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Can crude alkaloids extract of *Rhazya stricta* induce apoptosis in pancreatic cancer: In vitro study?

Nehad Abdulgader Shaer

Al Leith University College, Chemistry Department, Umm Al-Qura University, Saudi Arabia

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ABSTRACT

Cancer is a complicateddisease that reveals genetic variability even among the cells within the same tumor. Pancreatic cancer is the 12th cause of cancer deaths over the world. As a result of the incomplete recovery and the many side effects of current clinical treatment approaches, Herbal diet therapy as a single or adjuvant therapy show high significant output in cancer treatment. Our study focused on the role of the crude alkaloid extract of *Rhazya stricter (R. stricta*) on pancreatic cancer cells using MTT assay. The cytotoxic effect of different concentrations of *R. Strict* crude alkaloid on the pancreatic cancer cells showed significant decrease in cell viability with dose dependent manner and the effect was observed at higher concentration of crude *R. Stricta* alkaloids. On the other hand, no significant cytotoxic effect was observed with the normal WISH cells at all *R. Stricta* crude alkaloid concentrations with IC50. Moving on, in AsPC-1cells under the same concentrations mRNA expression was increased by 1.5 and 6 folds with 10 and 100 μg/ Ml treatment when compared with control. Under the same experimental conditions, the anti- apoptotic marker Bcl-2 showed high significant decrease in mRNA expression in both PANC-1 and AsPC-1 pancreatic cancer cells. The present study indicated that the crude alkaloids extract of *R. stricta* significantly induce apoptotic cell death in pancreatic cancer cells.

1. Introduction

Pancreatic cancer is a malignant neoplasm that either primarily arises from pancreatic tissue which is considered the commonest form, or metastatic which arise in any distant organ then spread to the pancreas [1]. Pancreatic cancer is the 12th cause of cancer deaths in the world, In Saudi Arabia; Al Ghamdi et al. [2] reported that pancreatic cancer is the 5th most prevalent cancer in patients where it represents about 1.75% of all cancers, where the percentage is high in males (2.5%) compared to females (1.1%). Because of symptoms of pancreatic cancer are nonspecific, pancreatic cancer is clinically silent and difficult to diagnose in early stages [3], The signs include digestive disorders, such as anorexia, nausea, diarrhea weight loss, fatigue and back pain are also among key clinical characteristic features in suspected pancreatic cancer patients [4].

Recently, considerable attention has been focused on Herbal medicine as one of the most commonly used complementary and alternative therapies by cancer people [5,6]. *R. stricta* is a native poisonous plant in Southern Iran, Afghanistan, Pakistan, India, Iraq, Oman, Yemen, and Saudi Arabia [7]. *R. stricta* is one of the most important medicinal plants in Saudi Arabia and it is grow in the desert areas in the Arabian Peninsula [8]. Ample data show that R. stricta has a high medical value due its chemical composition of Alkaloids, Flavonoids, Glycosides and Tannins [9]. Most biological activities of the R. stricta are due to its alkaloid fractions as Rhazimine, Sewarine, strictanol and tetrahydrosecaminediol which have many functions as antifungal, antioxidant, glucose homeostasis, effect on blood pressure, effects on central nervous system (CNS), arachidonic acid metabolism and antimicrobial [10]. The alkaloid extract of R. stricta show protective role against liver damage induced by paracetamol in mice and improve the liver functions [11]. It has also a significant effect on the metabolic disorders in rats by increasing the levels of insulin and liver enzymes and reduces the triglyceride levels [12]. So many people use the *R. stricta* to treat diabetes either alone or as adjuvant drug [13]. In this regard, it has reported that the crude alkaloid extract of R. stricta induce apoptotic human lung cancer cells (NSCLC line A549) death [14]. Another study showed that R. stricta induce (MCF-7 and MDA-MB-231) breast cancer cells death by apoptosis [15]. Many isolated alkaloids from R. stricta have been shown to exhibit antiproliferation and antimetastasis activity against many types of tumors in vitro and in vivo [16]. Finally, our recent study focused on the effect of *R. stricta* alkaloid extract on pancreatic cancer and explain the mechanism of cell deaths.

