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Gene Expression Analysis of *Aerva Javanica* and *Parkinsonia Aculeata* Synthesized Gold Nanoparticles in Cervical and Liver Cancer Cell Lines

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Abstract---Chemotherapy is effective in many cancer treatments compare to surgery and radiation. Due to its side effects and multidrug resistance, Nanoparticles synthesized from some of the medicinal plants are one of the prominent alternatives for the treatment of various diseases. In our present study, Synthesized the AuNPs from *Aerva javanica* and *Parkinsonia aculeata* extract and studied the p53, caspase-3, Caspase-9 and NF-kB gene expression in HeLa and HepG₂ cell lines by Real-time PCR. p53 gene down regulation was observed in *Aerva javanica* AuNPs treated HeLa cells compared to HepG₂ cells and caspase-3, Caspase-9 and NF-kB gene expression levels were not changed in both cell lines. *Parkinsonia aculeata* AuNPs treated HeLa and HepG₂ cells not showed any changes in gene expression levels of studied genes. Based on the present results concluding the *Aerva javanica* AuNPs are effective anti-cancer activity on cervical cancer cells.

Keywords---*Aerva javanica*, *Parkinsonia aculeata*, p53, HeLa, HepG₂.

Introduction

Globally cancer is a life-threatening disease in human population and there is a continuous demand for new therapies to prevent and treat (1). The plant producing secondary metabolites are great importance in treatment of various diseases and some of showing an anti-cancer activity (2). In developing countries

approximately 65-80 % of peoples still using a traditional medicine as their primary health care due to accessibility and economic (3). Cancer is an uncontrolled growth of cells and over 100 types of cancers are classified by the affected part type (4). Most of the available cancer drugs are synthetic compounds and showing the adverse effects to cancer patients. Chemotherapy is the widely using in the cancer treatments compare to other treatments like surgery and radiation but due to its multi drug resistance looking for the alternative to chemotherapy with less side effects.

All over the world, Cervical cancer is the second leading cancer in women with high mortality rate (5). Around 500000 new cases and 2,75,000 deaths are occurred approximately (6). In India, cervical cancer contributes to approximately 6–29% of all cancers in women (7). Worldwide Liver cancer mortality rate is increasing every year by 3% (8) with 8,41,000 new cases and 7,82,000 new deaths of liver cancers in 2018 (9).

Aerva javanica is a shrub and belongs to the Amaranthaceae family. whole plant of *Aerva javanica* is used for diarrhoea, diuretic and seeds are used for headache relief, flowers and leaves is used externally to heal the wounds and the inflammation of joints (10). In Egypt's this plant is used as antidiarrheal and anthelmintic medicine and anticancer agent in recent times (11).

Parkinsonia aculeata is a small tree up to 10 meters high and It belongs to the Fabaceae family (12) and used in traditional medicine of these plant parts seeds, leaves, flowers and bark (13-14). Extracts of this plant aerial parts showing the antidiabetic activity (15). In present study synthesized the gold nanoparticles from these two plant extracts and studied the gene expression in Cervical cell line HeLa and Liver cancer cell line HepG₂.

Chemicals

Aerva javanica and *Parkinsonia aculeata* plants were collected and authenticated by Taxonomist. Gold (III)chloride hydrate (Cat No 254169-500MG) purchased from Sigma Aldrich. Dulbecco's modified Eagle's medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Trypsin EDTA, PBS were purchased from HiClone and Fetal Bovine Serum (FBS) were purchased from Gibco. Culture flasks and 6 well plated purchased from Corning.

Preparation of Plant extracts

100 gm of dried plant powder soaked in 1000 ml water in conical flask for extraction and kept it for 72 hrs. with occasional shaking. After 72 hrs., the collected extracts were filtered and concentrated on rotary vapor using round bottom flask. Concentrated extract was stored at 4 °C used for further experiments.

Synthesis of Gold nanoparticles (AuNPs)

125 µl of plant extract (60 g/100 ml H₂O) was mixed with 10 ml of 10mM gold chloride solution at room temperature. The color change Observed that the

yellow-colored gold solution turned to wine red color within a minute. The synthesized Au-NPs were characterized and stored at 4°C until further experiments.

