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Purified solasodine from *Solanum mauritianum* Scop. and its molecular mechanism of antimetastatic potential

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ABSTRACT

Breast cancer has been identified as one of the most common type of cancer among female human population. General treatment approaches against breast cancer includes radio, chemo or hormonal therapy. Unfortunately, most of these approaches ameliorate the target and evoke adverse side effects. Phytochemicals including alkaloids are reported to alleviate ailments with no or little side effects. Bug weed (*Solanum mauritianum* Scop.), an evergreen medium sized tree of Solanaceae, a native of South America was used for treating different pain disorders. Glycoalkaloid solasodine was isolated and purified from *S. mauritianum* and was identified by ¹H NMR. Cytotoxic evaluation by MTT assay against MCF-7 breast cell lines revealed significant inhibition. In order to confirm the antimetastatic property and to elucidate the mode of action, molecular targets and signalling pathways, transmission electron microscopy and western blot analysis of the cell cycle regulatory proteins such as p53, p21, cyclin D1, cyclin E and molecules involved in apoptosis (PARP, procaspase 7, 8 and 9) and Bcl-2 family members (Bcl-2, Bcl-xL, Bax and Bak) were analysed. Solasodine treated MCF-7 cells showed varying degrees of cellular damage. Early apoptotic cells have degraded DNA with cell blebbing. Late apoptotic and necrotic cells showed fragmented DNA with reddish and green staining. Solasodine exhibited an upregulation of p53 and p21 proteins. Meanwhile, a decrease in the expression levels was noticed with cyclin D1 and E within 12 h of treatment. Procaspase-7, 8 and 9 showed reduced expression on solasodine application, suggesting the alkaloid has a triggering effect on the induction of both intrinsic and extrinsic apoptotic pathways. Interestingly, procaspase-9 was up regulated after 48 h following an initial down regulation. The results proposes that the activation of caspase-9 begins earlier compared to caspase-7 and 8. Further, solasodine elevated the expression of cleaved PARP moderately (24 h). In addition, solasodine treated MCF-7 cells showed significant decline in the expression of Bcl-2 and Bcl-xL (anti-apoptotic protein) after 48 h, with an increase in Bax and Bak (pro- apoptotic protein) expression. Therefore it can be interpreted that the solasodine inhibited the cell cycle at G2/M and the anti-apoptotic signals and promoted pro-apoptotic signals, thereby the mitochondrial permeability.

Keywords: Solasodine, MCF-7, Caspases, Cyclin, Apoptosis, MTT, TEM.

INTRODUCTION

Cancer a chronic disorder characterised by uncontrolled cell proliferation due to derailment of cell regulatory signaling pathways. Solowey *et al.*, reported that 8.2 million cancer deaths and 14.1 million new cancer cases from worldwide^[1]. Further, this is expected to go higher by 2030, with 26 million cancer cases and 17 million cancer deaths per year.

Major types of cancer reported were gastric cancer, pancreatic cancer, breast cancer and prostate cancer. Over the past few decades, there was a continuous and drastic increase in breast carcinoma (HCC) worldwide. Common treatments include radio, chemo or hormonal therapy. However, these methods evoke adverse side effects among patients. In addition, resistance gained by cancer cells against chemotherapy has posed a serious huddle. In this juncture, the need for designing novel drugs that function effectively with no or minimum side effects.

Herbals are natural store house of phytochemicals with immense therapeutic quality. Plant derived chemicals coupled with chemotherapy has gained much importance nowadays in alleviating the proliferation of various carcinomas with less side effects^[2]. The MTT *in vitro* cell proliferation assay is the most widely used assays for evaluating preliminary anticancer activity and gives an indication of cell cytotoxicity.

It is well established that p53, a tumour suppressor gene plays vital role in inducing the cell cycle arrest. Induction of p53 by the phytochemicals is a proven regulatory process associated with G1/G0, S, G2/M phase arrest in malignant cell lines^[3]. Hence, p53, p21Waf1/Kip1 (downstream transcriptional target

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gene of p53 and cyclin-dependent kinase inhibitor) and cyclin D1 and E the other cell cycle regulating proteins at the G1/S/G2 checkpoints were analyzed for confirming the antimetastatic potential.

Caspases belongs to endoprotease family which plays crucial role in cell regulatory networks and apoptosis. Caspases are synthesized in inactive procaspases monomer forms that require either di-merization or cleavage for activation. Initiator caspases (8 and 9) induce executioner caspases (3, 6 and 7) that subsequently coordinate events like destruction of key structural proteins and activation of apoptotic enzymes. The extrinsic apoptosis pathway is induced through binding of a ligand molecule to a death receptor, which further joins with adapter proteins like FADD/ TRADD that dimerizes and activate caspase-8. Activated caspase-8 initiates apoptosis directly through cleaving executioner caspase such as 3, 6, 7 or induces the intrinsic apoptotic (mitochondrial) mechanism via cleavage of BID to induce absolute cell death. The intrinsic apoptosis can be triggered via diverse cellular stresses that leads to the release of cytochrome c from the mitochondria to form apoptosome (consists of APAF1, cytochrome c, ATP + caspase -9). The chain of reactions leads to the activation of caspase-9 which further cleaves and trigger executioner caspases and thereby completing the apoptosis [4].

Bug weed (*Solanum mauritianum* Scop.) is an evergreen medium sized tree species of Solanaceae. It is a native to South America. During famine periods its ripe fruits were cooked and consumed by the local people. Similarly the leaf and fruit decoctions were used for curing different pain ailments [5]. These medical claims are not backed up by scientific data. In this junction, the present part of the study was carried to determine anticancer effects of purified alkaloid solasodine against MCF-7 breast cancer cell lines and to unravel its plausible mode of action.

