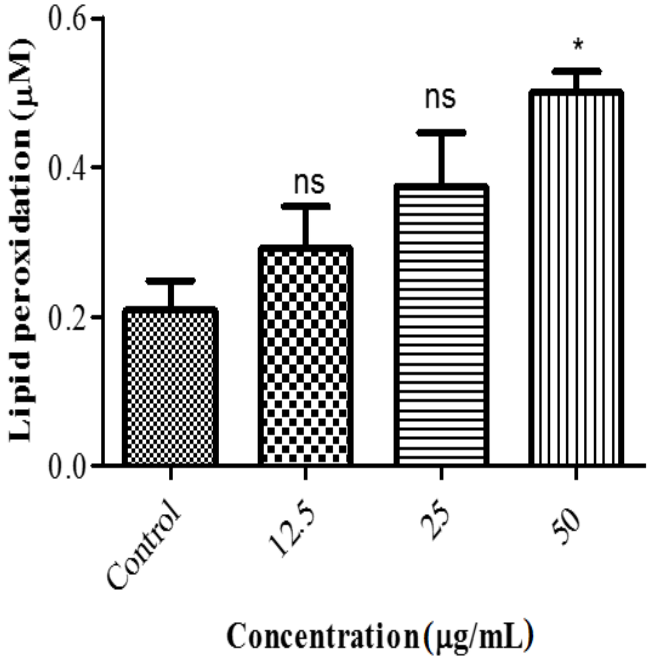

Figure 6: Lipid peroxidation assay. The graph showed the Lipid peroxidation level of HCT 116 treated with different concentrations of Gramine loaded PVA coated Iron Oxide NPs such as 12.5, 25, and 50 µg/ml. The LPO level was increased in a dose-dependent manner compared to the control. Data are represented as Mean ± SD. (p<0.01).

NO production increased remarkably in treated groups in a dose-dependent manner. Low and middle doses showed 1.64 ± 0.14 µM and 3.83 ± 0.23 µM of NO production, respectively. In contrast to the control group there was increased NO production noticed in high dosages (50 µg/mL) such as 5.83 ± 0.38 µM.

Lipid Peroxidation Assay

Lipid peroxidation release was estimated in HCT 116 cell line for both treated and untreated groups. Lipid peroxidation is the major factor in lipid metabolism and analysis of LPO release is the predominant factor. The LPO release estimate was depicted in Figure 6, which showed 0.20 ± 0.03 µM of LPO release. At 12.5 µg/ml concentration, LPO release was 0.29 ± 0.05 µM, whereas in middle and high dosages the LPO increase was moderate when compared to the control as 0.37 ± 0.07 µM and 0.5 ± 0.02 µM, respectively.

ROS Determination

Reactive oxygen species determination was performed using the Nitroblue tetrazolium method, which was reduced by free radicals and formed blue colored compound that was read spectrophotometrically at 620 nm. The Gramine loaded PVA coated Iron Oxide NPs treated and untreated HCT 116 cell line was subjected to ROS analysis. The results were shown in Figure 7 and ROS release was expressed in percentage. In contrast to the control group ROS release was higher in the treated groups such as 21.09 ± 1.97, 35.63 ± 1.76, and 60.89 ± 1.02 for 12.5, 25, and 50 µg/mL, respectively.

DISCUSSION

The synthesis of Gramine loaded PVA coated Iron Oxide NPs was verified using a variety of characterization approaches. The UV spectrum of Gramine loaded PVA coated Iron Oxide NPs indicated absorption peaks of 266.25 nm similar to previous literature.[26,27] The absorption peaks from UV Vis. spectrophotometer indicates that the synthesized NPs are uniformly dispersed throughout the aqueous medium. FT-IR is an efficient technique to identify Gramine conjugation and coating of PVA to Iron Oxide NPs. It is clear from the FT-IR data that the presence of peaks at 3232 cm^{-1} reveals -NH and -OH groups indicating the presence of indole alkaloid compounds. Since Gramine is also one of the indole alkaloids, peaks at 3445 cm^{-1} in the FT-IR of Gramine were comparable to peaks at 3232 cm^{-1} in the FT-IR of Gramine loaded PVA coated Iron Oxide NPs. It confirms the conjugation of Gramine to PVA-coated Iron Oxide NPs. Similarly, the band at 2946 cm^{-1}(-CH_3 group)[28], 1639 cm^{-1} (H-O-H stretching vibrations)[14], 1143 cm^{-1} (C-O stretching vibrations)[28], 500 cm^{-1} to 800 cm^{-1} iron-oxide bonding[29] also supports the formation of particles. Furthermore, the band at 572 cm^{-1} showed the presence of a distinctive Fe-O band completely in augment with previously reported work.[30] The PVA acted as stabilizing agent and the absorption band at 1143 cm^{-1} strongly indicates the incorporation of PVA into iron oxide nanoparticles which was consistent with