Cell lines and Culture conditions

Cervical cell line HeLa and Liver cancer cell line HepG₂ were procured from NCCS, Pune, India and monolayered maintained in DMEM supplemented with 10 % FBS and the antibiotics solution, in atmosphere of 5% CO₂ at 37 °C. Cell viability estimated by trypan blue assay and cells were seeded in 6 well plate. After 24 hrs treatment of AuNPs, cells were scraped and isolated the total RNA using TRIzol reagent, Takara Bio Inc and isolated RNA is converted to cDNA using the Takara Bio single strand synthesis kit.

Gene expression analysis by Real-Time Polymerase Chain Reaction (RT-PCR)

Gene expression studied were studied by SYBER green using Qiagen Rotor Gene Q real time PCR. The PCR conditions were Initial denaturation 2 mins at 94°C followed by 35 cycles denaturation 30 sec at 94°C, annealing 60°C and extension with 72°C. Final extension will be 72°C with 5 mins of reaction volume 20µl. All the reactions were run in triplicate, including no-template controls. The relative gene expression folds levels were calculated and tabulated.

Gene Name	Primer Sequence
p 53	F AGAGTCTATAGGCCACCCC R GCTCGACGCTAGGATCTGAC
Caspase-3	F AATTGCCTCCACACCTTCAC R TCACCAAGCTGCTCATCAAC
Caspase-9	F AAAGCCCCATCATTCTCCTT R CACCAGACTCGGCACAATC
NF-kB	F TAGCCACAGAGATGGAGGAG R CCGAGTCGCTATCAGAGGTA

Table 1: Primer sequences of Genes.

Statistical Analysis

Data were processed using GraphPad Prism 7 software and the results were provided as a mean ± standard deviation (SD). The significance of a difference was considered in p-value < 0.05.

Results and Discussion

Results

Aerva javanica and *Parkinsonia aculeata* gold nanoparticles (AuNPs) (**Figure 1**) treated with HeLa and HepG₂ cell lines to study the p⁵³, caspase-3, caspase-9 and NF-KB gene expression by quantitative Real Time-PCR. In *Aerva javanica* AuNPs treated HeLa cells showed the down regulation in P⁵³ compare to the HepG₂ cells and other genes expressions are not much changed in HeLa as well

as HepG₂. In *Parkinsonia aculeata* AuNPs treated HeLa and HepG₂ cells are not showed the downregulation in any of genes. (**Figure 2 &3**).

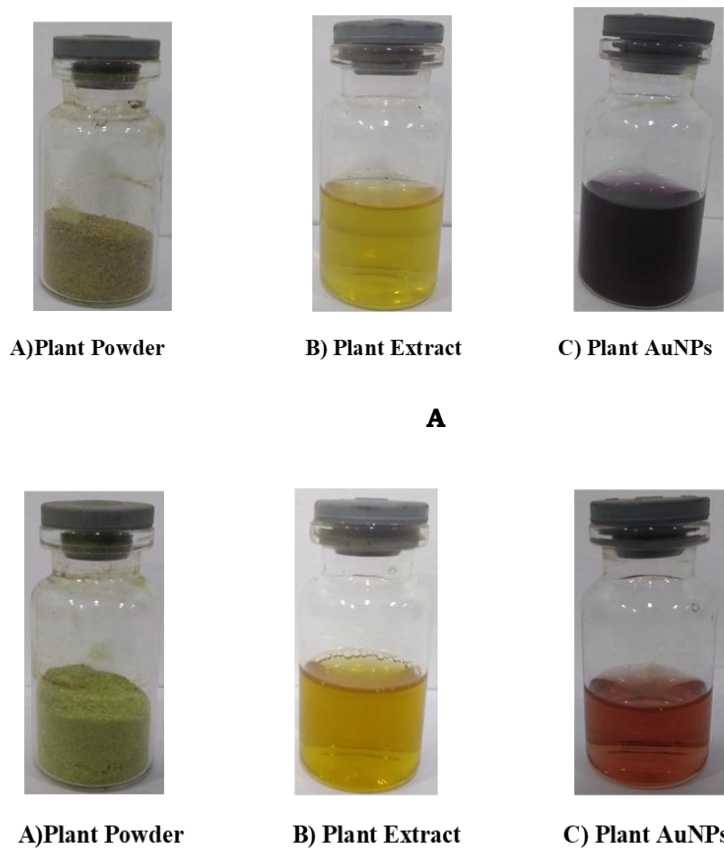


Figure 1: Synthesis of Gold nanoparticles (**A**) *Aerva javanica* (**B**) *Parkinsonia aculeata*.