MATERIALS AND METHODS

Plant material

Solanum mauritianum Scop. leaf samples for the present study were obtained from the wild habitat of Munnar hills, Idukki, Kerala. Confirmation of species identity was carried out by referring the herbarium at Jawaharlal Nehru Tropical Botanical Garden and Research Institute, (JNTBGRI) Palode, Trivandrum. The herbarium of the species was deposited in the department herbarium (UCT 1279).

Isolation and purification of solasodine

50 g of shade dried and powdered leaf samples of *S. mauritianum* were extracted by chloroform using soxhlet method. Lyophilised chloroform fraction was purified by using silica column (1 g) with a mesh size 60-120 for isolating the pure compound. Ethyl acetate (100 %) yielded yellowish brown fraction. This fraction was further reloaded at the top of freshly packed column for further purification. Chloroform and ethyl acetate (3:2) mobile phase yielded highly purified fraction. This fraction was subjected to acid hydrolysis as per the protocol of Manosroi *et al.*, to obtain crystalline solasodine [6]. Using ¹HNMR absorptions peaks, the identity and structure of the compound was confirmed as solasodine.

Malignant cell lines

Solasodine from *S. mauritianum* was screened against six major malignant cell lines such as human liver cancer cell line HLF

(ATCC), human colon adenocarcinoma cell line SW480, human ovarian carcinoma cell line NIH-OVCAR3, human lung cancer cell line PC-9 and two human breast cancerous cell lines, MCF-7 and MDAMB-231 for antimetastatic potentialities. Doxorubicin drug was used as positive control.

Culture medium

The selected malignant cell lines were grown in RPMI 1640 (Invitrogen) containing 10% fetal bovine serum (Invitrogen) with 50 U/mL of penicillin (Invitrogen) and 50 µg/mL of streptomycin (Invitrogen). Meanwhile, MCF-7 and MDAMB-231 were grown in DMEM (Invitrogen) supplemented with 50 U/mL of penicillin (Invitrogen) and 100 µg/mL of streptomycin (Invitrogen). All cells were maintained in a humidified incubator at 37°C and 5% CO₂.

MTT assay

Cytotoxic activity of solasodine was determined by MTT assay. Using different concentrations such as 5, 10, 25, 50 and 100 µg/mL incubated for 24, 48 and 72 h followed by the addition of 20 µL MTT (3-(4, 5-dimethylthiazol-yl)-2, 5-diphenyltetrazolium bromide) stock solution (5 mg/mL in PBS) and incubation for 5 h. The absorbance was recorded at 560 nm using a microplate reader. The percentage cytotoxicity was calculated with respect to control using the following formula

$$\% \text{ cytotoxicity} = \left\{ \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \right\} \times 100.$$

Transmission electron microscopy (TEM)

MCF-7 cells were cocultured with solasodine and incubated for 24, 48 and 72 h at 34°C for electron microscope analysis following the protocol of [7]. Resin blocks were cut using ultramicrotome into ultrathin sections and were placed into a grid and stained with uranyl acetate for 10 min followed by 50% acetone and finally stained using lead which was then washed thrice with distilled water. The stained samples were then viewed under TEM.

Western blot analysis of p53, p21, cyclin D1, cyclin E, PARP, procaspase 7, 8 and 9 and Bcl-2

Cells were seeded in six-well plates at 2×10^5 cells per well and followed by overnight incubation before treating with solasodine at the indicated concentrations and time intervals. Proteins from MCF-7 and treated cells were extracted with ice-cold cell lysis buffer (Cell signalling, Beverly, MA, USA) containing protease inhibitor cocktail. Protein lysates were centrifuged at 13,300 rpm for 5 min at 4°C to remove insoluble material. Protein concentration in the supernatants was determined by the Bradford protein assay kit according to the manufacturer's instructions. A microplate reader (TECAN Infinite M200, Mannedorf, Switzerland) was used to measure the absorbance at 595 nm and the concentration of the protein was calculated based on a bovine serum albumin (BSA) standard curve with a range of 0 to 1 mg/mL. Protein lysates were boiled in Laemmli sample buffer (1:1 dilution) (Bio-rad Laboratories, Hercules, CA, USA) at 100°C for 5 min and resolved by electrophoresis on 10% or 12% SDS polyacrylamide gels. After gel electrophoresis, the proteins were electrotransferred to a nitrocellulose membrane (0.45 µm) using Bio-Rad Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell at 15 V for 28 min. Subsequently, the membranes were then blocked with 5%

milk in TBS-Tween 20 for 30 min at 70 rpm, room temperature, followed by washing for 3x bufer, 10 min each at 110 rpm. Thereafter, the membranes were probed with corresponding primary antibodies (1:1000 dilution; e.g. PARP, p53, Bcl-2, Bcl-xL, Bax, Bak, p21, caspase-7, caspase-9, ER-a, p-Akt (Thr308), p-GSK3b (Tyr216) and total GSK3b) cyclin D1, E, beta actin) for proteins of interest overnight at 4°C, 70 rpm. After overnight incubation, the membranes were washed 3x buffer, 10 min each at 110 rpm before probing with corresponding secondary antibodies (chicken anti-rabbit or anti-mouse IgG conjugated with horse-radish peroxidase) diluted 1:10000 in blocking buffer (Santa Cruz, CA, USA) for 1 h at room temperature and followed by washing, 3x buffer, 10 min each. The immunoblots were examined with the enhanced chemiluminescence kit. β -actin was used in each blot as a loading control to ensure equal loading of proteins for each sample. Protein quantification was performed using ImageJ software (Java-based image processing program developed by National Institutes of Health).

