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Phytochemical-Induced Cytotoxicity and Apoptosis in CaSki Cells- Insights from Thymoquinone, Quercetin, Curcumin, and Resveratrol

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Abstract

Cervical cancer continues to pose a significant health challenge for women globally, with traditional treatments often showing limited effectiveness and causing undesirable side effects. Recently, naturally sourced phytochemicals have attracted considerable interest as potential anticancer agents due to their ability to target multiple pathways and their low toxicity. This study is aimed to assess and compare the cytotoxic effects of four phytochemicals thymoquinone, quercetin, curcumin, and resveratrol on CaSki human cervical cancer cells, and to further investigate the apoptotic mechanisms of the most effective compound. The cytotoxicity of thymoquinone, quercetin, curcumin, and resveratrol was evaluated using the MTT assay, wherein the cytotoxic potency was found to be in the order of thymoquinone (IC_{50} =63.408 µM)> Resveratrol (IC_{50} =115.83 µM) > Curcumin (IC_{50} =131.22 µM) > Quercetin (IC_{50} =141.74 µM). Since, thymoquinone (TQ), demonstrated the lowest IC_{50} value (63.408 µM), thereby indicating the highest potency, thus was chosen for further mechanistic exploration. Microscopic examination revealed morphological changes, while DAPI staining confirmed nuclear condensation and fragmentation. Acridine orange/ethidium bromide (AO/EtBr) dual staining successfully distinguished between live, apoptotic, and necrotic cells. Furthermore, the activities of caspase-3 and caspase-9 confirmed the induction of apoptosis via the intrinsic pathway. Our results suggest the activation of the mitochondrial apoptotic pathway. Our results are suggestive for the potential use of thymoquinone as a promising adjunct therapeutic option for managing cervical cancer.

Keywords: Thymoquinone; Resveratrol; CaSki; Cervical cancer; Natural compounds; Apoptosis

1. INTRODUCTION

Cervical cancer is the second most common cancer affecting women worldwide and remains a major contributor to illness and mortality [1]. While standard treatments such as surgery, radiotherapy, and chemotherapy have enhanced patient outcomes, their success is often hindered by the emergence of drug resistance and severe side effects, including nausea, hair loss, nerve damage, mouth sores and digestive issues [2]. High-risk strains of human papillomavirus (HPV), especially HPV-16 and HPV-18, are responsible for about 70% of cervical cancer cases. The viral oncoproteins E6 and E7 play a crucial role in cancer development by inactivating the tumor suppressors p53

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and pRb, leading to cell cycle disruption and unchecked cell growth [3]. Additional molecular changes, such as the overexpression of the epidermal growth factor receptor (EGFR), further promote tumor advancement and resistance to therapy.

In light of these challenges, there is a growing interest in alternative and complementary cancer management strategies. Phytochemicals, which are bioactive compounds from plants, have shown promise due to their ability to influence various cellular processes, including apoptosis, cell cycle regulation, oxidative stress, and critical signaling pathways involved in cancer development [4-7]. Unlike traditional chemotherapy drugs, many phytochemicals demonstrate lower toxicity to normal cells, offering safer and more cost-effective treatment options. Furthermore, combining phytochemicals can enhance anticancer effectiveness by targeting multiple pathways simultaneously, providing a multifaceted approach in overcoming drug resistance.

Thymoquinone, quercetin, curcumin, and resveratrol are among the compounds that have been extensively researched for their anticancer properties. Thymoquinone, a monoterpene quinone derived from Nigella sativa (black cumin) seeds, exhibits antiproliferative, pro-apoptotic, and anti-inflammatory properties across various cancer types [8-10]. Quercetin, a flavonoid found in high quantities in fruits and vegetables like onions, apples, and berries, is recognized for its ability to inhibit cell proliferation, induce apoptosis, and hinder metastasis [11,12]. Curcumin, a polyphenolic compound from Curcuma longa (turmeric), influences multiple signaling pathways to curb tumor growth, encourage apoptosis, and diminish metastatic potential [13,14]. Resveratrol, a stilbenoid polyphenol found in red grapes, berries, and peanuts, exhibits anticancer effects by modulating oncogenic signaling, promoting apoptosis, and inhibiting cancer cell proliferation [15-17]. Together, these phytochemicals demonstrate the therapeutic potential of natural compounds in managing cervical cancer, offering safer, multi-targeted, and cost-effective alternatives to traditional chemotherapy. Their capacity to influence key molecular pathways underscores the need for further exploration of phytochemical-based therapies as supplements or alternatives in cervical cancer treatment [18-22]. This study utilized four phytochemicals; Thymoquinone, resveratrol, curcumin, and Quercetin, to examine their anticancer effects on cervical cancer cells (CaSki).