E-mail address: nehadalshaaer@gmail.com

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2. Material and methods

2.1. Chemical and reagents

Dulbecco's Modified Eagle's Medium (DMEM) with L-glutamine growth medium, fetal bovine serum (FBS) and anti-biotic mix (Penicillin/Streptomycin) were bought from Gibco (Invitrogen, CA, USA). Micro-Amp R optical 96-well reaction plates for real time PCR, Micro-Amp R fast reaction 0.1 tubes for PCR were purchased from (applied biosystem). MTT (3-[4, 5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H tetrazolium bromide) and Triazol were obtained from Qiagen(Valencia, CA, USA). SYBR Greenmixwas purchased from advanced biotechnologies ltd (AB gene) UK. The cDNA Synthesis Kit was purchased from Agilent Technologies (Stratagene USA) and In Situ Cell Death Detection Kit(Roche Diagnostics GmbH, Mannheim, Germany).

2.2. Extraction of alkaloids from R. Stricta leaves

The leaves from R. Stricta, were collected and dried as previously described by Baeshen et al. [24]. Briefly, R. Stricta leaves were collected from a valley called "WadiFatma", which is located at a distance of 30 km from the city of "Makkah Al-Mukarramah" in Kingdom of Saudi Arabia. The freshly obtained leaves were allowed to dry at room temperature in darkness for 3 weeks. Once dried, the leaves were soaked in 70% ethanol for 3 days followed by filtration using simple filter paper. After the filtration process, ethanol was evaporated from the filtered mixture. This process was further repeated for 3 times to ensure maximum yield. The extract was diluted using 10% of HCl making final volume of 100 ml, followed by the addition of chloroform. By the use of a separation funnel, the mixture was separated into two layers, alkaloids and non-alkaloids. Ammonium hydroxide (NH4OH) was added to the alkaloids and pH was tested using Litmus paper. The alkaloids extract was re-suspended in chloroform, followed by another phase of separation. The purified alkaloid extract was stored at 4 °C.

2.3. Cell lines

Pancreatic human cancer cell lines PANC-1 and AsPC-1 and human normal cell lines WISH was gotten from VACSERA - Cell Culture Unit, Cairo, Egypt. Cells were originally obtained from ATCC (American Tissue Culture Collection). PANC-1, AsPC-1 and WISH cells were maintained in DMEM high glucose cell culture medium containing 10% inactivated FBS (fetal bovine serum) and provided with 1%penicillin/streptomycin and incubated for the following experiments.

2.4. MTT cell viability assay

Effect of *R. Stricta* alkaloid extract on PANC-1, AsPC-1 and WISH cells viability and toxicity was investigated using MTT cell viability assay as previously described [17]. In brief, a number of 1×104 cells/well were cultured in 96-well plates and then incubated overnight. On the following day, the drugs with (0, 6.25, 12.5, 25, 50 and 100 μg/Ml) were added to the cells at the indicated concentrations and negative control was included and then the cells were incubated for 24 h. Next, fifty μl of MTT solution (2 mg/ml of MTT in PBS) were added to each well and left for 3–4 hrs and then the supernatants were removed carefully and 150 μl DMSO were added to each well. Then, 96-well plates were shaken for 10 min and then were read at A570 with reference filter at A650 using Elisa plate reader.

2.5. Detection of DNA Fragmentation by terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay

The TUNEL assay was performed using the In Situ Cell Death Detection Kit, with fluorescein, according to manufacturer's instructions. Briefly, cells were trypsinized, washed then fixed with 100 μg/ml 4% paraformaldehyde for 1 h at room temperature on shaker to avoid clumping. Cells were washed in PBS and permeabilized with freshly prepared 0.1% Triton X-100 in 0.1% sodium citrate for 2 min on ice. Permeabilized cells were washed once again in PBS and incubated with TUNEL reaction mixture, containing terminal deoxynucleotidyl transferase (TdT) plus Deoxyuridine Triphosphate (dUTP) label, in the dark at 37 °C for 1 h. After labeling, the cells were washed twice in PBS. Control cells that were treated with the solvent only without the drug was included. In each sample, 10,000 cells were analyzed using a flow cytometer (FACS Calibur BD Biosciences, USA).

To investigate the apoptotic effect of the drug on cells, the fluorescence level was set using the control cells that were treated with the solvent. Cells above this fluorescence value in the control sample were considered apoptotic. The final percentage of cells with fragmented DNA was referred to as % TUNEL positive. Flow cytometric data were analyzed using Flow Jo software (version 6.1, Tree Star, Inc.; Ashland, OR).

