The Pharma Innovation Journal 2018; 7(7): 1014-1019

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(7): 1014-1019

www.thepharmajournal.com
Received: 20-05-2018
Accepted: 23-06-2018

Kishore VP
Department of Life Science/Biological Sciences, Bangalore University, Jnanabharathi Campus, Bangalore, Karnataka, India

Puttaraju HP
Department of Life Science/Biological Sciences, Bangalore University, Jnanabharathi Campus, Bangalore, Karnataka, India

Correspondence
Kishore VP
Department of Life Science/Biological Sciences, Bangalore University, Jnanabharathi Campus, Bangalore, Karnataka, India

Evaluation of action of *Wolbachia* surface protein (WSP) on DNA fragmentation caused by ethanol toxicity in hepg2 cell line

Kishore VP and Puttaraju HP

Abstract
The aim of this study was to evaluate the action of *Wolbachia* Surface Protein (WSP) on DNA fragmentation in HepG2 cells exposed to Ethanol. DNA fragmentation in HepG2 cells was caused by exposure of HepG2 cells to Ethanol. Action of *Wolbachia* Surface Protein (WSP) on DNA fragmentation was evaluated by flow cytometric study of TUNEL assay using FITC-d UTP stain. Flow cytometric study of TUNEL assay analysis of DNA fragmentation using FITC-d UTP stain showed that Ethanol exposure caused DNA fragmentation in HepG2 cells whereas, WSP treatment to Ethanol exposed cells decreased the DNA fragmentation. Thus these observations showed that the treatment with *Wolbachia* Surface Protein (WSP) suppressed DNA fragmentation in Ethanol exposed HepG2 cells. Finally these findings indicate that *Wolbachia* Surface Protein (WSP) exhibits suppression of DNA fragmentation. We can conclude that *Wolbachia* Surface Protein (WSP) exhibits cytoprotective action against DNA fragmentation caused by Ethanol in HepG2 cells by decreasing the DNA fragmentation. This will be of importance for the treatment of Ethanol-associated liver diseases. Thus *Wolbachia* Surface Protein (WSP) with potential protective action, might be used as an effective therapeutic drug to treat Ethanol-associated liver diseases.

Keywords: DNA fragmentation, ethanol-associated liver diseases, flow cytometric, hepg2 cells, therapeutic drug, *Wolbachia* surface protein (WSP)

Introduction
Wolbachia surface protein (WSP) is the most copiously expressed protein of a Gram-negative endosymbiotic bacteria *Wolbachia*. Earlier report revealed that r WSP results in apoptosis of monocytes and not lymphocytes [1]. Another report by Bazzocchi *et al.,* has showed that WSP, a surface protein from the endosymbiotic bacteria of filarial nematodes bears anti-apoptotic activity by postponing apoptosis in human polymorphonuclear cells PMNs [2]. Fascinating outcome of the former research promoted us to investigate the action of WSP on DNA fragmentation in Ethanol caused toxicity of HepG2 cells. Though information exists on the action of WSP on different kinds of human cells but then the information on the action of WSP (derived from *Wolbachia* of *Exorista sorbillans*) on Ethanol caused toxicity with respect to flow cytometric examination of DNA fragmentation in HepG2 cells is unknown until now.
DNA fragmentation is one of the subsequent stages in apoptosis which effects due to the activation of endonucleases that break down the greater order chromatin structure into fragments of ~300 kb and then into smaller DNA pieces of about 50 bp in length during apoptosis [3]. DNA fragmentation is a hallmark of Apoptosis [4]. DNA fragmentation assay is one of the generally used assays for detecting Apoptosis [5]. Flow cytometric procedures such as DNA strand breaks (TUNEL Assay), determination of Externalization of Phosphatidylserine, Dissipation of Mitochondrial Transmembrane Potential, Caspase activation etc., can be utilized to find out Apoptosis [6]. Therefore, in this study we have examined and described the action of WSP on DNA fragmentation in Ethanol-caused toxicity of HepG2 cells by analyzing DNA fragmentation using flow cytometer.
Since WSP will be derived from *Wolbachia*, the action of WSP could differ depending on the nature of *Wolbachia* being utilized that which can rely on the source of *Wolbachia* and the action of WSP could also differ relying on the nature of the cells in which that the WSP gets in contact. Thus here in the present work *Wolbachia* surface protein (WSP), a protein of *Wolbachia* endosymbiont derived from *Exorista sorbillans* was utilized. HepG2 cells were selected and utilized for investigating the action of WSP on Ethanol caused toxicity in HepG2 cells as HepG2 cell lines are utilized for the investigation of cytotoxic and cytoprotective

substances [7]. Ethanol was utilized for causing toxicity in HepG2 cells since Ethanol is identified to cause toxicity in HepG2 cells [8].

