RESEARCH PAPER

Synergistic anti-prostate cancer activity of Withaferin-A and Guggulsterone-Z from Ashwagandha and Guggul through inhibition of the NIK/NF-κB signalling pathway

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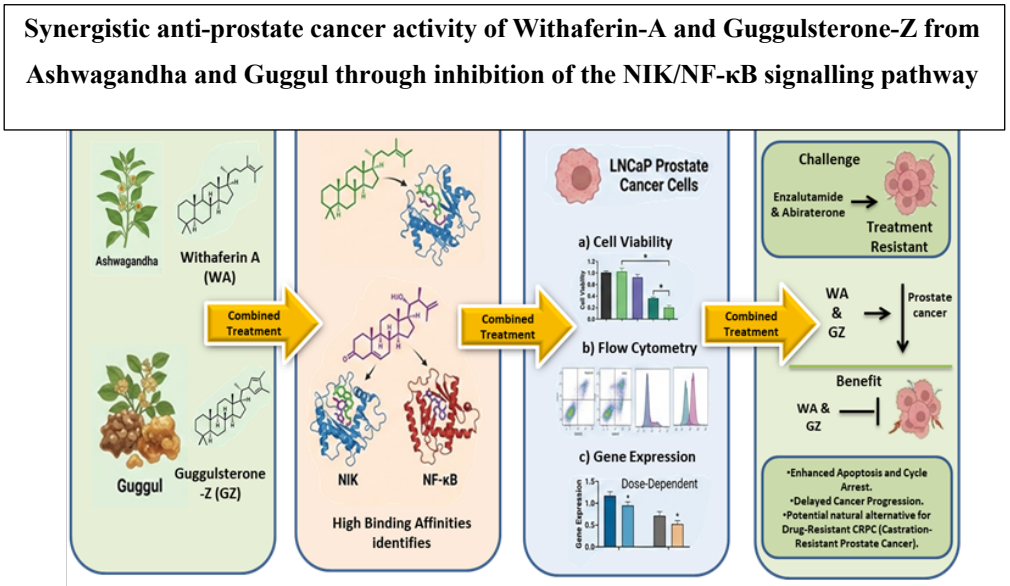
Graphical abstract



Highlights

- Withaferin-A (WA) and Guggulsterone-Z (GZ) target NIK/NF-κB signalling in prostate cancer
- Molecular docking reveals strong binding with NF-κB and NIK proteins
- Combination treatment shows synergistic cytotoxicity in LNCaP cells
- Flow cytometry confirms apoptosis induction by phytochemical combination
- Study validates traditional medicinal relevance of Ashwagandha and Guggul

Abstract

Traditional medicinal plants are valuable sources of bioactive compounds with potential therapeutic effects against chronic diseases including cancer. Ashwagandha (*Withania somnifera*) and Guggul (*Commiphora mukul*) are widely used in Ayurvedic medicine for treating inflammatory disorders and tumor-related conditions.

Aim of the study: The present study aimed to evaluate the synergistic anti-prostate cancer potential of the phytochemicals Withaferin-A and Guggulsterone-Z main bioactive compounds of Ashwagandha and Guggul, respectively using integrated computational and experimental approaches targeting the NIK/NF-κB signalling pathway.

Materials and methods: Molecular docking studies were conducted using AutoDock Vina to evaluate binding interactions of Withaferin-A and Guggulsterone-Z with prostate cancer targets including NF-κB and NF-κB-inducing kinase (NIK). Pharmacokinetic and toxicity profiles were predicted using SwissADME and ProTox-II. In-vitro