2. MATERIALS AND METHODS

2.1. Reagents

All reagents utilized in this study were of molecular grade. Dulbecco's Modified Eagle's Medium (DMEM) with high glucose concentration (4.5 g/L) and Penicillin-Streptomycin (10,000 U/mL) were from Gibco, Thermo Fisher Scientific (USA). Fetal bovine serum (FBS) and dimethyl sulfoxide (DMSO) were sourced from HiMedia, India, while Rhodamine 123 was from MedChemExpress, USA. Caspase activity assay kits were from Elabscience, USA. The compounds 2,7-Dichlorofluorescein diacetate (DCF-DA), Thymoquinone (TQ), Curcumin, Quercetin, Resveratrol, Propidium iodide (PI), 4,6-diamidino-2-phenylindole (DAPI), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were from Sigma-Aldrich, USA.

2.2. Cell culture

CaSki cells obtained from NCCS, Pune, were grown in Dulbecco's Modified Eagle Medium (DMEM) that was enriched with 10% Fetal bovine serum (FBS) and Penicillin-

Streptomycin (10,000 U/mL). The cultures were maintained at 37°C in a humidified environment with 5% CO_2.

2.3. MTT assay

The cytotoxic effects of Thymoquinone (TQ), Resveratrol (ReSV), Curcumin (CC), and Quercetin (QC), were evaluated in human cervical cancer CaSki cells using the MTT assay [23]. CaSki cells at 5 × 10^3 cells per well were placed in 96-well plates and incubated overnight under standard conditions (37°C, 5% CO_2, 95% humidity). Stock solutions of phytochemicals were prepared in DMSO and diluted with growth medium to achieve final concentrations of 1-100 µM, with the DMSO concentration not exceeding 0.1% (v/v). Untreated cells containing 0.1% DMSO served as the control group.

After 24 h of treatment, 20 µL of MTT reagent (5 mg/mL in PBS) was added to each well, and the plates were incubated for 4 hours at 37°C. The medium was aspirated, and 100 µL of DMSO was added to dissolve the purple formazan crystals formed by viable cells. The absorbance was measured at 570 nm using a microplate reader (Biorad). The percentage of viable cells was calculated using the formula:

$$\% \text{ Cell Viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100$$

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD).

2.4. Morphological analysis

In a 96-well plate, 1 × 10^4 CaSki cells were deposited and subsequently incubated overnight at 37°C under a 5% CO_2 atmosphere to facilitate optimal cell adhesion. The medium was then replaced with fresh DMEM containing thymoquinone at varying doses of 0, 20, 60, and 100 µM, and the cells were incubated for 24 hours. A phase-contrast inverted microscope (Nikon, Eclipse TS2) was employed to examine cellular morphology and assess treatment-induced changes at 20× magnification. Images were captured from random fields in each well, focusing on apoptotic features such as cell rounding, membrane blebbing, detachment, and cytoplasmic condensation. These observations were compared to untreated controls, which exhibited normal epithelial morphology.

2.5. DAPI staining

DNA fragmentation analysis was conducted using DAPI staining to evaluate cellular apoptosis and DNA damage. CaSki cells were seeded in 6-well plates at a density of 4 ×10^3 cells/mL and incubated at 37°C with 5% CO_2 for 24 hours [24]. Following a 24-hour incubation period, the cells were exposed to thymoquinone at concentrations of 0, 20, 60, and 100 µM for an additional 24 hours. Subsequently, the cells were washed with cold PBS and fixed in 70% methanol at ice-cold temperatures for 15 minutes. Cells were then stained with DAPI for 20 minutes in the dark at 37°C, washed with PBS, and observed under a fluorescence microscope using a DAPI filter (excitation at 358 nm and emission at 461 nm).

