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CYTOTOXIC ACTIVITY OF GALLIC ACID AND MYRICETIN AGAINST OVARIAN CANCER CELLS BY PRODUCTION OF REACTIVE OXYGEN SPECIES

LUIS VARELA-RODRÍGUEZ [1,*], PATRICIA TALAMÁS-ROHANA [2], BLANCA SÁNCHEZ-RAMÍREZ [3], VERÓNICA IVONNE HERNÁNDEZ-RAMÍREZ [2], HUGO VARELA-RODRÍGUEZ [1].

[1] Facultad de Enfermería y Nutriología (FEN-UACH), CP 31125, Chihuahua, CHH, México.
[2] Departamento de Infectómica y Patogénesis Molecular (CINVESTAV-IPN), CP 07360, CDMX, México.
[3] Facultad de Ciencias Químicas (FCQ-UACH), CP 31125, Chihuahua, CHH, México.
* Correspondence: lvrodriguez@uach.mx

INTRODUCTION

Ovarian cancer is the sixth most frequent tumor in women and represents the fourth cause of death in Mexico due to gynecological tumors [1]. The principal treatment for this disease is surgical resection, followed by a complementary treatment with chemotherapy [2]. However, chemotherapy for ovarian cancer has shown limited success and generation of resistance in neoplastic tissue, whereby search for alternative treatments or new therapeutic agents for this disease is necessary [2]. Some studies demonstrate that gallic acid (GA) and myricetin (MYR) identified in *Rhus trilobata*, could provide therapeutic activity against this pathology [3].

AIMS

To evaluate the cytotoxic activity of GA and MYR against ovarian cancer cells and determine the possible action mechanism present.

RESULTS

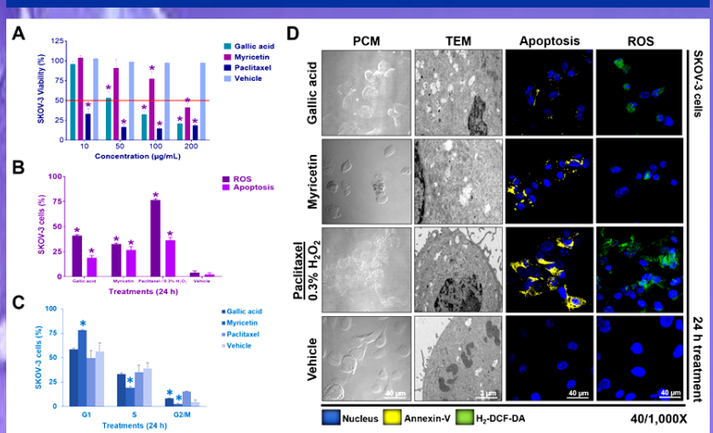

Figure 1. Biological activity of GA and MYR in SKOV-3 cells. Viability curves for 24 h by MTT assay (**A**). Induction of oxidative stress and apoptosis by H_2-DCF-DA and annexin-V assays, after 24 h of treatment with GA (50 µg/mL) and Myr (166 µg/mL) (**B**). DNA content by propidium iodide and flow cytometry in the same conditions mentioned above to determine phases of the cell cycle (**C**). Morphological and ultrastructural changes by phase-contrast microscopy (PCM) and transmission electron microscopy (TEM) (**D**). Results represent mean ± S.D. of 3 independent replicates (n = 3; triplicates); * Difference significative respect to vehicle group (cells treated with 0.5 % DMSO/1X PBS) ($p \leq 0.05$, ANOVA). Paclitaxel and H_2O_2 were positive controls used according to the assay.

Table 1. Therapeutic targets of GA and MYR.

Compound name	Target key	Target protein	Organism	Description	pKi (L.E.)	*P*-Value	Max Tc * (affinity)
GA (ZINC1504)	CAH9_HUMAN+5 (SP: Q16790)	Carbonic anhydrase-IX (CA9)	Eukaryote (Human)	Enzyme (E-lyase)	5.13 (0.60 kcal/mol/atom)	9.984 x10^{-5}	1 (6,990 nM)
MYR (ZINC3874317)	PIK3CG_HUMAN+5 (SP: P48736)	PI3K	Eukaryote (Human)	Enzyme (E-other)	5.33 (0.32 kcal/mol/atom)	0.5057	1

* Affinity binding of compound vs protein ≤ 10 µM. GA, gallic acid; MYR, myricetin; SP, Swiss-Prot protein sequence database (UniProt); PI3K, Phosphatidylinositol 4,5-bisphosphate 3-kinase.