Observing all the above points, we were interested to investigate the action of WSP (derived from *Wolbachia* of *Exorista sorbillans*) on DNA fragmentation in Ethanol caused toxicity of HepG2 cells. Thus in the present work, action of WSP on DNA fragmentation in Ethanol caused toxicity of HepG2 cells were investigated by flow cytometry.

In the present study, we determined the action of *Wolbachia* Surface Protein (WSP) on DNA fragmentation in HepG2 cells in response to Ethanol caused toxicity. To determine the action of *Wolbachia* Surface Protein (WSP), toxicity and DNA fragmentation in HepG2 cells was caused by exposing HepG2 cells to Ethanol. DNA fragmentation was analyzed by flow cytometry. DNA fragmentation was evaluated using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay based on terminal deoxynucleotidyl transferase (TdT) enzyme catalyzing the addition of FITC labeled dUTP (FITC-dUTP) nucleotides to 3′- hydroxyl (OH) termini of the fragmented DNA that are generated as a result of apoptosis [9]. Flow cytometric study of TUNEL assay analysis for labeling of DNA fragmentation using FITC-dUTP stain showed that Ethanol exposure caused DNA fragmentation in HepG2 cells whereas, *Wolbachia* Surface Protein (WSP) treatment to Ethanol exposed cells decreased the DNA fragmentation. *Wolbachia* Surface Protein's (WSP's) cytoprotective action was similar to that of Silymarin, a well-known hepatoprotective agent [10]. Thus these results showed that the treatment with *Wolbachia* Surface Protein (WSP) saved cells against DNA fragmentation by decreasing DNA fragmentation. Thus these observations show that *Wolbachia* Surface Protein (WSP) has the capacity to protect against DNA fragmentation in HepG2 cells exposed to Ethanol. These studies recommend that *Wolbachia* Surface Protein (WSP) with potential protective action, might be used as an effective therapeutic drug to treat Ethanol-associated liver diseases.

Materials and Methods

Chemicals and Materials

The APO-DIRECT Kit (Catalog No. 556381) was bought from BD Biosciences Pharmingen (Becton, Dickinson and Company BD Biosciences, San Jose, CA, USA).

Dulbecco's Modified Eagle's Medium (DMEM), Fetal Bovine Serum (FBS), L-glutamine, Penicillin, Streptomycin, Trypsin-EDTA and Phosphate Buffered Saline (PBS) were bought from Sigma Chemical Company, St. Louis, MO, USA. Cell culture flasks and culture plates were bought from Fisher Scientific (Pittsburgh, PA).

If not stated all other chemicals, reagents and materials mentioned in this article were bought from Sigma Chemical Company, St. Louis, MO, USA. Each one of the other chemicals stated in the present article were of the analytical grades.

Preparation of recombinant *Wolbachia* surface protein (rWSP) by cloning, expression and purification

Preparation of recombinant *Wolbachia* Surface Protein (rWSP) was necessary for investigating their action on DNA fragmentation in Ethanol caused toxicity of HepG2 cells.

The *Wolbachia* surface protein (WSP) of the *Wolbachia* from *Exorista sorbillans* was prepared in recombinant form by cloning, expression and purification as mentioned in our article (accepted, unpublished article – *In press*) [11] which is explained below.

