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LAWSONE INHIBITS CELL GROWTH AND IMPROVES THE EFFICACY OF CISPLATIN IN SKOV-3 OVARIAN CANCER CELL LINES

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Abstract

Background: Lawsone (LWS) is a colored napthoquinone moiety found in plant *Lawsonia inermis* L. (Lythraceae) it is used as precursor for synthesis some anticancer drugs. In present research we evaluate the effect of LWS alone and in combination with Cisplatin (CP) on SKOV-3 ovarian cancer cells.
Materials and Methods: Cell proliferation studies were done by MTT assay while the cell apoptosis studies were carried by performing nuclear Hoechst 33258 staining. Cell cycle analysis was done by Flow cytometric studies, Immunoblotting studies for protein expression was done, proteins controlling cell cycle such as cyclinD1, cyclin E, cyclin A, cyclin B1 and Cip1/p21 and p53 which also are cyclin dependent inhibitors of protein kinase were estimated. Annexin V staining was done to mark extent of apoptosis, expression of apoptotic protein such as Bcl-2, Bax, Bax/Bcl-2 ratio and activity of caspase 3.
Results: LWS alone and in combination with CP suppressed the growth of SKOV-3 cells in dose-dependent manner. Treatment inhibited SKOV-3 cells by arresting of G1/G0 phase in the cell cycle, by increasing the expression of p53 and Cip1/p21 followed by decreasing levels of two important proteins cyclin E and cyclin D1. LWS was found to induce apoptosis via decreasing the levels of Bcl-2, improving Bax:Bcl-2 ratio and activating caspase 3.
Conclusion: Results of this study clearly indicate LWS alone and in combination with CP have antiproliferative effect, causes apoptosis of SKOV-3 cells via suppressing Bcl-2. LWS could be a useful compound for treatment of ovarian cancer.

Keywords: Lawsone, Ovarian cancer, SKOV-3 cells, Cisplatin.

Introduction

Epithelial ovarian cancer (EOC) is reported to be affecting approximately 2 lakh women's approximately every year globally and is responsible for about 1.25 lakh deaths (Parkin et al. 2005). The major problem associated with treatment of EOC is that it shows very few symptoms till the end stage where it is found to metastasized in peritoneal cavity, this reduces the chances for its efficient cure. Currently the treatment options for EOC comprise of surgery followed by chemotherapy of platinum/taxane. Studies have been reported indicating good recovery in patients, but there after followed by relapse and development of a multifactorial drug resistance case (Mungenast et al. 2014; Siegel et al. 2014). The current chemotherapeutic strategies include platinum derivatives combined with anticancer drugs such as Paclitaxel which results in increased drug toxicity. The biggest disadvantage associated is that of toxicity and failure to distinguish between normal and cancer cells. Hence attempts are being made in searching for some new anticancer molecules that are efficient and cause fewer side effects.

Phytochemical products have always been compounds of interest due to their various pharmacological activities. Number of phytochemicals such as ellagic acid (Laura et al. 2016), corilagen (Luoqi et al. 2013) cucumin and triptolide (Ying-Ying et al. 2013) have been used in treating ovarian cancer and have also enhanced the activities of synthetic drugs

when used in combinations. These drugs of natural origin can manipulate molecular targets of cell proliferation such as the VEGFR family of receptor tyrosine kinases which are reported to be important for therapy or prevention of cancer (Aggarwal et al. 2006).

Lawsonia inermis L. (Lythraceae) a plant popularly called as '*Mehndi*' or '*Henna*' is well known for its cosmetic purpose and is also known for its medicinal properties from ancient times. It is reported to show activities such as antibacterial and antifungal by tanning effect (Habbal et al. 2011), antioxidant (Hsouna et al. 2010), immunomodulatory (Mikhaeil et al. 2004), analgesic and anti inflammatory (Luthfun et al. 2014) effect mediated by flavonoids The plant has a red coloring pigment lawsone (LS) chemically identified as 2-hydroxy-1,4-naphthoquinone (Lekouch et al. 2001) prompting this herb useful for producing a dying effect on hair surface (Cartwright 2006). LS also characterize its use in criminology for identification of finger prints (Khan 2010).

Lawsone (LWS) a compound having napthoquinone nucleus is found to be precursor for synthesis of anticancer drugs such as atovaquone, lapachol and dichloroallyl lawsone. The napthoquinone compound such as Juglone along with LWS is reported to possess inhibitory activity on HCT-15 (Human colon cancer cells) *in vitro* by blocking the S-phase in cell cycle (Pradhan et al. 2012) suggesting a potential anticancer activity of compound. However studies involving LS on Ovarian carcinoma are still unexplored. In the current research work we report molecular and cellular evidences responsible for inhibition of cell proliferation in ovarian cancer cell lines. The study also demonstrated a synergistic activity of LS in combination with Cisplatin. Current research evidenced potential use of this naphthoquinone compound in chemotherapy of ovarian cancer.