Statistical analysis

Each data point is the mean of three replicates obtained from 6 independent experiments. The data presented as mean value \pm SD. All experimental data were analyzed by an analysis of variance (ANOVA). After confirming the significance of F values, the significance of the differences between the mean values was tested using ANOVA. Significant differences were considered at $P < 0.01$ or $P < 0.05$ probability levels. All analysis were carried out employing the program SPSS 17.0 for windows.

RESULTS

Solasodine alkaloid fraction was extracted, purified by column chromatography from the leaves of *S. mauritianum* and the structure was confirmed by ^1H NMR analysis. The *in vitro* cytotoxicity tests using MTT assay revealed that solasodine exhibits remarkable cytotoxic potential against MCF-7 cells than other malignant cell lines in a concentration and time dependent manner (Table 1). Remarkable IC_{50} value was also noticed.

Table 1: Anti proliferative potentiality of solasodine from *S.mauritianum* in terms of viability (%) against MCF 7 cells using different concentrations and durations

Concentration (μg)	24 h	48 h	72 h
Control	100 \pm 1.9	100 \pm 3.3	98.9 \pm 2
5	88.6 \pm 6.3	71.2 \pm 2.6	64.3 \pm 3.3
10	80.1 \pm 2.5	67 \pm 1.8	59.1 \pm 4.8
25	74.4 \pm 4.1	59.2 \pm 2.7	50.2 \pm 6.6
50	69.5 \pm 1.7	43.4 \pm 1.6	30.9 \pm 1.3
100	39.3 \pm 2.1	35.7 \pm 0.8	33.8 \pm 2

MTT assay was based on the reduction of a soluble tetrazolium salt into a soluble coloredformazan compound by mitochondrial dehydrogenase activity and can be measured spectrophotometrically. The power for cytotoxicity for the crude extracts was established by the National Cancer Institute (NCI), in terms of IC_{50} value i.e.,

IC_{50} lower than 30 $\mu\text{g}/\text{mL}$ is optimal [8]. Alkaloids were widely distributed and documented in higher plants. They are unique chemical molecules that serve as source for drug discovery. Several alkaloids were isolated from plants exhibit antiproliferation and antimetastasis effects on diverse malignant cell lines both under *in vitro* and *in vivo* conditions [9].

TEM analysis

TEM examination of MCF-7 cells treated with 50 $\mu\text{g}/\text{mL}$ solasodine revealed significant structural deformities that further substantiate antiproliferation via apoptotic pathway. Morphological deformities visualized includes cytoplasmic condensation with pronounced reduction in cell volume, condensation of chromatin, fragmentation, blebbing of cell membrane, nuclear degeneration and the formation of apoptotic bodies (Fig. 1 b,c). Untreated cells displayed normal intact cellular features (Fig. 1a).

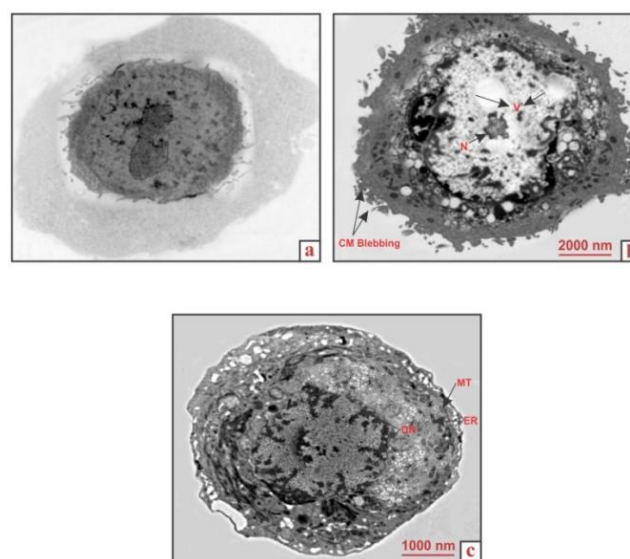


Figure 1: Transmission electron microscopic analysis of solasodine treated MCF-7 cells. a-control; b,c-treated cells showing cellular deformity

Analysis of cell cycle regulator proteins expression

The different cell cycle regulatory proteins were analyzed in order to unravel the plausible molecular process associated with solasodine (S) induced cell cycle arrest in MCF-7 cells. Figure 2 describes that solasodine (S) treatment soundly upregulated the expression of p53 in a time-dependent manner in MCF-7 cells. Concomitantly, the pronounced expression of p53 level was correlated with significant enhancement of its transcriptional target gene and p21 was also visualised. Meanwhile, the expression levels of cyclin D1 and E decreased remarkably within 12 h of solasodine treatment. Interestingly, the inhibition remained throughout the treatment periods. This substantiates further that the mode of action of solasodine against MCF-7 cells in the different phases of the cell cycle was through the induction of p53 and p21 genes and the down regulation of cyclin D and E cell cycle regulator proteins.

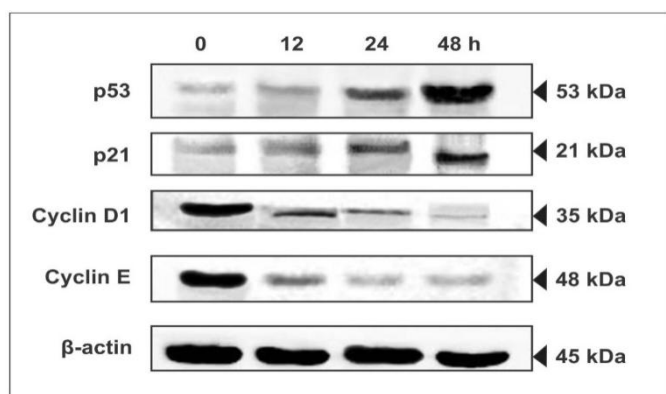


Figure 2: Western blot analysis reveals the effect of solasodine against various cell cycle regulatory proteins such as p53, p21, cyclin and β -actin

Expression analysis of caspases and Bcl-2 family protein in MCF-7 cells

The expression of diverse apoptosis regulating proteins like initiator caspases (caspase-8 and 9), effector caspases (caspase-3 and 7), poly-ADP ribose polymerase (PARP) and Bcl-2 family members such as Bcl-2, Bcl-xL, Bax and Bak were evaluated by western blot for elucidating their role in signaling pathway of apoptosis induced by solasodine (S) in MCF-7 cells.