The gene coding for the *Wolbachia* Surface Protein (WSP), got from genomic DNA taken out from *Exorista sorbillans*, was amplified through polymerase chain reaction (PCR) by using primers. Primer sequence of the forward primer utilized was 5' – CGA ATT CAT ATG GAT CCT GTT GGT CCA ATA AGT G – 3' and primer sequence of the reverse primer utilized was 5' – GCC TCG AGT CTA GAC CTA GAA ATT AAA CGC TAC – 3'. The forward primer and the reverse primer was added with NdeI and XhoI restriction enzyme sites correspondingly. Thus the amplified PCR product (WSP gene) got was further cloned into the pET 19b Expression vector. The pET 19b-WSP plasmid was shifted into the competent cells of *Escherichia coli* DH5-α. The shifted clones were checked for the existence of WSP gene by Electrophoretic mobility analysis. Delay in electrophoretic mobility was seen in the clones containing the WSP gene while compared with the clones devoid of WSP gene (data not provided). Delay observed in the electrophoretic mobility of the clones confirmed the existence of WSP gene. The existence of the WSP gene in the delayed clones were further checked and confirmed by PCR assay on the basis of amplification of the WSP gene using primers. Amplification of WSP gene by using primers confirmed the existence of WSP gene in the delayed clones (data not provided). Clones confirmed for the existence of WSP gene was shifted into *Escherichia coli* BL21 (DE3) and then the expression of the His-Tagged recombinant WSP (His-Tagged rWSP) was produced by 1 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG). After production, the cells were incubated for 4 hours at 37 °C, later 1.5 ml culture was taken and then centrifuged for 5 minutes at 6000 rpm. The cell pellet got was suspended in 50 µl of 1X TE and 5 µl of Cell Lysis buffer (contains 40 mg/ml Lysozyme along with 800 U/ml DNase and 24 U/ml RNase) was added. This was incubated for 30-60 minutes at 37 °C and centrifuged for 5 minutes at 8,000 rpm. To the pellet (insoluble protein) obtained, 6 M Urea was added. This produced and lysed culture, that is clones were checked for the expression by loading on 12% SDS PAGE (Sodium Dodecyl Sulphate Polyacryl Amide Gel Electrophoresis) gel and carrying out Sodium Dodecyl Sulphate Polyacryl Amide Gel Electrophoresis for 4 h at 100 V followed by staining and destaining (data not provided). Clones having very good expression was utilized for performing of Purification and Dialysis.

The recombinant His-tagged WSP expressed was purified by means of immobilized metal affinity chromatography (IMAC) using Nickel affinity absorption chromatography that is nickel- nitrilotriacetic acid (Ni-NTA) column. The cell pellet containing the recombinant protein was solubilized by means of sonication with 6 M Urea with sonication conditions of 30% ampt and 40 °C till the optical density (O.D) of cells attains 1/10th at 600 nm Absorbance. The soluble proteins were segregated by means of centrifugation for 20 minutes at 15,000 rpm at 4 °C and the supernatant was utilized for purification of the recombinant protein. Supernatant having the recombinant protein was purified by means of nickel-nitrilotriacetic acid (Ni-NTA) affinity column. Ni-NTA column was equilibrated by using Cell lysis buffer with 6 M Urea. The supernatant (soluble protein) was loaded on the pre-equilibrated column. Unbound protein was washed by the wash buffer comprising 10 mM Imidazole and the bound protein was eluted by using a step elution of Imidazole (an

imidazole gradient) (100 mM-300 mM Imidazole) in the Cell lysis buffer with 6 M Urea. The protein eluted is the purified protein. The purified protein was then dialyzed against 1X PBS buffer with 4 M Urea for 2 hours at 4 °C by using dialysis membrane. Buffer was replaced with new 1X PBS with 2 M Urea and dialyzed another time for 2 hours at 4 °C. Ultimate dialysis was carried out with buffer 1X PBS for 2 hours at 4 °C. After dialysis, the dialyzed protein was checked and confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) (data not provided). The dialyzed protein (*Wolbachia* Surface Protein) was shifted to a bottle and kept at 4 °C until further use.

The *Wolbachia* Surface Protein (WSP) was hence prepared by means of cloning, expression and purification (data not provided).

The recombinant *Wolbachia* Surface Protein (WSP) hence prepared was then used to investigate their action on DNA fragmentation in Ethanol caused toxicity of HepG2 cells.

HepG2 cell line and cell culture

HepG2 cell line used in the present study was got from the National Centre for Cell Science (NCCS) Pune, India. HepG2 cells were cultured and maintained in the cell culture flasks containing Dulbecco's Modified Eagle's Medium (DMEM) augmented with 10% Fetal Bovine Serum (FBS), Penicillin (100 units/mL), Streptomycin (100 µg/mL) and 2 mM L-glutamine at 37 °C in a CO_2 incubator containing 5% CO_2.

Treatment of cells

Cell stress was produced by culturing of HepG2 cells with Ethanol. Ethanol incubation of HepG2 cells were done after 12 h of seeding of cells. Cells were treated with 56.57 mM Ethanol (EtOH) for 24 h. Ethanol pretreated cells (56.57 mM Ethanol for 24 h) were treated with 150 µg/ml *Wolbachia* Surface Protein (WSP) and 250 µM Silymarin (SIL) separately for 24 h. Optimal concentration and time course of Ethanol, WSP and Silymarin were determined from preliminary experiments of MTT assay (data not provided). Treated cells were used for further experiments explained below.

