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Antiproliferative Potential of Biosynthesized Gold Nanoparticles in HepG2 Liver Cancer Cells

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ABSTRACT

OBJECTIVE: To assess the anticancer potential of biologically synthesized gold nanoparticles (AuNPs) through in vitro evaluation, elucidate their antiproliferative mechanisms, and examine their effects on the Hep G2 cell line.
METHODOLOGY: Gold nanoparticles (AuNPs) were biologically synthesized using the fungus *Aspergillus terreus* at the Microbiology Lab, IMBB Department, The University of Lahore. These nanoparticles were characterized to determine their size using X-ray diffraction (XRD) and Transmission Electron Microscopy (TEM). The IC50 of the synthesized AuNPs was evaluated using the MTT assay, which measures cell viability. A scratch assay was performed to check wound healing capacity. Additionally, gene expression analysis was performed using quantitative polymerase chain reaction (qPCR) to investigate the molecular mechanisms underlying the antiproliferative effects of AuNPs on the HepG2 liver cancer cell line.
RESULTS: X-ray diffraction (XRD) analysis revealed gold nanoparticles (AuNPs) with a size range of 18 ± 29 nm, while Transmission Electron Microscopy (TEM) measured them at 13 ± 8 nm. The AuNPs demonstrated significant anticancer activity against the Hep G2 cell line, as evidenced by cytotoxicity assays and qPCR analysis of altered gene expression patterns.
CONCLUSION: Biologically synthesized gold nanoparticles exhibit potent anticancer effects, suggesting their potential as an alternative therapeutic approach for liver cancer. Further investigations are necessary to elucidate their precise mechanisms of action and assess their clinical applicability.
KEYWORDS: Gold nanoparticles, Anticancer, Nanotechnology, Anti-proliferation, Transmission Electron Microscopy (TEM), X-ray diffraction (XRD), Liver Cancer.

INTRODUCTION

Nanotechnology has given a new sense to apply and study elements, especially valuable metals, for the sake of scientific research. The characteristic of nanotechnology to harness the innate properties of a component at the nanoscale level has been a strong factor in driving its application in the medical world. Due to significant advancements in research, nanotechnology has provided alternatives to traditional medicine and achieved notable progress in drug delivery and treatment. In this regard, diseases such as cancer, which is as prevalent as the common cold, are now in the spotlight as candidates for the application of nanotechnology products. Cancer is an uncontrolled division of somatic cells that can cause blockage of normal bodily functions and, in severe cases, even cause death. Even though treatments are available with full chances of recovery, the human body and period for recovery are larger due to side effects associated with the non-specific action of drugs, i.e. targeting organ systems as well as tumors. In this regard, nanotechnology has made significant strides in bringing targeted drug delivery and quicker responses in cancer treatment. Gold (Au) nanoparticles have been utilized in various aspects of bioscience and biomedical research. A product of nanotechnology, gold nanoparticles possess novel properties that make them suitable for use at the sub-cellular level.

The current consideration of AuNP in cancer treatments targets diverse stages of cancer. The use of gold nanoparticles in radiotherapy is of significance, as this metal is a good conductor of radiation and has been shown to induce tumour cell demolition in various treatments involving cancer patients. The effects of radiotherapy include the induction of apoptosis, leading to tissue necrosis, specifically in cancer cells, while sparing normal tissues to the extent possible through precise targeting and dose modulation. This factor is vital and shows promise in the treatment of malignant forms of cancer. An issue with effective treatment involves the in vivo stability of the therapeutic agent, which is compromised when it comes into contact with certain intra- and extracellular proteins and enzymes. In this regard, treatment utilizes binding drug-specific ligands to gold nanoparticles, which can aid in drug stability and delivery to tumour cells.[3] To reduce aggregation, the universal chemical surface of gold nanoparticles allows the coating of polymers, small molecules and bioreactors[4]. This surface modification allows the use of gold nanoparticles in a wide range of chemical, biological, technical and medical applications.