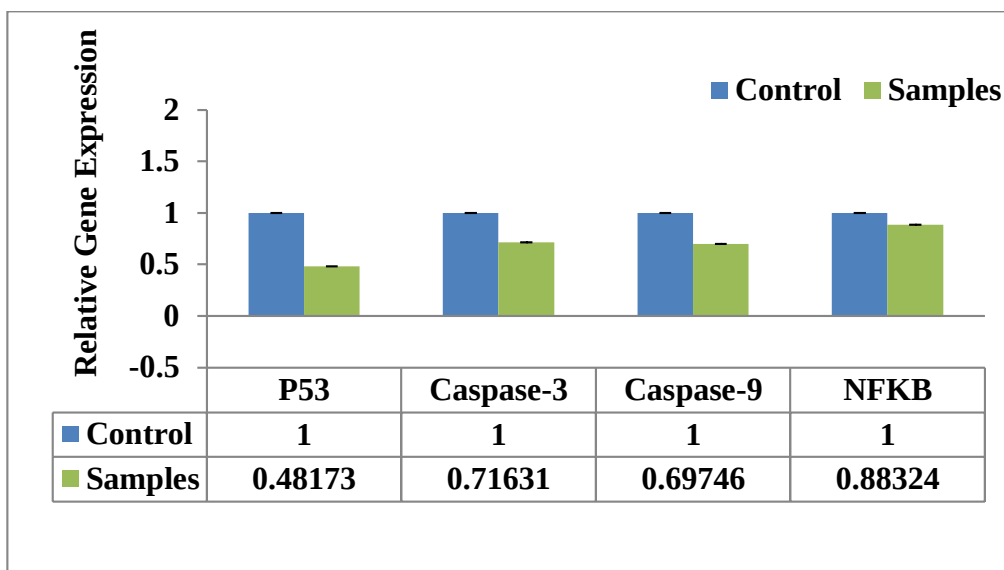
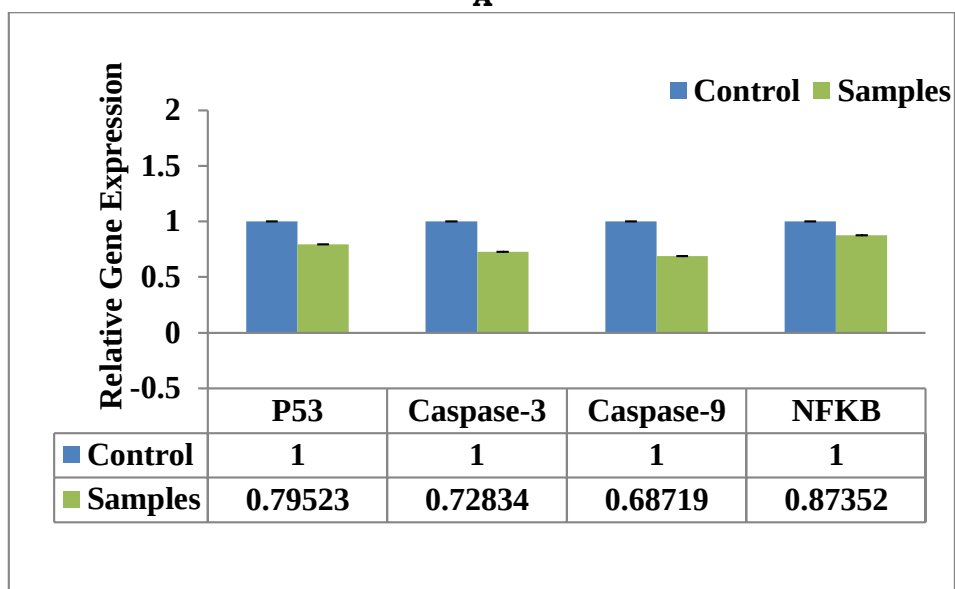
**A****B**

Figure 2: Relative gene expression *Aerva javanica* Gold nanoparticles. **(A)** HeLa **(B)** HepG₂ cells. Results are expressed in Mean $\pm$ SE (n=3).

