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by Ibrahim Hussein

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Gold nanoparticles (AuNPs) and *Rosmarinus officinalis* extract and their potentials to prompt apoptosis and arrest cell cycle in HT-29 colon cancer cells

Background: One of the significant morbidity and mortality causes is the colorectal cancer. *Rosmarinus officinalis* plant is utilized as food and medicine. Currently, nanoparticles are widely employed in medicinal preparations. This work aimed to explore the potentials of *R. officinalis* leaves acetone extract, alone or in combination gold nanoparticles (AuNPs), to kill colon cancer cells.

Methods: Fresh leaves of *R. officinalis* were collected from the Al Soudah, Saudi Arabia and then dried to prepare acetone extract. AuNPs were prepared utilizing the extract and portrayed using UV/Vis spectrophotometry and scanning electron microscopy (SEM). Active ingredients exist in the extract and extract+AuNPs were screened using FT-IR spectroscopy. Biological properties of the extract and extract+AuNPs including anti-cancer activity and apoptotic capacity were studied.

Results: Results of UV/Vis spectrophotometry and SEM demonstrated that AuNPs are of 79 nm in diameter. FTIR analysis revealed the existence of bioactive molecules in the extract. Extract and extract+AuNPs arrested HT-29 colon cancer cells at G2/M phase.

Conclusion: *R. officinalis* acetone extract and extract+AuNPs could arrest the proliferation of HT-29 colon cancer cells. Extract and extract+AuNPs actuated apoptosis in cancer cells as opposed to necrosis.

Keywords: HT-29 colon cancer cells; *Rosmarinus officinalis*; Apoptosis; Gold nanoparticles; Aseer.

1. Introduction

Normal cells divide under well-controlled manner according to host body needs. Cancer cells, the abnormal version of host cells, are characterized by their powerful capacity of proliferation with reduced capacity of apoptosis. The abnormal cell proliferation of cancer is due to the loss of perfect control mechanisms in the production of growth factors leading to imperfect cellular homeostasis and maintenance causing abnormal tissue architecture (Hanahan and Weinberg

2011). Several pharmaceutical derivatives have been obtained through the screening of plant natural compounds and showed anticancer properties (Da Rocha et al. 2001).

Rosmarinus officinalis (Rosemary) plant is one of the Lamiaceae (mint) family. It is widely existing in the Mediterranean region and distributed in many other locations through the world. Its leaves are utilized in the treatments of many disorders long time ago as well as food additives. Rosemary has many medicinal activities such as antibacterial (Nieto et al., 2018), the power to treat depressive behavior (MacHado et al. 2012), antitumor (Kontogianni et al., 2013), antioxidant (Nieto et al., 2018), hepato-protective power (Sotelo-Félix et al. 2002; Abdel-Wahhab et al. 2011; Rašković et al. 2014), anti-parasitic, wound-healing agent (Hamidpour, 2017), antispasmodic in renal colic, smooth muscle relaxant (Habtemariam, 2016), gastric ulcerative lesions (Corrêa Dias et al. 2000), control of hypercholesterolemia, relief of physical fatigue (Fernández et al. 2014), lipid peroxidation reduction (Posadas et al. 2009), radio-protective-antimutagenic capacities (Del Baño et al. 2006), treatment for cutaneous allergy (Tabassum and Hamdani 2014), anti-inflammatory (De Melo et al. 2011), antiviral (Nolkemper et al. 2006), antithrombotic (Yamamoto et al. 2005), and anti-hyperglycemic (Naimi et al. 2017). Several phytochemicals with biological characteristics were obtained from rosemary oil and extracts. These phytochemicals include acids (rosmarinic, ursolic, oleanolic, caffeic, and carnosic), eucalyptol, camphor, rosmadial, secohinokio, and eugenol/luteolin derivatives (Borges et al., 2019; Einbond et al., 2012; Gonçalves et al., 2019). Rosemary preparations (e.g. carnosic/ rosmarinic acids and diterpenes carnosol) are believed to apply strong biological effects (e.g. antitumor, anti-allergic, antibacterial and antioxidant) (González-Vallinas et al., 2015; Moore et al., 2016). Several studies demonstrated that *R. officinalis* exhibit good anti-cell propagation properties against some cancer cell lines (Kontogianni et al., 2013; Petiwala et al., 2013). Many

plants, including edible ones, have medicinal impacts against several diseases including colon cancer (Balakrishna and Kumar, 2015; Xu et al., 2015).

