Tumor Biology 43 (2021) 37–55
DOI:10.3233/TUB-200058
IOS Press

Annona muricata silver nanoparticles exhibit strong anticancer activities against cervical and prostate adenocarcinomas through regulation of *CASP9* and the *CXCL1/CXCR2* genes axis

Yahaya Gavamukulya[a,b,*], Esther N. Maina[b,c], Hany A. El-Shemy[b,d], Amos M. Meroka[c,e], Geoffrey K. Kangogo[f], Gabriel Magoma[b] and Fred Wamunyokoli[b,g]

[a]*Department of Biochemistry and Molecular Biology, Faculty of Health Sciences, Busitema University, Mbale, Uganda*
[b]*Department of Molecular Biology and Biotechnology, Pan African University Institute for Basic Sciences, Technology and Innovation, Nairobi, Kenya*
[c]*Department of Biochemistry, College of Health Sciences, University of Nairobi, Nairobi, Kenya*
[d]*Department of Biochemistry, Faculty of Agriculture, Cairo University, Giza, Egypt*
[e]*Department of Biochemistry, School of Medicine and Health Sciences, Kenya Methodist University, Meru, Kenya*
[f]*National HIV Reference Laboratory, Ministry of Health, Nairobi, Kenya*
[g]*Department of Biochemistry, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya*

Received 5 August 2020
Accepted 6 February 2021

Abstract.
BACKGROUND: Green synthesized nanoparticles have been earmarked for use in nanomedicine including for the development of better anticancer drugs.
OBJECTIVE: The aim of this study was to undertake biochemical evaluation of anticancer activities of green synthesized silver nanoparticles (AgNPs) from ethanolic extracts of fruits (AgNPs-F) and leaves (AgNPs-L) of *Annona muricata*.
METHODS: Previously synthesized silver nanoparticles were used for the study. The effects of the AgNPs and 5-Fluorouracil were studied on PC3, HeLa and PNT1A cells. The resazurin, migration and colonogenic assays as well as qRT-PCR were employed.
RESULTS: The AgNPs-F displayed significant antiproliferative effects against HeLa cells with an IC_{50} of 38.58 μg/ml and PC3 cells with an IC_{50} of 48.17 μg/ml but selectively spared normal PNT1A cells (selectivity index of 7.8), in comparison with first line drug 5FU and AgNPs-L whose selectivity index were 3.56 and 2.26 respectively. The migration assay revealed potential inhibition of the metastatic activity of the cells by the AgNPs-F while the colonogenic assay indicated the permanent effect of the AgNPs-F on the cancer cells yet being reversible on the normal cells in contrast with 5FU and AgNPs-L. *CASP9* was significantly over expressed in all HeLa cells treated with the AgNPs-F (1.53-fold), AgNPs-L (1.52-fold) and 5FU (4.30-fold). *CXCL1* was under expressed in HeLa cells treated with AgNPs-F (0.69-fold) and AgNPs-L (0.58-fold) and over expressed in cells treated with 5FU (4.95-fold), but the difference was not statistically significant. *CXCR2* was significantly

*Corresponding author: Yahaya Gavamukulya, Department of Biochemistry and Molecular Biology, Faculty of Health Sciences, Busitema University, P.O. Box, 1460 Mbale, Uganda. E-mails: gavayahya@fhs.busitema.ac.ug and gavayahya@yahoo.com. ORCID ID: https://orcid.org/0000-0001-6031-1642

over expressed in HeLa cells treated with 5FU (8.66-fold) and AgNPs-F (1.12-fold) but under expressed in cells treated with AgNPs-L (0.76-fold).

CONCLUSIONS: Here we show that biosynthesized AgNPs especially AgNPs-F can be used in the development of novel and better anticancer drugs. The mechanism of action of the AgNPs involves activation of the intrinsic apoptosis pathway through upregulation of *CASP9* and concerted down regulation of the *CXCL1/ CXCR2* gene axis.

Keywords: Silver nanoparticles, HeLa, PC3, PNT1A, *CASP9*, *CXCL1/CXCR2*

Abbreviations

AgNPs	Silver Nanoparticles
AgNPs-F	Silver Nanoparticles from fruits of *Annona muricata*
AgNPs-L	Silver Nanoparticles from leaves of *Annona muricata*
A570	Absorbance at 570 nm
A600	Absorbance at 600 nm
5FU	5-Fluorouracil
CC_{50}	Cytotoxic concentration required to kill 50% of the normal cells
DMEM	Dulbecco's modified Eagle medium
DMSO	Dimethyl Sulfoxide
FCS	Fetal Calf Serum
IC_{50}	Inhibitory concentration required to kill 50% of the cancer cells
RPMI	Roswell Park Memorial Institute
SD	Standard Deviation

1. Introduction

Cancer is reported to rank second among the top causes of death worldwide and was responsible for 8.8 million deaths in 2015, a figure expected to rise if no immediate interventions are put in place [1]. Cervical cancer remains the top cause of morbidity and mortality in women diagnosed with cancer in Eastern Africa while prostate cancer is the most prevalent cancer type in men within the region [2, 3]. Currently, there is a limited choice of treatments for these cancers [2–6], and therefore the need to continue searching for more effective treatment regimens.

Among the hallmarks of human cancers is the intrinsic or acquired resistance to apoptosis. Cancer cells resistance to apoptosis is therefore a critical rationale behind treatment failure [7, 8]. *CASP9* gene encodes Caspase 9 enzyme which can undergo autoproteolytic digestion and activation by the apoptosome activating factor 1 which is critical in the earliest steps in the caspase activation cascade of the intrinsic pathway [9, 10].

Chemokines are a group of low-molecular-weight chemotactic cytokines that participate in various cellular processes such as embryogenesis, angiogenic activity, leukocyte migration and tumor growth and metastasis [11–13]. *CXCLs/CXCR2* signaling is important in both cancer and different inflammatory diseases. Because the increased expression of CXCR2 and neutrophil/monocyte migration is critical in chronic inflammation and cancer, the need to focus on using CXCR2 antagonists in cancer and related diseases drugs development remains eminent [12, 13]. *CXCL1* gene as well as its receptor *CXCR2* gene have been found to be highly associated with tumorigenesis, angiogenesis, and metastasis [11, 12].

In recent times, traditional medicine has taken an important place especially in developing countries where limited health services are available [14, 15]. *Annona muricata* of the Annonaceae family is