2.6. DCFDA Assay

The generation of intracellular reactive oxygen species (ROS) was evaluated using the DCFDA assay. CaSki cells were plated and adhered for 24 hours before exposure to thymoquinone concentrations for 24 hours. Following this treatment, the cells were incubated with 10 µM DCFDA for 30 minutes at 37 °C in the dark. Intracellular esterases convert DCFDA into a non-fluorescent form, which is then oxidized by ROS to form the fluorescent compound DCF. After removing the excess dye with PBS, fluorescence images were captured using a Zeiss Axio Observer A1 microscope, equipped with a 495 nm excitation filter and a 529 nm emission filter.

2.7. AO/EtBr dual staining

To assess apoptotic and necrotic cell populations, acridine orange/ethidium bromide (AO/EtBr) dual staining was used [25,26]. CaSki cells co-cultured with varying doses of thymoquinone (0, 20, 60, and 100 µM) were rinsed with PBS and incubated with AO/EtBr solution (1 µg/mL each) for 5 minutes in dark. Under fluorescence microscopy, viable cells showed uniform green fluorescence with intact nuclei, while early apoptotic cells displayed bright green fluorescence with condensed nuclei. Late apoptotic and necrotic cells exhibited orange to red fluorescence due to ethidium bromide binding to fragmented DNA. Images were captured at 20× magnification.

2.8. Caspase-3 and Caspase-9 Activity Assays

To evaluate apoptosis induction via caspase activation, the enzymatic activities of caspase-3 and caspase-9 were assessed using colorimetric assay kits, as previously described [27]. These activities were measured in CaSki cells treated with Thymoquinone at its IC_{50} concentration (63.4 µM) and in untreated control cells. The treated cells were centrifuged at 6,000 g for 5 minutes, washed with PBS, and lysed using pre-chilled Cell Lysis Buffer. The mixture was kept on ice for 30 minutes, with stirring occurring 3 to 4 times, each lasting 10 seconds. The lysed sample was then centrifuged at 11,000 × g for 15 minutes at 4°C. Caspase-3 and caspase-9 activities in CaSki cells were assessed using the colorimetric substrates DEVD-pNA and LEHD-pNA, respectively. For each assay, 50 µL of cell lysate was mixed with 45 µL of reaction buffer and 5 µL of the respective substrate solution (4 mM), and incubated at 37°C for 1.5 hours. Cleavage of the substrates released p-nitroaniline (pNA), and the absorbance of pNA was measured at 405 nm using a microplate reader. Cells treated with the phytochemical at its IC_{50} concentration were compared to untreated controls. All experiments were performed in triplicate, and data were expressed as mean ± SD.

3. RESULTS AND DISCUSSION

3.1. Thymoquinone Reduces CaSki Cell Viability

Table 1 shows the comparative cytotoxicity results for the four phytochemicals obtained by MTT Assay. It presents the percentage of viable cells at varying concentrations of (0-100µM) along with their corresponding IC_{50} values. Thymoquinone exhibited the highest cytotoxicity, reducing cell viability by approximately 50.24 % at its IC_{50} concentration. Thymoquinone and Resveratrol showed moderate cytotoxic effects, while Quercetin and Curcumin had comparatively lower effects on cell viability (Table 1). These results established thymoquinone as the most potent compound among those tested.