2.6. Gene expression analyses

The effect of the different concentrations of *R. Stricta* alkaloid extract (0.00, 10 and 100 μg/ Ml) on the mRNA expression of **p53** and Bcl-2 genes in PANC-1 and AsPC-1 human pancreatic cancer cells was investigated using real-time quantitative PCR. Using **p53**: forward, 5′-AGGGTTAGTTTACAATCAGC-3′, reverse, 5′-GGTAGGTGCAAATGCC-3′; bcl-2: forward, 5′-TCGATGTGATGCCTCTGCGAA GAAC-3′; reverse, 5′- ATTGCACTGCCAAACGGAGCTG-3′; GAPDH (F) 5'AGATCATCAGCAATGCCTCCTG-3'and GAPDH (R) 5'-ATGGCATGGACTGTGGTCATG-3'[20].After performing the indicated treatments, RNA was extracted and then the corresponding cDNA was prepared and then real time PCR was applied for gene expression analysis as previously described [18]. In brief, all c DNA samples were processed in a 96-well plate using the following cycling conditions: 10 min at 95 °C, and 40 cycles at 95 °C for 15seconds ended byone min. at 60 °C. These data were analyzed according to Livak and Schmittgen [19].

2.7. Statistical analysis

Statistical Package for Social Science (SPSS) version 20 for windows program was applied to analyze the present data. The data were expressed as means +/- standard deviation (SD). Comparison of variables between groups was performed using One Way ANOVA test (LSD) and between same groups versus zero concentration using paired student ẗtest. Statistical significances were considered at P-value < 0.05.

3. Results

Effect of *R.stricta* alcoholic crude alkaloids extract on cell viability of different pancreatic cell lines (PANC-1 and AsPC-1) and human normal cells (WISH):The cytotoxic effect of different concentrations of R. Strict crude alkaloid on the pancreatic cancer cells PANC-1 and AsPC-1viability using MTT assay after 24 h of treatment show significant decrease in cell viability with dose dependent manner and the effect was observed at higher concentration of crude *R. Stricta* alkaloids (25, 50, and 100 μg/ml) with IC50 (78.77

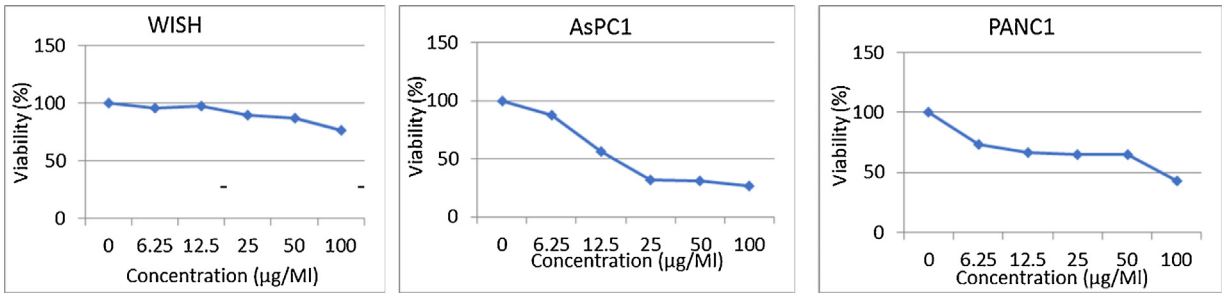

Fig. 1. Showed the cytotoxic effect of different concentrations of R. Strictacrude alkaloidon the normal Wish cells and AsPC-1 and PANC-1 pancreatic cancer cells after 24 h respectively, by MTT assay.