anticancer activity was evaluated in LNCaP prostate cancer cells using MTT assays. Apoptosis was assessed using Annexin V-FITC/PI flow cytometry, while gene expression changes were analyzed using conventional PCR.
Results: Docking results revealed strong binding affinities of Withaferin-A and Guggulsterone-Z toward NF-κB and NIK proteins, suggesting potential inhibition of inflammatory signalling pathways associated with tumor progression. *In-vitro* studies demonstrated dose-dependent inhibition of LNCaP cell proliferation. Combination treatment showed significantly higher cytotoxicity compared with individual treatments. Flow cytometry analysis confirmed enhanced apoptosis induction following combination therapy. Gene expression analysis further indicated modulation of apoptosis-related genes and androgen-associated signalling pathways.
Conclusion: The findings provide scientific validation for the traditional medicinal use of Ashwagandha and Guggul and suggest that their bioactive phytoconstituents may serve as promising multi-target therapeutic candidates for prostate cancer treatment.
Keywords- *Prostate cancer, Ashwagandha, Guggul, Molecular docking, Withaferin-A, Guggulsterone-Z, NF-κB signalling*
How to cite this article: Bhushan V, Kumar S, Gahlawat L, Sharma P, Singh B, Sindhu RK, Kashyap S. Synergistic Anti-Prostate Cancer Activity of Withaferin-A and Guggulsterone-Z From Ashwagandha and Guggul Through Inhibition of the NIK/NF-κB Signalling Pathway. Int J Drug Deliv Technol. 2026;16(8s): 1055-1065; DOI: 10.25258/ijddt.16.8s.118

1. Introduction

Prostate cancer is one of the most commonly diagnosed malignancies among men worldwide and remains a leading cause of cancer-related mortality (Shishodia et al., 2008). The disease is caused by dysregulated androgen receptor signalling, chronic inflammation, oxidative stress as well as changes in genetic mutations that collectively promote uncontrolled proliferation, tumor growth and therapeutic resistance (Gottesman, M. M. 2002). Although several treatment strategies such as hormone therapy, chemotherapy and radiotherapy have significantly increased the development of treatment resistance and associated adverse effects continue to limit long-term therapeutic success (Gottesman, M. M. 2002). These restrictions highlight the importance of alternative, more effective, and safer therapeutic approaches (Newman, D. J., & Cragg, G. M. 2020).

Phytoconstituents of medicinal plants had been an essential source of therapeutic agent especially in oncology treatment (Newman and Cragg, 2020; Cragg and Newman, 2005). A very high percentage of medicines that have been used in the treatment of cancer are the ones that were directly obtained in plants or were constructed based on the plant-based lead compounds (Newman and Cragg, 2020). These illustrations point to the fact that the research of bioactive substances of vegetable origin remains relevant in the search and development of new anticancer agents (Newman and Cragg, 2016; Atanasov et al., 2021).

Traditional medicine, including Ayurveda, is a great repository of information related to the medicinal use of plants, and it has made a lot of contributions to the current pharmacological agents (Pan et al., 2013; Petrovska, 2012). Ashwagandha (Withania somnifera) is one such widely known medicinal plant with a wide range of pharmacological effects such as adaptogenic, anti-inflammatory, antioxidant, immunomodulatory and anticancer (Mirjalili et al., 2009; Mishra et al., 2000). Withania somnifera has biological activities, which are largely due to a series of naturally occurring steroidal lactones referred to as withanolides (Mirjalili et al., 2009; Vanden Berghe et al., 2012). The most studied of these compounds is WA which has been reported to have important anticancer activity by modulating several cellular processes such as the induction of apoptosis, regulation of oxidative stress and the inhibition of inflammatory signalling pathways (Vanden Berghe et al., 2012; Dutta et al., 2019; Grover et al., 2012).
Guggul (Commiphora mukul), which is a resin-yielding plant is another significant Ayurvedic medicine used to treat inflammatory conditions, metabolic ailments, obesity, and arthritis (Sinal and Gonzalez, 2002; Shishodia and Aggarwal, 2004). GZ and Guggulsterone-E are the major bioactive constituents of Guggul that have been realized as being the main pharmacologically pertinent compounds of the resin (Urizar et al., 2002; Shishodia and Aggarwal, 2004). These phytochemicals were reported to have anti-inflammatory, anti-oxidant and anti-cancer effects upon regulation of various multiple molecular signalling pathways by which cell proliferation, apoptosis and survival are regulated (Shishodia and Aggarwal, 2004; Singh et al., 2007).

NF-κB signalling pathway is an important pathway in the development of cancer as it controls the process of inflammation, cell survival, proliferation and tumor development (Karin, 2006; Gupta et al., 2010). NF-κB (aberrant) activation of signalling has been closely linked to prostate cancer progression and resistance to therapy thus the advances that NF-κB signalling can be used as an anticancer treatment molecular target (Lessard et al., 2006; Jain et al., 2012). Due to the great biological potential of plant-derived compounds, and the key role of the NF-κB pathway in prostate cancer, it is possible that natural molecules with certain capabilities to target the signalling cascade would be desirable as potential agents of therapeutic activity (Newman and Cragg, 2020).

