WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 9, 984-1001. Research Article ISSN 2277– 7105

ANTI-NEOPLASTIC EFFECT OF *OCIMUM SANCTUM* METHANOL EXTRACT ON COLORECTAL CARCINOMA, GASTRIC CARCINOMA, HEPATOCELLULAR CARCINOMA & NORMAL CELLS

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Article Received on
04 June 2019,
Revised on 25 June 2019,
Accepted on 15 July 2019,
DOI: 10.20959/wjpr20199-15435

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ABSTRACT

The plant *Ocimum sanctum* (Holy Basil) is widely used in Indian traditional medicine as a broad spectrum medicine. This encouraged us to investigate the anti-cancer effect of the leaf of *Ocimum sanctum* in colorectal, gastric and hepatocellular carcinoma cell lines and delineate the apoptotic pathways. The cell cytotoxicity assay by MTT helped to evaluate the cytotoxic potential of the methanol extract *Ocimum sanctum* (OSME). To examine whether cell death occurred via apoptosis or necrosis, Annexin-V FITC double staining was done. Cell morphology and fragmented DNA was determined by fluorescence microscope and gel electrophoresis. The mitochondrial membrane potential assay was also performed by JC-1 staining. Cell cycle study helped to establish the phase distribution of the carcinoma as well as normal cells. OSME significantly inhibited the cell viability in a time and concentration dependent manner in all the carcinoma cells but in normal cells insignificantly inhibited. After treatment with the extract, hepatocarcinoma cells, showed several signs of apoptosis like chromatin condensation, nuclear fragmentation and formation of apoptotic bodies than control cells and degraded DNA bands in cancer cells. The flow cytometric analysis confirmed the presence of apoptotic cells in the early and late apoptotic stages whereas in normal cells apoptosis was not triggered. Cell cycle phase arrest was observed in the G0/G1 phase in only colorectal and hepatoma cancer cells. MMP shift

assay exhibited significant change in the OSME treated cells only in colorectal and hepatocellular carcinoma cells. These finding suggest that methanol extract *Ocimum sanctum* (OSME) possesses anti-neoplastic effects via apoptosis not necrosis without toxicity. Further mechanistic studies are going on to determine the different pathways contributing to apoptosis.

KEYWORDS: *Ocimum sanctum*, Colon, Hepatocellular, Carcinoma, Apoptosis.

INTRODUCTION

Cancer is a group of more than 100 different diseases. Cancer begins when genetic changes interfere with this orderly process. Some cancers may eventually spread into other tissues. Cells start to grow uncontrollably that can develop almost anywhere in the body. This escape from normal control mechanisms leads to the six hallmarks of cancer, which include sustaining proliferative signalling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis.[1] Colon carcinoma is the third most common cancer worldwide and the fourth most common cause of death.[2] Colorectal cancer is one of the most common cancer in people over 50 years both in the developed and less developed countries. It is estimated that worldwide over 862000 deaths occurred in the year of 2018 due to colorectal cancer (Global Health Estimates, WHO 2018).[3] Gastric cancer is the fourth most common cancer and the second leading cause of cancer-related death worldwide.[4,5] In 2000, about 880000 people were diagnosed with gastric cancer and approximately 650000 died of the disease.[6] Hepatocellular carcinoma (HCC), or hepatoma accounts for more than 90% of all cases of primary liver cancer.[7] It is the sixth most common type of cancer worldwide and has shown significant increase in its incidence, becoming third leading cause of cancer-related mortality.[8] Drug discovery from medicinal plants has played a very important role in the treatment of cancer. Tulsi has also been used in treatment of fever, bronchitis, arthritis, convulsions etc. However, very few chemical constituents and pharmacological activities have been reported on this species. Different parts of this plant have been reported to exhibit several medicinal properties.[9,10] Pharmacological properties like anabolic, hypotensive, cardiac depressant, smooth muscle relaxant, anti-fertility and anti-stress activity of this plant have been reported.[11,12] Administration of aqueous and ethanolic extracts of *Ocimum sanctum* to mice bearing Sarcoma-180 solid tumors mediated a significant reduction in tumor volume and an increase in lifespan.[13] *Ocimum sanctum* induces apoptosis in A549 lung

cancer cells and suppresses the *in-vivo* growth of Lewis lung carcinoma cells.[14] Reducing toxicity of chemo and radio therapies and providing better and healthier life style by Tulsi.[15] Therefore, we evaluated the effect of *Ocimum sanctum* methanol extract (OSME) against three types of colorectal carcinoma, one type of gastric adenocarcinoma, two types of hepatocellular carcinoma and two types of normal cells by using SW480, HCT116, HT29, AGS, Huh-7, HepG2 HEK293T and RAW264.7 cell lines respectively.

MATERIALS AND METHODS

Chemicals

DMEM, RPMI1640 medium, Trypsin (Gibco, USA), Fetal Bovine Serum(FBS), Penicillin-streptomycin (Biowest, Germany), Gentamycin (Nicholas, India), HEPES, L- glutamine, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)], Acridine orange, Ethidium bromide, RNAse, JC-1 dye, Propidium iodide (Sigma), Agarose (Puregene), Ethylene diamine tetra acetic acid (EDTA), Proteinase k (SRL), DMSO (dimethylsulphoxide),Chloroform, isoamyl alcohol, Methanol (Merk), and all other chemicals and reagents were of analytical grade and procured locally.

Cell culture

SW480, HCT116 and HT29 (Colorectal carcinoma), AGS (Gastric adenocarcinoma) Huh-7, and HepG2(Hepatocellular Carcinoma), Hek293T (Normal Human Kidney Transgenic) and Raw264.7 (Murine Macrophage) cells were obtained from National Centre for Cell Science, Pune, India. The cells were cultured and routinely maintained in DMEM and RPMI1640 medium medium and the medium was supplemented with 10% heat inactivated fetal bovine serum (FBS), penicillin (100 units/ml), streptomycin (100µg/ml), gentamycin (100µg/ml) and were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 inside a CO_2 incubator. All cell lines were adherent in nature.