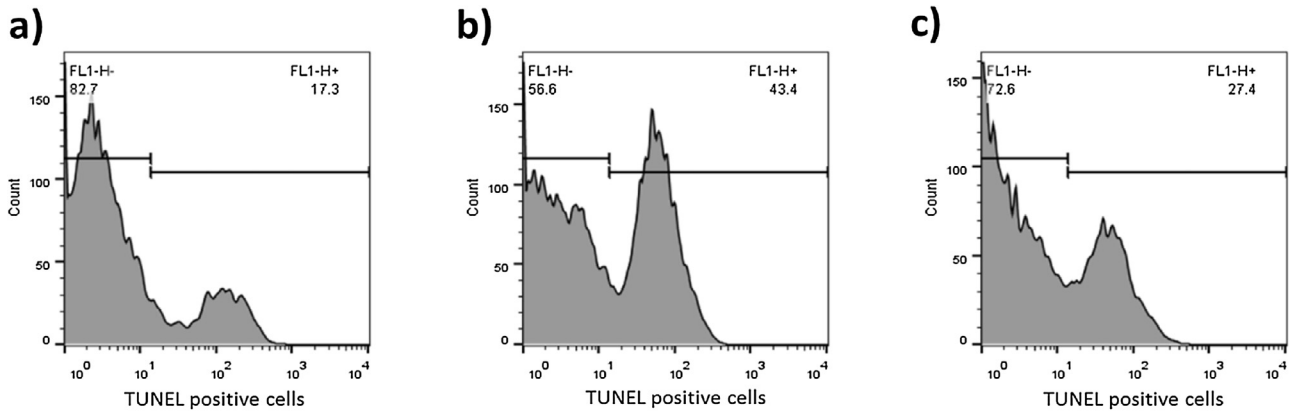

Fig. 2. Analysis of *R. Stricta* crude alkaloid extract induced apoptosis in PANC-1 cells by flow cytometry using the TUNEL assay. a) Cells were treated with solvent control, b) Cells were treated with 100 µg/ml and c) Cells were treated with 10 µg/ml. Subsequently, apoptotic cells were labeled and analyzed as described in material and methods section.

and 41.4 µg/ ml) on PANC-1 and AsPC-1; respectively. On the other hand, no significant cytotoxic effect was observed with the normal WISH cells at all *R. Stricta* crude alkaloid concentrations with IC50 (215.5 µg/ ml) as shown in Fig. 1.

3.1. Apoptotic cell death by FACS analysis

R. Stricta crude alkaloid extract induce apoptotic cell death in PANC-1 pancreatic cancer cells:

The TUNEL assay was used to assess DNA damage after treatment of PANC-1 cells with different concentrations of *R. Stricta* crude alkaloid extract. Control cells treated with solvent showed a fragmented DNA pattern in 17.3% of cells. However, treating cells with 100 µg/ Ml resulted in the highest rate of DNA fragmentation (43.4%) and treatment with 10 µg/ml resulted in 27.4% apoptotic cells as shown in (Fig. 3).

3.2. R. Stricta *crude alkaloid extract induced apoptotic cell death in AsPC-1 pancreatic cancer cells*

DNA damage analysis after treatment with (0, 10 and 100 µg/Ml of the extract for 24 h. Control cells treated with solvent showed a fragmented DNA pattern in 16.2% of cells. However, treating cells with 10 µg/Ml induce (32.7%) cell death and treatment with 100 µg/ml resulted highest apoptotic cell death 57.5% as shown in (Fig. 4A–C).

a) Cells were treated with solvent control, b) Cells were treated with 10 µg/ml and c) Cells were treated with 100 µg/ml for 24 h. Subsequently, apoptotic cells were labeled and analyzed as described in material and methods section.

3.3. Gene expression analysis by qPCR

The effect of *R. Stricta* crude alkaloid extract on the apoptotic marker **p53** mRNA expression levels on the pancreatic cancer cells PANC-1 and AsPC-1 were investigated using quantitative real time PCR analyses. In PANC-1cells, real time PCR results showed that 10 and 100 µg/ Ml treatment for 24 h of the alkaloid crude extract increased the**p53** expression about 7 and 5 folds; respectively when compared with control cells (Fig. 2A). Moving on, in AsPC-1cells under the sameconcentrations mRNA expression was increased by 1.5 and 6 folds with 10 and 100 µg/ Ml treatment when compared with controls (Fig. 2B). Under the same experimental conditions, the anti- apoptotic marker Bcl-2 show high significant decrease in mRNA expression in both PANC-1 and AsPC-1 pancreatic cancer cells (fig. C and D).

4. Discussion

Pancreatic cancer as reported by Al Ghamdi et al. [2] is the 5th most common cancer in patients where it is represent around 1.75% of all cancer cases in Saudi Arabia and the incidence is higher in males (2.5%) than females (1.1%). Many of current treatment therapies as chemotherapeutic drugs, immune-based therapeutics, radiation and stem cell transplantation failed to achieve complete cure of cancer patient which make many studies focusing on Natural Medicines (NMs) as a single or adjuvant therapy to obtain the highest cure rate of cancer [20,21]. In our study we focused on the R. Stricta as a nature product because it contains many bio active compounds as Alkaloids, Flavoinids, Glycosides and Tannins [9]. Specifically, we used in our study the crude alkaloid extract which contain many biological important compounds as Rhazimine, Sewarine, strictanol and tetrahydrosecaminediol [22,23].