Interestingly, 50 μ g/mL solasodine treated MCF-7 cells for 48 h resulted in the reduced expression of procaspase-7, 8 and 9 indicating the caspase dimerization or cleavage and thereby activating both intrinsic and extrinsic apoptotic pathways. Strikingly, the down regulated expression of procaspase-9 initially (from 12 to 24 h) was up regulated after 48 h. The reduced expression of procaspase-7 and 8 was less obvious compared to that of procaspase-9 (at 12 h), proposing that the caspase-9 was activated earlier than caspase-7 and 8 (Fig. 3).

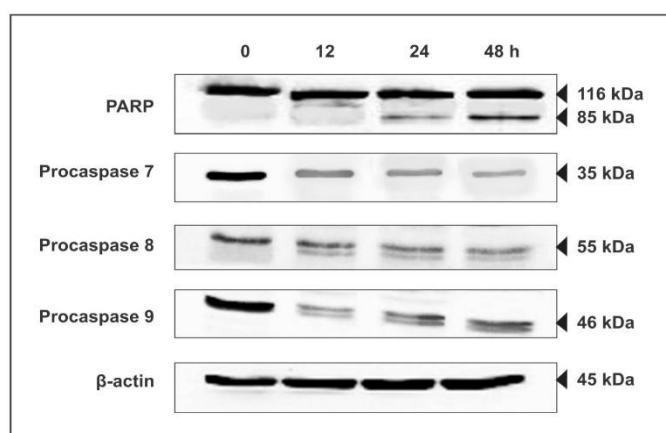


Figure 3: Effect of solasodine induced time dependent apoptosis in MCF-7 cells in terms of cleavag of PARP and down regulation of pro caspases as revealed by western blot

In addition, solasodine triggered the expression of cleaved PARP moderately (24 h) and this continued and became significant at 48 h when compared to that of control. PARP is considered as a biochemical indicator of apoptotic events in cells, therefore, it can be interpreted that the process of apoptosis was induced via caspase-7 which triggers the proteolytic cleavage of PARP. Western blot analysis of expression level of caspase-7 confirms this.

Role of Bcl-2 family of regulators proteins of the apoptotic pathway vssolasodine was further examined. Solasodine treated MCF-7 cells (48 h) exhibited a remarkable decline in the expression of anti-apoptotic protein (Bcl-2 and Bcl-xL), concomitant with an increase in pro- apoptotic protein expression (Bax and Bak) (Fig. 4). Interestingly, down regulation of Bcl-2 was drastic while, Bcl-xL decreased steadily with duration. Meanwhile, proapoptotic protein Bax was effectively upregulated when compared to that of Bak. This time-dependent expression of Bax/Bcl-2 ratio provides positive sign towards intrinsic apoptotic pathway induced by solasodine in MCF-7 cells. Thus, caspase dependent apoptosis by solasodine in MCF-7 cells may be substantiated.

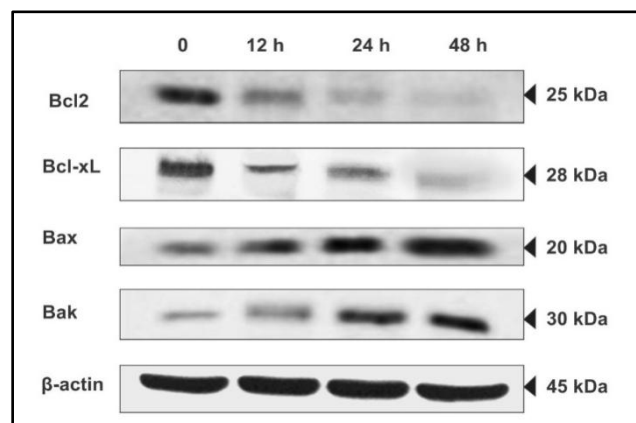
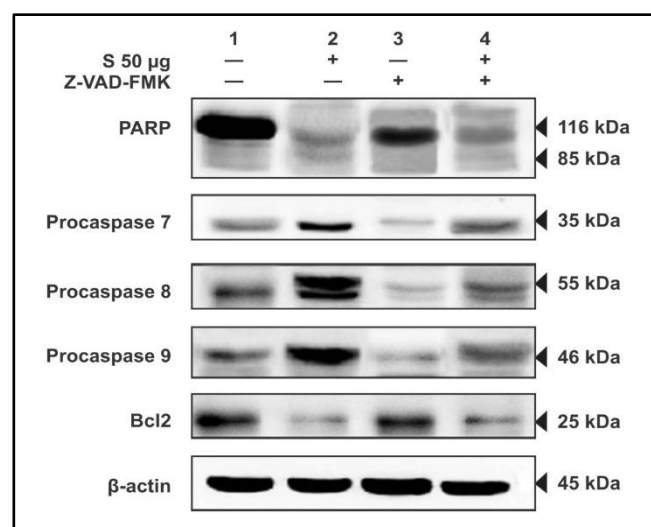


Figure 4: Solasodine induced apoptosis in MCF-7 cells by down regulating anti apoptotic and up regulating pro apoptotic proteins

The role of caspases in solasodine induced apoptosis was further evaluated using a general caspase arrestor, Z-Val-Ala-Asp-fluoromethyl ketone (z-VAD-fmk). Annexin V-FITC/PI analysis revealed that z-VAD-fmk considerably reduced the solasodine triggered apoptosis in MCF-7 cells, but not to the ground level (Fig. 5). This provides additional evidence that suppression of caspases may divert the inhibition of apoptosis induced by the alkaloid solasodine.