Examination of DNA Fragmentation by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay using Flow cytometry

DNA fragmentation was examined by FACSCalibur Flow cytometer (Becton Dickinson (BD) Biosciences, San Jose, CA, USA) using an APO-DIRECT Kit (BD Biosciences Pharmingen) (Becton, Dickinson and Company BD Biosciences, San Jose, CA, USA) as per the instructions of the manufacturer. DNA fragmentation was examined using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay by labeling DNA fragments with FITC-dUTP, followed by flow cytometric analysis.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay determines DNA fragmentation on the basis of terminal deoxynucleotidyl transferase (TdT) enzyme catalyzing the addition of FITC labeled dUTP (FITC-dUTP) nucleotides to 3′- hydroxyl (OH) termini of the fragmented DNA that are generated as a result of apoptosis [9]. DNA fragmentation was examined by TUNEL assay using FITC-dUTP and PI stains. FITC-dUTP is used for labeling DNA breaks and PI is used for staining total DNA [9]. The DNA fragments were then analyzed by flow cytometry.

To evaluate DNA fragmentation, HepG2 cells were cultured in a 6-well plate at a density of 3×10^5 cells/2 ml and incubated in a CO_2 incubator for 24 h at 37ºC. The used medium was removed and washed by using 1ml 1X PBS. Thereafter the cells were incubated for 24 h with and without 56.57 mM Ethanol (EtOH) separately and later they were treated for 24 h with 150 µg/ml *Wolbachia* Surface Protein (WSP) and 250 µM Silymarin (SIL) separately. After treatment, the medium was taken away from all the wells, put into 12 x 75 mm polystyrene tubes and washed by 500 µl PBS (the PBS was preserved within the same tubes). Cells left untreated (UT) were used as control. The PBS was taken out, 180 µl of trypsin-EDTA solution was put and incubated in 37ºC for 3-4 minutes. The culture medium was put back into their particular wells, the cells were taken and put into the tubes. The tubes with the cells were subjected to centrifugation at 300 g at 25 ºC for 5 minutes. The supernatant was decanted carefully and the pellet was washed with PBS two times. The PBS was poured out entirely.

Cells were fixed by suspending cells in 1% (w/v) paraformaldehyde in PBS (pH 7.4) at a concentration of 1-2 x 10^6 cells/ml. The cell suspension was placed on ice for 30-60 minutes. The cells were centrifuged for 5 minutes at 300 g and the supernatant was discarded. The cells were washed in 5 ml of PBS, then the cells were pelleted by centrifugation. The wash and the centrifugation were repeated. The cell pellet was resuspended in the residual PBS in the tube by vortexing the tube gently. The cells were permeabilized with ice-cold 70% ethanol [at a concentration of 1-2 x 10^6 cells/ml in 70% (v/v) ice-cold ethanol]. The cells were let to stand for a minimum of 30 minutes on ice or in the freezer (-20 °C). Cells were stored in 70% (v/v) ethanol at -20 °C until use.

The cells were resuspended by swirling the vials. 1 ml aliquots of the cell suspensions (approximately 1 x 10^6 cells/ml) were removed and placed in 12 x 75 mm centrifuge tubes. The cell suspensions were centrifuged for 5 minutes at 300 g and the 70% (v/v) ethanol was removed by aspiration carefully without disturbing the cell pellet. Wash Buffer treatment was given to each tube of cells by resuspending with 1.0 ml of Wash Buffer (51-6548AZ) for each tube followed by centrifugation for 5 minutes at 300 g and removal of the supernatant by aspiration. The Wash Buffer treatment was repeated. Each tube of the cell pellet was resuspended in 50 µl of the DNA Labeling Solution (prepared by adding 10.00 µl Reaction Buffer, 0.75 µl TdT Enzyme, 8.00 µl FITC dUTP, 32.25 µl Distilled water for a total Volume of 51.00 µl for 1 Assay). The cells were incubated in the DNA Labeling Solution for 60 min at 37 °C. At the end of the incubation time, 1.0 ml of Rinse Buffer (51-6550AZ) was added to each tube and centrifuged each tube at 300 g for 5 minutes. The supernatant was removed by aspiration. The cell rinsing was done again with 1.0 ml of the Rinse Buffer, centrifuged and the supernatant was taken out by aspiration. The cell pellet was resuspended in 0.5 ml of the PI/RNase Staining Buffer (51-6551AZ) (The amount of PI/RNase Staining Buffer was decreased to 0.3 ml, if the cell density was low). The cells were incubated in the dark for 30 minutes at Room Temperature. The cells were analyzed in PI/RNase solution by FACSCalibur flow cytometry within 3 hours of staining (to avoid deterioration of cells if left overnight before analysis). Green fluorescence indicates TUNEL positive cells. The percentage of cells with fragmented DNA, stained by the TUNEL method in each sample was assessed.