Materials and Methods

For growth of cancer cell lines RPMI-1640 media, MEM (Minimum Essential Medium), FBS (fetal bovine serum), antibiotics, trypsin and L-glutamine were procured from Gibco Co. Ltd. (UK). Lawsone (LWS), cocktail of proteinase, MTT [3-(4,5-cimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], Triton X-100, sodium o-vanadate, acridine orange, sodium fluoride, *N,N,N′,N′*– tetramethylethylenediamine (TEMED), Tween 20, sodium pyrophosphate and ammonia persulfate were obtained from Sigma Aldrich Co. The protein assay reagent Bicinchoninic acid (BCA) was sourced from Sigma Aldrich Co. Acrylamide was procured from Fisher Sci. Co. Polyvinylidene fluoride (PVDF) membrane (Immobilon-P) was obtained from Bio-Rad (Hercules). The mouse monoclonal anticaspase 3, cyclin D1, cyclin B1, cyclin E and B cell lymphoma 2 (Bcl- 2) antibodies were procured from Enzo Biochem Inc. (China). PARP the goat polyclonal poly [ADP-ribose] polymerase, Cip1/p21, anti-p53 and Bcl-2 antibodies (Bax) associated with protein X. Goat anti-rabbit and anti-mouse antibodies, Rabbit anti-goat secondary horseradish peroxidase conjugated antibodies (HRP) were obtained from Abcam co. U.S.A.

Cell Lines

For the study SKOV-3 human ovarian cancer cells and MRC-5 normal human lung fibroblast cells were used, all the cell lines were collected from Inst. of Biochem. and Cell Bio., Chinese Acad. of Sci. For the study both cell lines were subjected to culture separately. SKOV-3 cells were allowed to culture in 90% RPMI medium and were supplemented with FBS 10% previously inactivated by heating. The normal control MRC-5 cells were cultured in medium similar to that used for SKOV-3. For all the cell lines subjected for culture the media used were supplemented with antibiotics penicillin having concentration of 25 U/mL and streptomycin having 25μg/mL. The incubation of cell lines was done at 37°C.

MTT assay for Cell proliferation studies

The cytotoxic effect of LWS on human ovarian cancer cells was evaluated by MTT assay. MTT chemically is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide. The assay mainly depends on reduction of the mitochondrial enzyme by tetrazolium dye to detect and determine cell viability. In this study, both SKOV-3 and MRC-5 cells were subjected to seeding in 96-well microtiter plate having flat bottom at a density of $1x10^5$ cells. The cells in the wells were exposed to LWS (Prepared by dissolving in 3% tween-80 and 0.9% saline solution) 10, 20, 40, 80 and 160mg/ml. Similarly Cisplatin (CP) was also diluted to obtain concentrations of 10, 25, 50, 75 and 100µM and combination of LWS+CP for time period of 24h. After the defined treatments the plates were added with 10 µl working solution of MTT prepared in PBS having concentration 5mg/ml and were kept aside for another 4 h. After 4 h the formed formazan insoluble blue crystals were made soluble by addition of 50µl of DMSO followed by Spectrophotometric quantification at 540 nm by an ELISA reader (Bio-Tek Instrument Co., USA). The percentage inhibition was computed by formula (absorbance of treated cells/ absorbance of control cells) x 100.

Apoptosis studies by nuclear staining with Hoechst 33258

The ovarian cancer cell lines SKOV-3 and the normal MRC-5 cells were plated at 7×10^5 cells per 6-cm dish for 24 h. The cell lines were then exposed to CP 10-100 μM, LWS at 10-160mg/ml and combination CP+LWS (100μM+160mg/ml) for 48 h. after exposure for 48 h each dish was added with 5mM Hoechst 33258 followed by incubation of 5 min. in dark. The cells were then washed with PBS before putting them under observation using a fluorescent microscope (Zeiss, Axio observer Germany). Untreated cells were treated as negative control.

Cell studies for synchronization.

Cell synchronization experiment was done by synchronizing SKOV-3 cells opting double thymidine block to the G1/S boundary. The cells were subjected to pre-synchronization for 15h by incubating them in 2mM thymidine (Wako, Japan), the medium was changed with a medium without thymidine and cells were incubated for 8h. The cells were again resynchronized by incubating them in 2mM thymidine for next 15h followed by washing and immediately incubating in fresh independent culture medium containing LWS, CP and mixture of CP+LWS for promoting entry of cells into cell cycle.

Cell cycle analysis.

Flow cytometric studies were carried out for evaluating cell cycle analysis of SKOV-3 and MRC-5 cells. For the study harvested SKOV-3 cells were fixed for 1h in ethanol (70%, ice cold). The cells were then exposed to RNaseA (10 μg/ml) for 30 min at 37˚C followed by treatment of propidium iodide (20μg/ml) (PI). The cells were analysed by FACSC alibur flow cytometer manufactured by Becton Dickinson Bioscience, USA. The percentages of cells in various phases (i.e. G1, S, and G2/M) of cell cycle were determined using Modfit software (U.S.A).

