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Original Article

# Reserpine Induces Apoptosis in Drug-Resistant Cancer through Modulating STAT3 and NF-κB Signaling

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## ABSTRACT

**Introduction:** A major feature of drug-resistant cancer is the overexpression of transcription factors. During the study, reserpine, an indole alkaloid, was examined for its anticancer effects on cancer with drug resistance. **Objectives:** The objective of this study was to explore the anti-apoptotic impacts of reserpine in drug-resistant cancer cells by impeding the nuclear translocation of NF-κB and STAT3. **Materials and Methods:** For the *in vitro* study of reserpine's anticancer activity, human drug resistance cancer cell line KB-ChR-8-5 was selected. A MTT assay was used to evaluate the cytotoxicity of reserpine. Reactive Oxygen Species (ROS), comet test, Mitochondrial Membrane Potential (MMP) and AO/EtBr staining were used to assess cell proliferation, DNA damage and apoptotic activation. Conducting western blot analysis was employed in this research study to examine the effect of reserpine on KB-ChR-8-5 cells express levels of proteins related to apoptosis markers and transcription factor expression. **Results:** We found that, reserpine decreased cell viability, increased ROS levels, enhanced DNA damage and decreased mitochondrial membrane potential due to its antioxidant properties. Moreover, the suppression of NF-κB and STAT3 facilitates the upregulation of specific apoptotic proteins, such as Bax, cytochrome C, Caspase-9 and Caspase-3, which simultaneously inhibit cancer growth. **Conclusion:** These results imply that reserpine suppressed NF-κB and STAT3 nuclear translocation process, which led to ROS-induced apoptosis and tumour cell death.

**Keywords:** Reserpine, STAT3, NF-κB, ROS, Apoptosis.

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## INTRODUCTION

Globally, cancer-related mortality is largely attributed to Multidrug Resistance (MDR), more than 90% of the deaths of patients who received traditional chemotherapy were caused by this condition.[1] Tobacco smoking, chewing, excessive alcohol, genetic susceptibility are major risk factors.[2] Despite some progress even surgery treatment achieved often diagnosed at last decades, the 5-year survival associated was <50%.[3] Accumulating evidence has indicated that drug resistance was from dysregulation of multiple signaling pathways, which disrupt balance between oncogenes and suppressor genes that cause enhanced cell proliferation and reduced cell death.

Dysregulation of transcription factors is one of the most common genetic aberration ultimately affect multiple signalling pathways. Recently, numerous cancers cases can be found drug resistance, including bone, breast, head and neck, lung, are appearing to depend upon transcription factors upregulation.[4] According to the recent findings NF-κB and STAT3 contributes to the growth and cells developments which is often overexpressed in variety of cancers.[5,6] The activated transcription factor NF-κB and STAT3 trigger gene involved proliferate, invade cells, promote angiogenesis, leads to develop tumor growth and metastasis.[7] Thus, novel inhibitors targeting NF-κB and STAT3 and their ability to regulate signaling in cancer, offer great potential for developing effective therapeutic approaches to treat cancer with resistance.

The use of phytomedicine has increased significantly in recent years as an effective bioactive and significant chemopreventive agents for treatment of various type of the cancers, due to inhibiting multiple signaling pathways.[8] In the year 1940 to 2002, more

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than 60% natural products and its formulations were approved for cancer treatment owing to low toxicity, anti-proliferative and pro-apoptotic properties represents a long-term resource for cancer treatment.[9] The identification of the indole alkaloid reserpine within *Rauvolfia serpentina* has been reported to have anti-oxidant, anti-bacterial and antihypertensive.[10,11] Recent studies have been reported that reserpine have exerts its anticancer effects in skin, oral, endometrial and hepatocellular carcinoma through subduing the stimulation of cellular proliferation, cell cycle arrest and facilitating of apoptosis promotion.[12,13] However, reserpine comprehensive evaluation on drug resistance cancer is lacking. Therefore, present study was designed to evaluate reserpine treatment inhibits proliferation and enhance apoptosis via interacting with NF-κB and STAT3.

## MATERIALS AND METHODS

### Chemicals

Dulbecco's Modified Eagles Medium (DMEM), Hoechst 33342, 2,7-diacetyl-Dichlorofluorescein (DCFH-DA), Phosphate Buffered Saline (PBS), penicillin streptomycin solution (antibiotics), Acridine Orange (AO), Ethidium Bromide (EtBr), Trypsin EDTA, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl Tetrazolium bromide (MTT), Rhodamine 123, Fetal Bovine Serum (FBS), 2-mercaptoethanol and glutamine were obtained from Hi-media. Reserpine (98%), monoclonal anti-bodies Caspase-3, cytochrome C, Caspase-9, IκBα, Bcl-2, STAT3 NF-κB, Bax and polyclonal antibody IgG-HRP have been collected from Sigma Chemicals Co., St. Louis, USA.

### Cell culture

Cancer cell lines (KB-ChR-8-5) exhibiting drug resistance were obtained from the National Centre for Cell Science (NCCS) in Pune, India. The cultivation cells were developed in Dulbecco's Modified Medium Eagle (DMEM), enhanced by 10% foetal bovine serum and 100 U/mL penicillin and streptomycin. Cells kept at 37°C under humidified environment containing 5% $CO_2$.