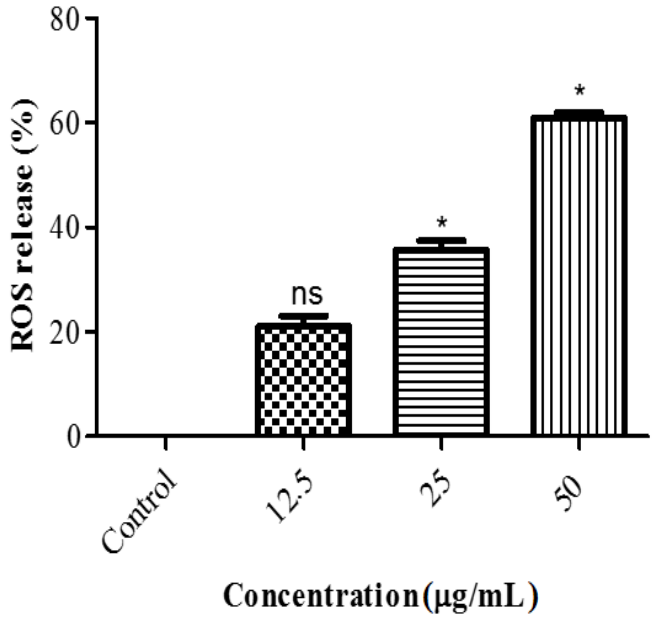

Figure 7: ROS assay. The graph showed the ROS level of HCT 116 treated with different concentrations of Gramine loaded PVA coated Iron Oxide NPs such as 12.5, 25, and 50 µg/ml. The ROS level was increased in a dose-dependent manner compared to the control. Data are represented as Mean ± SD. (P<0.0001).

previous data.[28] In the XRD profile, Gramine loaded PVA coated Iron Oxide NPs are found to be crystalline and tetragonal in structure. The absence of (210) and (300) planes correlates to γ-Fe_2O_3 exemplifies the phase purity of complexes.[19] The SEM data reveal tetragonal-shaped, rough-surface nanoparticles which correlate to XRD. The darker sites in SEM micrograph are due to the presence of PVA.[31]

The cancer therapeutic strategy involves the combination of anticancer drugs and radiation to enhance the efficacy of treatment outcomes. However, selective killing of cancer cells does not occur, creating intrinsic oxidative stress, which activated ROS scavenging and results in resistance to stress. Moreover, exposure of normal cells to cytotoxic antitumor agents leads to increased lethality with serious negative effects on normal tissue and which alarm about the safety of the drugs.[32] Gramin is a simple indole alkaloid with several curative properties such as anti-inflammatory, antiviral, anticancer, and anti-angiogenesis effects.[18,33,34] Gramine's strong hydrophobicity, however, poses a significant barrier to its successful application as a therapeutic agent. Drug delivery or surface modification can be used to overcome the above limitation. Iron nanoparticles can be used as a carrier to deliver chemotherapeutics, peptides, and active compounds, to tumour cells. Though iron oxide nanoparticles are used for delivering of drugs, because of their toxic nature it needs to be coated with polymer to render them biocompatible. The PVA coating exerts non-antigenic, protein-resistant, prolongs blood span time and prevents agglomeration.[35,36] To improve Gramine bioavailability and biocompatibility, a current investigation was made on Gramine-loaded PVA-coated Iron Oxide NPs against HCT 116 cell line.