Hence, the current paper was intended to examine how WA and GZ anticancer active compound may be used to treat prostate cancer through an integrated approach that involves employing computational molecular docking to assess the compounds with the subsequent experimental validation. The experiment particularly determines the activity of these phytochemicals with main molecular target of the NIK/ NF-κB signalling pathway and their impact on prostate cancer cell viability and apoptosis.

2. Materials and Methods

2.1 In Silico

2.1.1 Selection of Phytoconstituents

The bioactive compounds of Ashwagandha (Withaferin-A) and Guggul (Guggulsterone-Z) were chosen in accordance with the earlier reported anticancer and anti-inflammatory potential (Brauchle et al., 2014). PubChem retrieved reported structures and optimized them to be used in computational analyses based on standard energy minimization protocols. (Newman, D. J., & Cragg, G. M. 2020).

2.1.2 Target Identification and Protein Preparation

Androgen receptor (AR), NF-κB and NIK have been chosen as prostate cancer molecular targets of central interest in tumour proliferation, survival, and inflammation (Shishodia et al., 2008; Gottesman, M. M. 2002). The structures of proteins for NF-κB (PDB ID: 3GUT) and NIK (PDB ID: 4G3D)] were retrieved in the Protein Data Bank (PDB), this was purified by removing water molecules, adding hydrogen atoms and optimizing side chains using the standard protocols (Newman, D. J., & Cragg, G. M. 2020). Molecular Docking AutoDock Vina was used to perform molecular docking with the preparation of receptor, ligand preparations and conduct simulations (Zayed, 2025; Naeem, 2024). Withania somnifera and Commiphora wightii produced 2 major phytochemicals, Withaferin-A and Guggulsterone-Z (Figure 1(a) &(b), respectively), were docked into prostate cancer-related targets NF-Kb: 3GUT and NIK: 4G3D. Non-essential heteroatoms were removed and the structure of receptors minimized to optimize and minimize the energy to precisely predict the pose. PDBQT files have been prepared with ligands and receptors and subsequently Vina macros were run to determine the most favorable binding poses. The docking results were analyzed in terms of binding affinity, hydrogen bonding interactions, and active-site residues.

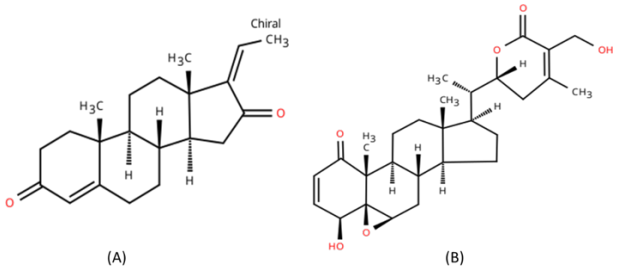


Figure 1(a–b) represents the optimized steroidal frameworks of GZ (GZ) and WA (WA), which served as starting conformers for all docking simulations.

2.1.4 ADME and toxicity prediction

Pharmacokinetic properties including absorption, distribution, metabolism, and excretion were predicted using the SwissADME web server (https://www.swissadme.ch/). Toxicity predictions were performed using ProTox-II (Newman, D. J., & Cragg, G. M. 2020).

2.2 Cell culturing

LNCaP prostate cancer cells were procured from NCCS, Pune**,** and maintained in RPMI-1640 high-glucose medium supplemented with 10% FBS and 1% antibiotic solution. Experimental groups included**:** Control, Positive Control (Sodium Salicylate (10Mm/L)), Negative Control DMSO 0.1%, GZ (12.5µmol/L), WA (12.5µmol/L), Low-dose GZ+WA (12.5µmol/L), and High-dose GZ+WA (25µmol/L),. Cells were incubated at 37°C, 5% CO_2 in a humidified incubator (Heal Force HF-90). Prepared treatment solutions (5–50 µL) were added to each well, followed by a 24-hour incubation before downstream assays.