Collection, Extraction and preparation of Test Sample

The leaves of *Ocimum sanctum* were collected from the nursery of Ramakrishna Mission Ashram, Narendrapur. The plant was identified by Dr. K. Karthigeyan, scientist C, Central National Herbarium, Indian Botanical Garden, Howrah, India.

Leaves of *Ocimum sanctum* were collected, shade dried and grinded into fine dust. 200gm of *Ocimum sanctum* leaf powder was taken in a conical flask and soaked in 500ml hexane for 3 days with occasionally shaking for removal of fat. After 3 days, the mixture was filtered and

the filtrate was evaporated by Rotary evaporator. After evaporation 2gm of hexane extract was obtained and was stored in air tight container. After removal of fat from *Ocimum sanctum* leaves, it was soaked in 3x500 ml of methanol for one week with occasional shaking. The mixture was filtered and the filtrate was evaporated by Rotary evaporator. After evaporation 3.4gm of sticky methanolic extract was obtained finally and designated as OSME and kept in air tight container at 4^0C. Stalk solution was prepared as 1mg/ml in PBS from here desired concentrations (25,50, 100,200 µg/ml) was used for *in-vitro* experiments.

Detection of Cytotoxicity by MTT assay

SW480, HCT116, HepG2, Hek293T and Raw264.7 cells ($1x10^5$) were separately seeded in 96-well sterile plates for 24, 48 and 72 hrs, whereas HT29, Huh-7 and AGS cells were treated for 24 and 48hrs respectively. All the treated cells were grown in humidified atmosphere containing 5% CO2 in an incubator at 37℃ and the untreated cells were considered as control. After desired incubation 20µl of MTT (4-5mg/ml in PBS as a stock solution) was added to each well and incubated again for 3 to 4hrs at 37℃. The MTT assay is a colorimetric assay for assessing the metabolic activity of the cells or cell viability of NADPH dependent cellular oxidoreductase enzymes, and represents number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT, which is yellow in colour, to insoluble purple colored formazan. The intensity of the colour was measured at 570nm by micro-plate manager (Reader type: Model 680XR Bio-Rad Laboratories Inc.). The IC_{50} values were determined for the all the carcinoma cells.

Detection of cell morphological by Fluorescence Microscope

Huh-7 and HepG2 cells($1x10^6$) were treated with IC_{50} of OSME for 24 h were observed using a fluorescence microscope for morphological changes. The untreated control cells and the OSME treated cells were harvested separately, washed with PBS and then stained with acridine orange (100 µg/ml) and ethidium bromide (100 µg/ml) (1:1). The cells were then immediately mounted on slides and observed under a fluorescence microscope in (Olympus, Fluoview FV10i) at 60x. for the morphological determination of the cells undergoing apoptosis.

Detection of DNA Fragmentation by Agarose Gel Electrophoresis

SW480, Huh-7, HepG2, cells($1x10^6$) were treated with IC_{50} dose but Hek293T and Raw264.7 cells treated with 100 µg/ml dose of OSME for 24 h. The cells were harvested and washed

twice with PBS. The cells were resuspended in 500 µl of lysis buffer (50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 0.5% SDS), 100 µg/ml of proteinase K was added and incubation was done at 55 °C for 1 h and 37 °C overnight respectively. DNA extraction was done by following the general phenol-chloroform extraction procedure and kept at -20 °C overnight. After centrifugation, DNA precipitates were washed with 70% chilled ethanol, dried and evaporated at room temperature and dissolved in TAE buffer (pH 8.0) at 4 °C overnight. To detect the DNA fragments, the isolated DNA samples were electrophoresed overnight at 20 V in 1% agarose gel and stained with ethidium bromide. DNA fragmentation was observed in UV transilluminator. (GENEI, Bangalore Genei Pvt. Ltd.).

Detection of mitochondrial membrane potential (Δψm) assay

SW480, Huh-7, HepG2, Hek293T and Raw264.7 ($1x10^6$) cells were treated with OSME with desired dose and untreated as control for 24 hours to assay the mitochondrial membrane potential activity of cell in a flow cytometer. Cell were washed with PBS, pelleted down and eventually stained with JC-1 stain. The sample were incubated at 37ºC for 15 min. Shift in the mitochondrial membrane potential was determined by FACS (Becton Dickinson FACS Fortessa 4 leaser cytometer), Fluorescence detector equipped with 520 nm argon laser light source and 623 nm band pass filter (liner scale) with the help of BD FACS Diva software (Becton Dickinson).

Detection of Apoptosis by Flow Cytometric analysis

In order to investigate the type of cell death induced by OSME, flow cytometric analysis was done by performing dot plot assay. SW480, Huh-7, HepG2, Hek293T and Raw264 cells ($1x10^6$) were treated with desired dose of OSME for 24 h. The cells were pelleted down, centrifuged at 2000 rpm for 8 min at 4 °C and washed with Annexin V FITC binding buffer (10 mM HEPES, 140 mM NaCl and 2.5 mM CaCl2 2H2O; pH 7.4). Again after centrifuging at 2000 rpm at 4 °C, the cell pellets were dissolved in Annexin V FITC binding buffer containing annexin V FITC and propidium iodide. After 15 min incubation in dark at room temperature flow cytometric analysis was done. All data were acquired with a Becton-Dickinson FACS Caliber single laser cytometer. Flow-cytometric reading was taken using 488 nm excitation and band pass filters of 530/30 nm (for FITC detection) and 585/42 nm (for PI detection). Live statistics were used to align the *X* and *Y* mean values of the Annexin-V FITC or PI stained quadrant populations by compensation. Data analysis was performed with Cell Quest (Macintosh platform) program.