Data analysis of DNA fragmentation by Flow cytometry

Flow cytometric (FACS) analysis of DNA fragmentation in HepG2 cells were carried out using FITC-dUTP stain by TUNEL assay using a FACSCalibur flow cytometer (Becton Dickinson (BD) Biosciences, San Jose, CA, USA). Data attainment was done and evaluated using FACSCalibur flow cytometer equipped with a 15 mW, 488 nm air-cooled argon-ion laser as the light source and Cell Quest Pro software (Becton Dickinson BD Biosciences, San Jose, CA, USA). Debris was gated out by establishing a region around the population of interest (samples). The gates were set for samples, based on light scattering properties in the forward light scatter (FSC) and the side light scatter (SSC). Cells were analyzed for each sample using a flow cytometer.

DNA fragmentation was examined by TUNEL assay using FITC-dUTP and PI stains. FITC-dUTP is used for labeling DNA breaks and PI is used for staining total DNA [9]. The green fluorescence (FITC-dUTP) and red fluorescence (PI) were examined using FACSCalibur flow cytometer by filtration using a 530 ± 30 nm emission filter on the FL1 detector for cells labeled with FITC-dUTP and a 585 ± 42 nm emission filter on the FL2 detector for cells labeled with PI. Then, an FL-2/FL-1 dot plot of green on the Y-axis and red on the X-axis was generated. Two dual parameter and two single parameter displays were created. The gating demonstration was the standard dual parameter DNA doublet differentiation display with the DNA Width on the X-axis and the DNA Area signal on the Y-axis. From this demonstration, a gate was plotted throughout the non-clumped cells and the second gated double parameter display was created. Display of DNA (Linear Red Fluorescence) on the X-axis and the FITC-dUTP (Log Green Fluorescence) on the Y-axis was set. Histograms were examined for FITC-dUTP stained cells. Histograms show log scale of fluorescence intensity on the X-axis and cell count on the Y-axis. The green and red fluorescence were measured using FACSCalibur flow cytometer. Green fluorescence indicates TUNEL positive cells.

Statistical analysis

Statistical analysis of all the data was done using the statistical software Graph Pad Prism 6.0 Software (Graph Pad Software, Inc., San Diego, CA, USA). Data were stated as mean ± Standard Deviation (SD) of three independent experiments. Data were statistically evaluated by Analysis of Variance (ANOVA) followed by Tukey's test. The values of p less than 0.05 (p<0.05) were regarded as statistically significant.

Results and Discussion

Analysis of action of *Wolbachia* Surface Protein (WSP) on DNA fragmentation in Ethanol exposed HepG2 cells by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) Assay using flow cytometry

In this study, *Wolbachia* Surface Protein's (WSP's) action on DNA fragmentation was evaluated by incubating HepG2 cells with and without 56.57 mM Ethanol (EtOH) separately for 24 h followed further by treatment with 150 µg/ml *Wolbachia* Surface Protein (WSP) and 250 µM Silymarin (SIL) separately for 24 h. Then the DNA fragmentation was examined using FITC-dUTP stain by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay using FACSCalibur flow cytometer (Becton Dickinson (BD) Biosciences, San Jose, CA, USA).

Cells were picked up for study by Cell Quest Pro software (Becton Dickinson (BD) Biosciences, San Jose, CA, USA) and determination of cells having DNA fragmentation, as the percentage of the whole cell population, was done. We examined the DNA fragmentation using FACSCalibur flow cytometer by means of detection of green and red fluorescence. The level of green fluorescence represented cells with DNA fragmentation.

We noticed that DNA fragmentation was minute in Untreated (UT) HepG2 cells. DNA fragmentation was seen notably in HepG2 cells after exposure to Ethanol (EtOH) for 24 h. *Wolbachia* Surface Protein (WSP) treatment for 24 h decreased DNA fragmentation in HepG2 cells exposed to Ethanol and also Silymarin (SIL) treatment for 24 h decreased DNA fragmentation in HepG2 cells exposed to Ethanol as assessed by TUNEL assay using flow cytometer (Fig. 1, 2, 3, 4) (Table 1) (Fig. 5).