2.2.1 Gene Expression Analysis

2.2.1.1 RNA Isolation

Total RNA was extracted from treated and control LNCaP cells using TRIzol reagent (Thermo Scientific) following manufacturer protocols. RNA purity was verified via 1.5% agarose gel electrophoresis**.**

2.2.1.2 cDNA Synthesis

cDNA was generated using the PrimeScript™ First-Strand cDNA Synthesis Kit, with 2 µg RNA, random hexamers, and oligo-dT primers. Reaction conditions:

Table 1*: RT-PCR conditions.*

Stage	Temp (°C)	Time	Cycles
cDNA synthesis	42°C	60 min	1
Enzyme inactivation	95°C	5 min	1

2.2.1.3 PCR Amplification

Target genes were amplified using a Bio-Rad T100 Thermocycler (USA) under optimized cycling conditions.

2.2.2 Apoptosis Analysis via Flow Cytometry

The Cell lines had been retrieved from NCCS Pune were cultivated as well as treated with the sample dose specified in the excel sheet. After treatment, cytological specimens were sequential lavage bipartite with frigid-equilibrated saline-electrolyte matrix along titrated frequency of A mega-unit magnitude entities per milliliter of the aqueous substrate. Biological substrates remained separated into 5 groups: untreated, control, annexin only, PI only, as well as treated. Annexin V FITC (Elabscience- E-CK-A111) and PI were added to their respective labelled tubes. Subsequent to a quarter-hour duration of vortexing and temporal exposure at ambient thermal magnitude under non-elevated caloric conditions, each tube received 1X Binding buffer along with this it was analysed within 1 hour using a BD FACS LyricTM flow cytometer. Results were analysed using FCS Analyser software (Widodo, N et al, 2010).

Cytotoxicity assay (MTT)

Culturing of human prostate cancer cell lines was done in accordance to standard protocols (Widodo, N et al., 2010; Shishodia, S., et al., 2003). WA, GZ, and their combinations were evaluated using the MTT test using cytotoxic effects. The cells in LNCaP (10,000 cells/well) were placed in 96-well plates and subjected to a given concentration of the treatment after 24 hours. Controls, positive control: sodium salicylate 10 mM and negative control: DMSO 0.1% were included following incubation cells were incubated with different concentrations of phytoconstituents of interest, and cytotoxicity was measured with MTT assay. Divided into the following groups - Control, Positive Control, Negative Control, GZ, WA, GZ+WA (Low Concentration), and GZ+WA (High Concentration) groups. At 90% cell confluency, the flasks were also incubated with RPMI-1640 h.g. (high glucose) medium (Roswell Park Memorial Institute - AT162- 10X1L-HIMEDIA) with 10% FBS (Foetal Bovine Serum - HIMEDIA-RM 10432) and 1% antibiotic solution at 37° C with 5% CO_2.

When removing the media, place fresh culture medium to every well of the plate. Prepared treatment volumetric attenuation to 5 and 50 µl (10% of total medium) of prepared treatment in spatial delimitation and incubate 24 hrs pneumatically-insulated hypercapnic gaseous medium thermostatic proliferation-vessel Heal Force - HF90. It is a cytoplasmic cytochrome culture that is therefore prepared to proceed with the next phase of experiments. The 5 mg/mL MTT reagent was added during 2 hours. Formazan crystals were dried by placing 100 µL of DMSO and the absorbance of the solution measured at 540 nm with a Bio-Rad iMark ELISA reader.

The results were obtained on the basis of calculating IC_{50} in terms of GraphPad Prism 6 and presented as the mean + SEM (Singh, 2025; Vishwakarma, 2025; Mohite, 2025) to define the antiproliferative potency (Widodo, N et al, 2010).

2.5 Apoptosis and Cell Cycle Analysis

Apoptosis (Annexin V/PI staining) and cell-cycle arrest were determined by flow cytometry. The expression of the apoptosis related markers (BAX, BCL-2, caspases) were determined using RT-PCR or Western blotting, according to the earlier established protocols of phytochemical anticancer identification (Widodo, N et al, 2010).