### Cytotoxic assay and cell viability

Colorimetric MTT technique assessed the influence of reserpine on cell viability. After trypsinization, a density of 1 to $10^4$/mL was obtained and seeded on a culture plate of 96 well plates (100 µL each well). The liquid was removed after 24 hr of culture and the reserpine dosage was added to 0 to 100 µM. The adherent cells were being processed using MTT at a concentration of 0.5 mg/mL treatment for the 4 hr after 24 hr of treatment, removed the medium and added 100 µL dimethyl sulfoxide. Microplate Reader (BIO-RAD) at 570 nm was used to measure the wavelength for absorb of each well.

### Determine the role of ROS in cellular processes

The properties and applications of DCFH-DA (2,7-diacetyldichlorofluorescein) as a cell-permeable redox, that has the potential to enter the intracellular matrix and is utilised to monitor the role of oxygen-containing molecules in cellular processes like Reactive Oxygen Species (ROS); it is assumed that ROS are involved in the oxidation of DCFH-DA to fluorescent DCF.[14] Human multi drug resistance cancer cells KB-ChR-8-5 were seeded on a 6-well plate at 24 hr were spent maturing in a Carbon Dioxide ($CO_2$) incubator. The cells underwent then stained and incubated through DCFH-DA for 10 min after being 24 hr reserpine treatment. The percentage (%) of fluorescence intensity has increased as a result of the experiment. Finally, evaluate the fluorescence intensity using an filter that selects the light source for fluorescence set at 485±10 nm or an filter with emission and wavelength of 530±12.5 nm.

### Observe mitochondrial membrane potential loss

The cationic lipophilic dye Rhodamine 123 assessed Mitochondrial Membrane Potential (MMP).[15] Human drug resistance type of cancerous cells known as KB-ChR-8-5 are located to a plate containing 6 well ($1\times10^6$ cells/well), then treated varying reserpine dosages. After 24 hr of treatment, cells incubate 30 min with Rh-123 dye. A fluorescent microscope assessed the membrane potential change, the excitation filter was 485±10 nm and the emission filter was 530±12.5 nm.

### Identification of morphological alterations associated with apoptosis

In order to stain cancerous cells known as KB-ChR-8-5 twice, we employed the dyes that stain: Acridine Orange and Ethidium Bromide (AO/EtBr).[16] Healthy and dead cells are permeated with AO, whereas only dead cells can be stained by EtBr. A density of $3x10^4$ cells/well was achieved by seeding cells on a 6-well cell culture plate after adhesion cells had been treated with reserpine for a duration of 24 hr and the Cells were fixed using a mixture of methanol and glacial acetic acid (3:1) as predetermined at a temperature of 4°C for a duration of 30 min. After that, 1:1 ratio of AO and EtBr is used to stain cells for apoptosis. The mixture should be incubated at 37°C for duration of 30 min. Examine the stained cells under a fluorescence microscope to determine the cells nature (Olympus, Japan).

### Detection and investigation of DNA damage

Centrifuge the control and reserpine-treated cancerous cells KB-ChR-8-5 and re-suspend the pelleted cells in low melting agarose (0.5%) at 37°C. The slides were covered with normal melting agarose to produce a foundation layer and the second layer was a combination of cells containing agarose. Then hold the slides at a temperature of 4°C for 5 min. Immerse the comet slide in cold fresh lysate (A solution with 10% DMSO, Tris pH 10 buffer, 100 mM EDTA chelate, 1% Triton X-100 detergent and 2.5

M NaCl.) for 2 hr at 4ºC.Transfer to electrophoresis (25 V) 4ºC for 30 min to denature the double helix structure of DNA, wash the slides 3 times with a buffer for neutralisation (0.4 M Tris, pH 5) and stain for 15 min with solution of 20 µg/mL EtBr to stained cells is and kept in the dark place for analyse DNA damage. After using a fluorescence microscope (Olympus, Japan), comets were finally observed.

## Immunoblot analysis

The cells were subjected to protein extraction by applying RIPA buffer, Electrophoresis was used to separate the samples on a 10% polyacrylamide gel and then semi-dry blotting was used to transfer the samples into PVDF membrane. 1% BSA in TBS solution were used to block the membrane and add specific antibody against the proteins of interest. protein was exposed to 4ºC for an hour at room temperature, followed by a wash with 1xTBST of the membrane and incubated with the secondary antibody conjugate in 1xTBS and underwent another hour of incubation at room temperature, lastly the membrane was washed with 1xTBST and detected using a chemiluminescence detecting system. As a loading control, GAPDH was utilized.

## Statistical analysis

The ANOVA test in the SPSS 11.0 software programme was used to conduct the statistical analysis. Further, the Duncan's Multiple Range Test (DMRT) was utilized to assess potential statistically significant differences among means ($p$<0.05).