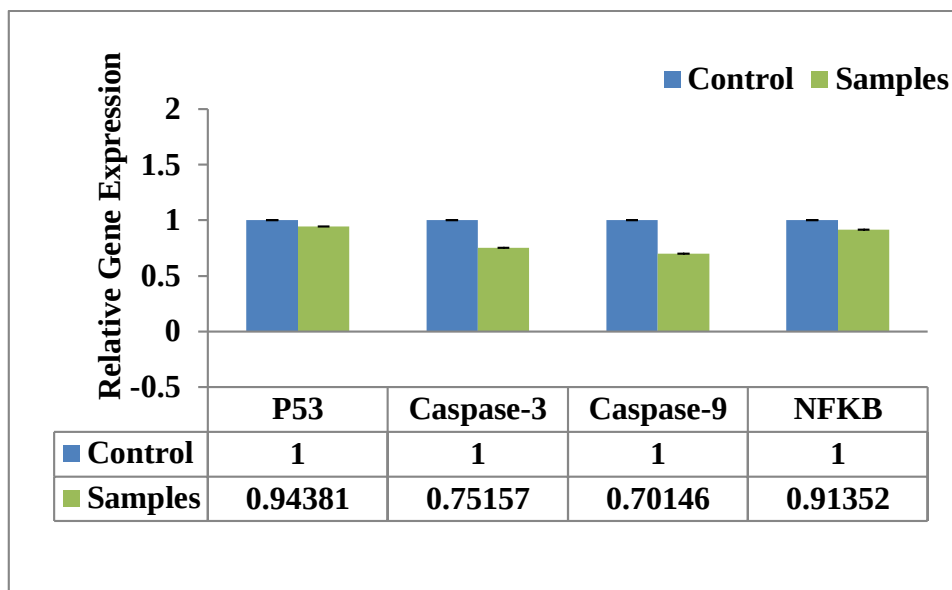
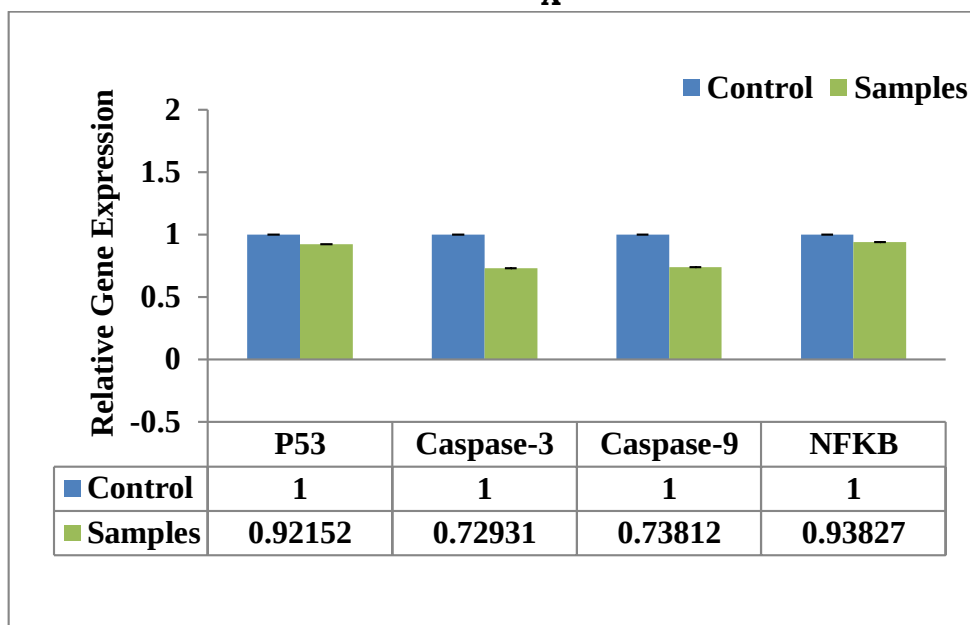
**A****B**

Figure 3: Relative gene expression *Parkinsonia aculeata* Gold nanoparticles. **(A)** HeLa **(B)** HepG₂ cells. Results are expressed in Mean $\pm$ SE (n=3).