2.6 Statistical Analysis

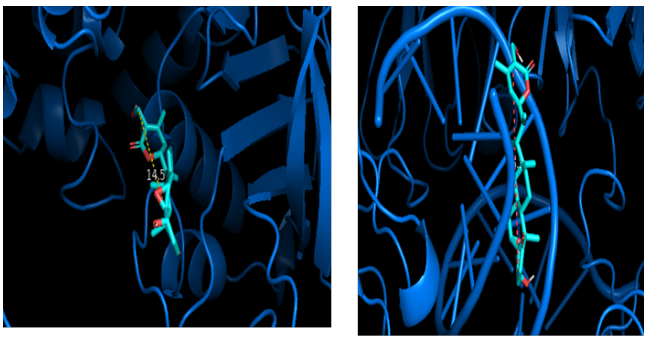
Each experiment was done three times. ANOVA was used to analyze the results with a significance level of $p < 0.05$, which is the standard practice of biological assays (Gottesman, M. M. 2002).

2 RESULTS AND DISCUSSION

3.1 *In-Silico* Molecular Docking Analysis

AutoDock Vina was used with the aid of YASARA in molecular docking of receptors with ligands, which supports receptor–ligand preparation and simulation processes (Zayed, 2025; Naeem, 2024). Two phytochemicals, WA of Withania somnifera and GZ of Commiphora wightii were docked using prostate cancer associated targets NFkB (3GUT) and NIK (4G3D). Cleaning receptor structures was performed through the process of eliminating heteroatoms that were not essential, and optimization and minimization of energy was achieved to be able to predict the correct poses. Ligands and receptors were prepared into PDB files and Vina macros were run to find the most desirable binding conformation. Docking variables were binding energies, hydrogen-bond patterns and interactions at the active site. WA when participating

GZ demonstrates a strong binding affinity integration of WA with NIK as the outcome of NFkB will provide a maximum binding energy as -75 along with -88 kcal/mol and subsequently GZ will be seen to show the maximum binding energy when paired with NIK along with NFkB at the -32 to -185 kcal/mol as shown in the results below in figure 2.



(A) (B)

"Figure 2: 2D-Docked structures of WA and GZ in binding with NFkB & NIK such as 2(a) WA with NIK, (b) WA with NFkB, (c) GZ with NIK, (d) GZ with NFkB.

The group falls under basic phytochemicals, which possess significant docking score for NFkB as well as NIK is depicted in Table 2.

Table 2 - List of phytochemicals with docking score for NFkB and NIK.

Compounds	Docking score value
Withaferin A (WA) +NIK	-7.5
Withaferin A (WA) + NFkB	-8.8
Guggulsterone Z (GZ) + NIK	-7.1
Guggulsterone Z(GZ) + NFkB	-8.2

Simultaneously, the steric and electrostatic complementary were assessed using QSAR-based predictions and it allows identifying viable inhibitory scaffolds acting on NIK-mediated NF-kB pathway. The binding affinity of WA was highest and multiple hydrogen bonds and hydrophobic interactions were demonstrated in the NF- kB inhibitory domain. GZ demonstrated strong binding to the activation loop of NIK, which is in line with its reported capability of controlling inflammatory signalling. These associations were further supported by steric/electrostatic analysis using the QSAR, indicating a high level of favorable complementation and location of pharmacophoric sites in key kinase conformational alterations. These discoveries have a strong indication that WA and GZ are able to disrupt the NIK → NF-KB signalling pathway, which is one of the key induces of prostate cancer growth and survival.

3.2 In-Vitro Cytotoxicity Analysis (MTT Assay)

Cytotoxic tests conducted using LNCaP prostate cancer cell (obtained through the National Centre of Cell science) showed a distinct dose- dependence decrease in cell viability. Following 24-hour exposure: WA demonstrated the strongest cytotoxicity amongst the individual phytoconstituents, GZ demonstrated moderate antiproliferative and in (GZ+WA) combination demonstrated a significantly greater potency of cytotoxicity, which is a survival of synergy as shown in figure 3 Assay sensitivity was confirmed through positive control (10 mM sodium salicylate), and negative control (0.1% DMSO) had no toxicity. The combination therapy in GraphPad Prism made the calculations of IC 5 and found that lower concentrations were needed to produce 50 percent inhibition percentage making the combination therapy better in its therapeutic potential.