Based on IC_{50} values, our results depicted in Table 1, exhibited the order of the effectiveness of the selected phytochemicals as TQ (IC_{50} =63.408 µM)> ReSV (IC_{50} =115.83 µM) > CC (IC_{50} =131.22 µM) > QC (IC_{50} =141.74 µM). These results established thymoquinone (TQ) as the most potent compound among those tested, exhibiting the lowest IC_{50} value (63.408 µM). It was therefore selected for further analysis of apoptosis-related processes, as described in the subsequent sections. Next, the data in (Fig.1) illustrate the cytotoxic impact of thymoquinone at varying concentrations (0-100 µM). Cells in the control group (0 µM) maintained full viability. When exposed to 20 µM, there was a slight decline in cell viability to 80.36%, suggesting minimal cytotoxicity. A significant reduction in viability was noted at 60 µM, with only about half of the cells (50.24%) remaining viable. At the highest concentration of 100 µM, thymoquinone showed maximum cytotoxicity, with just 28.94% of cells surviving. These findings indicate that thymoquinone causes a concentration-dependent decrease in the viability of CaSki cells, underscoring its strong cytotoxic effect against cervical cancer cells. This decrease in viability suggests mitochondrial dysfunction and metabolic suppression, consistent with previous studies demonstrating the antiproliferative potential of TQ through inhibition of key survival signaling pathways such as PI3K/Akt and NF-κB [28,29].

Table 1 Percentage cell viability and corresponding IC_{50} values (µM) of phytochemicals in CaSki cells following 24 h treatment, as determined by MTT assay.

Concentration (uM)	Thymoquinone	Resveratrol	Curcumin	Quercetin
0	98.24	99.28	98.34	98.02
1	98.34	98.04	99.24	98.08
1.5	97.28	97.26	97.28	99.34
2	96.22	98.42	99.02	97.38
5	93.12	96.82	97.22	98.16
10	83.18	98.24	97.84	98.02
20	80.36	97.28	98.26	97.48
30	74.22	96.78	90.76	93.12
40	66.12	84.24	88.24	90.48
50	60.26	78.12	81.38	86.12
60	50.24	73.52	78.42	80.55
70	42.86	77.12	70.18	74.22
80	35.98	65.68	71.24	70.38
90	30.36	60.12	66.78	69.04
100	28.94	51.11	59.06	61.44
IC_{50}	63.408	115.83	131.22	141.74

3.2. Thymoquinone Alters Cell Morphology

Thymoquinone induced progressive morphological changes in CaSki cells with increasing concentrations. At 20 µM, cells began to show slight shrinkage and rounding. At 60 µM, a greater number of cells exhibited shrinkage, membrane blebbing, and detachment from the culture surface. At 100 µM, the majority of cells were rounded, shrunk, and detached, indicating extensive apoptotic features. Control cells (0 µM) maintained normal morphology, adhered to the surface, and displayed typical spreading (Fig. 2). These characteristic morphological alterations clearly distinguish apoptotic death from necrotic changes and correlate with the observed reduction in viability.

3.3. Thymoquinone Causes Nuclear Condensation and Fragmentation

DAPI staining demonstrated concentration-dependent apoptotic nuclear changes. At a concentration of 20 µM, slight chromatin condensation was detected. When the concentration reached 60 µM, cells exhibited significant nuclear condensation along with fragmented nuclei. At 100 µM, there was extensive nuclear fragmentation, and the nuclei appeared bright and condensed. Control cells displayed uniform, round nuclei with no signs of apoptosis (Fig. 3). These nuclear changes clearly indicate chromatin condensation and DNA fragmentation, further validating the induction of apoptosis by thymoquinone, which is in accordance with earlier reports in other cancer model systems [30].

3.4. Thymoquinone Increases ROS Levels

To investigate whether oxidative stress plays a role in TQ-induced apoptosis, reactive oxygen species (ROS) generation was assessed using the DCFDA staining method. As evident from (Fig. 4) control cells displayed weak green fluorescence, indicative of basal ROS levels, whereas TQ-treated cells showed a pronounced increase in fluorescence intensity in a dose-dependent manner. This suggests that TQ significantly raised intracellular ROS levels, resulting in oxidative stress-mediated damage. Excessive ROS can lead to mitochondrial dysfunction and cytochrome-c release, subsequently activating the intrinsic apoptotic pathway. Thymoquinone caused a dose-dependent rise in ROS levels. In control cells, fluorescence was barely detectable. At a concentration of 20 µM, there was a slight rise in DCFDA fluorescence. When the concentration reached 60 µM, a significant increase in ROS accumulation was observed, and at 100 µM, the majority of cells exhibited strong fluorescence, indicating a high level of oxidative stress. (Fig. 4). The observed increase in ROS levels, as measured by the DCFDA assay, is consistent with previous reports demonstrating thymoquinone-induced oxidative stress and apoptosis in various other cancer cell lines [34-36]