Discussion

Green synthesis of nanoparticles is eco-friendly, less toxic and showing the some of metallic nanoparticles are antibacterial, anti-oxidant and anti-cancer agents. Synthesis and applications of green synthesis nanoparticles is increasing day by

day due to drawbacks chemical synthesis (16). To evaluate their biological properties of synthesizing the silver nanoparticles from plants and carrying out the testing to evaluate their biological activities (17). Due to the formation of biofilm layer above the micro-organisms, most of the microorganisms are resistant to antibiotics due to their heavy molecular weight (18). The advantage of nanoparticles size antibiotics converts to nanoparticle size easily entered to the biofilm and help in killing the microorganisms. Ahmed H. Arbab et al studied the oxidative and hepatoprotective of ethanolic extract of *Aerva javanica* aerial parts and reported the attenuation of ex vivo and in vivo hepatotoxicity and oxidative damage (19). Liaqat Hussain and Khizar Abbas et al reported the aqueous-methanolic extract of *aerva javanica* showed the analgesic activity and anti-inflammatory activity (20). Desingu Kamalanathan and Devarajan Natarajan conclude the in vitro plant sample having more potent anticancer property (21). Mohammed Al-Shehri, and Mahmoud Moustafa studied and reported the *aerva javanica* methnolic extract showed the effect in treat breast cancers compared to the prostate cancers (22) In our present work studied the *Aerva javanica* AuNPs and *Parkinsonia aculeata* AuNPs effect in cervical and liver cancer genes of p53, caspase-3, caspase-9 and NF-KB. p53 gene down regulation were observed in the *Aerva javanica* AuNPs treated HeLa cells compared to HepG₂ cells. Other gene expression levels were not much changed in *Aerva javanica* AuNPs treated HeLa and HepG₂ cells. *Parkinsonia aculeata* AuNPs were not showed any affect in the gene expressions levels in HeLa and HepG₂ cells.

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Conflict of interest statement: There are no conflict of interest

Conclusion

Based on our present study we are concluding the *Aerva javanica* AuNPs showed the effect on cervical cancer cells and down regulated the p53 gene expression. *Parkinsonia aculeata* AuNPs not effective like *Aerva javanica* AuNPs in cervical and liver cancers. Further in-vivo studies to be need to conclude the statement.

References

1. M. Greenwell and P.K.S.M. Rahman* Medicinal Plants: Their Use in Anticancer Treatment. Int J Pharm Sci Res. 2015 Oct 1; 6(10): 4103–4112.
2. Faik Gokalp. The effective natural compounds for inhibiting Cervical Cancer. Medical Oncology Volume 38, Article number:12 (2021).
3. ANITHA JOSEPH, KIRUTHIKA DHANRAJ, SIVAKUMAR RAMALINGAM, RENUKA SARAVANAN. TREATING CERVICAL CANCER WITH HERBS: A REVIEW. Asian J Pharm Clin Res, Vol 11, Issue 7, 2018, 4-10
4. Subhankari Prasad Chakraborty. Medicinal plants and cervical cancer therapy: An overview. Journal of Pharmacognosy and Phytochemistry 2019; 8(3): 3633-3641.
5. Medina-Alarcón KP, Voltan AR, Fonseca-Santos B, Moro IJ, de Oliveira Souza F, Chorilli M, et al. Highlights in nanocarriers for the treatment against cervical cancer. Mater Sci Eng C Mater Biol Appl 2017; 80:748-59.