CONCLUSION

GA and MYR demonstrated are capable of act against SKOV-3 ovarian adenocarcinoma cells through ROS production, which modifies the actin/tubulin cytoskeleton, induces cell cycle arrest, and activation of cell death by apoptosis, mainly. In silico studies with the SEA model allowed us to propose that carbonic anhydrase-IX and PI3K enzymes could be the targets for GA and MYR, respectively. However, docking and experimental studies are necessary to confirm this proposal. Therefore, GA and MYR could be considered as base compounds for the development of new treatments in the chemotherapy of ovarian cancer.

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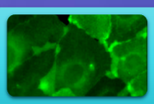
METHODS

Compounds studied
- GA and MYR (≥ 96 % purity, HPLC-grade)

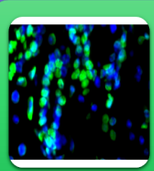
MilliporeSigma



Cell culture protocol
- SKOV-3, ovarian adenocarcinoma
- Conditions: 37 °C and 5 % CO_2

ATCC®

Cell viability by Formazan salts [3]
- Cultures: 20,000 cells/well, 200 µL supplemented medium, 24 h.
- Treatments: 10 to 200 µg/mL of samples/controls, 24 h
- MTT: 5 mg/mL, 4 h
- λ: 590 nm
- IC_{50}: % survival vs [log]



ROS and apoptosis by immunofluorescence [3]
- Cultures: 20,000 cells/well, 200 µL of supplemented medium, 24 h.
- Treatments: IC_{50} samples/controls for 24 h.
- ROS: 25 µM H_2-DCF-DA, for 15 min, at 37 °C, and λ: $488_{ex}/529_{em}$ nm
- Apoptosis: AnV-FITC, for 15 min, at 37 °C, λ: $485_{ex}/538_{em}$ nm

Cell cycle and morphology [3]
- Cultures: 50,000 cells/well, 2 mL of supplemented medium, 24 h.
- Treatments: IC_{50} samples/controls for 24 h.
- Cell cycle: flow cytometry with propidium iodide
- Cell morphology: immunofluorescence with Rhodamine-Phalloidin (actin), α-tubulin antibody (tubulin), and DAPI (nucleus)
- Cell ultrastructure: transmission electron microscopy (TEM)

***In-silico* analysis [4]**
- Identification of target pharmacophores: Similarity Ensemble Approach-model
- Parameters: *pKi*, *P-Value*, *Max TC*

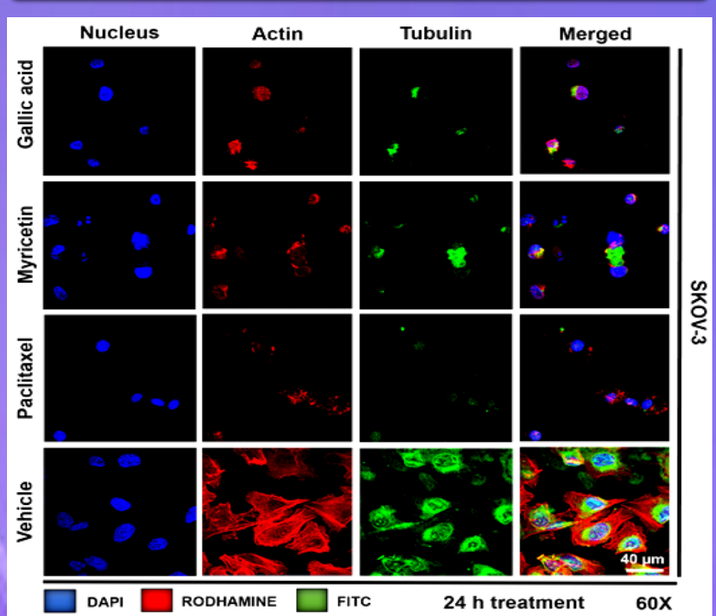


Figure 2. Cytoskeletal alterations in SKOV-3 cells during treatments with GA and MYR. Morphological changes observed in actin microfilaments or tubulin microtubules in SKOV-3 cells after 24 h of treatment with GA (50 µg/mL) and Myr (166 µg/mL) were by immunofluorescence with Rhodamine-Phalloidin and α-tubulin primary polyclonal antibody. Preparations were mounted with VectaShield®/DAPI and observed in confocal microscopy at 60X magnification. Results are representative of 3 independent replicates (n = 3; triplicates). Paclitaxel and 0.5 % DMSO/1X PBS were positive and negative controls used in studies.

REFERENCES

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