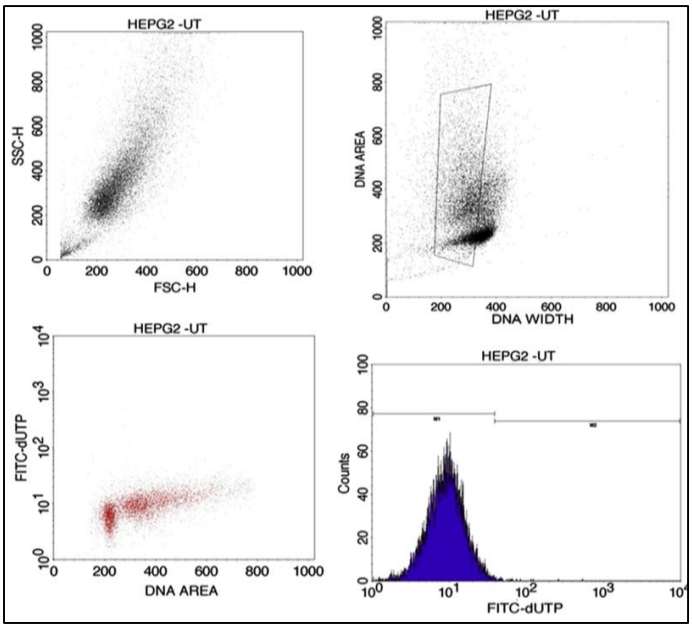

Fig 1: Representative figure of Flow cytometric evaluation result of DNA Fragmentation for the Untreated (UT) HepG2 cells Flow cytometric evaluation result shown is the representative of 3 independent experiments.

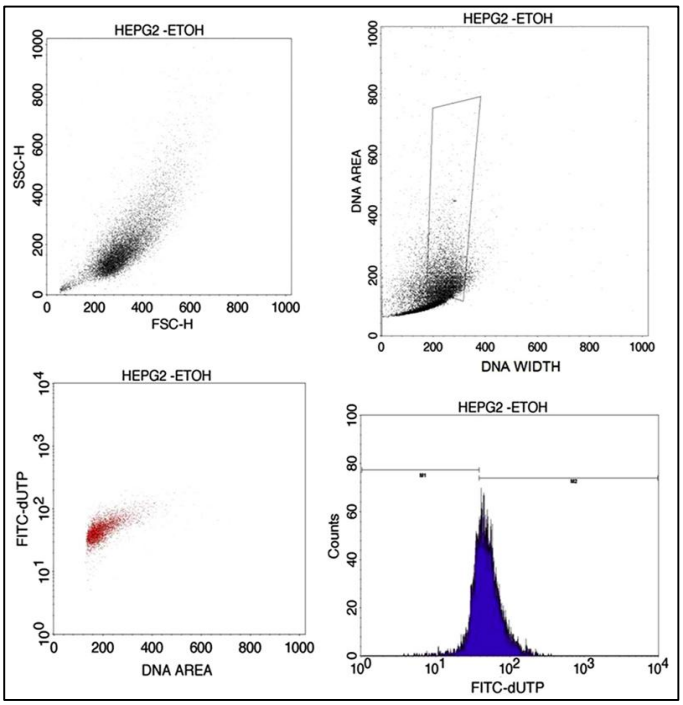

Fig 2: Representative figure of Flow cytometric evaluation result of DNA Fragmentation for Ethanol (EtOH) exposure to HepG2 cells for 24 h Flow cytometric evaluation result shown is the representative of 3 independent experiments.