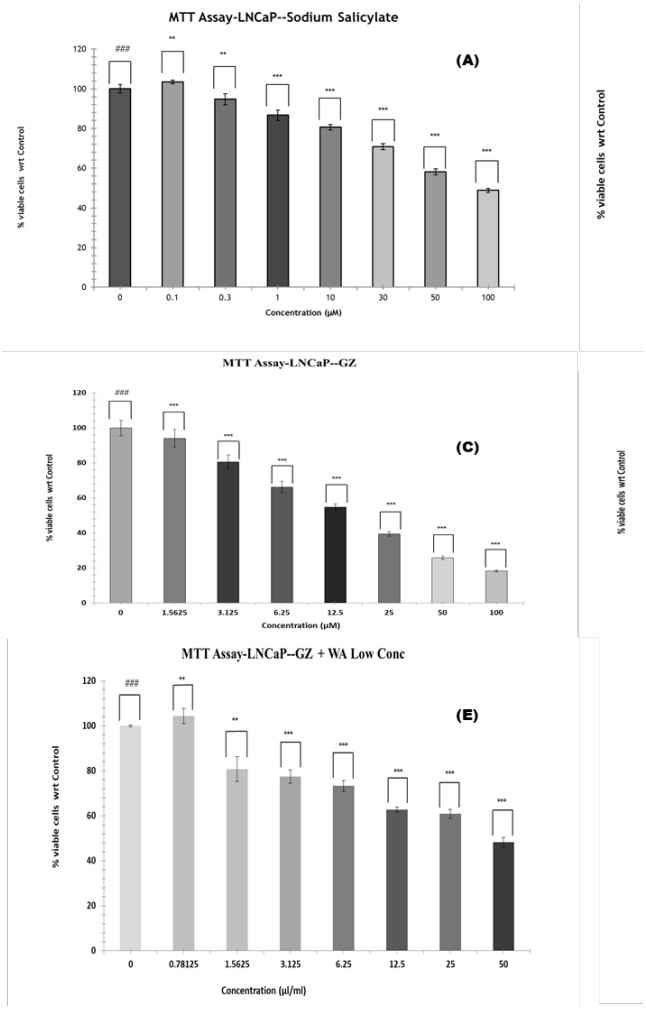


Figure 3: Cytotoxicity assay shown excellent cytotoxic effects, that were observed instantaneously in androgen-sensitive LNCaP prostate cancer cells in response to the test formulations, that included Control, Positive Control, Negative Control, GZ, WA, GZ+WA (Low Concentration) and GZ+WA (High Concentration) groups, Magnitudes are articulated as of independent triplicate iterations± standardized stochastic deviation of the mean (SEM), ****p < 0.001, **p<0.05, ###p < 0.001 vs. control*

Cellular viability on receiving an assessment of the application of MTT assay that showed a high and dose dependent cytotoxic reaction in the androgen sensitive LNCaP prostate cancer cell line immediately following treatment with the test formulations.

The inhibition of the activity of mitochondrial metabolic activity of the cells in particular occurred in all of the analyzed samples for viability and the proliferative capability. It is interesting to note that, in the levels of cytotoxicity we observed that the difference between the treatment and non-treatment groups is significant; hence there is formulation-specific alternative variability in anti-proliferative activity.

3.3 Apoptosis Determination via Flow Cytometry

Flow cytometric analysis which was conducted on the staining of Annexin V FITC and PI revealed that both early and late apoptotic populations significantly increased in both WA-treated and GZ-treated groups. WA induced severe early apoptosis, and GZ induced late apoptosis mostly. It is interesting to note that GZ+WA samples gave the best result in terms of apoptotic index, which was way above individual samples. The higher PI positivity signified the loss of membrane integrity, which is in line with ultimate apoptotic transition. These findings are effectively associated with the projected interaction of phytochemicals with the apoptotic NF-KB. The results in respect to the study performed when the cells were allowed to get exposed to the sample effective dose indicated that treated sample-GZ +WA Low Conc. had 89.4% viable cells, 6.2% cells in incipient apoptotic phase and 3.5% cells in terminally apoptotic phase and 0.96% cells in necrotic phase respectively as compared to 49% viable cells, 49.4% cells in early apoptotic phase, 0.6% cells in terminally apoptotic phase and 0. The sample-GZ +WA High Conc. had 66.4% viable cells, 8.3% cells in incipiently apoptotic phase, 23.5% cells in terminally apoptotic, and 1.8% cells in necrotic stage. (Rehan shaikh, 2024) Sample-GZ demonstrated 82.8 percentage viable cells, 6.3 percentage cell in early apoptotic phase, 10.6 percentage cytological units in terminal apoptotic phase and 0.3 percentages necrotic phase cytological unit.