Detection of Cell Cycle Arrest by Flow Cytometric Analysis

In order to study the stage of cell cycle arrest in flow cytometry, SW480, Huh-7, HepG2, Hek293T and Raw264.7 cells ($1x10^6$) were treated with desired of OSME for 18 h. Cells were washed with PBS, fixed with cold methanol by adding methanol drop-wise and kept at -20 °C for 3 min. They were then resuspended in cold PBS and kept at 4 °C for 90 min. Cells were pelleted down, dissolved in cold PBS, treated with RNase A for 30 min at 37 °C and stained with propidium iodide (20 µl from 50 µg /ml) and kept in dark for 15 min. Cell cycle phase distribution of nuclear DNA was determined on BD FACS Diva software (Becton Dickinson FACS).

Statistical Analysis

Percentage of cell growth inhibition was calculated by the following formula: % Cell Inhibition= 10 X (O.D of Control –O.D. of treated /O. D. of Control), O. D= Optical Density.

Percentage of cell viability was calculated as follows: Viable Cells (%) = (Total number viable cells per ml/Total number of cells per 1ml) x100.

RESULTS

Cytotoxicity study by MTT assay

Treatment with OSME by MTT assay showed significant reduction in the O.D values in the colorectal carcinoma cells SW480, HCT116, HT29, hepatocellular carcinoma cells HepG2 and Huh-7 cells along with AGS gastric carcinoma cells in a time and concentration dependent manner. HEK293T and RAW264.7 cells exhibited insignificant reduction in the O.D values. These observations provided proof for cytotoxic nature of OSME towards only on all the carcinoma cell lines. The IC50 value of OSME treatment for 24hrs was calculated to be 15.81µg for SW480, 93.3 µg for HCT116 and 70.0µg for HepG2 cells. Whereas 48 hrs OSME treatment yielded an IC50 value of 91.21µg for HT29, 47.39µg for AGS and 91.75µg for Huh-7 cells respectively.

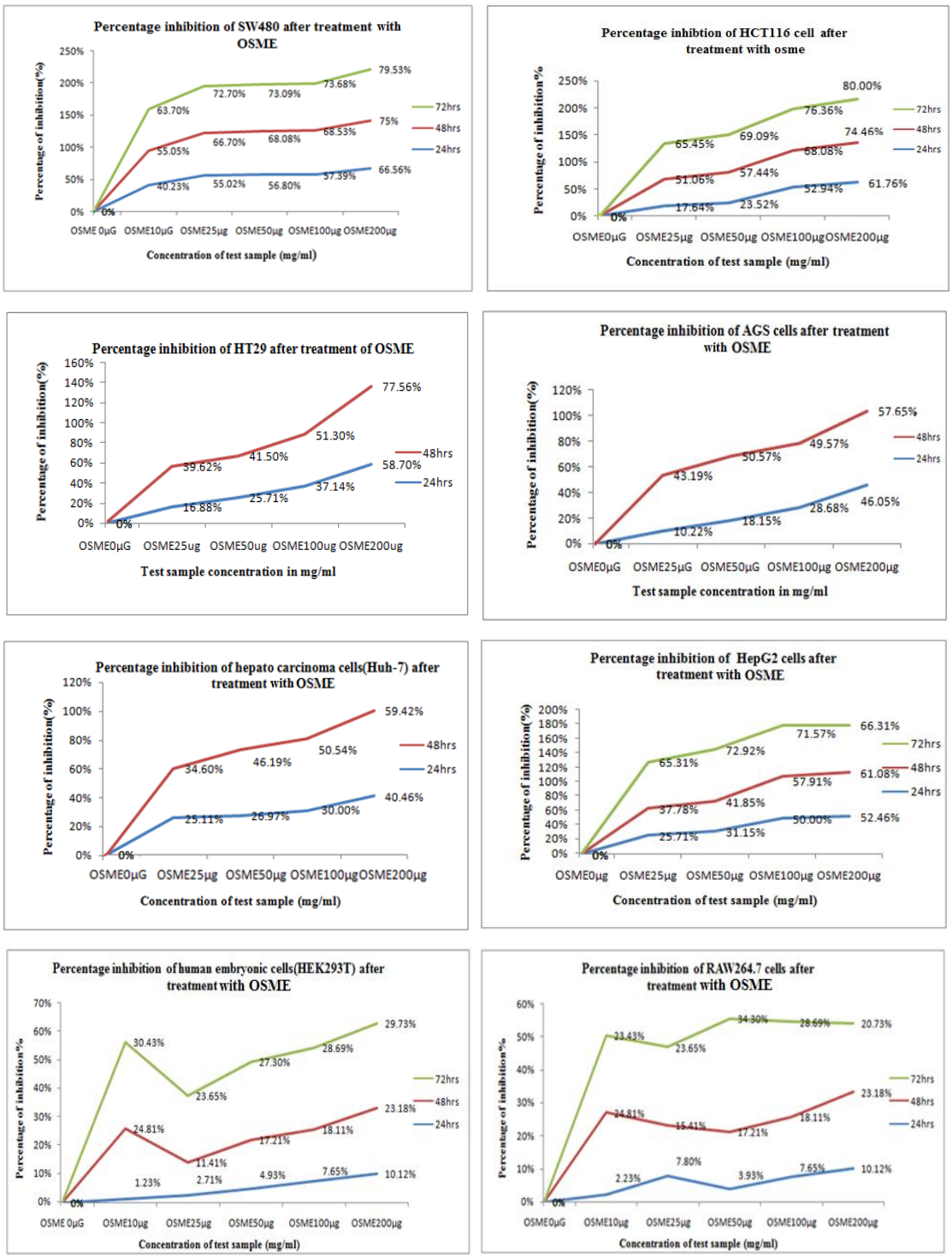


Fig 1: All the line graph showing the percentage inhibition of viable cells in SW480, HCT116 and HepG2 cells treated with OSME for 24, 48,72hrs and HT29, AGS and Huh-7 cells were treated for 24 and 48 hrs. The OSME treated cells were significantly increases the % inhibition in a time and concentration dependent manner. The IC_{50} value of OSME were calculated for SW480 15.81µg, HCT116 93.3 µg and HepG2 91.21 µg in 24 hrs and HT29 91.21µg, AGS 47.39µg and Huh-7 91.75 µg for 48hrs respectively.