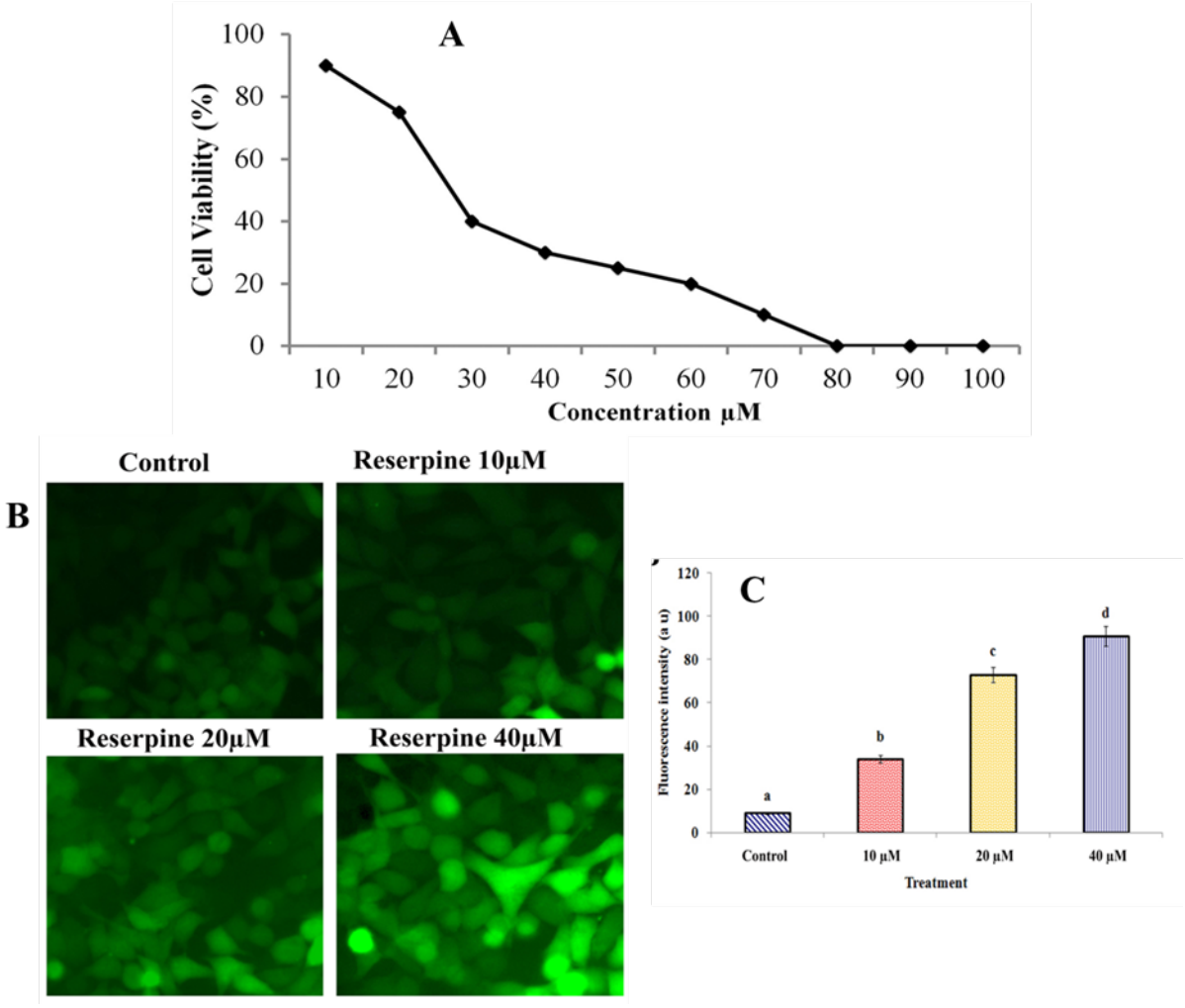


**Figure 1A:** The MTT test was used to determine the effect of reserpine on KB-ChR-8-5 cell viability. (A) Reserpine impact on KB-ChR-8-5 cells is that it inhibits cell proliferation. Values are reported as the mean±SD of each group of six experiments. Figure 1B: The effect of reserpine on the production of intracellular ROS in KB-ChR-8-5 cells was investigated in order to determine the DCFH-DA staining technique. (A) On the basis of the micrographs, it was discovered that untreated KB-ChR-8-5 cells had weak DCF fluorescence and that different doses of reserpine (10, 20 and 40 µM) resulted in enhanced generation of ROS, suggesting a significant amount of DCF fluorescence intensity. Floid cell imaging station captured this picture of cell. (C) A spectrofluorometer may be used to determine the proportion of ROS production. Except as otherwise stated, All experiments were conducted in triplicate and the results were represented as the mean standard deviation (mean±SD). The statistical significance of the results was determined using an analysis of variance followed by DMRT. Comparing values that do not share common superscript letters (a-d) and are basically distinct at $p$≤0.05 compared to the control (DMRT).

## RESULTS

### Effectuation of reserpine regarding the viability of cell

To assess the impact of reserpine on the proliferation of drug resistance cancerous cell known as KB-ChR-8-5 at concentrations ranging from 0-100 µM as demonstrated in Figure 1A. The MTT assay was utilized to determine the $IC_{50}$, which is the concentration at which reserpine reduces cell viability by 50%, indicating the maximum potential response to treatment. The evaluation of KB-ChR-8-5 cell viability revealed that a specific dosage of reserpine 80 µM greatly increased the maximal cell mortality, hence we chose the 10, 20 and 40 µM dosages for future investigation.