The sample-WA also had 83.3 percentage viable cells and 10.5 percentage cells in early apoptotic phase and 5.8 percentage cells in terminal apoptotic phase and 0.4 percentage cells in necrotic phase. Sample-Negative Control had 82.3 percentage viable cells, 11.1 percentage cells in early apoptotic phase and 6.3 percentage cytological units in terminal incipiently terminal phase and 0.3 percentage cytological units in passive cytolytic specimens phase. Sample-Sodium Salicylate had a percentage viable of 81.1 and early apoptotic 8.6 percentage of cytological unit in terminal apoptotic and passive cytolytic specimens, and cytological unit in early apoptotic phase respectively was 9.8 and 0.5 respective.

This method will particularly benefit organizations that are relatively new and unable to utilize traditional study techniques to assess their performance.

This approach will be especially useful to organizations, which are comparatively new and cannot employ traditional study methods to evaluate their performance.

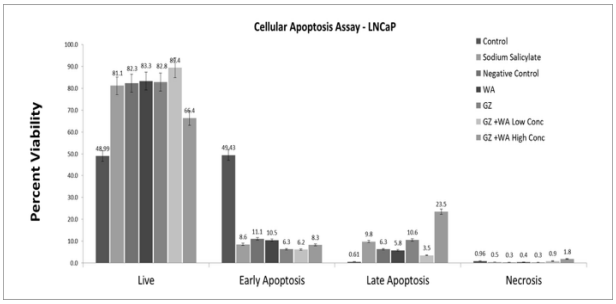


Figure 4- Flow cytometry–based apoptosis profiling reveals concentration-dependent modulation of cell survival and apoptotic signalling by Guggulsterone - Z (GZ), Withaferin - A (WA), and their combinatorial treatment (GZ+WA), as evidenced by altered distributions of viable, early apoptotic, late apoptotic, and necrotic cell populations relative to control groups.

3.4 Gene Expression Profiling (Conventional PCR)

RNA isolated using TRIzol and reverse-transcribed to cDNA displayed clear amplification patterns in downstream PCR analysis. Treatment with WA, GZ, and especially their combination resulted in d**ownregulation of NF-κB–associated pro-survival genes, Altered expression of apoptotic regulatory markers (BAX, BCL-2, caspases), suppression of NIK-linked transcriptional drivers.** These molecular changes strongly support the docking predictions and confirm the mechanism of action at the gene-expression level.

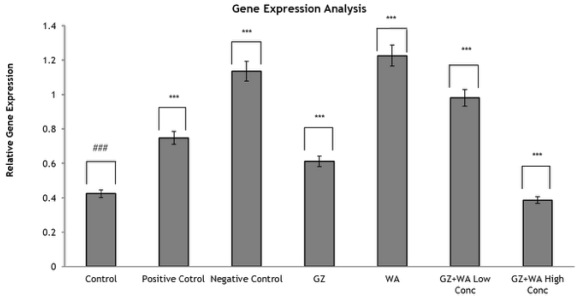


Figure 5- Relative gene expression of *HSD17-B* normalized to GAPDH determined by conventional PCR. Bars represent relative expression levels in Control, Positive Control, Negative Control, GZ, WA, GZ+WA (Low Concentration), and GZ+WA (High Concentration) groups. Expression was lowest within the non-interventional reference cohort as well as markedly increased in the WA-exposed experimental cohort, while high-concentration GZ+WA showed pronounced down-regulation. Magnitudes are articulated as of independent triplicate iterations± standardized stochastic deviation of the mean (SEM), ***$p < 0.001$, ###$p < 0.001$ *vs. control*

Quantitative assessment of HSD17-B transcript levels by conventional PCR normalized against the GAPDH housekeeping gene, demonstrated pronounced treatment-dependent transcriptional modulation (Figure 5). Baseline HSD17-B expression was minimal in the untreated control group, reflecting low endogenous gene activity. WA exposure elicited a significant and robust transcriptional induction of HSD17-B, yielding the highest relative expression among all experimental conditions (***P < 0.001). In contrast, GZ treatment alone resulted in only a modest elevation in transcript abundance, while positive and negative control groups exhibited intermediate expression profiles. (Souza, 2024) The combinatorial regimen of GZ and WA at low concentration produced a moderate up- regulation of HSD17-B, whereas high-concentration co-treatment caused a marked transcriptional repression, indicating a concentration-dependent antagonistic regulatory effect. Collectively, these data suggest that HSD17-B expression is highly sensitive to WA-mediated signalling and is differentially regulated by GZ–WA combinatorial dosing. Data represent mean ± SEM from three independent biological replicates.