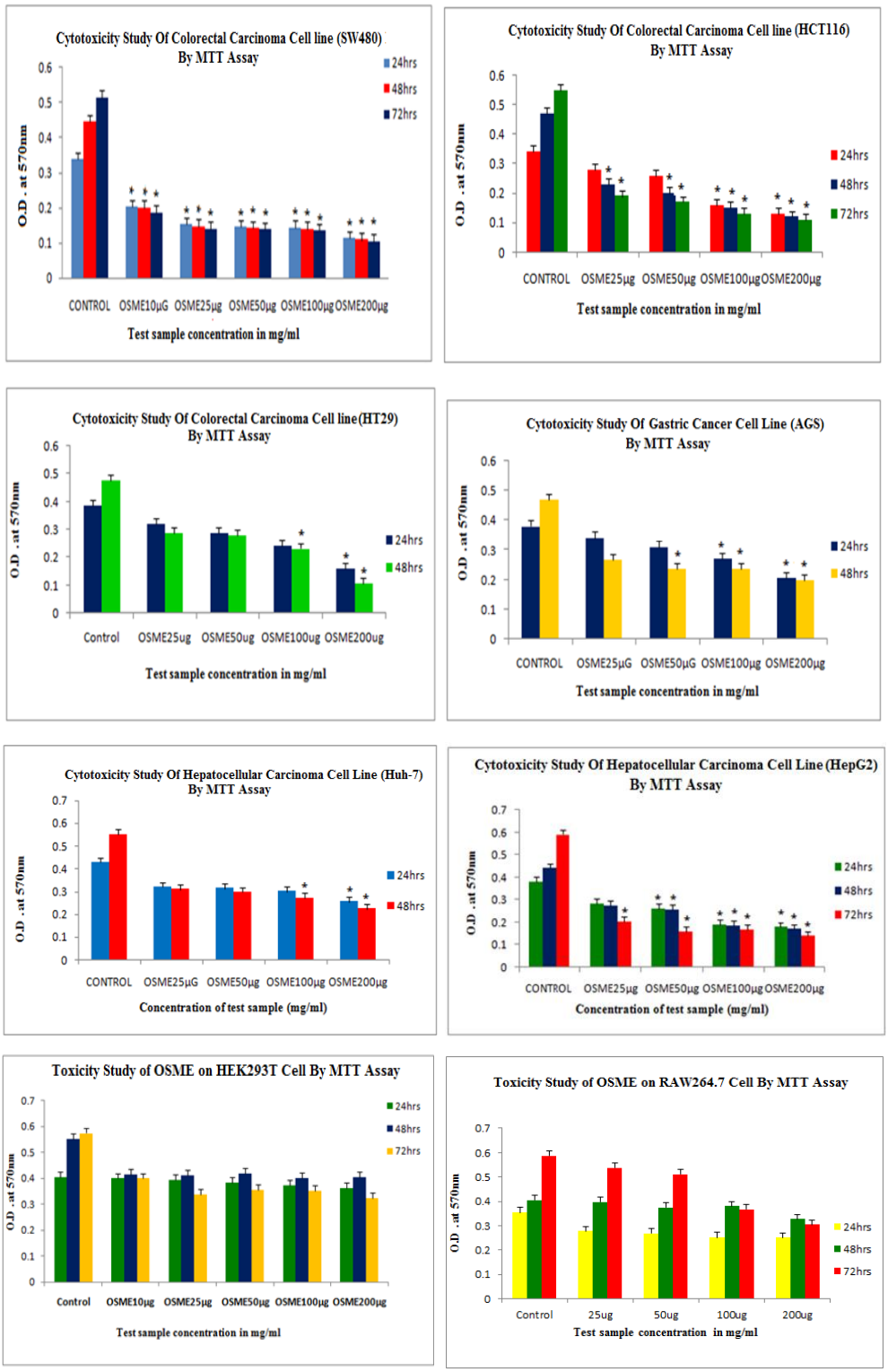


Fig.2: Histogram Showing the O.D. values of SW480, HCT116, HT29, AGS, Huh-7, HepG2, Hek293T and Raw264.7 cells treated with OSME. OSME treated all the carcinoma cells showed significant reduction in O.D. value in a time and concentration dependent manner whereas in normal cells showed insignificant reduction in the O.D. values up to 72 hrs. Data are mean ± S.E.M.

Detection of cell morphological by Fluorescence Microscope

Huh-7 and HepG2 cells were treated with OSME and stained with both acridine orange and ethidium bromide. OSME treated cells confirm the presence of early and late apoptotic cells as compared to the untreated control cells. In this study nuclear changes were observed including chromatin condensation and apoptotic body formation that are the indication of apoptotic processes.