The science concerning with study and manufacturing of nano-dimension materials is called nanotechnology (Rajeshkumar, 2016). Gold nanoparticles (AuNPs) production employing plant extract is clean, ecofriendly, and cost effective method comparing to other methods (chemical and physical). Gold nanoparticles are reported to have anti-bacterial (Mohamed et al. 2017), anticancer (Rajeshkumar 2016b) and immunomodulatory (Dykman and Khlebtsov 2017) activities.

In the current work, biological proprieties (anticancer, antibacterial, effects on cell cycle/apoptosis) of *R. officinalis* leaf acetone extract and its synthesized AuNPS on HT-29 colon cancer cells were examined. In addition, the power of the extract to produce AuNPs was evaluated.

2. Experimental methods

2.1. Rosemary leaf extract preparation

Leaves of *R. officinalis* (Fig. 1) were collected in August 2019, from Abha, Aseer, Saudi Arabia. The leaves extract was prepared as described elsewhere (Ibrahim et al. 2021). A stock preparation (1%) was designed in acetone, sterilized (0.45 μm filter, Fisher Scientific) and stocked at -20 °C.

Fig. 1: *R. officinalis* plant collected from Al Soda mountain, Aseer, KSA.

2.2. AuNPS synthesis/characterization and functional group analysis

AuNPs preparation, characterization, size/morphology, and plant extract functional group analysis were done according Ghramh et al. (2019).

2.3. Maintenance, preparation of cells and cytotoxicity tests

HT-29 cancer cell line was maintained utilizing the same methods and reagents described by Ganesan et al. (2020) The investigation of the extract and extract/AuNPs cytotoxicity towards HT-29 cells was done according to Ghramh et al. (2021) at concentration of 0, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0 132.0, 264, 528.0 and 1056.0 µg/mL.

2.4. Apoptotic effects of extract and extract/AuNPs

The extract and extract containing AuNPs at IC_{50} concentrations were separately added to HT-29 cells and incubated for 48 h. The apoptotic effect was tested using similar reagents and methodology described by Ibrahim et al. (2021).

3. Results

3.1. Gold nanoparticles production

Gold nanoparticles synthesis was observed through the color alteration of the mixture ($AuCl_4$/ extract, Fig. 2A). After color alteration (Fig. 2C), AuNPs assembling was reviewed spectrophotometrically (Fig. 2D). Results uncovered the manufacturing of AuNPs at a specific peak (520 nm).

Fig. 2: UV/Vis light monitoring of AuNPs synthesis by *R. officinalis*; A: Extract; B: extract light absorbance; C: extract after synthesis of AuNPs; D: extract+AuNPs light absorbance.

3.2. Functional groups

The FTIR spectral analysis of the R. *officinalis* extract is displayed in figure 3. Strong broad band (characteristic to alcoholic) in the range of 3633-3300 cm-1, concerned to stretching vibration of O-H groups. Two bands (2938 and 2851 cm-1) are due to stretching vibrations of the groups CH2 and CH3. Bands present at 1717, 1696 and 1661 cm-1 are due to stretching vibration of C=O/C=C groups of flavonoids and amino acids. A peak ranged from 1457-1276 cm-1 is attributed to aromatic C=C and due to the presence of series of small peaks in the area 1400 – 2000 cm-1. Numerous peaks found at 1030- 523 cm-1 represent C-O stretch of acid, ester, ether, alcohol, anhydride, monosubstituted alkene and halo compound.

Fig. 3: FTIR spectrum of *R. officinalis* extract.

3.3. Characterization of AuNPs

SEM examination divulged that the manufactured AuNPs are nearly uniform spherical in shape with median size of 79 nm.

3.4. Extract cytotoxicity toward HT-29 cells

R. officinalis extract inhibited HT-29 cell line growth at a significant ($p > 0.0001$) levels at the concentration 32-1000 µg/mL. Extract containing AuNPs also inhibited HT-29 cells growth at a significant ($p > 0.0001$) levels but only up to concentrations ranged 32-1000 µg/mL (Fig. 4).

Fig. 4: Effects of *R. officinalis* extract and extract+AuNPs on HT-29 cell growth.