Gold nanoparticles (AuNPs) are approximately inert in biological experimentation and carry many physical characteristics relevant to different biomedical functions. For multiple-targeting and therapeutic ligands that bind to gold nanoparticles, the gold nanoparticles must first pass through a polymeric stabilizer to enhance their stability and functionality. Subsequently, the anti-estrogen molecule is conjugated with thiolated polyethylene glycol (PEG) via an Au-S bond, forming a stable and functional gold-thiolate nanoparticle complex for targeted therapeutic applications[5]. Successful use of the colorimetric AuNP in clinical diagnostics is a sensitive test for clinical specimens of *Myco-bacterium* tuberculosis, a cause of tuberculosis in Homo sapiens[6].

The primary goal of cancer treatment is to provide improved efficacy. A cancer diagnosis is often considered critical, but if detected at an early stage, the prognosis is generally more favorable, allowing for effective treatment and better survival outcomes. A large number of cancer patients are asymptomatic before being at an advanced stage. Current treatment is limited to chemotherapy, radiotherapy and surgery[6]. Due to the limitations of existing treatments and clinical procedures in addressing numerous drug-resistant cancers, the development of innovative technologies for accurate detection and effective treatment has become essential in improving patient outcomes[7]. Several fungi have been used for nanoparticle synthesis; Aspergillus terreus is less commonly reported compared to Aspergillus niger or Penicillium species. We used Aspergillus niger for the synthesis of gold nanoparticles.

METHODOLOGY

Synthesis of nanoparticles (gold and Aspergillus terreus): Gold nanoparticles (AuNPs) were biologically synthesized using the fungus *Aspergillus terreus* at the Microbiology Lab, IMBB Department, The University of Lahore. The cultural flask was incubated at 28˚C on a shaker with 150 rpm for 7 days. Fungal biomass was separated from the medium with the help of the Whatman filter paper. Fungal biomolecules were extracted by washing the biomass several times with sterile water to remove residual media components. The biosynthesis of gold nanoparticles was achieved using an Aspergillus terreus biomass extract as a reducing agent. A 1 mM solution of hydrogen tetra-chloroaurate(III) was prepared in deionized water **Lodhi MS et al.**[12]. The fungal extract was added to 100 mL of 1 mM hydrogen tetrachloroaurate(III) in a 500 mL beaker, with continuous stirring, to synthesize gold nanoparticles. Different concentrations of fungal biomass were used to optimize the reaction until the desired wine-red colour of the solution was achieved. The size range of the resulting particles was 18±29 nm, as determined by XRD, and 13±8 nm by TEM, respectively.

Culturing of the Cell Line: The HepG2 cell line was maintained in the CRIMM department at the University of Lahore. Thecancer cells were inoculated into a DMEM medium and cultured at 37 °C in a 5% CO2 incubator. After 24 hours of culture, the bound cells were examined under a phase-contrast microscope. The cell line was further maintained in a CO_2 incubator, and the DMEM medium was periodically replaced over time.

MTT Assay: When cells reached 70-80% confluency, they were trypsinized and harvested into a 96-well plate at a density of 10,000 cells per well. Drug dilutions (1–200 µg/ml) were applied after 24 hours of culturing the cells. Then, 25 µl of MTT (5 mg/ml) reagent was added to measure cell viability via mitochondrial activity. Formazan crystals were measured at 570 nm by an ELISA plate reader.

Scratch Assay: HepG2 cells were grown in the presence of DMEM supplemented with 10% FBS in a 6-well plate. An 80% to 90% confluency assay was performed using a 10 µL tip, and the sample was observed under a FLOID cell imaging station microscope. The sample was then left for 0 hours and 48 hours for later estimation. No closure shows our novel drug stops the proliferation of thecells. Statistical analysis is accomplished utilizing statistical analysis (SPSS) version 17.0.

RNA extraction: RNA isolation was performed using Trizol reagent. HepG2 cells in Trizol were vortexed, incubated, and then mixed with chloroform. The mixture was centrifuged to separate the aqueous RNA phase. RNA was precipitated with isopropanol, pelleted by centrifugation, washed with 75% ethanol, and air-dried. Finally, RNA was dissolved in 40 µl of nuclease-free water for downstream applications.