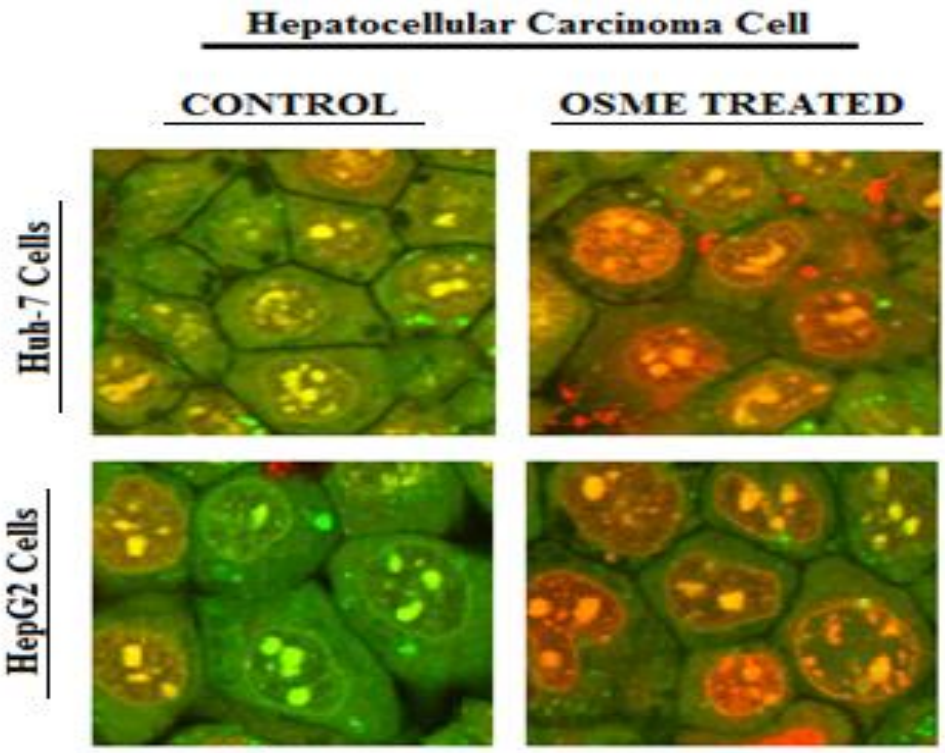


Fig 3: Fluorescence microscopic images of both the hepatocellular carcinoma cells untreated control and OSME treated with IC50 dose. The control cells give a bright green fluorescence whereas the treated cells show an orange-red colour, demarking the occurrence of Apoptosis in hepatocellular carcinoma, Huh-7 and HepG2 cells.

Detection of DNA Fragmentation by Agarose Gel Electrophoresis

Agarose gel electrophoresis of the DNA samples isolated from untreated and control SW480, Huh-7 and HepG2 cells showed intact DNA bands, whereas OSME treated SW480, Huh-7 and HepG2 cells showed degraded DNA bands in the form of ladders. Whereas the gel pattern of the DNA samples isolated from OSME treated Raw264.7 showed no change. So, the observations confirmed that the treatment with OSME in all the three cells caused apoptosis.

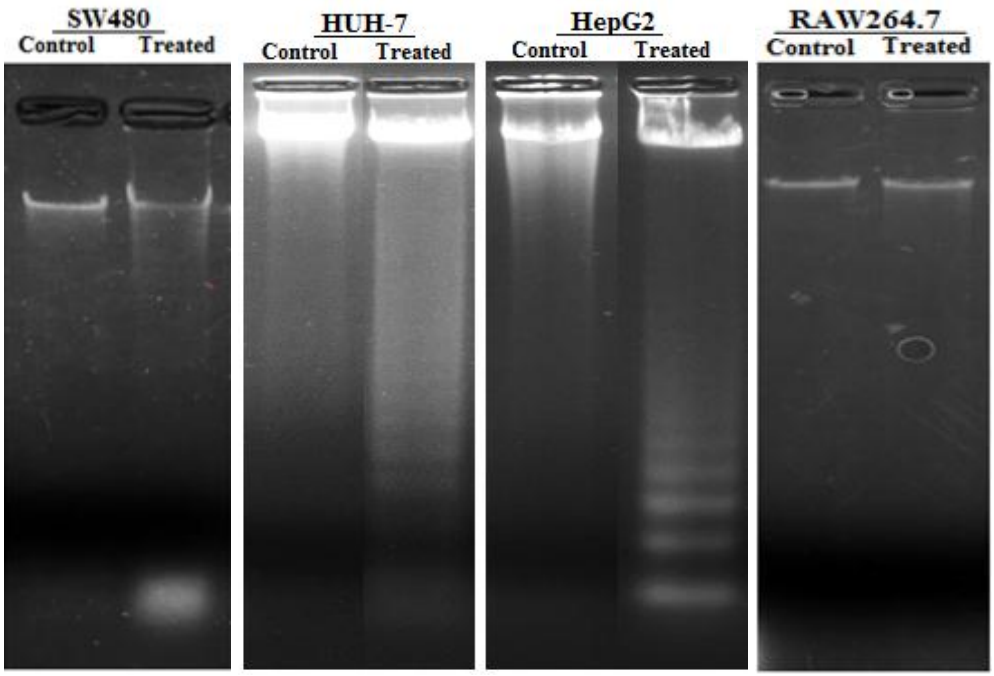


Fig.4: DNA fragmentation by agarose gel electrophoresis in SW480, Huh-7, HepG2 and Raw264.7 cells. Lane 1 represent control cells which shows intact DNA whereas Lane 2 represent OSME desired dose treated cells in which DNA fragments are clearly visible in carcinoma cells but in Raw264.7 cells shows intact DNA like control cells.