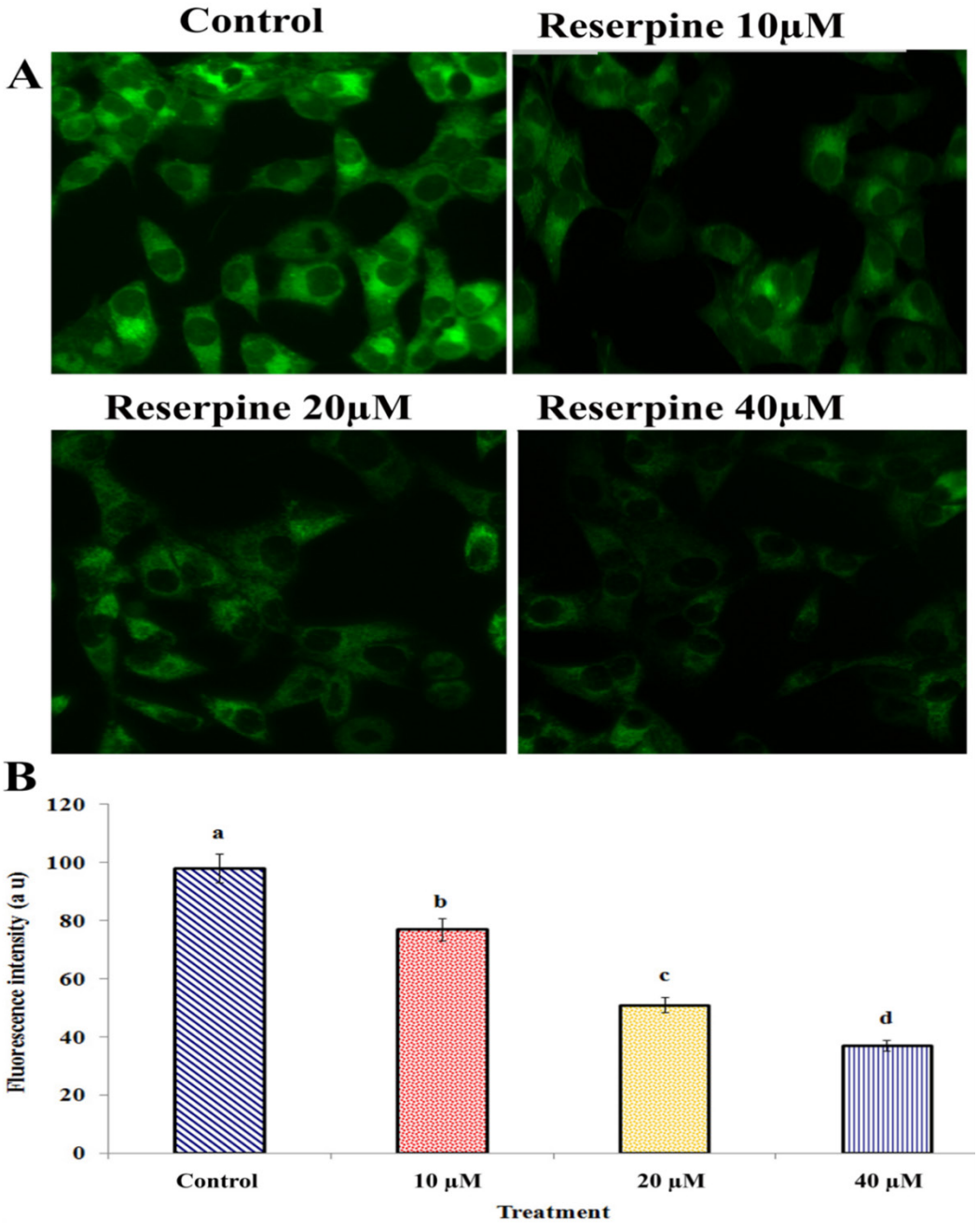


**Figure 2:** To investigate the effects of reserpine on Mitochondrial Membrane Potential (MMP) in KB-ChR-8-5 cells, Rhodamine 123 staining was used. (A) The KB-ChR-8-5 untreated cells, strong fluorescence was seen, indicating that the mitochondrial membrane polarization. Reserpine different doses (10, 20 and 40 µM) administered over a 24 hr period suggest the collapse of the mitochondrial matrix. Floid cell imaging station captured this picture of cell. (B) The spectrofluorometer was used to determine the intensity of the fluorescence. All experiments were conducted in triplicate and the results were represented as the mean standard deviation (mean±SD). The statistical significance of the results was determined using an analysis of variance followed by DMRT. Comparing values that do not share common superscript letters (a-d) and are basically distinct at $p \leq 0.05$ compared to the control (DMRT).

## Impact of reserpine on the generation of ROS

The Figure 1B illustrates the exposure of ROS generation under the influence of reserpine. We found that reserpine concentrations (10, 20 and 40 µM) considerably enhanced the ROS formation in KB-ChR-8-5 cells after 24 hr exposure in comparison to the control as measured by the fluorescence intensity of 2′,7′-Dichlorofluorescein (DCF). It demonstrates that the generation of ROS is the most important element in the initiation of cell death.

## Effect of reserpine on Mitochondrial Membrane Potential (MMP)

The effect of reserpine on MMP levels in drug resistance cancerous cells KB-ChR-8-5 was exposed in Figure 2. We studied