Immunoblotting

Both cell lines i.e. cancerous SKOV-3 and normal MRC-5 were subjected to washing with phosphate-buffer saline (ice-cold) and were lysed in buffer composed of 10mM Tris-HCl having pH 7.4, 2mM EDTA, 50mM NaCl, 1mM EGTA, 1% Triton X-100, 20mM sodium pyrophosphate, 50mM NaF, 1mM sodium orthovanadate, and 1 : 100 proteinase inhibitor cocktail) for homogenization in a ice bath for 0.5h. The lysed cells were centrifuged for 0.5h at 12,000 rpm in a cooling centrifuge at 4ºC resulting in complete removal of insoluble materials. Obtained lysate was evaluated for protein content using a BCA protein estimation kit followed by separation using SDS-PAGE.

Immunoblotting was initiated by electro-transferring resolved bands to PVDF membranes with the aid of semidry blot apparatus (Bio-Rad). The PVDF membranes were incubated with Tris buffer saline in 5% nonfat milk the media was supplemented with Tween 20 (TBST, 10mM Tris, pH 7.4, 150mM NaCl, 0.2% Tween 20) for 60 min at room temperature. The PVDF membrane was incubated in 3% non-fat milk with different primary antibodies in TBST at 4ºC for 18h followed by incubation with secondary antibody in conjugation with HRP after washing repeatedly with TBST. Enhanced chemiluminescence was done to obtain Immunoblots, the luminescence was imaged on X-ray film or opting a chemoluminescence detection system (Bio-Rad).

Cell apoptosis study using Annexin V staining

Cell apoptosis measurement study was done by annexin V as labeling agent on surface of cells undergone apoptosis's having phosphatidylserine (Shao-Hung et al. 2015). Briefly, the LWS, CP and LWS+CP treated cells were subjected to trypsinization and washing two times with phosphate-buffered saline and finally suspended in binding buffer (10mM HEPES, pH 7.4, 140mM NaCl, and 2.5mM CaCl2). The cells were stained using annexin V (2μg/mL) in conjugation with fluorescein isothiocyanate (FITC) for 30 min in dark and room temperature condition. The cells stained with annexin V were analyzed using a flow cytometer; parameter such as FL-1H was chosen to evaluate the apoptotic cells. In the study untreated SKOV-3 cells served as the negative control.

Statistical Analysis

All data were obtained as average of three experiments performed independently; the results are represented as the means ± standard deviation (SD). Differences were calculated using Student's unpaired *t*-test. Values of $P < 0.05$ were considered significant statistically. All analyses were performed using graph pad software.

Result

Evaluation of anti-proliferative effect on SKOV-3 and MRC-5 cells

Antiproliferative studies were done on ovarian cancerous SKOV-3 cells and normal MRC-5 cells by MTT assay to measure cell viability. The assay is based upon formazan formation due to cleavage of MTT by living and active mitochondria; the reaction is quantitative and is proportional directly to the number of living cells. In the process cultures of SKOV-3 and normal MRC-5 cells were exposed to LWS (Prepared by dissolving in 3% tween-80 and 0.9% saline solution) 10, 20, 40, 80 and 160 mg/ml. Similarly CP was also diluted to obtain concentrations of 10, 25, 50, 75 and 100 µM and combination of CP+LWS (10µM+10mg/ml to 100µM+160 mg/ml) for time period of 24h. The results suggested the effect of all the 3 treatments was dose dependent (figure 1). The exposure of CP at 10µM showed 37.25 % inhibition and increased to 90.25% on exposure to 100µM. LWS also demonstrated the antiproliferative activity causing 33.6% inhibition at 10 mg/ml and 83.76% at 160mg/ml. Combination of CP+LWS (100µM+160mg/ml) showed maximum % inhibition values of 96.28% thereby demonstrating synergistic effect of LWS with CP. Interestingly LS treatment exhibited no significant proliferation on normal MRC-5 cells.