1-without solasodine and inhibitor, 2-with solasodine and without inhibitor, 3-without solasodine and with inhibitor, 4-with solasodine and inhibitor

Figure 5: Western blot analysis of role of caspases in solasodine induced apoptosis in MCF-7 cells Z-VAD-FMK: z-Val-Ala-Asp-fluoromethylketone, PARP-poly ADP ribose, Bcl2-B-cell lymphoma 2

Western blotting of the PARP suggests that pre-treatment of z-VAD-fmk redirect the solasodine mediated apoptosis in the MCF-7 cells i.e., the inhibition of active caspase prevented the cleavage of PARP (Fig. 5). Z-VAD-fmk also reverses the solasodine induced Bcl-2 expression level, suggesting strongly that the inhibition of active caspases affect the intrinsic apoptotic pathway. In toto, the results suggest that solasodine induced mechanism of apoptosis in MCF-7 cells is caspase dependent. Solasodine inhibited the proliferation of MCF-7 cells in a time and dose dependent manner. Further, it remarkably arrested cell-type specific G2/M growth in MCF-7 cells through down regulating the expression of cyclin D1, E and also via p53 dependent pathway. In addition, the solasodine remarkably down regulated anti-apoptotic proteins like Bcl-xL and Bcl-2 and up regulated pro-apoptotic proteins such as Bax and Bak. Simultaneously, procaspase-8 and 9 were dimerized and thereby activated which in turn induce caspase-3 and 7, resulting in cleavage of PARP. Similarly, the proven caspase inhibitor z-VAD-fmk inhibited the caspases and there by solasodine induced apoptosis in MCF-7 cells was inhibited.

DISCUSSION

Initial MTT assay results of solasodine tempted to analyze their mode of action against cell cycle regulatory proteins. Western blot analysis has clearly revealed that solasodine triggered p53 and p21 protein expression and inhibited that of cyclin D1 and E in MCF-7 cells. This provides further ample proof that solasodine arrested G1 phase in MCF-7 cells, thereby arresting their progression to the S phase.

Solasodine treatment inhibited the Bcl-2 and Bcl-xL expression levels whereas Bax and Bak were upregulated in MCF-7 cells. Therefore it can be interpreted that the solasodine inhibited the anti-apoptotic signals and promoted pro-apoptotic signals, thereby the mitochondrial permeability. Molecular analysis showed the activation of caspase-9 induced by solasodine may stimulate the release of cytochrome c, which results in the formation of apoptosome complexes. This is substantiated by the level of caspase-7 in MCF-7 cells and the cleavage of PARP. Another plausible mechanism by which solasodine triggers apoptosis is via modulating the expression of Bcl-2 family members to affect membrane permeability, leads to sequential activation of caspase-9, 3 and/or caspase-7 and ultimately cleavage of PARP. These observations are in agreement with *Vernonia amygdalina* extract which altered the cell membrane permeability in MCF-7 cells related with anticancer potentiality^[10].

Similarly, the solasodine induces apoptosis partly through the activation of caspases in MCF-7 cells. However, the mode by which solasodine triggers caspases still to be elucidated. The mode of solasodine induced apoptosis via the induction of the extrinsic apoptotic pathway is to be confirmed. It is yet to find out whether solasodine induced activation of caspases can break Bid, which is a BH3 pro-apoptotic protein that can induce the mitochondrial pathway. Similarly, analysis related with the expression of Bid is needed to elucidate whether there is any cross-talk exist between the mitochondrial and the death-receptor-mediated pathway in solasodine induced apoptosis.

Although caspase may be the critical factor in the execution of programmed cell death, the event of caspase activation is not the only the sole mechanism of induction of apoptosis. Wang *et al.*, reported caspase independent cell death model among various cancer cell lines such as Jurkat, MCF-7 and Hela cells^[11]. Therefore, it is essential to

find out solasodine induced apoptosis can still function in the presence of z-VAD-fmk, the caspase inhibitor. Z-VAD-fmk is a cell-permeable tripeptide inhibitor which possess aspartate fraction and fmk group, mimicking the cleavage region of caspase and forming a covalent inhibitor/enzyme complex. The mode of functioning is by binding irreversibly to the catalytic site of caspases. The present analysis also provides an insight in to the role of caspases in solasodine induced apoptosis in human breast cancer cell lines MCF-7 i.e., the inhibition of caspase activity by z-VAD-fmk blocked the cleavage of PARP and there by suppressing the entire spectra of apoptosis induced by solasodine. Thus, it may be possible to suggest that solasodine inhibits the proliferation of MCF-7 cells via the induction of caspase dependent apoptosis.

p53 the tumour suppressor i.e., the safe guardian of the integrity of genetic information from genomic toxicities has been the pivotal molecule of interest in oncology research^[12]. This plays dual role such as suppressing the growth arrest and the induction of apoptosis through which it exerts its tumour suppressing potential. p53, as a transcription factor, induces apoptosis via the transcription of its targeted genes like Bcl-2 members.