6. Viswanathan AN, Dizon DS, Gien LT, Koh WJ. Chapter 58–Cervical Cancer. Clinical Radiation Oncology. 4th ed. Philadelphia, PA: Elsevier; 2016. p. 1173-202.
7. SaurabhBobdey, Jignasa Sathwara, Aanchal Jain, and Ganesh Balasubramaniam.. Burden of cervical cancer and role of screening in India. Indian J Med Paediatr Oncol. 2016 Oct-Dec; 37(4): 278–285.
8. Siegel, R. L., Miller, K. D., Jemal, A. (2017). Cancer Statistics 2017. *CA Cancer J. Clin.* 67, 7–30
9. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 68, 394–424.
10. Samejo MQ, Memon S, Bhanger MI, Khan KM. Chemical compositions of the essential oil of *Aerva javanica* leaves and stems. *Pak J Anal Environ Chem.* 2012;13: 48–51
11. Noha A. El-Tayeh, Hanaa K. Galal, Magda I. Soliman, Hoida Zaki. Association of Morphological, Ecological, and Genetic Diversity of *Aerva javanica* Populations Growing in the Eastern Desert of Egypt. *Agronomy* 2020, 10, 402.
12. Osman Taha Elzaki, Nawal Ibrahim Idris, Mohamed Elsanosi Adam Habib, Tarig Osman Khider Suitability of *Parkinsonia aculeata* (L.) Wood Grown as an Architectural Landscape Tree in North Darfur State for Interior Design and Furniture. Research Square.
13. Baumer, M. 1983. Trees and Shrubs of Arid and Semi-arid Regions. FAO, Rome. 270 pp.
14. Baja, Y. 1988. Medicinal and Aromatic Plants. In: Biotechnology in Agriculture and Forestry, p24.
15. Ana Catarina Rezende Leite, Tiago Gomes Araújo, Bruno de Melo Carvalho, Maria Bernadete Souza Maia, Vera Lúcia de Menezes Lima. Characterization of the Antidiabetic Role of *Parkinsonia aculeata* (Caesalpineaceae). Evidence-Based Complementary and Alternative Medicine Volume 2011.
16. Arpita Roy Synthesis of silver nanoparticles from medicinal plants and its biological application: A review. *Res Rev Biosci.* 2017;12(4):138
17. S. S. Mahipal, M. Manokari, N. Kannan, J. Revathi, and R. Latha, “Synthesis of silver nanoparticles using *Cardiospermum halicacabum* L. leaf extract and their characterization,” *Journal of Phytopharmacology*, vol. 2, no. 5, pp. 15–20, 2013.
18. HARSHITA AGARWAL, MAHALINGAM GAYATHRI BIOLOGICAL SYNTHESIS OF NANOPARTICLES FROM MEDICINAL PLANTS AND ITS USES IN INHIBITING BIOFILM FORMATION. *Asian J Pharm Clin Res*, Vol 10, Issue 5, 2017, 64-68.
19. Ahmed H. Arbab , Mohammad K. Parvez1 , Mohammed S. Al-Dosari , Adnan J. Al-Rehaily , Khalid E. Ibrahim , Perwez Alam , Mansour S. Alsaid1, Syed Rafatullah Therapeutic efficacy of ethanolic extract of *Aerva javanica* aerial parts in the amelioration of CCl4-induced hepatotoxicity and oxidative damage in rats. *Food & Nutrition Research* 2016, 60: 30864.
20. Liaqat Hussain, Khizar Abbas , Bilal Ahmad , Muhammad Baber, Syed Aun Muhammad Muhammad Imran Qadir. Analgesic and anti-inflammatory

- activity of aqueous-methanolic extract of *Aerva javanica* Pak. J. Pharm. Sci., Vol.30, No.1, January 2017, pp.213-215.
21. Desingu Kamalanathan, Devarajan Natarajan Anticancer potential of leaf and leaf-derived callus extracts of *Aerva javanica* against MCF-7 breast cancer cell line. Journal of Cancer Research and Therapeutics - Volume 14 - Issue 2 - January-March 2018.
 22. Mohammed Al-Shehri, Mahmoud Moustafa Anticancer, Antibacterial, and Phytochemicals Derived From Extract of *Aerva javanica* (Burm.f.) Juss. ex Schult. Grown Naturally in Saudi Arabia. Tropical Conservation Science Volume 12: 1-10