Detection of mitochondrial membrane potential (Δψm) assay

Mitochondrial dysfunction is an essential target for induction of apoptosis. The colorectal cancer (SW480), hepatocellular carcinoma (Huh-7and HepG2) and normal (Hek293T & Raw264.7) cell lines when treated with desired dose of OSME, showed a loss of Mitochondrial Membrane Potential (Δψm). The JC-1 stain cannot accumulate in the mitochondria of the apoptotic cells, as the mitochondrial membrane potential collapses, hence showing green fluorescence (P4) denotes apoptotic cells and red fluorescence (P3) denoting healthy cells where JC-1 stain accumulates. Depolarization in mitochondrial membrane potential was observed by staining untreated and treated cell by JC-1 dye. The depolarization led to a transmembrane shift from red to green fluorescence leading to release of cytochrome c. A significant transmembrane shift of 12.4% to 45.9%, 15.7% to 52.9% and 15.8% to 57.1% were observed when SW480, Huh-7and HepG2 cells were treated with the IC_{50} value of OSME for 24 hours respectively. But an insignificant transmembrane shift of 14.6% to 20.1% and 9.0% to 3.0% were observed when HEK293T and RAW264.7 were treated with the desired dose of OSME for 24 hours.

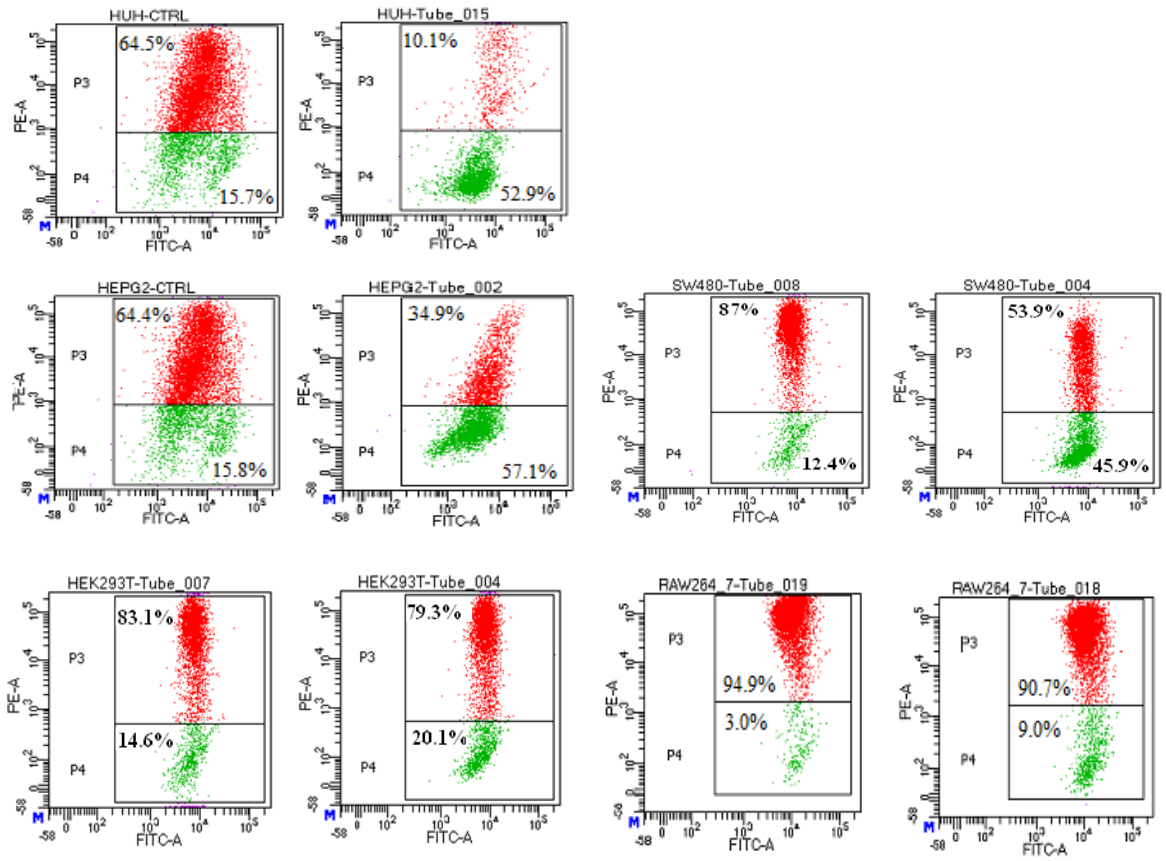


Fig.5: Flow cytometric analysis of mitochondrial membrane potential (Δψm) on SW480, Huh-7, HepG2, HEK 293T and RAW264.7 cell line in both control and OSME treated cells respectively after 24 hrs of treatment. Transmembrane shift was significantly increases in SW480, Huh-7 and HepG2 cells whereas in both the normal cells insignificant increase observed when treated with desired dose of OSME.

Detection of apoptosis by flow cytometry

In the flow cytometric analysis, double labeling technique, using Annexin V-FITC and propidium iodide, was utilized. Lower left (LL) quadrant (Annexin V-/PI-) is regarded as the population of live cells, lower right quadrant (LR) (Annexin V+/PI-) is considered as the cell population at early apoptotic stage, upper right (UR)quadrant (annexin V+/PI+) represents the cell population at late apoptotic stage. Flow cytometric data analysis revealed that after 18 h of treatment with desired dose of OSME for quantification of apoptosis was observed for SW480 0.096% against 22.1%, for Huh-7 0.7% against 36.7%, HepG2 8.4% against 18.8%, cells were in upper right quadrant which implies apoptotic cells thereby, showing apoptotic inducing property of OSME on colorectal and hepatoma cells. In Normal cells HEK293T 8.4% against 11.9% and Raw264.7 2.4% against 3.6% cells were in upper right quadrant does

not implies apoptotic cells thereby, OSME showing non-apoptotic inducing property towards both the normal cell lines.