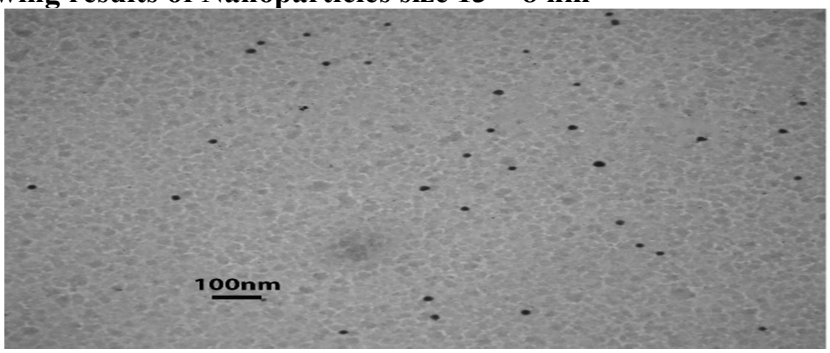
cDNA synthesis: cDNA synthesis was performed using the OneScript cDNA synthesis kit (cat. no. G236). The synthesized cDNA was stored at -20°C or used immediately for downstream applications. Quantitative PCR (qPCR) was performed using SYBR Green 2X qPCR Master Mix in a 10 µl reaction containing master mix, forward and reverse primers (10 µM each), template DNA (≤500 ng), and nuclease-free water. The cycling program included enzyme activation at 95°C for 10 minutes, followed by one cycle of denaturation at 95°C for 15 sec and annealing/extension at 60°C for 10 min. Fast cycling was also employed, with 95°C for 3 seconds and 60°C for 30 seconds. Reactions were prepared on ice, thoroughly mixed, and run according to the protocol.

Step	Temperature (°C)	Time	Cycles
Initial Enzyme Activation	95°C	10 minutes	1
Denaturation	95°C	15 seconds	1
Annealing/Extension	60°C	10 minutes	1
Fast Cycling Protocol			35
Denaturation	95°C	3 seconds	Repeated
Annealing/Extension	60°C	30 seconds	Repeated
Final Hold (if applicable)	4°C	Indefinite	1

RESULTS

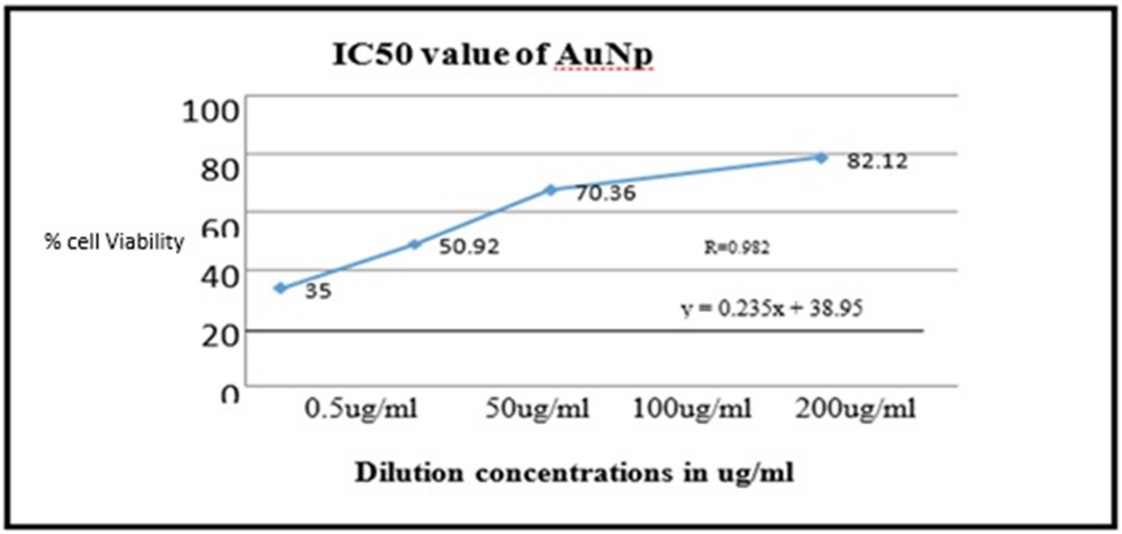
TEM analysis of the gold nanoparticles revealed their size, shape, and distribution. The images showed that the nanoparticles were predominantly rounded, with a narrow size difference. The average diameter of the nanoparticles was observed to be around 10 nm, with a range from Y nm to Z nm. The nanoparticles were well-dispersed without significant aggregation, indicating stable colloidal dispersion. Additionally, the particles exhibited a uniform surface morphology, with no significant defects or irregularities observed in the crystalline structure, as shown in **Figure 1**.