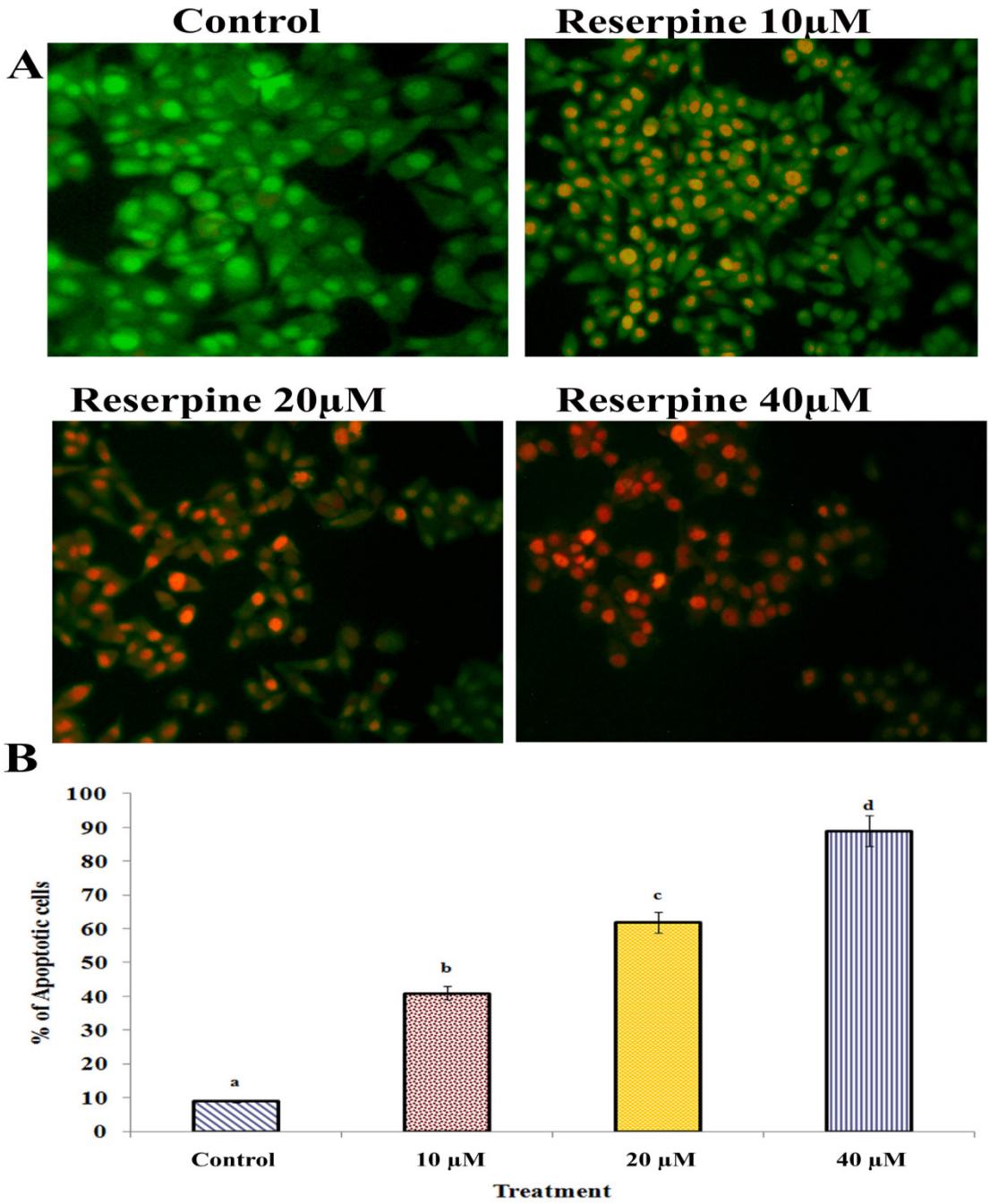


**Figure 3:** Apoptosis was seen in the presence of reserpine, as determined by dual staining (AO/EtBr) in the presence of reserpine. (A) KB-ChR-8-5 cells were treated with reserpine at varied doses (10, 20 and 40 µM) and the percentage of apoptotic cells significantly increased as compared to untreated cells. (B) To show that the proportion of apoptotic cells has been calculated. The data are given as the mean standard deviation of three Separate Experiments (SD). All experiments were conducted in triplicate and the results were represented as the mean standard deviation (mean±SD). The statistical significance of the results was determined using an analysis of variance followed by DMRT. Comparing values that do not share common superscript letters (a-d) and are basically distinct at $p \leq 0.05$ compared to the control (DMRT).

the intact mitochondrial membrane and exposure to varying doses of reserpine (10, 20 and 40 µM) using rhodamine 123. It shows that the integrity of the membrane was lost proportionally across to the doses that verifies a continuous reduction in green fluorescence relative to control, which suggest release of Cyt-C from mitochondria.

## Effect of reserpine on apoptotic morphological changes

The nucleus was fragmented into smaller fragments, showing that reserpine induces apoptosis in the cells (Figure 3). Control cells show normal nuclear chromatin with green fluorescence, whereas treatment with reserpine varying doses (10, 20 and 40 µM) enhances apoptosis as a result of damage to the cell membrane