3.5. Apoptotic effects of *Rosmarinus officinalis* acetone extract

Evaluation of the extract and extract containing AuNPs was done using Annexin V staining (Table 1). The extract caused significant ($p<0.05$) apoptosis (9.44%) in HT-29 cells, while extract+AuNPs treated HT-29 cells showed higher effect (16.04%) over the untreated cells (1.92%).

Table 1: Apoptic effects of *R. officinalis* extract and extract+AuNPs on HT-29 cells.

Treatment	Apoptosis (%)			Necrosis (%)
	Total	Early	Late	
Extract	9.44	1.52	6.31	1.61
Extract+AuNPs	16.04	6.17	8.21	1.66

Discussion

The use of therapeutic medicinal plants to fight cancer is a good approach. *R. officinalis* is an edible and also used in cosmetics. In the current work, we targeted the valuation of the biological effects *R. officinalis* when combined with gold nanoparticles. In addition, we investigated the impact of the extract on HT-29 cells.

In the current study, we utilized *R. officinalis* leaf extract to create AuNPs. The biomolecules contained in the extract, and shown by RTIR analysis, could reduce and cap gold ions forming the AuNPs. Many studies also reported the presence of active biomolecules in *R. officinalis* leaves (Cheung and Tai 2007; Wang et al. 2012; González-Vallinas et al. 2015; Moore et al. 2016). The created AuNPs appeared as spheres of average size of 79 nm. Many researchers were able to create nanoparticles with diverse sizes utilizing *Rosmarinus officinalis* leaf extracts (Ghaedi et al. 2015; Soltanabad et al. 2018; Hadi Soltanabad et al. 2020).

Typically, the cell cycle of cells passes through four consecutive stages starting from the quiescence stage (G_0) to the propagation (G1, S, G2, and M) stage, and return back to either the G_0 or G1 stage (Jingwen et al. 2017). Genes controlling the cell cycle are always mutated in cancers, causing uncontrolled cell division and tumor development (Williams and Stoeber 2012). Abnormal (cancer) cells start over G1 straightforwardly after M stage leading to abnormal cell division. The target of anticancer medicines is to induce the cell cycle arrest at the M phase (American Cancer Society 2015).

Extract and extract+AuNPs prepared in the current work demonstrated growth suppressive impacts on human colon cancer HT-29 cells. Vasanth (2014) stated that nanoparticles included in *R. officinalis* extract had the power to induce apoptosis in cervical cancer cells through the

elevation in ROS levels and its subsequent action. Extract and extract+AuNPs revealed non-toxic potential or cell cycle interference impacts. This distinctly mentions that extract+AuNPs interferes the cell cycle of cancer cells.

Programmed cell death or apoptosis works efficiently in the equilibrium of healthy cells by removing the cancer cells (Levine et al. 2001; Wang et al. 2015). It is documented that the bioactive materials exist in *R. officinalis* have the power to initiate apoptosis in cells of the tumor. Bioactive materials exist in the plant's extract (e.g. carnosol, carnosic & rosemarinic acids) have been shown to induce apoptosis in cells of the tumor, possibly through the synthesis of nitric oxide (Dilas et al. 2012; Tai et al. 2012; Kontogianni et al. 2013; Petiwala et al. 2013). p53, a tumor suppressor, exerts a critical part in the apoptosis inducement and affects the mitochondrial intrinsic apoptosis pathway (Vaseva and Moll 2009; Nieminen et al. 2013).
In the current study, HT-29 cells were arrested by the extract and extract+AuNPs at G2/M stages. Other *in vitro* ivestigations utilized several cancer cell lines (DLD-1, CaCo-2, SW480, and SW620 colon cancer cells) concluded that *R. officinalis* has anticancer properties (Slameová et al. 2002; Yi and Wetzstein 2011; González-Vallinas et al. 2013).

Conclusion

R. officinalis leaf acetone extract could synthesize AuNPs with 79 nm diameter. Extract and extract+AuNPs could arrest HT-29 cancer cell proliferation. Extract and extract+AuNPs actuated apoptosis in cancer cells as opposed to necrosis. The edible plant *R. officinalis* can be utilized in the preparation of anticancer formulas, at least against colon tumors, either alone or in combination with gold nanoparticles.

Conflicts of interest

The authors declare that there are no existing competing interests.

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