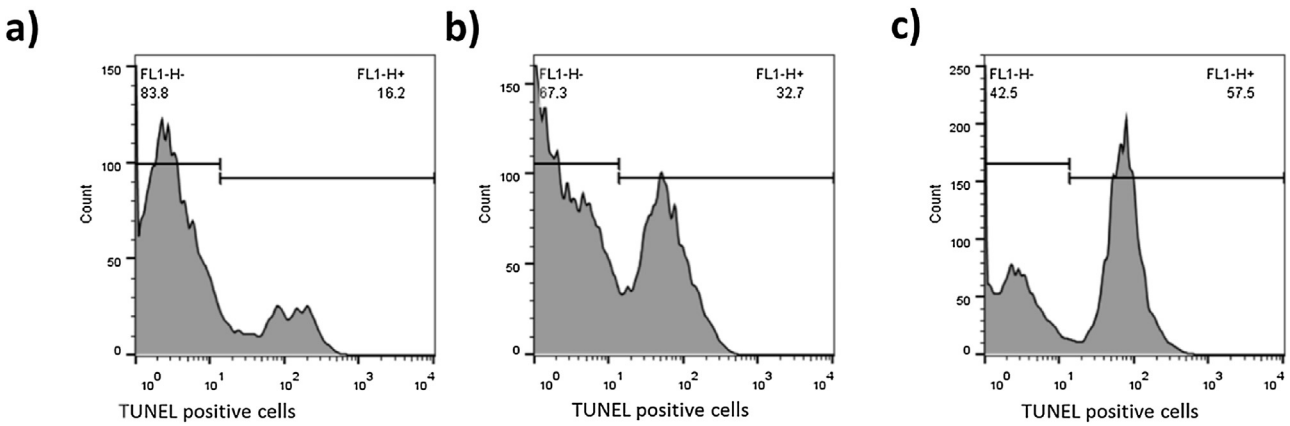

Fig. 3. Analysis of RS induced apoptosis in AsPC-1 cells by flow cytometry using the TUNEL assay.
a) Cells were treated with solvent control, b) Cells were treated with 10 μg/ml and c) Cells were treated with 100 μg/ml for 24 h. Subsequently, apoptotic cells were labeled and analyzed as described in material and methods section.