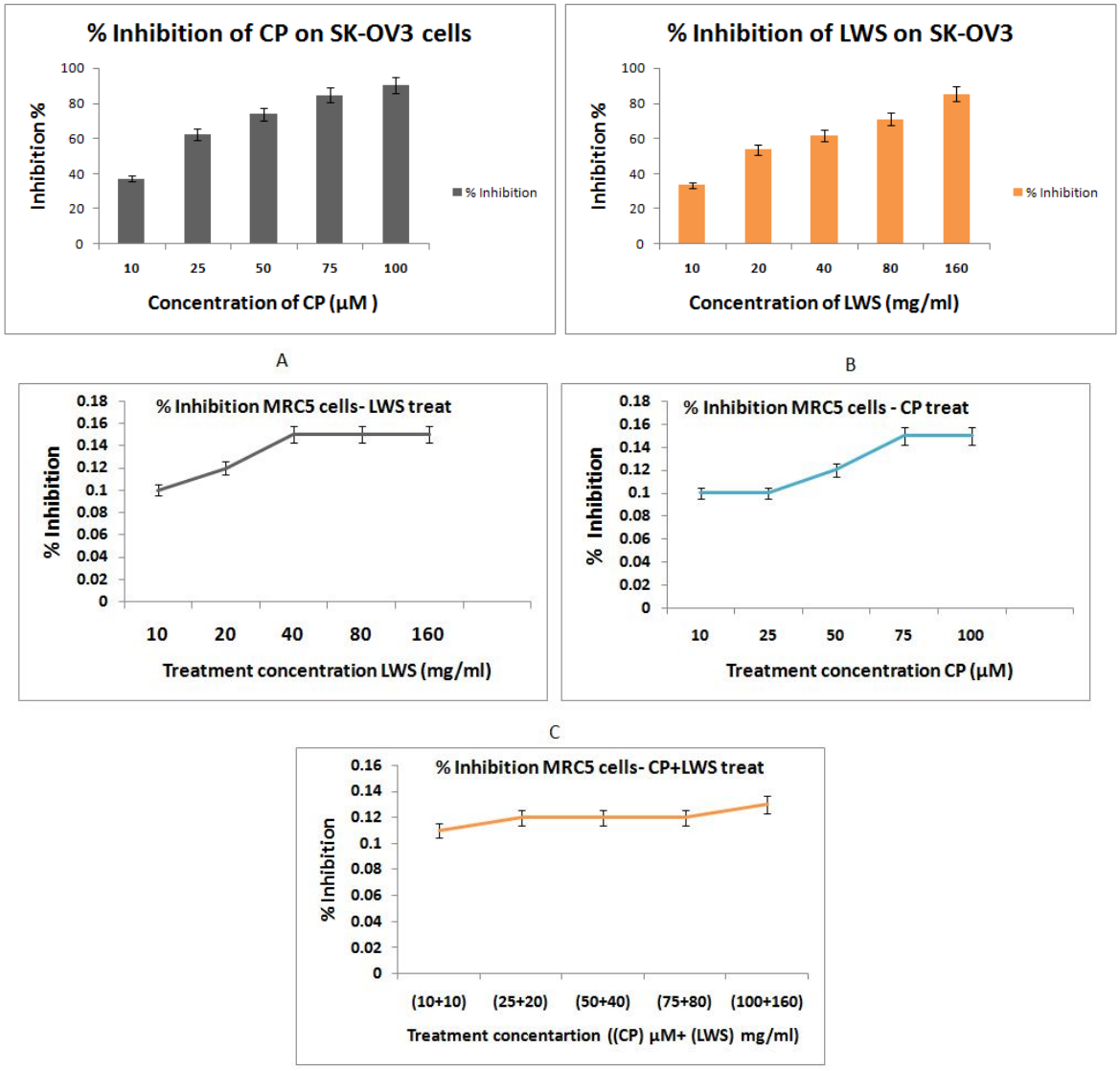

Figure 1: Effect of CP and LWS alone on SKOV-3 cell. A dose dependent proliferation of SKOV-3 cells was observed both for CP and LWS. A, B and C represents % inhibition on normal MRC5 cells showing insignificant cell inhibition.

Nuclear Morphological Changes of Cancerous SKOV-3 cells exposed to CP and LWS

Apoptosis followed by any morphological changes in cancerous and normal cells was detected by Hoechst 33258 staining in both selected cancerous SKOV-3 cells and normal MRC-5 cells. It is well evidenced about the classical characters of cells undergone apoptotsis such as chromatin condensation and nuclear fragmentation (Lo et al. 2013). In process of investigating role of apoptosis in cytotoxicity of LWS on cancerous and normal cells we incubated SKOV-3 and MRC-5 with LWS, CP and combination of both (CP+LWS) separately for time of 48h. Post treated cells were stained using Hoechst 33258 followed by fluorescence microscopy to identify any notable morphological changes. The cells exhibiting bright color were considered to be apoptotic. Figure 2 shows the fragmentation of nucleus and chromatin condensation, in the CP+LWS treated cells exhibiting brighter color significantly than the groups treated with one of the two i.e. CP and LWS. However no visible staining was observed in normal MRC-5 cells and the untreated cells (negative control).

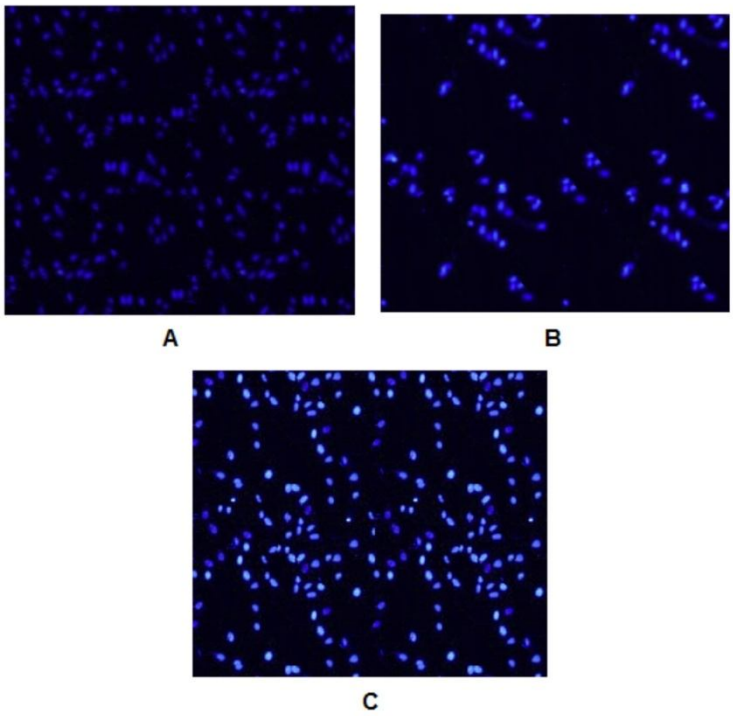

Figure 2: Results of Hoechst 33258 staining of SKOV-3 cells A: LWS treated cells, B: CP treated and C: CP+LWS treated