3.5. Thymoquinone Compromises Cell Membrane Integrity

AO/EtBr dual staining revealed progressive membrane damage with increasing thymoquinone concentrations. At 20 µM, a few cells exhibited yellow/orange fluorescence, indicating early apoptosis. At 60 µM, the proportion of apoptotic cells increased, and some cells exhibited red fluorescence, indicative of late apoptosis. At 100 µM, most cells exhibited orange to red fluorescence, indicating extensive apoptosis and compromised membrane integrity. Control cells fluoresced green, indicating intact membranes (Fig. 5). These results strongly support the notion that TQ initiates apoptosis in CaSki cells rather than causing nonspecific necrosis and, in turn, are in accordance with earlier findings in other cancerous cells [31-33].

3.6. Thymoquinone Activates Caspase-3 and Caspase-9 to Trigger Apoptosis

The effect of thymoquinone on apoptosis was assessed by measuring caspase-3 and caspase-9 activities in CaSki cells at 0, 60, and 100 µM (Fig. 6). Baseline caspase-3 activity in control cells (0 µM) was normalized to a fold-change of 1(Fig. 6a). Treatment with 60 µM thymoquinone resulted in a substantial increase in caspase-3 activity, reaching 24.16-fold, indicating the activation of the execution phase of apoptosis. At 100 µM, caspase-3 activity increased further to 33.52-fold, demonstrating a pronounced dose-dependent induction of apoptosis.

Caspase-9 activity also followed a similar dose-dependent pattern (Fig. 6b). Control cells exhibited minimal activity (fold change 1). Treatment with 60 µM thymoquinone elevated caspase-9 activity to 19.11-fold, reflecting activation of the intrinsic apoptotic pathway. At 100 µM, caspase-9 activity reached 26.82-fold, confirming strong mitochondrial pathway-mediated apoptosis.

These results collectively indicate that thymoquinone induces intrinsic apoptosis in CaSki cells through significant, dose-dependent activation of both caspase-3 and caspase-9 (Fig. 6). The enhanced activity of both caspases indicates activation of the intrinsic apoptotic pathway, where caspase-9 acts as the initiator and caspase-3 functions as the executioner, confirming that thymoquinone-induced cell death occurs through a mitochondria-mediated apoptotic mechanism. These findings collectively highlight the consistent role of thymoquinone in inducing apoptosis across various cancer cell lines through the activation of caspase pathways. Such mechanistic insights, as observed in CaSki cells, align with previously reported studies in other cancer cell lines, reinforcing thymoquinone's potential as a promising therapeutic adjunct in cervical cancer treatment [37,38].

4. CONCLUSION

Our data illustrate that thymoquinone effectively triggers apoptosis in CaSki cervical cancer cells through a complex mechanism that includes increased production of reactive oxygen species (ROS), mitochondrial dysfunction, nuclear condensation, and activation of the caspase cascade, particularly caspase-9 and caspase-3. The combination of morphological alterations, biochemical evidence, and molecular markers confirms that thymoquinone initiates programmed cell death via the intrinsic mitochondrial pathway rather than inducing nonspecific necrosis. Our results are suggestive of the potential of thymoquinone, which could be employed as a natural, multi-targeted compound, that can selectively inhibit cancer cell growth while minimizing toxicity. Due to its capacity to enhance apoptotic signaling and impair tumor cell survival, thymoquinone shows promise as a therapeutic adjuvant in cervical cancer treatment, potentially complementing traditional chemotherapeutic agents to improve treatment effectiveness, overcome resistance, and reduce adverse side effects. Further in vivo studies and clinical evaluations are necessary to fully explore its potential in the treatment of integrative cervical cancer.