To investigate the current hypothesis, the cytotoxic effect of Gramine loaded PVA coated Iron Oxide NPs was studied in HCT 116 cells. The IC_{50} of Gramine loaded PVA coated Iron Oxide NPs was noticed at 25 µg/mL and a cytotoxic effect was observed in higher dosages and modest inhibition was noticed in a lower dosages. Microscopic analysis of cytotoxic effect was observed as rounding of cells, shrinkage, and necrosis. These results confirm that bioactive compounds with PVA coated Iron Oxide NPs exerted anticancer activity by inhibiting cell proliferation in a dose-dependent manner.

The imbalance of oxidative stress and anti-oxidant systems within the cell is hampered by ROS, which results in excessive cellular destruction.[37] Hence, the intracellular deposition of ROS within cells disrupts the function of cell organelles and results in cytotoxic effects. On treatment with various doses of Gramine loaded PVA coated Iron Oxide NPs, there was an increase in oxidative stress markers like LPO and NO in HCT 116 cell lines. Moreover, compared to control cells, Gramine loaded PVA coated Iron Oxide NPs treated cells showed a statistically prominent increase in LPO. As a result of the treatment, lipid peroxides may develop because of the release of ROS and the impairment of antioxidants.[38] Proteins and other cellular macromolecules can be oxidised by excessive ROS production, and fatty acids produce excessive amount of reactive radicals, which in turn raise LPO levels.[39] In the present investigation, the concentration of NO was considerably higher in the treated HCT 116 cells. NO controls signaling and the equilibrium of redox pathways, but abnormally excessive NO production can influence a series of cellular events, including ROS generation, disruption of mitochondrial membrane integrity, and ultimately resulting in apoptosis.[40]

CONCLUSION

The present study reported the synthesis of Gramine loaded PVA coated Iron Oxide NPs confirmed by UV-Vis spectrum, FT-IR, XRD, and SEM. Furthermore, the MTT assay showed inhibition of cell proliferation which is well correlated with trypan blue exclusion assay and phase contrast microscopic analysis. The increased NO, LPO, and ROS levels of oxidative stress indicators in treated groups confirm the anticancer activity of Gramine loaded PVA coated Iron Oxide NPs in HCT 116 cells. The current findings suggest that PVA coated Iron Oxide NPs could be effective in delivering bioactive compounds which suppress the proliferation of cancer cells through apoptosis. Further research is needed to explore the nano-pharmacological properties of PVA-coated Iron Oxide NPs.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

ABBREVIATION

PVA: Polyvinyl alcohol; **FTIR:** Fourier Transform Infrared Spectroscopy; **SEM:** Scanning Electron Microscopy; **ROS:** Reactive oxygen species; **NO:** Nitric oxide; **TBARS:** Thiobarbituric acid; **DMEM:** Dulbecco's Modified Eagle Medium; **FBS:** Fetal bovine serum; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **DMSO:** Dimethyl sulfoxide; **NED:** N-(1-Naphthyl)ethylenediamine; **SDS:** Sodium dodecyl sulphate; **PBS:** Phosphate buffered saline

SUMMARY

Gramine-loaded PVA coated Iron Oxide NPs inhibit the proliferation of HCT 116 cells after being treated for 24 hr. Gramine causes the elevation of oxidative stress indicators such as NO, and LPO and the inflation of ROS levels disrupts cellular function and mitochondrial membrane integrity ultimately leading to cytotoxic effects. Gramine-loaded PVA coated Iron Oxide NPs possess remarkable anti-cancer activity. PVA coated Iron Oxide NPs could be a potent drug delivery method for targeting Gramine in cancer treatment.

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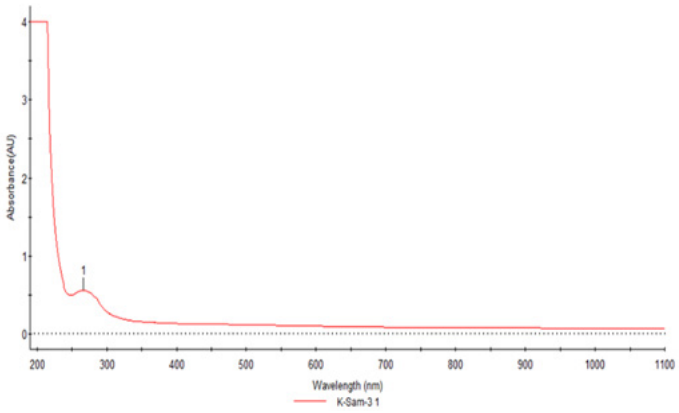

Figure S1: UV-spectrum of Gramine-loaded PVA-coated Iron Oxide NPs.