Progression of cell cycle and protein expression by immunoblotting in SKOV-3 cells

In the process of establishing the mechanism behind the LWS mediated inhibition of Ovarian cancer cells, cell cycle progression and immunoblotting studies were carried out. Results of cell cycle progression depicted in Figure 3 indicated an increase in distribution of the G0/G1 phase of the cell cycle while decrease in S and G2/M phases with increase in concentration of all the three treatments i.e. the LWS, CP and CP+LWS. However the results indicated a significant increase in the distribution of G0/G1 phase and decrease in S and G2/M phase in the cells exposed to combination of CP+LWS (Figure 3) as compared to controls.

In order to establish mechanism by which LWS produces a synergistic effect, protein expression by immunoblotting was done. As observed in cell cycle analysis, exposure of LWS on cancerous SKOV-3 ovarian cancer cells exhibited block of G0/G1 phase hence the levels of proteins controlling cell cycle such as cyclinD1, cyclin E, cyclin A, cyclin B1 and the two Cip1/p21 and p53 (cyclin dependent protein kinase inhibitor) were estimated. The levels were estimated by immunoblotting technique. As observed (Figure 4), the treatment of LWS enhanced the expression of p53 significantly in combination with CP. Also the cells treated with CP+LWS exhibited a concentration dependent increase in levels of Cip1/p21 levels as compared to alone treated cells. Treatment of LWS in combination with CP decreased the expression levels of cyclin B1 significantly in dose dependent manner as compared to LWS and CP alone treated cell lines.

Expression levels of protein cyclin D1 decreased in SKOV-3 cells when exposed to CP+LWS at higher concentration levels of 160mg/ml+100μM of LWS and CP respectively and not at lower concentrations. Levels of protein cyclin E decreased in SKOV-3 cells exposed to LWS but only at higher concentrations (160mg/ml) and not at lower, where as levels decreased in CP+LWS treated cells after a combining concentration of 40mg/ml of LWS+50μM CP and remained

unaffected till the last higher concentration of both. Expression of cyclin A was found to unaffected by any of the three exposed treatments i.e LWS, CP and CP+LWS.

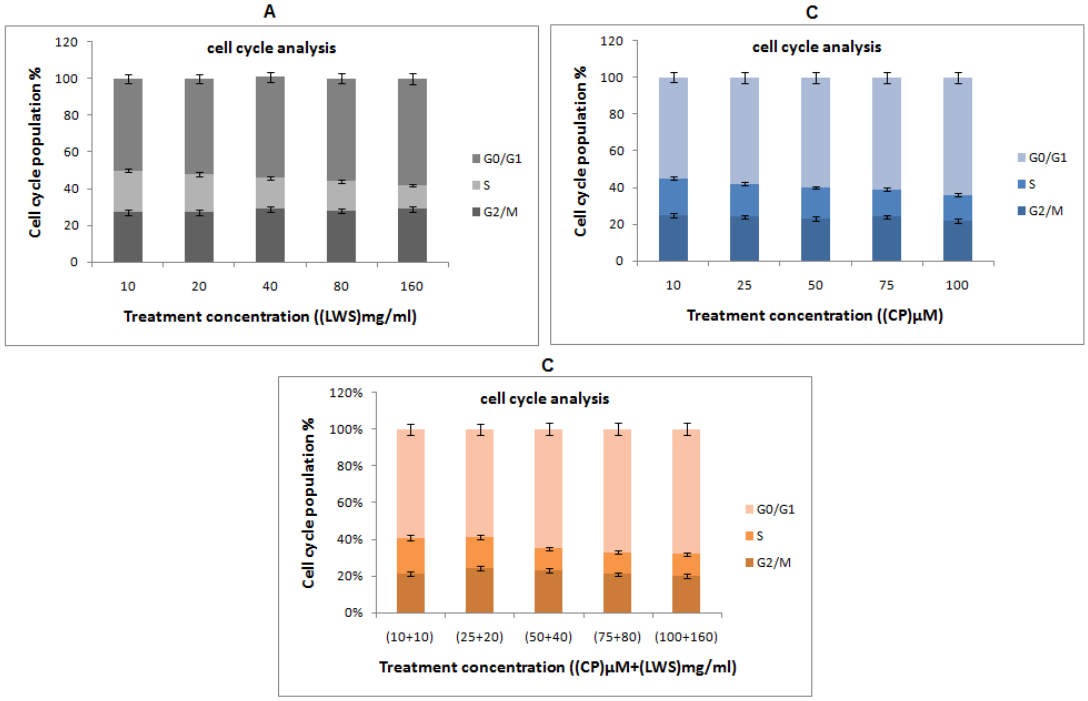

Figure 3: Results of Cell-cycle arrest in SKOV-3 ovarian carcinoma cells. The LWS, CP and CP+LWS treated cells were stained with propidium iodide then analyzed by flow cytometry. The data represented are the percentages of total cells from the means of three independent experiments and are expressed as means ± SD, $*P < 0.01$.