In overall discussion, the integration of computational and biological experiments provides a powerful validation framework for assessing Ashwagandha- and Guggul-derived phytoconstituents. The converging evidence—from docking, QSAR, cell viability, flow cytometry, and PCR—demonstrates that WA and GZ directly target key survival pathways by binding to NIK and NF-κB indicates strong potential to inhibit cancer-driving inflammatory signalling. Experimental assays confirm predicted biological effects to reduced viability, enhanced apoptosis, and suppressed pro-survival gene expression all align with the proposed molecular mechanism. Combination therapy produces

synergistic effects showed dual treatment consistently outperformed individual phytoconstituents, suggesting therapeutic complementarity. NF-κB is a promising therapeutic target for the prostate cancer treatment. Targeting this pathway via natural steroidal scaffolds may offer a safer and effective alternative to conventional chemotherapeutics.

4. Discussion

The current research combines both the computational and experimental methods to assess anticancer property of WA and GZ obtained as Ayurvedic plant traditional medicine.

Molecular docking study indicated that WA and GZ showed high binding affinities to crucial signalling proteins involved in the development of prostate cancer especially NF- k B and NF- k B-inducing kinase (NIK). These proteins have very important roles in the regulation of inflammatory signalling, cell growth and apoptosis. NIK/NF- kB pathway inhibition has been largely identified as an effective approach towards tumour growth and metastasis suppression. The binding interactions which are observed indicate that both phytoconstituents might disrupt the activation of this pathway and hence the downstream pro-survival signalling pathways. The cytotoxicity findings of the in-vitro test (MTT assay) revealed a dose effect of a decrease in the viability of LNCaP prostate cancer cells after the use of WA and GZ. Interestingly, the combinatorial treatment had been found to have much greater cytotoxic effects than that of the individual compounds, thus there could be a synergistic interaction between the two phytochemicals. This type of synergistic action is especially beneficial in the field of phytochemical-based therapies, where one can easily afford to use lower concentrations.

Flow cytometric analysis went a step further to establish the decline of cell viability was linked to an increase in the number of apoptotic cells. Both early and late apoptotic cells were increased in combination treatment than in the single-compound treatment. These results indicate that the combination of the phytochemicals induces programmed cell death in prostate cancer cells. Mechanism Gene expression analysis offered more mechanistic information. The phytochemicals treatment led to the regulation of genes related to apoptosis, androgen-associated, and changes in the levels of BAX, BCL-2 and HSD17-B. Applett (2011) found that the down-regulation of the pro-survival genes along with the activation of the apoptotic pathways to go hand-in-hand with the suggested mechanism of action as it was predicted by docking analysis.

The overall findings indicate that computational predictions are well correlated with the experimental validation. The synergizing phytochemicals has the capability to work on molecular processes leading to inflammation-mediated carcinogenesis, specifically the operation of the NIK/NF- kB signal cascade. The findings reveal that Ashwagandha and Guggul have scientific medicinal relevance and demonstrate potential therapeutic value of the phytoconstituents as multi-targeted therapies to treat prostate cancer. Nevertheless, more research such as in-vivo and pharmacokinetic studies, as well as clinical studies, are needed to establish their potential in translation.

4 Conclusion

The study conclusively demonstrates that WA and GZ exhibit potent anti-prostate-cancer activity through multi-targeted suppression of the NIK/NF-κB signalling cascade. In-silico ligand-receptor interrogation confirmed superior binding to targets, while MTT assays revealed robust cytotoxicity and synergy in LNCaP cells. Flow cytometry evidenced apoptosis (with G0/G1 arrest, corroborated by RT-PCR showing downregulation of *HSD17-B* expression. The strong correlation between in-silico predictions and in-vitro outcomes supports further exploration through advanced proteomic, mechanistic, and preclinical studies. These phytoconstituents hold significant promise as plant-derived, multi-targeted therapeutic candidates for prostate cancer management.

Funding declaration

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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