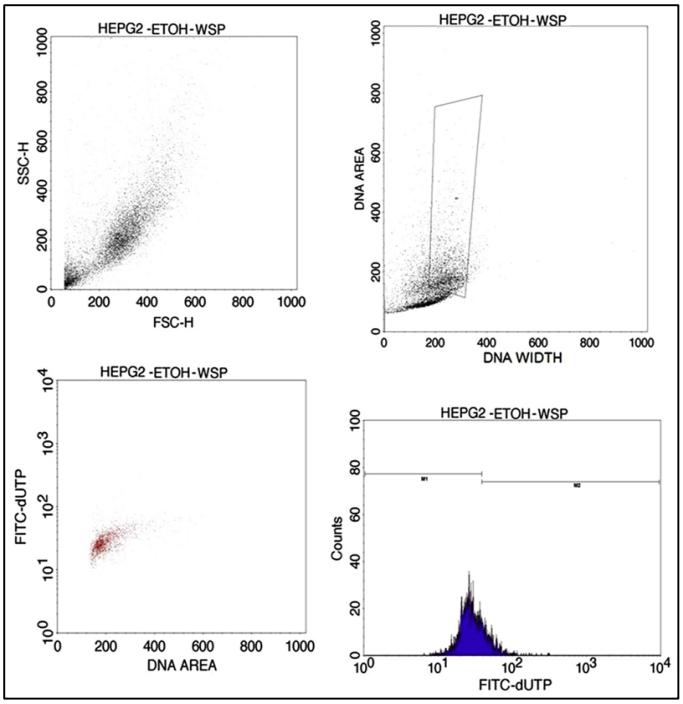

Fig 3: Representative figure of Flow cytometric evaluation result of DNA Fragmentation for *Wolbachia* Surface Protein (WSP) treatment to Ethanol-exposed HepG2 cells for 24 h Flow cytometric evaluation result shown is the representative of 3 independent experiments.

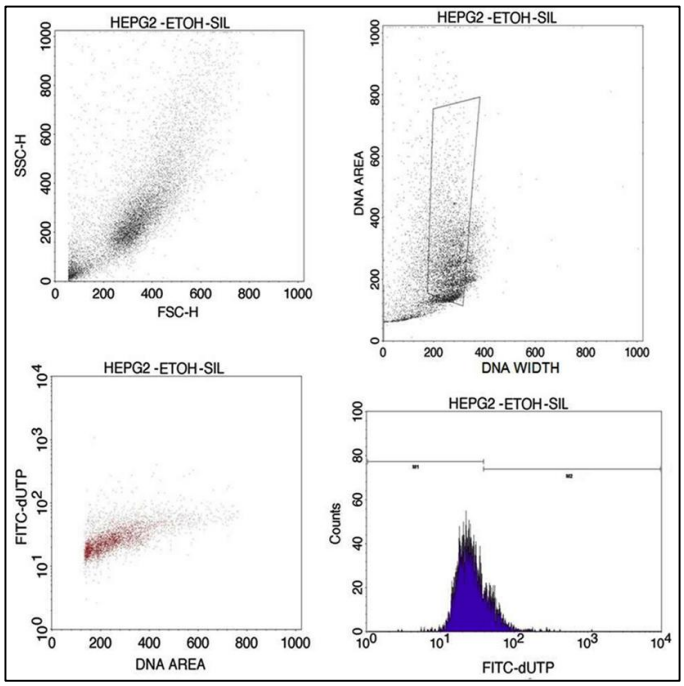

Fig 4: Representative figure of Flow cytometric evaluation result of DNA Fragmentation for Silymarin (SIL) treatment to Ethanol-exposed HepG2 cells for 24 h Flow cytometric evaluation result shown is the representative of 3 independent experiments.

Table 1: Data for percentage of DNA Fragmentation in HepG2 cells

Groups	Percentage of DNA Fragmentation
Untreated (UT)	0.4367 ± 0.05508
EtOH	73.17± 1.001
EtOH-WSP	24.96 ± 1.573
EtOH-SIL	22.44 ± 1.068

Values expressed are the mean ± Standard Deviation (SD) of three independent experiments.

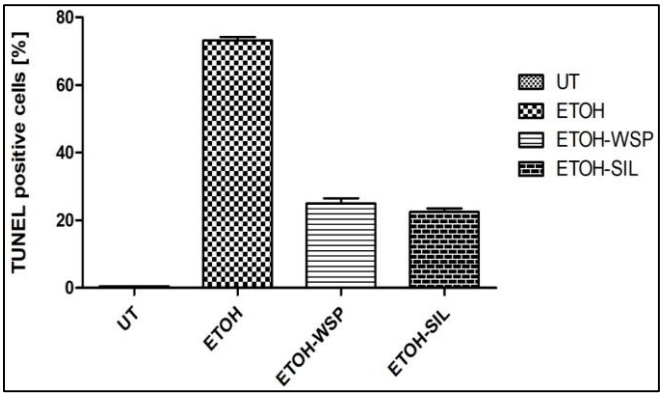

Fig 5: Graphical diagram of data for percentage of DNA Fragmentation in HepG2 cells Values plotted in the bar graph represents the mean ± SD of three independent experiments (n=3). Significance: EtOH was identified to be highly significant when compared to UT with a p-value of $p<0.0001$. EtOH-WSP and EtOH-SIL were found to be highly significant when compared to EtOH with the p-value of $p<0.0001$.