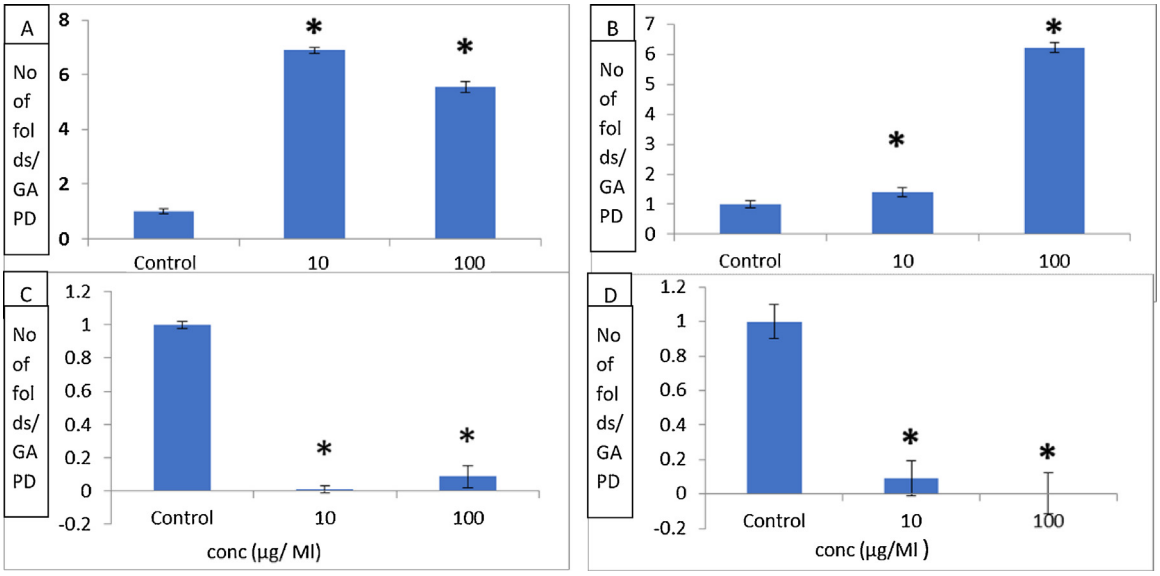

Fig. 4. Show the mRNA expression by qPCR. (A) And (B) **p53** mRNA expression in PANC-1 and AsPC-1 cells under indicated doses of the alkaloid crude extract. (C) And (D) Bcl-2 mRNA expression in PANC-1 and AsPC-1 cellsunder the same experimental conditions.

Many published data previously describedthat the alkaloid extract exert antifungal, antioxidant, glucose homeostasis, effect on blood pressure, effects on central nervous system (CNS), arachidonic acid metabolism, antimicrobial activity and improvement in liver function [12,24,25].

In consistent with the many published data which clearly proved that the alkaloid extract suppress cell growth and proliferation of many tumors cells [14,16,25,26], our cytotoxicity results show that the alkaloid extract of R. Strict exert significant inhibition of cell growth and proliferation of PANC-1and AsPC-1 pancreatic cancer cell lines in a concentration-dependent manner with IC_{50} (78.77 and 41.4 μg/ Ml);However,the same concentrations of R. Stricta show unobserved cytotoxic effect on the human normal cells WISH cells with IC_{50}(215.5 μg/ Ml).Apoptotic DNA fragmentation is a key feature of apoptosis, a type of programmed cell death which is characterized by the activation of endogenous endonucleases, particularly the caspase-3 activated DNase (CAD) with subsequent cleavage of nuclear DNA into internucleosomalfragments of roughly 180 base pairs (bp) [27]. Ample published data demonstrated that the apoptotic DNA fragmentation is being used as a marker of apoptosis and for identification of apoptotic cells we used the TUNEL assay [28,29]. Our TUNEL assay results clearly show that the pancreatic cancer cells treated with (10 and 100 μg/Ml) induce (27.5% and 43.4%)cell death; Respectively, while in case of AsPC-1 pancreatic cancer cells show (32.7% and 57.5%) under the same treatment which proved the anti-cancer role of the alkaloid extract of R. Stricta.

Most anticancer agents, which resulted in the DNA fragmentation induce apoptosis through, induce stress-response signaling pathways that trigger apoptosis by activating the mitochondria lapoptotic pathway by releasing of mitochondrial proteins, such as cytc and initiation of apoptotic cascades. However, the intrinsic mitochondria pathway is controlled by Bcl-2 family proteinswhich have opposing roles in initiating mitochondrialapoptotic signaling [30–33].The ability of alkaloid extract of R. Stricta to inducePANC-1and AsPC-1 pancreatic cancer cell death were examined in detail in order to elucidate the molecular mechanism by which this extract exert its role in inhibition of pancreatic cancer growth by study gene expesion of p53 as apoptotic marker and Bcl-2 as ant apoptotic gene and our results proved that using (10 and 100 μg/Ml) of the alkaloid extract increase the expression of p53 mRNA while Bcl-2 mRNA expression markedly decreased which strongly activate the apoptosis cell death which supported with many previous studies [16,25,26,34].Finally, our simple study show the promising

role of the alkaloid extract of R. Stricta as inducer of pancreatic cancer cell death via DNA damage and activation of intrinsic apoptotic pathway.

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Conflicts of interest

"The author declares no conflict of interest."