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Supplementary Information None
Author contributions U.H. designed and performed the experiments, analyzed the data, and contributed to the writing of the manuscript. S.N. provided guidance as and when needed. N.I. conceptualized and designed the study, supervised the experimental work, and critically reviewed and revised the manuscript for intellectual content.

Competing interests The authors declare no competing interests.

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FIGURE LEGENDS

Figure 1. Effect of thymoquinone (TQ) on CaSki cell viability. Cells were treated with increasing concentrations of TQ for 24 h, and viability was measured using the MTT assay. Data represent mean ± SD of three independent experiments.

Figure 2. Morphological changes in CaSki cells following TQ treatment. (a) Control cells showing normal spindle-shaped morphology. (b-d) TQ-treated cells at 20, 60, and 100 µM, respectively, exhibited apoptotic features, including cell shrinkage, rounding, membrane blebbing, and detachment. Images were captured under an inverted microscope at 20× magnification.

Figure 3. DAPI staining of CaSki cells showing nuclear morphology. (a) Control cells exhibit intact nuclei with uniform staining. (b-d) Thymoquinone (TQ) treatment at 20, 60, and 100 µM induces nuclear condensation and fragmentation, indicating apoptosis. Images were captured at 40× magnification using fluorescence microscopy.

Figure 4. Detection of reactive oxygen species (ROS) in CaSki cells using DCFDA staining. (A) Control cells show low green fluorescence, indicating basal ROS levels. Thymoquinone (TQ) treatment at (B) 20 µM, (C) 60 µM, and (D) 100 µM induces a dose-

dependent increase in green fluorescence, reflecting elevated intracellular ROS. Images were acquired at 20× magnification using fluorescence microscopy.

Figure 5. Acridine orange/ethidium bromide (AO/EB) staining of CaSki cells after thymoquinone (TQ) treatment. Control cells show predominantly green fluorescence, indicating viable cells. TQ-treated cells at increasing concentrations display orange to red fluorescence, representing early and late apoptotic populations. Images were captured at 20× magnification.

Figure 6. Caspase activity in CaSki cells following thymoquinone (TQ) treatment. (a) Caspase-3 activity and (b) Caspase-9 activity were measured using colorimetric assays after 24 h treatment with 0, 60, and 100 µM TQ. The fold increase relative to the untreated control (fold 1) is shown. Data are presented as mean ± SD of three independent experiments (**p < 0.01, ***p < 0.001 compared to control).