However, other studies reported the existence of a transcription-independent pathway i.e. direct localization of p53 in the mitochondria can leads to direct interaction with Bcl-2 or Bcl-xL to promote apoptosis^[13,14]. Thus, it is utmost important to determine whether the drug plays a p53 transcription-dependent or independent pathway in the induced apoptosis using pi fithrin-mu (PFT- μ) and PFT- α . PFT- μ functions by decreasing the binding capacity of p53 to Bcl-2 and Bcl-xL, thereby blocking its binding to mitochondria without affecting its transactivation role^[15]. This can sort out whether the apoptosis is independent of p53 transcriptional activity or not. If both PFT- α and PFT- μ fail to reverse the drug induced apoptosis, it clearly suggests that the phytochemical induced apoptosis is independent of p53. Mrozek-Wilczkiewicz *et al.*, proved *in vitro* activity of styrylquinolines via p53 independent mechanism against human colon carcinoma cell lines such as normal HCT116 p53+/+ and mutants with disabled TP53 gene (HCT116 p53-/-)^[16].

Hormonal receptor mechanism has been known as the unique prognostic and predictive factor for oncological and hormonal therapy. Estrogen receptor (ER) level is a unique key component for the breast cancer treatments and drugs that can compromise ER signaling can clinically an effective therapeutic drug^[17]. Berger *et al.*, reported that ER- α isoform of ER can functions as a transcription factor to initiate the transcription of specific target genes through estrogen activation^[18]. Nearly, 70% of reported breast cancers were ER-positive which express ER- α , if solasodine inhibit ER- α expression, then it will be a potential clinical significance.

Mirzayans *et al.*, analyzed the increasing complexity of onco cell response to DNA damaging agents leading to cell survival or death^[19]. Wild-type p53 either initiates the activation of cell cycle checkpoints to induce DNA repair and cell survival or coordinates apoptosis via anticancer drugs. This reigning paradigm has been argued by oncologists in tumor-derived cell lines. In addition, caspase-3, the executor of apoptosis facilitate DNA damage and genomic instability in carcinogenesis

Makarevic *et al.*, examined amygdalin effects in UMUC-3, TCCSUP and RT112 bladder tumour cells by adhesion, invasion and integrin α , β subtypes linked kinase (ILK) and focal adhesion kinase (FAK). i.e.,

integrin expression followed by anti-integrin-linked kinase involved activation of FAK^[20]. Anticancer activities of alkaloids derived from Amaryllidaceae members were evaluated against L5178 mouse lymphoma cells by Zupko and co-workers^[21]. MTT assay revealed that the alkaloids such as pretazettine, trisphaeridine and 2-O-acetyllycorine showed potent antiproliferative effects.

Lu *et al.*, reviewed alkaloids isolated from herbals such as berberine, evodiamine, matrine, piperine, sanguinarine, tetrandrine, chelerythrine, chelidonine, fagaronine, lycorine, nitidine and solanine as antitumour agents^[22]. Role of four African herbal extracts on MCF-7 cell lines were analyzed by Engel *et al.*, in terms of cell death and IC₅₀ values (23–38 µg/mL)^[23]. This was further substantiated by apoptosis using annexin staining and DNA fragmentation results. Decreased β1- integrin expression and phosphorylation of the FAK were the mechanism noticed in them related with pro-apoptotic and anti-adhesive activities. Biswas *et al.*, attempted in designing anticancer drug based on the above concepts with plant based chemicals^[24]. Huska *et al.*, proved an electrochemical interaction of an anticancer alkaloid ellipticine with DNA^[25].

Wu *et al.*, reported anticancer potential of the alkaloid casticin from *Fructus viticis* against A375.S2 human melanoma cell through migration/invasion coupled with down regulation of NF-κB and matrix metalloproteinase-2 and -1 (MMP)^[26]. Cell migration ability was analyzed by wound healing assay and cross checked with transwell-migration assay. Inhibitory effects on MMP-2 and 9 activities followed by decrease in p-EGFR; Ras and p-ERK1/2 activities leads to down-regulation of migration and invasion of cells. The NF-κB p65 and p-ERK levels in nuclear proteins were also decreased. EMSA assay also confirmed the decrease of NF-κB p65 and DNA content. Cytotoxicity of aporphine alkaloids from the roots of *Annona reticulata* against human cancer cell lines was evaluated by Suresh and his team^[27]. The IC₅₀ values reported was ranging from 7.4 to 8.8 µg/mL. Interestingly, the alkaloid showed low activity against Vero cell lines in comparison with the cancer cell lines. Sathya and Muthuchelian proved antimetastatic potential of alkaloid extract from *Tabebuia rosea* leaves on MOLT-4 cells by MTT assay and also the genotoxic potential using cytokinesis block *in vitro* micronucleus assay^[28]. GI₅₀ values were 46.95 (24 h), 36.05 (48 h) and 25.75 (72 h) µg/mL/1×10⁶ cells against cancer cells. Similarly, *Citrullus colocynthis* fruit alkaloid was effective against MCF-7 and HEPG-2 cell lines^[29]. The cytotoxic effect in terms of LC₅₀ values were 3.30, 12.54 µg/mL respectively confirming the potent anticancer property of the alkaloids. In the present study, the alkaloid solasodine also showed a similar trend.