References

[1] M. Kanai, K. Yoshimura, M. Asada, A. Imaizumi, C. Suzuki, S. Matsumoto, T. Nishimura, Y. Mori, T. Masui, Y. Kawaguchi, A phase I/II study of gemcitabine-based chemotherapy plus curcumin for patients with gemcitabine-resistant pancreatic cancer, Cancer Chemother. Pharmacol. 68 (2011) 157–164.
[2] H.J. AlGhamdi, S.A. Alfaifi, A.A. Alolayan, S. Musaad, A.M. Jazieh, Pancreatic cancer in Saudi patients treated at tertiary institution. Ten years retrospective study, Saudi Med. J. 34 (2013) 604–608.
[3] Q.J. Wu, L. Wu, L.Q. Zheng, X. Xu, C. Ji, T.T. Gong, Consumption of fruit and vegetables reduces risk of pancreatic cancer: evidence from epidemiological studies, Eur. J. Cancer Prev. 25 (3) (2016) 196–205.
[4] E.A. Gale, GLP-1–based therapies and the exocrine pancreas: more light, or just more heat? Diabetes 61 (2012) 986–988.
[5] M. Russo, C. Spagnuolo, I. Tedesco, G.L. Russo, Phytochemicals in cancer prevention and therapy: truth or dare? Toxins (Basel). 2 (2012) 517–551.
[6] S. Prasad, B.B. Aggarwal, Chronic diseases caused by chronic inflammation require chronic treatment: Anti-inflammatoryRole of dietary spices, Clin Cellular Immunol. 5 (2014) 1–11.
[7] V. Mozzaffarian, Farhang moaser publication, Tehran, Iran, in: A Dictionary of Iranian Plant Names, 1998.
[8] E.-T. Adel, E.-A.A. Mohamed, A.O. Attia, D. Salman, E. Dessoky, In Vitro Multiplication of the Important Medicinal Plant, Harmal (*Rhazya stricta* Decne), vol. 6, 2012, pp. 3586–3590.
[9] S.H. Gari, Cytogenetic and Molecular Evaluation of Genetic Effects of Leaves Extract of *Rhazyastricta* (Dence). PhD Thesis, King Abdulaziz University, Jeddah, Saudi Arabia, 2006.
[10] S.K. Marwat, Fazal-ur-Rehman, K. Usman, S.S. Shah, N. Anwar, I. Ullah, A review of phytochemistry, bioactivities and ethno medicinal uses of*Rhazyastricta* Decsne (Apocynaceae), Afr. J. Microbiol. Res. 6 (2012) 1629–1641.
[11] B. Ali, A. Bashir, R. Rasheed, Effect of the traditional medicinal plants *Rhazyastricta*, Balanitisaegyptiaca and Haplophylumtuberculatum on paracetamol-induced hepatotoxicity in mice, Phytother. Res. 15 (2001) 598–603.
[12] N.A. Baeshin, S.S. Yaghmoora, H.M.A. Ashmaouia, T.A. Kumosanib, K.S. Sainia, The Indole-Alkaloid Fraction of *Rhazyastricta* Improves Key Biochemical Parameters Associated With Metabolic Syndrome in Rats, vol. 44, 2014, pp. 358–371.
[13] Z.M. Al-Hasawi, H.A.A. Al-Harbi, Effect of *Rhazyastricta* Dense Leaf Extract on the Liver and Kidney Tissue Structure of Albino Mice, 2014.
[14] A.I. Elkady, Crude alkaloid extract of *Rhazyastricta* inhibits cell growth and sensitizes human lung cancer cells to cisplatin through induction of apoptosis, Genet. Mol. Biol. 36 (2013) 12–21.
[15] J.A. Bernstein, N. Liu, B.A. Knorr, S.S. Smugar, W.D. Hanley, T.F. Reiss, S. Greenberg, MK-0633, a potent 5-lipoxygenase inhibitor, in chronic obstructive pulmonary disease, Respiratory medicine 105 (2011) 392–401.
[16] J. Lu, L. Bao, X.P. Chen, M. Huang, Y. Wang, "Alkaloidsisolated from natural herbs as the anticancer agents,", Evidence based Complementary and Alternative Medicine 2012 (2012), 485042, 12 pages.
[17] I.A. Ismail, H.S. Kang, H.J. Lee, J.K. Kim, S.H. Hong, DJ-1 up regulates breast cancer cell invasion by repressing KLF17 expression, Br. J. Cancer 110 (2014) 1298–1306.
[18] S. Rongli, X. Mengzhen, Z. Juan, Y. Lihong, P. Yuepu, Apoptosis and Altered Expression of p53, Bax and bcl-2 Induced by Formaldehyde in HELF Cells, ISIAQ. Org., 2018.