Figure I: Showing results of Nanoparticles size 13 ± 8 nm



The MTT assay was conducted to assess the viability of HepG2 cells and determine the IC50 value following treatment with varying concentrations of AuNPs for 48 hours. The IC50 value, representing the concentration required to inhibit 50% of cell viability, was calculated to evaluate the potency of AuNPs in suppressing cellular activity. The nanoparticles exhibited an IC50 value of 47.02 µg/mL.

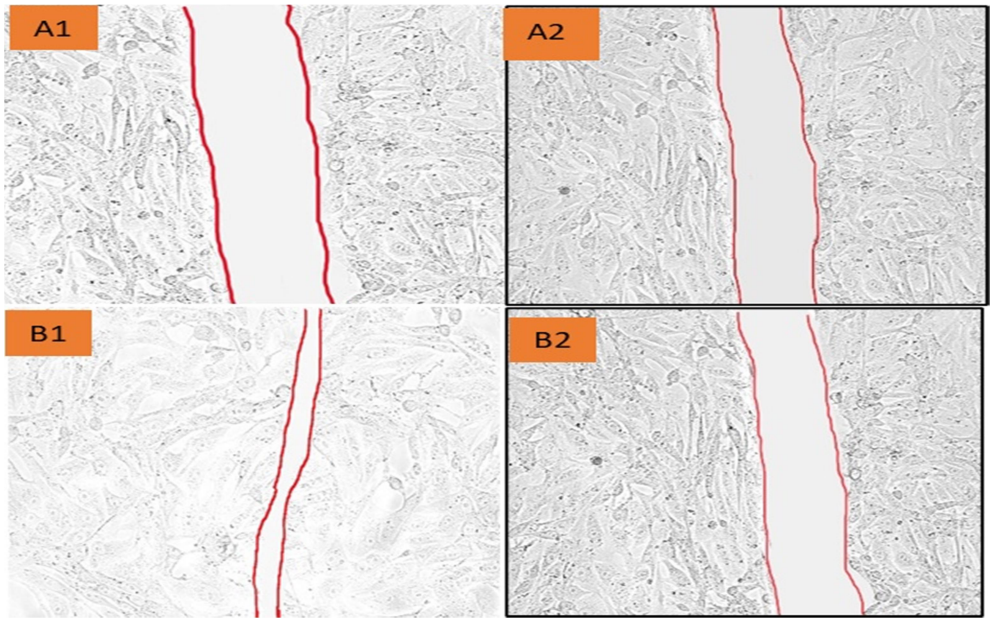
Figure II: Graphical representation of IC 50 value of AuNp



The calculated IC50 value is 47.02 µg/ml, indicating that this concentration is required for 50% inhibition of HepG2 viability. IC 50 Calculations Y=0.235x+38.97 50=0.235x+38.95, 50-38.95=0.235x, 11.05=0.235, X=11.05/0.235X=47.02uM

Microscopic analysis of wound healing, depicted in Figure 3, shows wound healing in the control and treated groups at 24 hours of treatment (A1-A2) and 0 hours (B1-B2) after 48 hours of treatment, respectively. We see no closure of wounds describing the effects of AuNPsin wound healing.