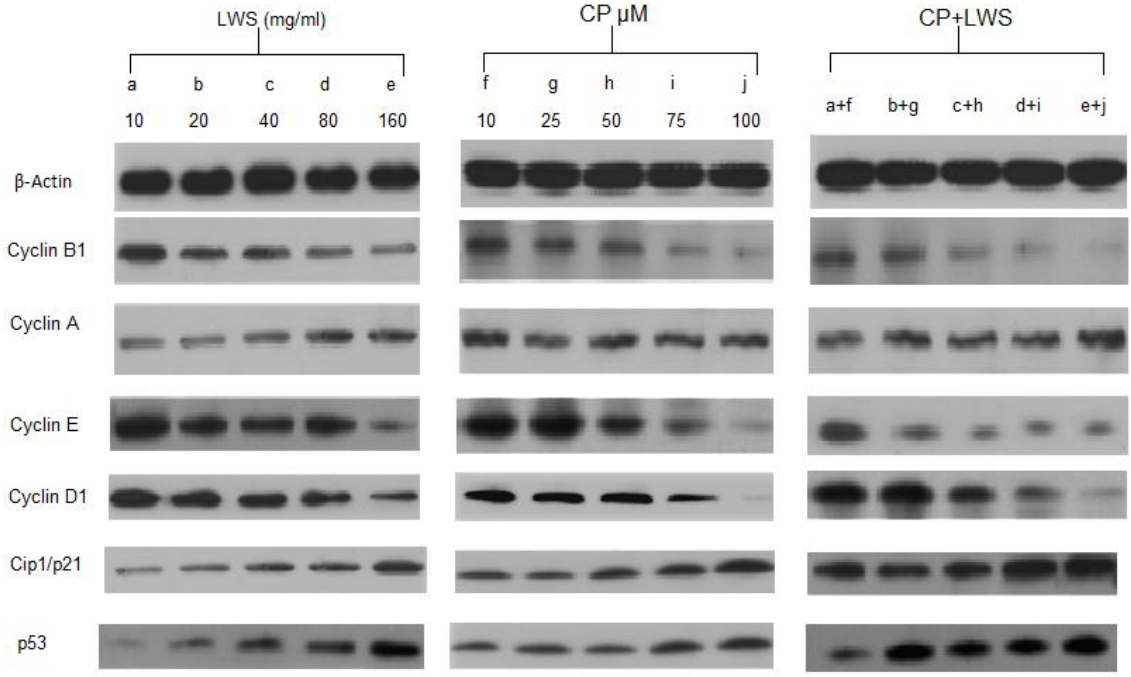

Figure 4: Results of immunoblotting of lysed cells after treatment of LWS, CP and combination CP+LWS. The proteins from treated cells were separated with the help of SDS-PAGE followed by immunoblotting to show expression of proteins as indicated, β-actin was used as loading control. The results of protein levels were evaluated using Image Lab software (Bio-Rad) recording the density of bands on the immunoblotting, the results were normalized with the reference band of β-actin.

Annexin V staining studies for cell apotosis

Presence of Phosphatidylserine on apoptotic cell surface was assessed in LWS, CP and CP+LWS treated SKOV-3 ovarian carcinoma cells by annexin V staining conjugated with FITC. It was found that the annexin V-positive SKOV-3 cell number increased with treatment of more than 20mg/ml of LWS and 10 µM of CP. However the results were highly significant in CP+LWS (100µM+160mg/ml) as compared to untreated cells (Figure 5). Subsequent immunoblotting of treated cells showed increase in levels of Procaspase 3 and decrease in expression of Bcl-2 under treatment with more than 40 mg/ml of LWS, 50µM of CP and 25µM+20mg/ml of CP+LWS (Figures 6). It was seen that Bax levels do not changed significantly whereas the substrate of caspase 3 i.e. PARP was found to be cleaved in cancerous SKOV-3 cells. It was found that Bax : Bcl-2 ratio increased under all treatments (Figure 6).