FIGURES

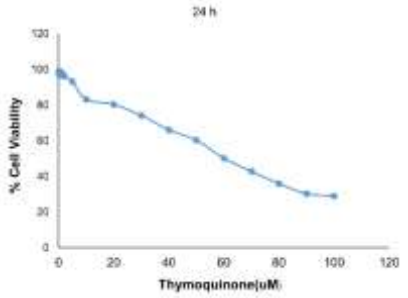

Figure 1

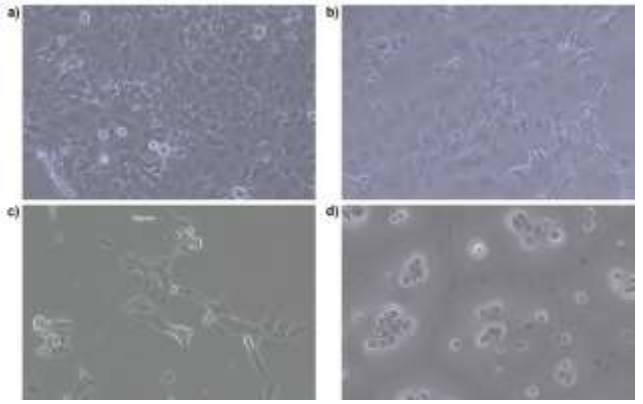

Figure 2

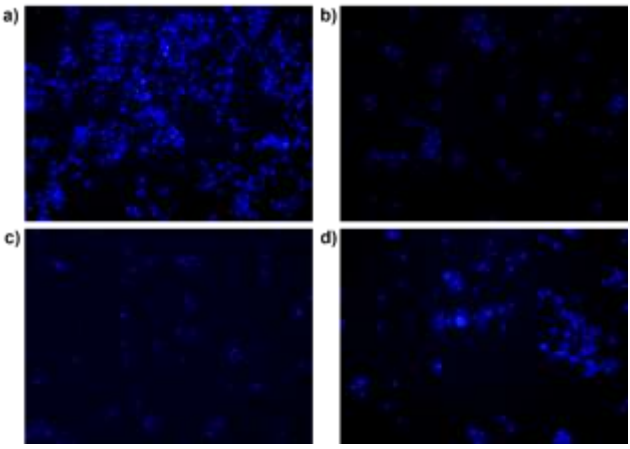

Figure 3

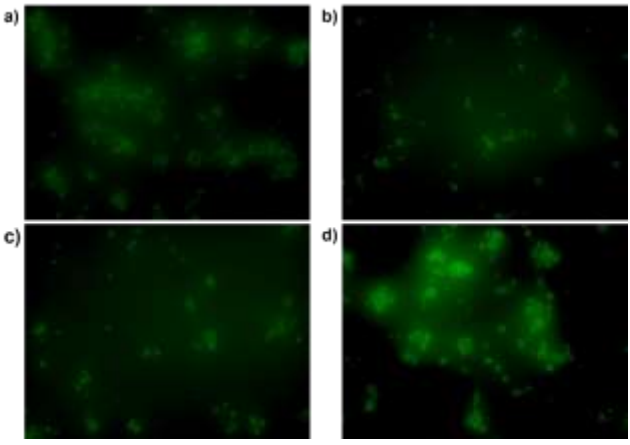

Figure 4

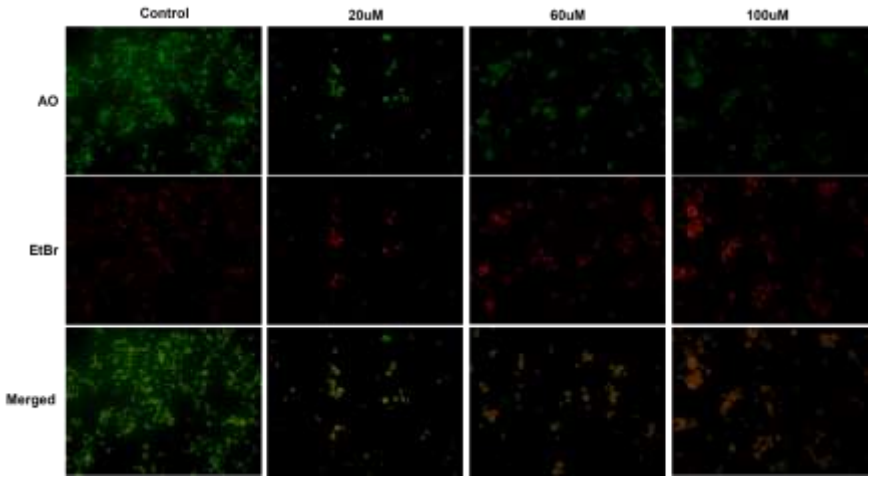

Figure 5

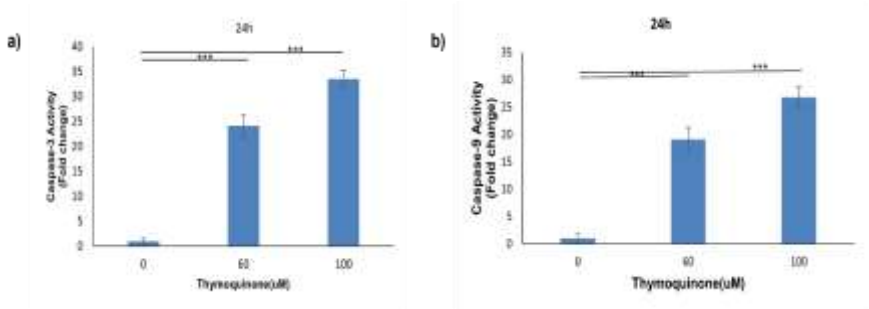

Figure 6

AREA OF RESEARCH INTEREST OF CORRESPONDING AUTHOR:

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My research focuses on the exploration of a wide spectrum of natural compounds that may help in the better understanding and management of various diseases like Cancer, Cardiac disorders, Tuberculosis and Osteoporosis. Emphasis is on the use of natural antioxidants and phytochemicals as therapeutic agents / adjuncts. I have demonstrated the role of organosulfur compounds and catechins in modulating apoptosis, oxidative stress, and inflammatory pathways in cancer and infectious models. The published research work from my laboratory appears to have contributed to understanding how bioactive compounds can regulate gene expression, inhibit pathogenic activity, and enhance cellular defense mechanisms.