Figure III: Shows the difference in microscopic analysis of wound healing structures in the HepG2 cell line, markedwith gold nanoparticles

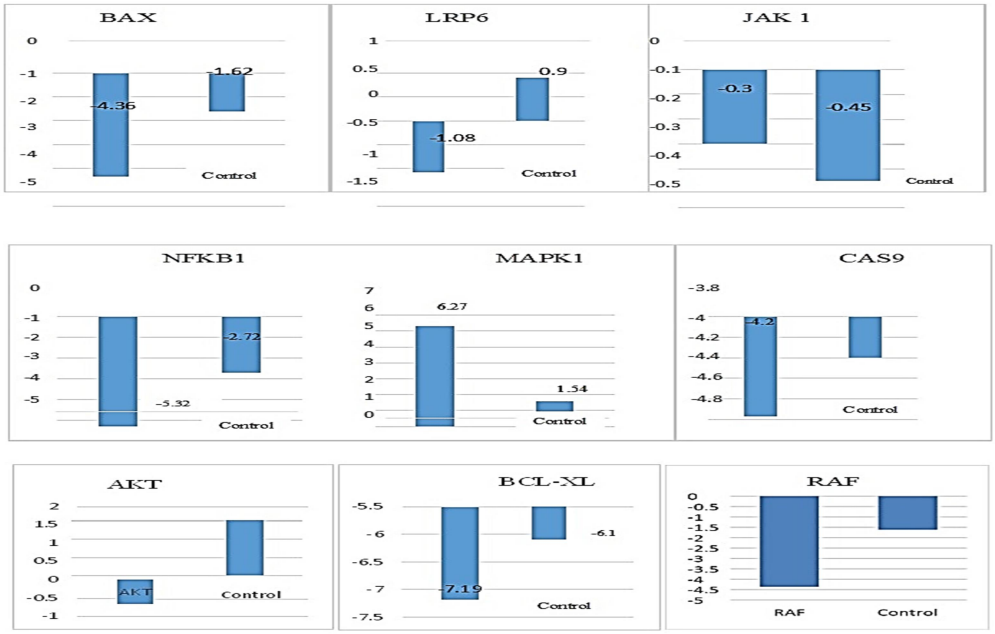


The images illustrate the wound healing process after 48 hours. Panels A1 and B1 depict untreated cells, while panels A2 and B2 show cells treated with the IC50 dose.

RT PCR RESULT

These results validate that, after treatment with gold nanoparticles (a), Bax is downregulated (-4.97) relative to the control sample (-3.96). (b) LRP6 is downregulated (-1.08) compared to theuntreated one (0.9). (c) JAK1 is overexpressed (-0.3) as compared to its control sample, which shows down expression (-0.45) (d) The gene NFKB1 shows down-regulation (-5.32) relative to the control sample (-2.72). (e) MAPK1 overexpressed (6.27) as compared to its control sample (1.54) (f) CAS9 is downregulated in treated one (-4.77) and upregulated in the control (-4.2). (g) AKT is downregulatedin a treated sample of gold nanoparticles (-0.66) and over-expressed in control (1.54) (h) BCL- XL shows downregulation in treated (-7.19) and upregulation in the control sample (-6.1). (i) RAF is down expressed (-4.36) as compared to control one (-1.62)

Figure IV: This graph shows the y-axis representing the ΔCT value of calibrated and Δtest values, and the x-axis indicates gene symbols



DISCUSSION

Cancer is an abnormal growth of cells that tend to proliferate in an uncontrolled manner and, in some cases, to metastasize. Cancer is a leading cause of death in economically developed as well as underdeveloped countries[8]. In this study, we utilized the HepG-2 cell line to evaluate the anticancer activity of biologically synthesized gold nanoparticles. To date, no study has assessed the potential anticancer activity of biologically synthesized gold nanoparticles. The MTT assay was perfumed, which showed IC50 47.02ug/ml. Real-time PCR was performed to determine the expression levels of various genes, including BAX, LRP6, JAK1, NFKB1, MAPK1, RAF, CAS9, AKT, and BCL-XL[9]. *JAK1* and *MAPK* showed differential expression in particular.