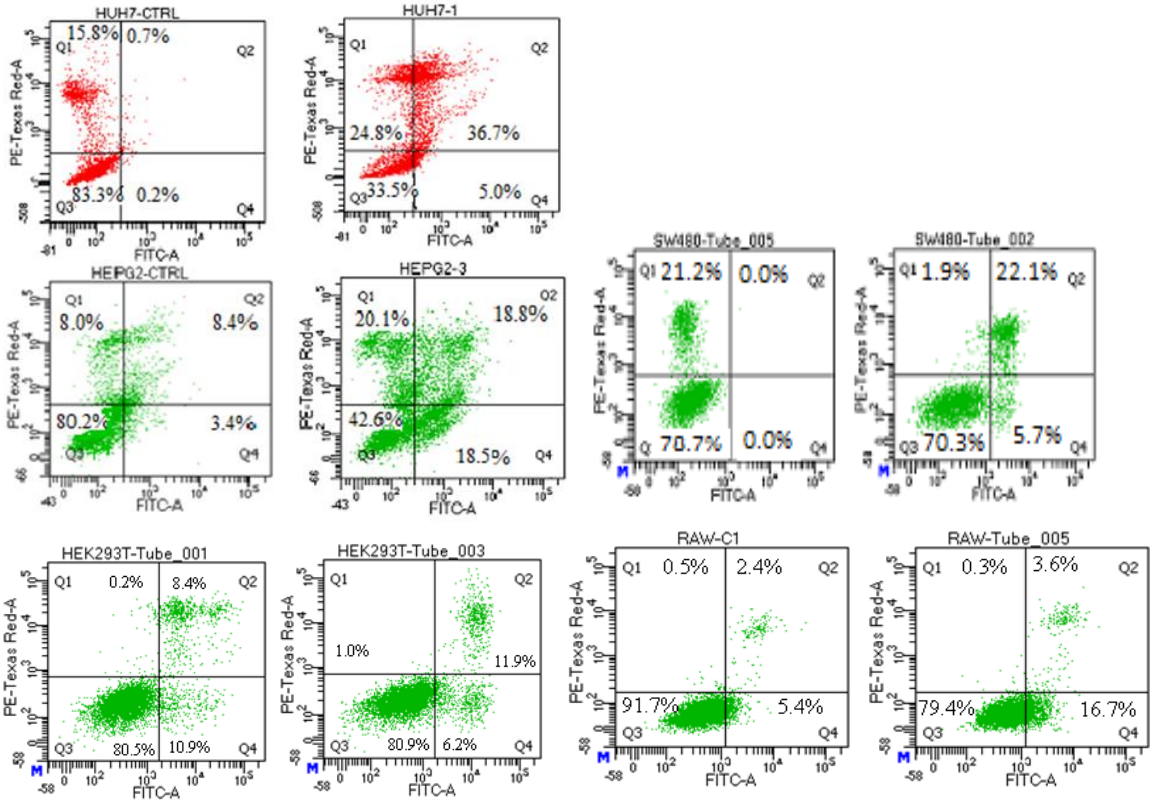
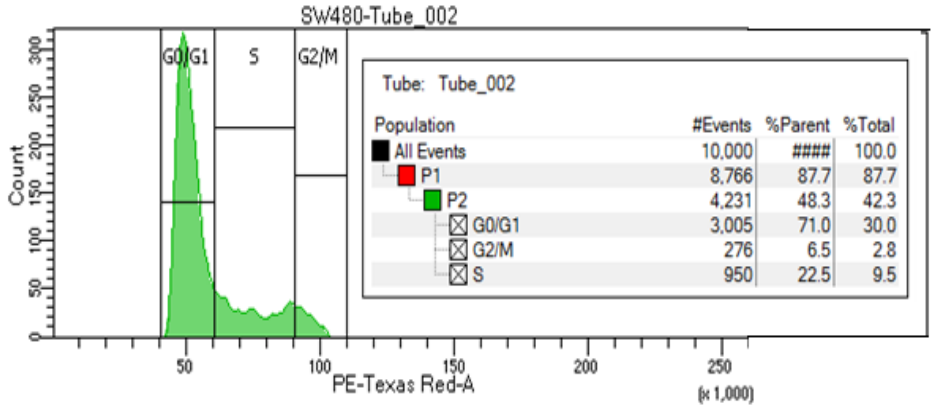
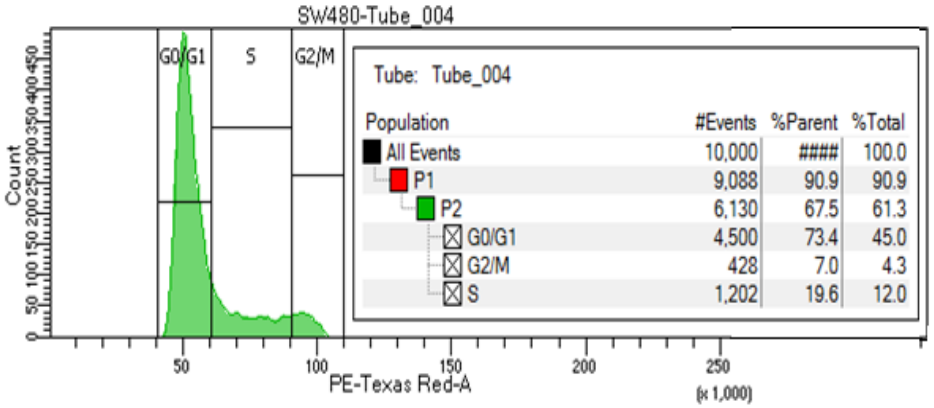