Recent Publications

1. Ansari, M. A., Shoaib, S., Chauhan, W., Gahtani, R. M., Hani, U., Alomary, M. N., Alasiri, G., Ahmad, N., JahanR., Yusuf, N. and **Islam, N. (2024)** Nanozymes and carbon-dots based nanoplatforms for cancer imaging, diagnosis and therapeutics: Current trends and Challenges. ***Environmental Research***. *241: 117522*
2. Ansari, M. A., Shoaib, S., Alomary, M. N., Ather, H., Ansari, S. M. A., Hani, U., Jamous, Y. F., Alyahya, S. A., Alharbi, J. N., Imran, M. A., Wahab, S.., Ahmad, W., and **Islam, N. (2023)** Deciphering the emerging role of phytocompounds: Implications in the management of drug-resistant tuberculosis and ATDs-induced hepatic damage, ***Journal Infection and Public Health, 16(9): 1443-1459.***
3. Shoaib S, Ansari MA, Ghazwani M, Hani U, Jamous YF, Alali Z, Wahab S, Ahmad W, Weir SA, Alomary MN, Yusuf N. and **Islam N. (2023)** Prospective Epigenetic Actions of Organo-Sulfur Compounds against Cancer: Perspectives and **Molecular** Mechanisms. ***Cancers* (Basel). 23;15(3):697. doi:10.3390/cancers15030697. PMID: 36765652; PMCID: PMC9913804.**
4. Shoaib S, Ansari MA, Fatease AA, Safhi AY, Hani U, Jahan R, Alomary MN, Ansari MN, Ahmed N, Wahab S, Ahmad W, Yusuf N. and **Islam N. (2023)** Plant-Derived Bioactive Compounds in the Management of Neurodegenerative Disorders: Challenges, Future Directions and Molecular Mechanisms Involved in Neuroprotection. ***Pharmaceutics.* 23;15(3):749.** doi:10.3390/pharmaceutics15030749. PMID: 36986610; PMCID: PMC10057544.
5. Shoaib S, Ansari MA, Kandasamy G, Vasudevan R, Hani U, Chauhan W, Alhumaidi MS, Altammar KA, Azmi S, Ahmad W, Wahab S. and **Islam N. (2023)**An Attention towards the Prophylactic and Therapeutic Options of Phytochemicals for SARS-CoV-2: A Molecular Insight. ***Molecules.* 2023 13;28(2):795.** doi:10.3390/molecules28020795. PMID: 36677853; PMCID: PMC9864057.
6. Shoaib S, **Islam N,** Yusuf N. **(2023)** Phytocompounds from the Medicinal and Dietary Plants: Multi-target Agents for Cervical Cancer Prevention and Therapy. ***Curr Med Chem.* 2022;29(26):4481-4506.** doi: 10.2174/0929867329666220301114251. PMID: 35232338.
7. Shoaib, Tufail, S., Sherwani, M. A., Yusuf, N. and **Islam, N. (2021)** Phenethyl Isothiocyanate Induces Apoptosis Through ROS Generation and Caspase-3 Activation in Cervical Cancer Cells, **Front. Pharmacol., 12:673103. (doi:10.3389/fphar.2021.673103)**
8. Tufail, S., Sherwani, M.A., Shoaib, S. Azmi, S., Owais, M. And **Islam, N.** (2018) Ovalbumin self-assembles into amyloid nanosheets that elicit immune responses and facilitate sustained drug release. **J. Biol. Chem. 293(29) 11310-11324.**