Flow cytometer analysis of TUNEL assay showed that *Wolbachia* Surface Protein (WSP) treatment effectively reduced Ethanol-caused DNA fragmentation in Ethanol exposed HepG2 cells. Therefore, this TUNEL assay data recommends that *Wolbachia* Surface Protein (WSP) has a protective effect against Ethanol-induced DNA fragmentation in HepG2 cells. The observations of the present study recommend that WSP might function as a cytoprotective agent.

The literature review revealed that *Wolbachia* Surface Protein (WSP) suppresses apoptosis in the human Polymorphonuclear cells (PMNs) [2]. But, there was no experimental report concerning the determination of protective action of WSP (of *Wolbachia* of Uzifly) against Ethanol caused DNA fragmentation in HepG2 cells using flow cytometery. These results demonstrated that WSP could defend HepG2 cells from DNA fragmentation and cytotoxic action of Ethanol. WSP shows similar action like that of Silymarin, a well-known hepatoprotective agent [10]. This study recommends that *Wolbachia* Surface Protein (WSP) might be used as an effective therapeutic drug to treat Ethanol-associated liver diseases.

Conclusion

This study revealed that WSP shows cytoprotective action against DNA fragmentation caused by Ethanol in HepG2 cells by reducing the DNA fragmentation. Thus this study recommends that *Wolbachia* Surface Protein (WSP) might be used as an effective therapeutic drug to treat Ethanol-associated liver diseases.

References

1. Haripriya Kalyanaraman, Kamalakannan Vijayan, Kirthika Sreenivas, Subash Babu, Rangarajan Badri Narayanan. Recombinant *Wolbachia* Surface Protein from *Brugia malayi* modulates Human Monocyte functions *in vitro.* International Trends in Immunity. 2014; 2(1):53-61.
2. Bazzocchi C, Comazzi S, Santoni R, Bandi C, Genchi C, Mortarino M. *Wolbachia* surface protein (WSP) inhibits apoptosis in human neutrophils. Parasite Immunology. 2007; 29(2):73-79.
3. Roy Walker P, Julie Leblanc, Brandon Smith, Siyaram Pandey, Marianna Sikorska. Detection of DNA Fragmentation and Endonucleases in Apoptosis. METHODS: A Companion to Methods in Enzymology 1999; 17:329-338.
4. Zbigniew Darzynkiewicz, Dariusz Galkowski and Hong Zhao. Analysis of apoptosis by cytometry using TUNEL assay. Methods. 2008; 44(3):250-254.
5. Susan Elmore. Apoptosis: A Review of Programmed Cell Death. Toxicol Pathol. 2007; 35(4):495-516.
6. Donald Wlodkowic, William Telford, Joanna Skommer and Zbigniew Darzynkiewicz. Apoptosis and Beyond: Cytometry in Studies of Programmed Cell Death. Methods Cell Biol. 2011; 103:55-98.
7. Volker Mersch-Sundermann, Siegfried Knasmuller, Xin-jiang Wu, Firouz Darroudi, Fekadu Kassie. Use of a human-derived liver cell line for the detection of cytoprotective, antigenotoxic and cogenotoxic agents. Toxicology. 2004; 198(1-3):329-340.
8. Neuman MG, Koren G, Tiribelli C. *In Vitro* Assessment of the Ethanol-Induced Hepatotoxicity on HepG2 Cell Line. Biochemical and Biophysical Research Communications. 1993; 197(2):932-941.
9. Mirella Nardini, Finkelstein EI, Reddy S, Valacchi G, Traber M, Cross CE *et al*. Acrolein-induced cytotoxicity in cultured human bronchial epithelial cells. Modulation by alpha-tocopherol and ascorbic acid. Toxicology. 2002; 170:173-185.
10. Feher J, Deak G, Muzes G, Lang I, Niederland V, Nekam K *et al*. Liver-protective action of silymarin therapy in chronic alcoholic liver diseases. Orv Hetil. 1989; 130(51):2723-2727.
11. Kishore VP, Puttaraju HP. Analysis of effect of *Wolbachia* Surface Protein (WSP) on Apoptosis and Mitochondrial Membrane Potential in Ethanol-induced toxicity of HepG2 cell line. International Journal of Pharmacy and Biological Sciences In press (accepted, unpublished article), 2018, 8(3).