Hadjiakhoondi *et al.*, compared cytotoxicity of alkaloids from two species of *Glaucium* from Iran^[30]. IC₅₀ values confirmed that the growth and proliferation of NIH/3T3 cells were less affected in comparison to human colon adenocarcinoma cell lines (HT-29, Caco-2). A novel group of phytochemicals viz. dienamide -kunstleramide was isolated from the bark of *Beilschmiedia kunstleri* together with neolignan: (+)-kunstlerone and seven other alkaloids: (+)-normuciferine, (-)-isocaryachine, (+)-cassythicine, (+)-laurotetanine, (+)-boldine, noratherosperminine, (+)-*N*-demethylphylllocryptine. Structural elucidation was carried by different spectroscopic techniques. The alkaloids showed low DPPH activity, with an IC₅₀ value of 179.5 ± 4.4 µg/mL and also cytotoxic effect against A375, A549, HT-29, PC-3 and WRL-68 in terms of EC₅₀ values by MTT assay^[31]. Afzaliet *al.*, showed non-addictive opium alkaloids - noscapine and papaverine selectively promote apoptosis in cancer

cells compared to normal cells i.e., papaverine induces apoptosis in all cancer cell lines while, noscapine was active only in T47D and HT29 cells^[32]. New cytotoxic pyridoacridine alkaloid was isolated from *Oceanapia* and analyzed by 1D and 2D NMR as kuanoniamine C and sagitol. Cytotoxicity against L5178Y, PC12 and HeLa cell lines gave 93, 88 and 76% growth inhibition at a concentration of 24.6 µM and 81, 74 and 37% at a concentration of 12.3 µM with ED₅₀(s) of 0.7, 0.9 and 2.3 M, respectively^[33]. Friedman analyzed chemistry and anticarcinogenic mechanisms of glycoalkaloids produced by egg plants, potatoes and tomatoes^[34]. An *et al.*, reviewed about the mechanisms of anti-cancer potentiality of *Solanum nigrum*^[35]. Crude alkaloid treatment resulted into structural and functional deformity of tumor cell membrane, disturbed synthesis of DNA and RNA, changed the cell cycle and there by inhibiting tumor cells. Mechanism involved includes blocking the anti-apoptotic pathway of NF-κB, activating caspase cascades reaction and increasing the production of ROS-nitric oxide. Wu *et al.*, confirmed that *Solanum incanum* extract induces apoptosis in human cutaneous squamous cell carcinoma via modulating tumor necrosis factor receptor signaling pathway via TNFRs, Fas and downstream adaptors FADD/TRADD of the TNF-α and Fas ligand signaling cascades^[36]. Further, mitochondrial apoptotic pathway was regulated by up-regulation of cytochrome c and Bax and down-regulation of Bcl-X(L).

Solamargine (SM), a steroidal glycosidic alkaloid from *Solanum incanum* exhibited remarkable cytotoxic potential via extrinsic and intrinsic pathway compared with that of synthetic drugs such as cisplatin (cDDP), methotrexate (MTX), 5-fluorouracil, epirubicin and cyclophosphamide against human breast cancer cell lines. Molecular mechanism involved was by inducing the over expressions of external death receptors like tumor necrosis factor receptor I (TNFR-I), Fas receptor (Fas), TNFR-I-associated death domain and Fas-associated death domain. SM also triggered the intrinsic ratio of Bax to Bcl-2 by increasing Bax and decreasing Bcl-2 and Bcl-xL expressions. Further, the release of mitochondrial cytochrome c and activation of caspase-8 and 9 in the onco cells leads to apoptosis. Similarly, SM, cis-diamminedi chloride platinum (cDDP) causes cancer cell apoptosis though caspase-8/3 and Bax/cytochrome c, but the resistance to cDDP was correlated with Bcl-2 and Bcl-xL up regulation. However, the over induction of Bcl-2 and Bcl-xL can be inhibited by SM. The combination of SM + cDDP significantly decreases Bcl-2 and Bcl-xL expressions and increase Bax, cytochrome c, caspase-9 and -3 expressions in breast cancer cells. Thus, SM + cDDP may be potential in cDDP-resistant breast cancer treatment^[37].

Similarly, *Solanum lyratum* alkaloids showed efficacy against HeLa cell lines through apoptosis pathway. Cytotoxicity was analyzed through growth inhibition assay (IC₅₀ value was 82 µg/mL). Apoptosis was detected by Hoechst33324 and Tdt-mediated dUTP nick end labeling (TUNEL) staining assays (cells in the G2/M phase). The *in vitro* cytotoxic studies were supported by the cell cycle results and caspase-3 activity. RT-PCR showed an induction of apoptosis-associated genes. Upregulation of p53 and Bax gene concomitantly with the activity of caspase-3 without much change in Bcl-2 expression compared to the normal cells were noticed^[38]. Bhattacharya *et al.*, isolated solasodine from the unripe fruits of *Solanum xanthocarpum* and proved anti cancer activity against HeLa and U937 cancer cell lines^[39]. *In vitro* and *in vivo* anticancer activity of the fruit peels of *S. melongena* against hepatocellular carcinoma was also proved^[40]. Mechanisms of solamargine alkaloid for the cytotoxicity were through interfering with the cell membrane via