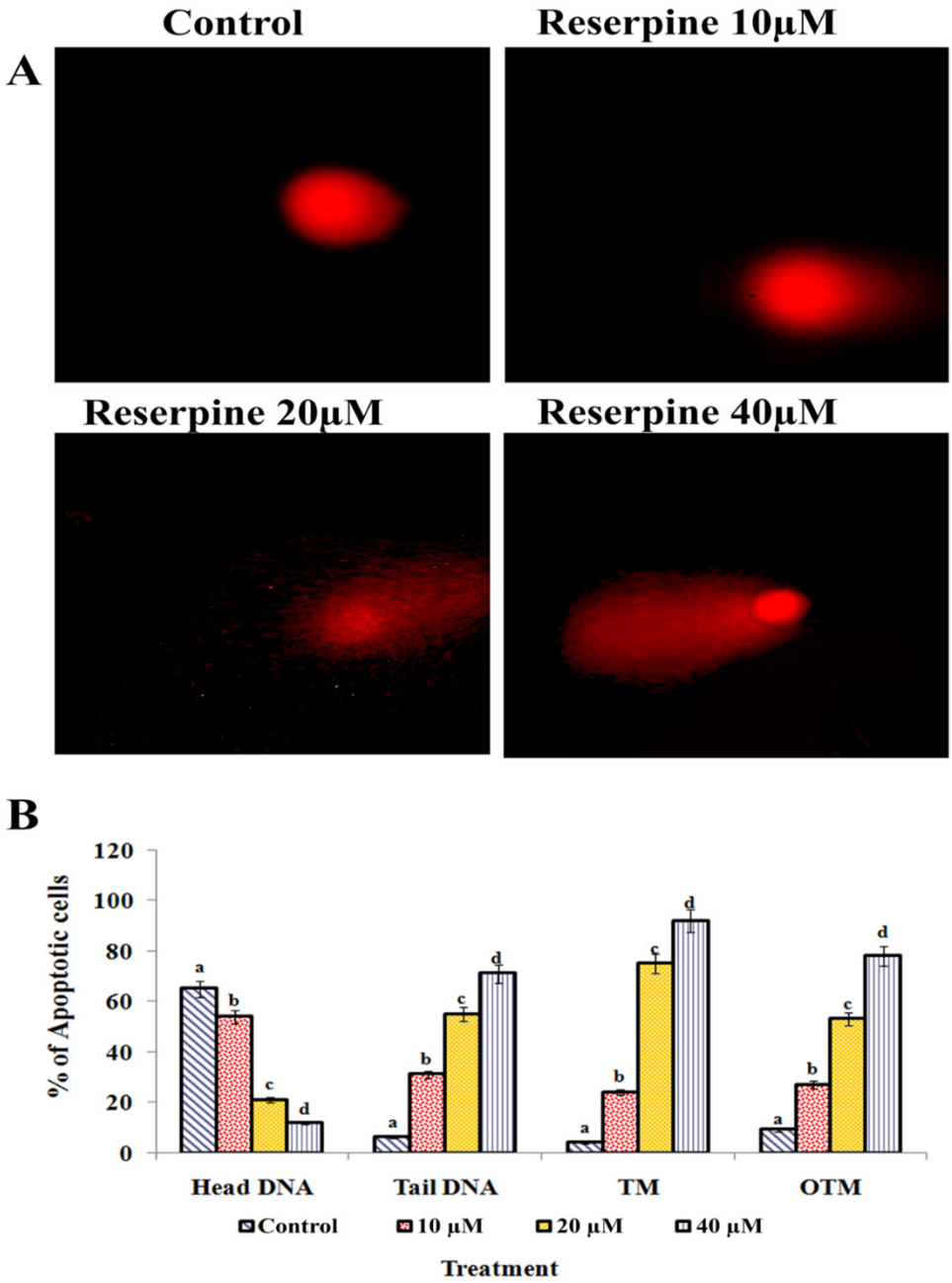


**Figure 4:** Effect of reserpine on the single stand break of DNA in KB-ChR-8-5 cells. Single-cell gel electrophoresis was used to separate the cell lysates and the break that occurred in the DNA molecule's two liner strands was detected by ethidium bromide staining. (A) As KB-ChR-8-5 cells were treated with reserpine at different concentrations (10, 20 and 40 µM), significant differences in comet shape were found when compared to untreated cells. (B) Results revealed that DNA damage in cells treated with reserpine was defined by the head DNA %, DNA tail, DNA tail moments, as well as movement of the olive tail, as compared to untreated cells. All experiments were conducted in triplicate and the results were represented as the mean standard deviation (mean±SD). The statistical significance of the results was determined using an analysis of variance followed by DMRT. Comparing values that do not share common superscript letters (a-d) and are basically distinct at $p \leq 0.05$ compared to the control (DMRT).

and DNA, respectively (orange-red shade). Our findings show that reserpine has a significant influence on the morphological alterations of the nucleus, which are strongly associated with cell death.

### Effect of reserpine on DNA fragmentation

Reserpine treatment impact on DNA damage was assessed as the % of head, tail and OTM determined by the comet test. The dense DNA in the nucleus of the head cell is separated and dispersed into fragments, which constitute the DNA tail in various reserpines (10, 20 and 40 µM) treated with a certain concentration of KB-ChR-8-5 cells. The findings revealed that substantial dosage of reserpine, increased % of tail DNA and OTM dramatically compared with control (Figure 4). These results clearly suggesting that cells are sensitive to death because of apoptosis.

### Effect of reserpine on promote apoptosis

Pro-apoptotic and anti-apoptotic markers expression were observed in reserpine-treated cells and control cells by western blotting (Figure 5A). It was discovered that there has been a substantial reduction in the modulation of the anti-apoptotic protein Bcl-2 expression. The pro-apoptotic proteins Bax, caspase-9, cytochromic C and caspase-3 expression in cells treated with reserpine (10, 20 and 40 µM) was shown to be considerably higher than that in control cells, that is plausibly associated with DNA damage and apoptosis.

### Effect of reserpine on STAT3 and NF-κB and transcription protein signaling

According to studies, the STAT3, NF-κB factors is crucial in regulating cell proliferation and invasion in KB-ChR-8-5, a cancer cell line with resistance to drugs. Treatment with reserpine (10, 20 and 40 µM) recover IκBα substantially decreased STAT3, NF-κB expression in drug resistant cancer cells (Figure 5B). In this research, we showed that reserpine substantially downregulates the STAT3 and NF-κB, thereby inhibits cell growth, enhanced programmed cell death in resistance cancerous cells.