Previous studies have demonstrated that various cytokines and mediators play a crucial role in the development, regulation, and progression of cancer, with significant increases in the levels of these markers. Members of the *BCL-2* gene family are the most important regulators of apoptosis[10]. *BAX* is a pro-apoptotic gene involved in the conserved apoptosis pathway, which positively regulates cancer cell apoptosis. The purpose of our study was to evaluate the expression of BCL-XL and BAX genes in predicting proliferation[11]. Other studies have also shown that the overexpression of BCL-XL is responsible for the higher tumour sphere formation capacity, and exposure to a BCL-XL-specific inhibitor therapy produces marked therapeutic effects[12].

In our study, the mRNA expression levels of *BCL-2* and *BAX* in the treated group were also evaluated compared to the normal control group. Our results showed that the expression levels of BCL-XL and BAX were significantly lower in the treated group than in the normal control, following a similar trend to other studies. Akt is a serine/threonine kinase that plays a central role in the pleiotropic control of cell proliferation by insulin and most other growth factors[13]. AKT promotes cell survival by inhibiting apoptosis[14]. AKT1 is amplified or activated almost ubiquitously in prostate cancer[15] as well as many different types of cancer[16,17]. Recent research has uncovered considerably more information on the role of Akt signaling in prostate tumorigenesis. Upregulated Akt serine/threonine kinase, one activity has been observed in multiple tumor types such as brain, breast, ovary, pancreatic and colorectal cancer[15]. In our study, we evaluated the downregulation of RAF and AKT genes in treated groups compared to the control. It has been shown that *LRP6* is upregulated in a subpopulation of human breast cancers[16]. Our findings also showed a similar trend, i.e., downregulation in the treated group. *NF-κB* regulates the expression of genes involved in the development and progression of cancer, including those related to proliferation, migration, and apoptosis. Abnormal and persistent activation of NF-κB has been demonstrated in numerous human malignancies[17]. In our study, gold nanoparticles resulted in a reduction in gene expression levels in the treated samples compared to the control[18]. In this study, we found that treatment with gold nanoparticles improved the parameters of all changes in the treatment groups[19]. A wound-healing assay was also performed to check the invasion and motility of cancer cells and their inhibitory effects. Gold nanoparticles exhibited inhibitory effects on the treated samples in the scratch space, resulting in no cell proliferation[20].

CONCLUSION

Gold nanoparticles (AuNPs) exhibit promising anticancer activity and antiproliferative effects on the Hep-G2 cell line. Conjugation with drug-specific ligands enhances the stability and targeted delivery of these drugs to tumours. Biologically synthesized gold nanoparticles (AuNPs) are environmentally friendly and offer a viable alternative to lipid-based micelles. Combined with medications like AuNPs, it could enable practical and sustainable cancer treatments.

Ethical permission: University of Lahore, Lahore, Pakistan, IRB letter No. IMBB/BBBC/22/129.
Conflict of interest: There is no conflict of interest between the authors.
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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHOR CONTRIBUTION

Azhar MM: Literature search, review, study design
Maqbool T: Designing of questionnaire, data collection
Akbar A: Statistical data analysis
Raza M: Literature search
Rizvi SA: Data interpretation
Ali F: Drafting