[19] I.A. Ismail, H.S. Kang, H.J. Lee, H. Chang, J. Yun, C.W. Lee, N.H. Kim, H.S. Kim, J.I. Yook, S.H. Hong, B.M. Kwon, 2-Hydroxycinnamaldehyde inhibits the epithelial-mesenchymal transition in breast cancer cells, Breast Cancer Res. Treat. 137 (3) (2013) 697–708.
[20] M. Russo, C. Spagnuolo, I. Tedesco, G.L. Russo, Phytochemicals in cancer prevention and therapy: truth or dare? Toxins (Basel) 2 (2012) 517–551.
[21] S. Prasad, B.B. Aggarwal, Chronic diseases caused by chronic inflammation require chronic treatment: anti-inflammatory role of dietary spices, Clin Cellular Immunol. 5 (2014) 1–11.
[22] B.H. Ali, A.A. Al-Qarawi, A.K. Bashir, M.O. Tanira, Phytochemistry, pharmacology and toxicity of Rhazyastricta Decne: a review, Phytother. Res. 14 (2000) 229–234.
[23] S.A. Gilani, A. Kikuchi, Z.K. Shinwari, Z.I. Khattak, K.N. Watanabe, Phytochemical, pharmacological and ethnobotanical studies of Rhazyastricta Decne, Phytother. Res. 21 (2007) 301–307.
[24] N.A. Baeshen, S.A. Lari, H. Al Doghaither, H.A. Ramadan, Effect of *Rhazya stricta* extract on rat adiponectin gene and insulin resistance, J. Am. Sci. 6 (2010) 1237–1245.
[25] B. Ali, A. Al-Qarawi, H. Mousa, A. Bashir, M. Tanira, M. Patel, R. Bayoumi, Concentration of amino acids in brains of mice treated with the traditional medicinal plant *Rhazyastricta* decne, Indian J. Pharmacol. 32 (2000) 253.
[26] N.A. Baeshen, A.I. Elkady, O.A. Abuzinadah, M.H. Mutwakil, Potential anticancer activity of the medicinal herb, Rhazyastricta, against human breast cancer, Afr. J. Biotechnol. 11 (37) (2012) 8960.
[27] H. Sakahira, M. Enari, NagataS, Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis, Nature 391 (6662) (1998) 96–99, Erratum in: Nature. 2015 Oct 29;526(7575):728. PMID 9422513.
[28] W. Gorczyca, S. Bruno, R. Darzynkiewicz, J. Gong, Z. Darzynkiewicz, DNA strand breaks occurring during apoptosis - their early insitu detection by the terminal deoxynucleotidyltransferase and nick translation assays and prevention by serine protease inhibitors, Int. J. Oncol. 1 (November (6)) (1992) 639–648, PMID 21584593.
[29] Jump up, Y. Gavrieli, Y. Sherman, S.A. Ben-Sasson, Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation, J. Cell Biol. 119 (3) (1992) 493–501, http://dx.doi.org/10.1083/jcb.119.3.493, PMC 2289665. PMID 1400587.
[30] D.R. Green, Apoptotic pathways: paper wraps stone bluntsscissors, Cell 102 (2000) 1–4.
[31] R.S.Y. Wong, Apoptosis in cancer: from pathogenesis totreatment, Wong J. Exp. Clin. Cancer Res 30 (2011) 87.
[32] M. Edderkaoui, C. Chheda, B. Soufi, F. Zayou, R. Hu, V.K. Ramanujan, X. Pan, L.G. Boros, J. Tajbakhsh, A. Madhav, N. Bhowmick, Q. Wang, M. Lewis, R. Tuli, A. Habtezion, R. Murali, S.J. Pandol, An inhibitor of GSK3B and HDACs kills pancreatic Cancer cells and slows pancreatic tumor growth and metastasis in mice, Gastroenterology (August) (2018), http://dx.doi.org/10.1053/j.gastro.2018.08.028, pii: S0016-5085(18)34893-5.
[33] C.H. Lu, S.H. Lin, C.H. Hsieh, W.T. Chen, C.Y. Chao, Enhanced anticancer effects of low-dose curcumin with non-invasive pulsed electric field on PANC-1 cells, Onco Targets Ther. Aug 10 (11) (2018) 4723–4732.
[34] A. Kaur, M.S. Riaz, S.K. Singh, U. Kishore, Human surfactant protein d suppresses epithelial-to-Mesenchymal transition in pancreatic Cancer cells by downregulating TGF-β, Front. Immunol. 15 (August (9)) (2018) 1844, http://dx.doi.org/10.3389/fimmu.2.01844, e Collection 2018.