## DISCUSSION

Inhibition of proliferation and promoting apoptosis are complicated process, there are a number of transcription factors implicated in these functions. Hence, novel agents that inhibit cancer-related transcription factors can promote apoptosis in cancer cells and to inhibit tumor development. There are many herbal phytochemicals that can be used instead of conventional cancer treatments due to these compounds exhibit strong biological activity, they are effective in the fight against cancer.[17] Plant-derived anticancer medicines used in chemotherapy treatments around the world include camptothecin, vinblastine, vinorelbine, paclitaxel and epipodophyllotoxin.[18] Research by Hong *et al.*, 2016 indicates that reserpine exhibits cancer preventive effects by reactivating Nrf2 and inducing the expression of genes involved in cellular protection, suggesting a potential role for reserpine in altering cellular responses.[19] The aim of this study was to investigate whether reserpine induces apoptosis and suppress tumor growth by inhibiting NF-kB and STAT3 in multidrug resistant cancerous cells.

Cancer cells frequently exhibit elevated levels of Reactive Oxygen Species (ROS); however, surplus ROS will inhibit their growth and induce apoptosis. As therapeutic strategies for targeting the destruction of cancer cells, ROS altering drugs are becoming predictable.[20] In recent years, many natural products have been found to increase ROS levels especially in cancerous cells. In this research investigation, we found that reserpine significantly induced ROS levels in human multidrug resistant cancer cells KB-ChR-8-5 when administered in a manner contingent on concentration levels. Lim *et al.*, 2021, reported that the indole alkaloid indole-3-carbinol prompts apoptosis in H1299 lung cancer via increased ROS levels, which, in turn, leads to caspase-3

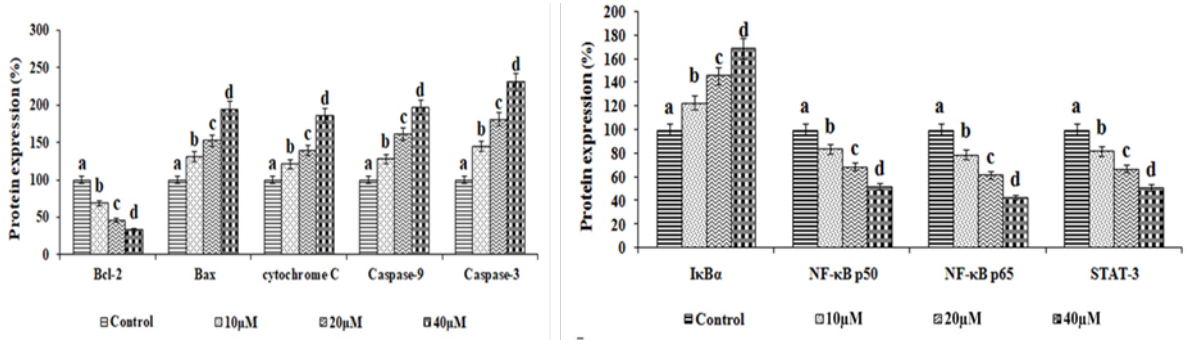


**Figure 5A:** The effect of reserpine on the expression of the apoptotic protein. The expression of Bcl-2, Bax, cytochrome C, Caspase-9 and Caspase-3 in KB-ChR-8-5 cells after 24 hr of treatment with and without reserpine is shown in the representative immunoblot study. GAPDH serves as a loading control protein. Densitometric analysis. Protein expression in the control lysates was assessed six times and was represented as 100% in the graph. When comparing values that do not share common superscript letters (a-d) and are essentially unique at $p \leq 0.05$ compared to the control (DMRT). Figure 5B: Effects of reserpine on transcription protein IκBα, NF-κB p50, NF-κB p65 and STAT3 expression. The expression of IκBα, NF-κB p50, NF-κB p65 and STAT3 in KB-ChR-8-5 cells after 24 hr of treatment with and without reserpine is shown in the representative immunoblot study. GAPDH serves as a loading control protein. Densitometric analysis. Protein expression in the control lysates was assessed six times and was represented as 100% in the graph. When comparing values that do not share common superscript letters (a-d) and are essentially unique at $p \leq 0.05$ compared to the control (DMRT).

activation.[21] As well, our study revealed that the administration of reserpine led to an increase in Reactive Oxygen Species (ROS) generation within the KB-ChR-8-5 cells and this subsequently stimulated caspase-3 expression to prompts apoptosis. The indole alkaloid yohimbine, a structurally similar to reserpine, induces caspase-9 activity through ROS production by changing the mitochondrial membrane potential.[22] There is an effective correlation exists between the production of Reactive Oxygen Species (ROS) and the activity of apoptosis that is in agreement with our results, which provides evidence that reserpine increases ROS production and, as a result, apoptosis is increased.

The mitochondria play crucial role in facilitating the energization of apoptosis within the presence of anticancer drugs which achieve their antitumor effects by triggering ROS-dependent apoptotic signaling. Numerous studies have shown that high amounts of Reactive Oxygen Species (ROS) in cells cause mitochondrial membrane potential loss, which releases pro-apoptotic proteins, DNA damage, nuclear chromatin condensation and apoptosis.[23] We have observed that treatment of human multi drug resistance cancerous cells KB-ChR-8-5 with reserpine resulted in the mitochondrial dysfunction, accompanied with ROS augmentation. These findings are consistent with earlier research on cathachunine, a novel indole alkaloid that stimulates programmed cell death in leukaemia cells by reducing $\Delta\Psi m$, causing the discharge of mitochondrial Cytochrome C activation that triggers the activation of caspase.[24] Consequently, reserpine could induce apoptosis by reducing MMP through ROS mediated mechanisms.