REFERENCES

1. Harun-Ur-Rashid M, Jahan I, Foyez T, Imran AB. Bio-inspired nanomaterials for micro/nanodevices: a new era in biomedical applications. Micromachines. 2023; 14(9): 1786.
2. Kesharwani P, Ma R, Sang L, Fatima M, Sheikh A, Abourehab MA et al. Gold nanoparticles and gold nanorods in the landscape of cancer therapy. Mol Cancer. 2023; 22(1): 98.
3. Javid H, Oryani MA, Rezagholinejad N, Hashemzadeh A, Karimi-Shahri M. Unlocking the potential of RGD-conjugated gold nanoparticles: a new frontier in targeted cancer therapy, imaging, and metastasis inhibition. J Mater Chem B. 2024.
4. Hammami I, Alabdallah NM. Gold nanoparticles: Synthesis, properties, and applications. J King Saud Univ Sci. 2021; 33(7): 101560.
5. Esmailzadeh F, Taheri-Ledari R, Salehi MM, Zarei-Shokat S, Ganjali F, Mohammadi A et al. Bonding states of Gold/Silver plasmonic nanostructures and sulfur-containing active biological ingredients in biomedical applications: a review. Phys Chem Chem Phys. 2024.
6. Wikantyasning ER, Wibowo HA. Application of gold nanoparticle-based colorimetric methods in virus detection: a narrative review. In: Proceedings of the 4th International Conference Current Breakthrough in Pharmacy (ICB-Pharma 2022). Atlantis Press; 2022. p. 14–28.
7. Garg P, Malhotra J, Kulkarni P, Horne D, Salgia R, Singhal SS. Emerging therapeutic strategies to overcome drug resistance in cancer cells. Cancers (Basel). 2024; 16(13): 2478.
8. Maqbool T, Awan SJ, Malik S, Hadi F, Shehzadi S, Tariq K. In-vitro antiproliferative, apoptotic and antioxidative activities of medicinal herb Kalonji (Nigella sativa). Curr Pharm Biotechnol. 2019; 20(15): 1288–1308.
9. Özdemir ÖÜ, Yurt K, Pektaş AN, Berk Ş. Evaluation and normalization of a set of reliable reference genes for quantitative sgk-1 gene expression analysis in Caenorhabditis elegans-focused cancer research. Nucleosides Nucleotides Nucleic Acids. 2024; 1-20.
10. Hadi F, Awan SJ, Tayyeb A, Maqbool T, Shehzadi S, Malik S et al. Hepato-protective role of itraconazole mediated cytochrome P450 pathway inhibition in liver fibrosis. Pak J Pharm Sci. 2020; 33: 1–9.
11. Kalsoom A, Altaf A, Ashraf M, Ali MM, Aftab S, Sattar H et al. In vitro evaluation of cytotoxic potential of Caladium lindenii extracts on human hepatocarcinoma HepG2 and normal HEK293T cell lines. Pak J Pharm Sci. 2022; 35(1): 1–9.
12. Lodhi MS, Khan MT, Aftab S, Samra ZQ, Wang H, Wei DQ. A novel formulation of theranostic nanomedicine for targeting drug delivery to gastrointestinal tract cancer. Cancer Nanotechnol. 2021; 12: 1–27.
13. Qian S, Wei Z, Yang W, Huang J, Yang Y, Wang J. The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. Front Oncol. 2022; 12: 985363.
14. Jiang N, Dai Q, Su X, Fu J, Feng X, Peng J. Role of PI3K/AKT pathway in cancer: the framework of malignant behavior. Mol Biol Rep. 2020; 47(6): 4587–4629.
15. Torres DG, Paes J, da Costa AG, Malheiro A, Silva GV, Mourão LPDS et al. JAK2 variant signaling: genetic, hematologic and immune implication in chronic myeloproliferative neoplasms. Biomolecules. 2022; 12(2): 291.
16. Omokehinde TN. GP130 regulation of breast cancer signaling and bone dissemination [dissertation]. Nashville (TN): Vanderbilt University; 2022.

17. Mishra RC, Kalra R, Dilawari R, Goel M, Barrow CJ. Bio-synthesis of Aspergillus terreus-mediated gold nanoparticles: antimicrobial, antioxidant, antifungal and in vitro cytotoxicity studies. Materials (Basel). 2022; 15(11): 3877.
18. Abid F, Saleem M, Muller CD, Asim MH, Arshad S, Maqbool T et al. Antiproliferative and apoptosis-inducing activity of Acacia modesta and Opuntia monocantha extracts on HeLa cells. Asian Pac J Cancer Prev. 2020; 21(10): 3125–3133.
19. Ahmad I, Maqbool T, Naz S, Hadi F, Atif M. Apoptotic potential of geranyl acetate in HepG2 liver cancer cells. Int J Appl Exp Biol. 2023; 2(2): 89–96.
20. Kurmi BD, Patel P, Paliwal R, Paliwal SR. Molecular approaches for targeted drug delivery towards cancer: a concise review with respect to nanotechnology. J Drug Deliv Sci Technol. 2020; 57: 101682.