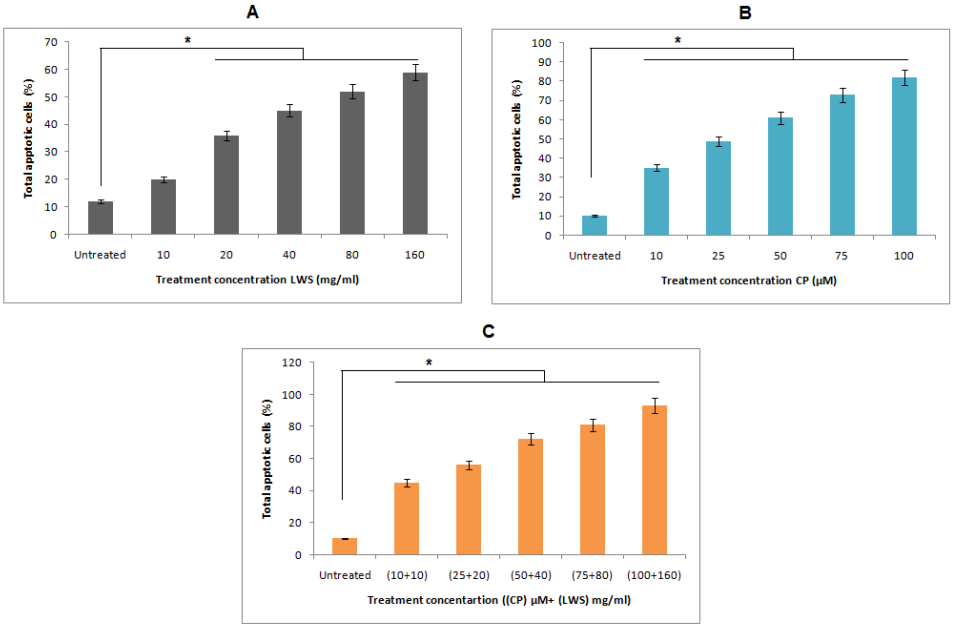

Figure 5: Effect of LWS, CP and CP+LWS induced apoptosis in SKOV-3 ovarian carcinoma. The treated cells were incubated and stained with Annexin V in conjugation with FITC followed by flowcytometry studies. The data reported are the mean of three independent experiments and are expressed as means ± SD. *$P < 0.01$. A: LWS, B: CP and C: CP+LWS treated cells

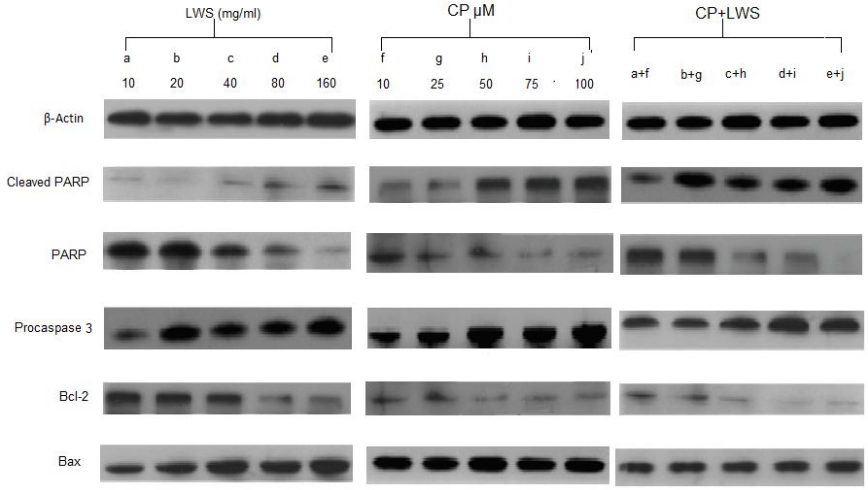

Figure 6: Results of immunoblotting studies, expression of proteins in LWS, CP and CP+LWS treated cells. The proteins from cell lysates were separated with aid of SDS-PAGE and transferred to PVDF membranes followed by immunoblotting to mark expression of proteins.

Discussion

Ovarian cancer remains one of the most occurring cancers globally in females. Treatment strategies for ovarian cancer involve use of chemo therapeutic agents which include platinum-based drug molecule such as Cisplatin (CP) with taxanes derivatives Paclitaxel (Taxol) or Docetaxel (Taxotere). The first line agent CP reacts mainly at the N7 position of guanine resulting in formation of variety of monofunctional and bifunctional adducts which further leads to formation of intrastrand or interstrand cross-links. This alterations lead to prevention of normal DNA function (Johnson et al. 2001; Eastman 1987). One of the major aspects of platinum based therapy is resistance; mechanisms indicate decreased accumulation of drug followed by drug inactivation via cellular proteins which often leads to increased dose causing severe side effects. Thus some more active agents are needed severely which could potentiate the efficacy of first line agents like CP without increasing the dose in treating ovarian cancer moreover the treatments should not damage normal cells and tissues also the agents need to be non toxic systemically. Phytochemicals from natural origin found in fruits, vegetables, spices and herbs or from any other natural source are assumed to be suitable for the purpose (Mishra and Vinayak 2015; Ruhul et al. 2009). Phytochemical compounds have been evaluated from number of years for their various pharmacological activities including anticancer due to their safety, deceased toxicity and easy in availability (Yang et al. 2011). These agents have been extensively used alone or in combination with chemotherapeutic agents for treating different types of cancer.