branched extensions of the connected sugars. This feature disrupt the integrity of the cells by entering the morphology of the cell and its DNA content leading to apoptosis^[41,42]. In addition, the uniqueness from solamargine was its glucose fraction directly attached to the 3-β-OH + two attached rhamnose group which may bind into the tumour cell leading to phagocytosis via rupturing of lysosomes^[43,44]. Similarly, the alkaloid reduces the DNA content and enhances apoptosis via cell fragmentation. The 2 rhamnose moiety seems to be crucial for apoptosis induction. Meanwhile, solasonine possess an attached galactose moiety, a rhamnose and a terminal glucose revealed comparatively a poor activity. Moreover, β-sitosterol-3-O-β-D-glucoside and poriferasterol-3-O-β-D-glucoside were also showed promising cytotoxic potentials against MCF-7 and colon HCT116 cell lines. The alkaloids showed significant *in vivo* activity against the hepatocellular carcinoma in a concentration dependent manner through verification of reducing α-fetoprotein (AFP) (a liver tumor indicator), in addition to regaining the levels of AST, ALT and albumin in a concentration dependent manner. Histopathology of liver tissues was normal with usual structural features like portal tracts, absence of fibrosis and inflammation. Wu *et al.*, confirmed that the alkaloids from *S. incanum* down regulates aldehyde dehydrogenase 1-mediated stemness and blocks tumor formation in ovarian cancer cells^[45]. Solamargine effectively induces apoptosis in many tumour cell lines but not in bone marrow cells, fibroblasts and normal hepatocytes, suggesting its preferential activity against malignant cell lines only^[46]. In the present study also fibroblast cells are not affected by the solasodine alkaloids. Many anticancer reports reveal that solamargine promotes cell death through the activation of TNFRs^[47] and the mitochondrial apoptotic pathway^[48]. SR-T100 was shown to induce cutaneous squamous cell carcinoma cell apoptosis via similar pathways and suppresses tumor growth in mouse xenografts and patients with actinic keratosis. Wu *et al.*, revealed the novel molecular pathway underlying induced cytotoxicity, via inhibition of ALDH1 induced stemness and COL11A1-regulated chemoresistance^[45]. Similarly, cancer stem cells (CSC), which were featured with enhanced tumour formation ability and drug resistance, share some stem cell markers, including ALDH1 and Notch1^[49,50]. ALDH1 expression was closely linked with tumorigenic potential in ovarian cancer cell lines via downregulating FoxM1 and Notch1 effectors^[51]. Further, FoxM1 coordinates epithelial-mesenchymal transition, stemness and resistance in epithelial ovarian carcinoma cells^[52]. Wu *et al.*, confirmed the inhibition of CSC marker, FoxM1 expression and sphere production in ovarian cancer cells in mouse xenografts^[45]. Chemoresistance often causes patient death, due to a lack of effective treatments. Xia *et al.*, proved that solamargine effectively induces apoptosis in multidrug-resistant onco cells, through actin disruption mechanism^[53]. Moreover, reduced stemness, regulates cell sensitivity to anticancer molecules via down streaming of c/EBPβ and COL11A1. Induction of COL11A1 in ovarian cancer cells by synthetic drugs like cisplatin and paclitaxel confers chemoresistance via inducing the Akt/c/EBPβ and stabilizing phosphoinositide-dependent kinase-1. Cham reviewed the modern chemotherapeutic possibilities of solamargine and other solasodinerhamnosyl glycosides as potential anticancer agents^[54].

Xia *et al.*, analyzed the effects of solamargine (SM) on cell membrane blebbing of human K562 leukemia and squamous cell carcinoma KB cells^[53]. Treatment of SM with these cancer cell lines was featured by marked changes in shape and volume of cells. Blebs were formed on the cell membranes followed by uneven swelling of the mitochondria, the nuclear content aggregation and the cells death. It was suggested that apoptosis and oncosis shared certain pathways and alterations

within the cells before they died apoptotically. Poor concentrations of SM killed cancer cells by apoptosis and at increased concentrations SM killed cancer cell by oncosis and both types of cell death were triggered by intermediate doses of SM. Li *et al.*, showed that SM substantially decrease cell viability and induce apoptosis in osteosarcoma U2OS cells^[55]. SM enhanced the mRNA and p53 and Bax protein expression (a pro-apoptotic protein downstream p53). The expression of Bcl-2 was also decreased. SM triggered mitochondrial translocation of p53, loss of mitochondrial potential, release of cytochrome c and induction of caspase-9 and -3. Inhibitors of p53 transcriptors or mitochondrial translocation will partially reverse SM-triggered apoptosis. It was confirmed that *Solanum* alkaloids activate the mitochondria-induced apoptotic mechanism in U2OS cells *via* both p53 transcription-dependent and-independent pathways.

In situ visualization of the retinol binding protein (RBP) receptor on onco cell lines surfaces was revealed by biotinylatedrhamnose and chacotriose (the triglycoside of SM). BEC was powerful in killing onco cells. These reports support previous observations signifying the potential of RBP to screen neoplastic cells and the plausible design of novel antineoplastic drugs that will specifically interact and eliminate onco cells. Cham and Chase reported that in addition to apoptosis and perhaps as a consequenc, Beckman Coulter Inc (BEC) also has an effect on stimulating lasting immunity against tumor as shown in mouse model and the terminal cancer S180 cell lines^[56]. BEC could play a crucial role in clinical management of diseases like malignancy and also be used as a preventative therapy. It has also been proved that BEC was effective in treating herpes simplex, herpes zoster, genital herpes and other viral infections and similarly, patients with no resurrences^[57,58]. It was also suggested that BEC may exert activity and immunological impacts with similar mechanism of action on a number of diverse diseased states including tumor. Ding *et al.*, proved that SM remarkably inhibit the growth of human hepatoma SMMC-7721 and HepG2 cells and induce cell apoptosis through cell cycle arrest at the G2/M phase by over expressing of caspase-3^[58]. All the above outputs substantiate the efficacy showed by the alkaloid solasodine as anticancer drug against MCF 7 cancer cell lines.

CONCLUSION

Solasodine exhibited concentration dependent inhibition of cell proliferation of the breast onco cell lines MCF-7. The underlying mechanism of this growth inhibition is likely to be caspase and p53 dependent pathways. Bcl family members include pro or anti-apoptotic proteins are differentially controlled by the solasodine via down or up regulating their expression levels. Doxorubicin was used as positive control. TEM data further reveals structural deformities in the treated MCF 7 cells compared to control. The entire results suggesting that solasodine can complement current chemotherapeutic treatment leading the way for future research in the field of anti-cancer drug discovery.

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