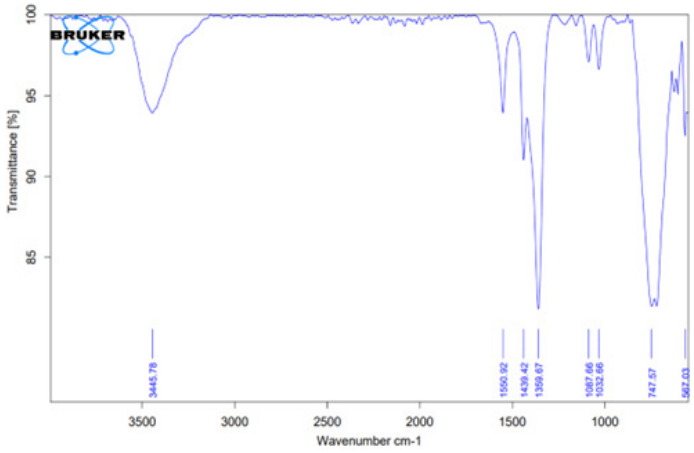

Figure S3: FT-IR profile of Gramine.

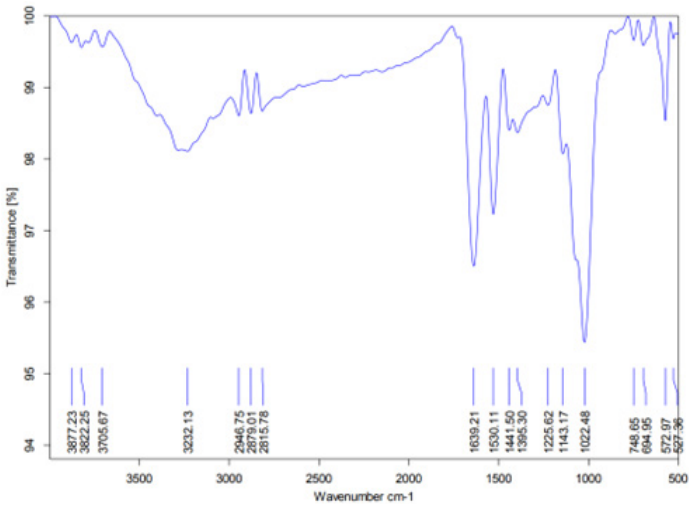

Figure S2: FT-IR profile of Gramine-loaded PVA coated Iron Oxide NPs.

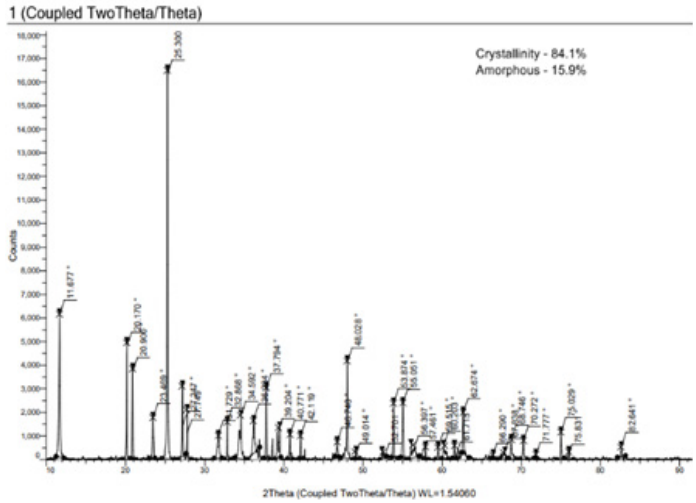

Figure S4: XRD diffraction of Gramine-loaded PVA-coated Iron Oxide NPs.