Study showed that all the three treatments i.e. LWS, CP and combinations of CP+LWS inhibited proliferation of cancerous SKOV-3 cell lines in a dose dependent manner. However it was found that LWS did not altered the growth of MRC-5 the normal cell line concluding a selective inhibitory role of LWS. The inhibition of proliferation on cancerous SKOV-3 cells was maximum with CP+LWS (100μM+160mg/ml). The study of cell apoptosis done by Hoechst assay on SKOV-3 cells suggested a higher rate of cell death in CP+LWS treated group as compared to CP and LWS alone, however the LWS treatment also resulted in apoptosis.

The inhibitory effect of LWS, CP and combination of CP+LWS was further evaluated and correlated with cell cycle analysis. Results of cell cycle suggested all three treatments caused arrest of cell cycle at the G1/G0 phase. The study was evidenced by elevation in levels of p53 and Cip1/p21 followed by suppressing expression of cyclin D1 and cyclin E, results were significant in combination treated cells as compared to LWS and CP alone treated. LWS with CP induced apoptosis which was evidenced by up-regulation of Bax:Bcl-2 ratio and the caspase 3 activity. Outcomes of present study conclude that LWS produced antiproliferative effect on ovarian carcinoma cells and potentiated the activity of CP.

Results of cell cycle analysis and immunoblotting study concluded that LWS alone and in combination with CP elevated the expression of the two important tumor suppressor proteins Cip1/p21 and p53 in cancerous cells. It has been reported theoretically that cells with vandalized DNA undergo a fresh start to synthesis of protein p53 which is accounted to be as tumor suppressor. The p53 protein leads to activation of Cip1/p21 expression for repairing DNA damage or activates expression of Bax for inducing apoptotic death of cells (Li et al. 2005).

The results of present study showed that treatment of LWS alone and in combination with CP increased the levels of p53 with increasing concentrations, confirming that LWS may induce DNA damage in SKOV-3 cell lines. The increased expression of Cip1/p21 in ovarian cancer cells was correlated to variation in expression of p53 and Bax levels. This can be considered as one of the reason leading to arrest in G1/G0 phase of cell cycle and ease the repair process of DNA damage (Weiss 2003). Cyclin D1 and cyclin E are reported to be the governing proteins of CDK4/6 activity which is responsible for inactivation of retinoblastoma proteins and lead to activation of E2F factor responsible for expression of DNA synthesis enzymes (Zhang et al. 1994). It was observed that LWS treatment alone and on combining with CP decreased levels of proteins cyclin D1, cyclin B1 and cyclin E in SKOV-3 cells, this suggested possible role of this phytochemical in G1/G0 phase arrest of cell cycle. Cyclin B1 found to be associated with cdc2, contributes to the entry of cells into mitosis, theoretically it has been documented that decreased levels of cyclin B1 guides to arrest of cells at the G2/M phase (Taylor et al. 2001). On the contrary results of study were not parallel to this theory, LWS alone do not exhibited a significant decrease in levels of cyclin B1 but when combined with CP showed a makeable decline in expression of protein cyclin B1.

According to literatures the predicted possible reason is elevated levels of cyclin B1 in G2/M phase cells and decreased levels in G1 or S phase of cells in cell cycle (Miyazaki et al. 2007). The results of cell cycle study suggest that the population of cells in G1/G0 phase was high hence leading to dilution of the cyclin B1. Hence results of immunoblotting and cell cycle analysis suggest rise of G1/G0 phase arrest of SKOV-3 cells mainly from up regulation of p53 and Cip1/p21 and secondly by suppressing the expressions of cyclinD1 and E.

The treatments of LWS along with CP showed apoptosis in SKOV-3 cells which was marked by cells with increased annexin V. The outcome may be due to decreased expression of the antiapoptosis protein Bcl-2. Mano et al., reported expression of Bcl-2 associated with chemo resistance in ovarian cancer (Mano et al. 1999). The results of immunoblotting carried for expression of Bcl-2 suggested decreased levels and increased Bax:Bcl-2 ratio in SKOV-3 cells treated with LWS and its combination. The study hence concludes that the antiproliferative effect of LWS and its

combination treatments with CP on SKOV-3 ovarian carcinoma cells is mainly due to p53/p21 arbitrated arrest of G1/G0 phase of cell cycle and Bax/Bcl-2 ratio caspase-3 caused apoptotic cell death.

In conclusion, present study demonstrated that LWS alone and in combination with CP inhibits cell proliferation in SKOV-3 ovarian carcinoma cells. The inclusion of LWS with CP potentiated the effect of platinum derivative CP. LWS inhibited ovarian SKOV-3 cancer cells primarily through arrest of G1/G0 phase in the cell cycle, via increasing the expression of proteins p53 and Cip1/p21 followed by decreasing the levels of two important proteins cyclin D1 and cyclin E. LWS was found to induce apoptosis via decreasing the levels of Bcl-2, improving Bax:Bcl-2 ratio and activation of caspase 3. Overall we found that LWS showed antiproliferative property and induced apoptosis alone and on combination potentiated the effect of cisplatin on ovarian SKOV-3 cancer cells, proposing its potentiality as a novel chemo-assisting agent in treating ovarian carcinoma.

Conflicts of Interest: The authors declare that this research presents no conflicts of interest.

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