A well-known characteristic of AO is that it intercalates double stranded DNA into all cells, emitting green fluorescence, while EB can only stain nuclear DNA in cells that have lost their membrane integrity.[25] Reserpine treatment of KB-ChR-8-5 cancerous cells revealed that viable cells consistently stained green, late stage apoptotic and the dead cells-stained orange in the multidrug resistant cancer cells. Therefore, the results of the staining using Acridine Orange/Ethidium Bromide (AO/EB) revealed that resulted treatment nearly twice as many apoptotic cells as the control group. Similarly, indole alkaloid aaptamine treatment activated apoptotic responses in HepG2 human liver carcinoma cells, indicating the need of indole drugs to combat this disease.[26] Additionally, DNA damage analysis using the comet test and fragmentation verified the apoptotic cell death process. DNA strand breaks and oxidative damage are also caused by excessive ROS production. As a result of our successful comet assay, it was found that reserpine induced higher levels of ROS in drug resistant cancerous KB-ChR-8-5 cells, which led to higher DNA damage. Consistent with our findings Ding *et al.*, 2016 observed that Alantolactone overload ROS contribute to cell death and dysfunction in colorectal cancer.[27] Our study showed that reserpine causes structural damage to the DNA double helical structure in treated cells by increasing DNA oxidative damage compared with untreated cells, as indicated the formation of DNA breaks. We observed similar results in previous studies where various anticancer agents generated high levels of intracellular ROS, which induced programmed cell death in cancer cells.[28-30] Thus, reserpine induces cell death as a result of its pro-oxidant stimulate properties.

In a protein expression study, reserpine led to a significant reduction in Bcl-2 expression and an increase expression of Bax, thus favouring apoptosis. Reserpine lessened the MMP, causing the release of cytochrome C from the mitochondria into the cytoplasm. As a result, reserpine upregulated cytosolic cytochrome C stimulates caspase 9 as well as caspase 3, which triggered DNA damage. Therefore, in drug resistance cancer cells, the natural product reserpine inhibits the transcription factor cascade that causes apoptosis. Notably, reserpine has previously been demonstrated to induce apoptosis in leukemia cells with multidrug resistance, such as CEM/ADR5000 cells.[31] It appears that reserpine increases intracellular ROS, leading to increase the expression of apoptosis, however its exact mechanism remains unclear.

A majority of proliferative and angiogenic cytokines have been shown to be activated when the transcription factors are overexpressed. Researchers have reported that overexpression of NF-κB and STAT3 can enhance tumor growth by translocating into the nucleus and binding to certain DNA sequences.[32] This makes NF-κB and STAT3 one of the most prominent causes of cancer and an important target for cancer treatment with therapeutic implications. The inhibition of transcription factors increases in mitochondrial ROS production, which eventually results in apoptotic cell death. We observed that reserpine treatment inhibits NF-κB and STAT3 expression downregulating tumor progression and induce ROS production may promote the apoptosis. Our findings align with the observations reported by Shen *et al.*, 2019 who documented that betulinic acid induced ROS mediate apoptotic cell death in human multiple myeloma cancer cells via the inhibition of NF-κB signaling.[33] In addition, the MCF-7 breast cancer cells were found to be inhibited by Brevilin A, which resulted in the induction of ROS-dependent apoptosis via the suppression of STAT3 signaling.[34] Based on our analysis, it is evident that reserpine impedes tumorigenesis and triggers apoptosis by inhibiting the NF-κB and STAT3 pathways.

## CONCLUSION

Overall, reserpine inhibited NF-κB and STAT3 nucleus translocation, which decreased proliferation and increased Bax mediated intracellular apoptosis in human multidrug resistant cancer cells. The findings of this investigation illustrated the promise of reserpine as a potential cancer therapeutic agent and also demonstrated that the suppression of the NF-κB and STAT3 signaling pathways is a vital mechanism that assists the activation of apoptosis.

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## FUNDING

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**STAT3:** Signal transducers and activators of transcription 3; **NF-κB:** Nuclear factor kappa B; **DMEM:** Dulbecco's Modified Eagle Medium; **ROS:** Reactive oxygen species; **FBS:** Fetal Bovine Serum; **$CO_2$:** Carbon dioxide; **AO/EtBr:** Acridine orange/Ethidium bromide; **MMP:** Mitochondrial Membrane Potential; **PBS:** Phosphate buffer saline; **IκBα:** Inhibitor of nuclear factor kappa B.

## SUMMARY

The study explores the plant-derived natural product reserpine potential, as a cancer therapeutic agent, targeting NF-κB and STAT3. Reserpine treatment generates excessive Reactive Oxygen Species (ROS) in cancerous KB-ChR-8-5 cells, can inhibit growth and induce apoptosis due to mitochondrial dysfunction. The acridine orange/ethidium bromide staining and comet test to assess apoptosis and DNA damage in reserpine treatment, revealing increased apoptotic cells and DNA damage. Reserpine suppress anti-apoptotic Bcl-2 protein expression causes increasing Bax expression, mitochondrial membrane potential decline, cytochrome C release, caspase 9 and 3, DNA damage and apoptotic cell death. The study investigates reserpine's effect on NF-κB and STAT3 transcription factors, suggesting that inhibiting these pathways is a key mechanism for tumor suppression and apoptosis, paving the way for future clinical applications.

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