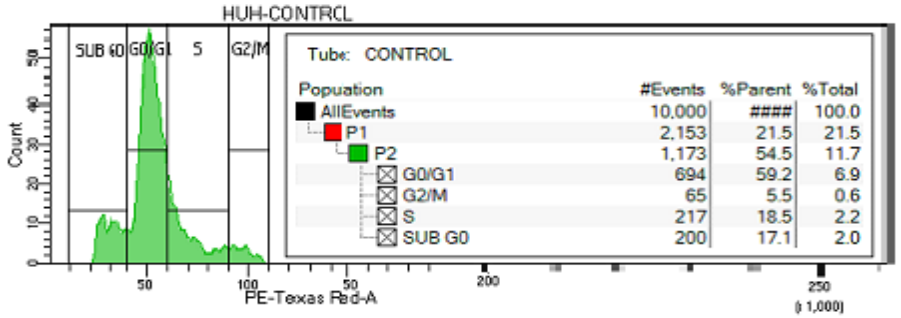
Fig.6: Flow cytometric analysis of untreated control and OSME treated of Huh-7, HepG2, Sw480, HEK 293T and RAW264.7cells respectively stained with Annexin V FITC and propidium iodide. Dual parameter dot plot of FITC fluorescence (*x*-axis) *vs* PI-fluorescence (*y*-axis) shows logarithmic intensity.

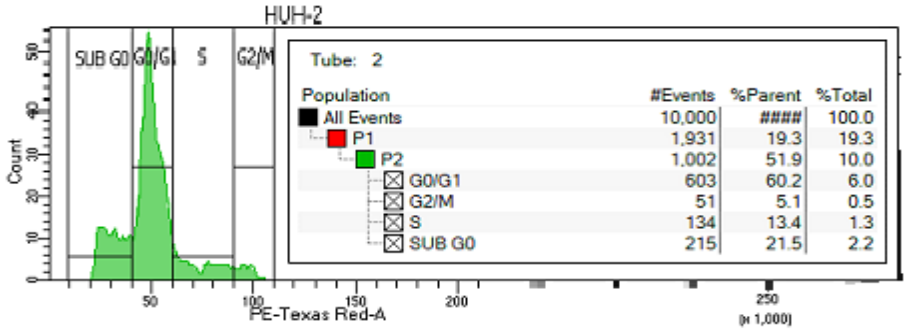
Detection of Cell Cycle Arrest by Flow Cytometric Analysis

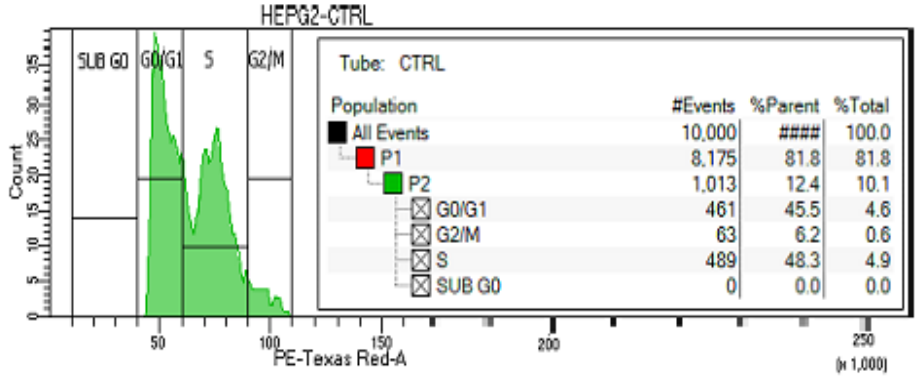
Flow cytometry analysis showed that after 24hrs treatment of SW480, Huh-7and HepG2cell lines with OSME at desired dose, sub-G1 peak was markedly changed, but in case of Hek293T and Raw264.7 cells insignificant change at sub-G1 peak was observed. The DNA content increase in OSME treated cell 73.4% against 71.0% in SW480, 60.2% against 59.2% in Huh-7, 69.8% against 45.5% in HepG2 76.5% against 76.4% in HEK293T and 71.2.% against 71.3% in RAW264.7 cells. These observations revealed that OSME significantly inhibited the growth of SW480, Huh-7 and HepG2 carcinoma cells by arresting the cell populations in the sub-G0/G1 phase of the cell cycle.


SW480-Tube_002
G0/G1
S
G2/M
Count
PE-Texas Red-A
(x 1,000)
Tube: Tube_002
Population #Events %Parent %Total
All Events 10,000 #### 100.0
P1 8,766 87.7 87.7
P2 4,231 48.3 42.3
G0/G1 3,005 71.0 30.0
G2/M 276 6.5 2.8
S 950 22.5 9.5


SW480-Tube_004
G0/G1
S
G2/M
Count
PE-Texas Red-A
(x 1,000)
Tube: Tube_004
Population #Events %Parent %Total
All Events 10,000 #### 100.0
P1 9,088 90.9 90.9
P2 6,130 67.5 61.3
G0/G1 4,500 73.4 45.0
G2/M 428 7.0 4.3
S 1,202 19.6 12.0


HUH-CONTRCL
SUB G0
G0/G1
S
G2/M
Count
PE-Texas Red-A
Tube: CONTROL
Popuation #Events %Parent %Total
AllEvents 10,000 #### 100.0
P1 2,153 21.5 21.5
P2 1,173 54.5 11.7
G0/G1 694 59.2 6.9
G2/M 65 5.5 0.6
S 217 18.5 2.2
SUB G0 200 17.1 2.0


HUH-2
SUB G0
G0/G1
S
G2/M
Count
PE-Texas Red-A
(x 1,000)
Tube: 2
Population #Events %Parent %Total
All Events 10,000 #### 100.0
P1 1,931 19.3 19.3
P2 1,002 51.9 10.0
G0/G1 603 60.2 6.0
G2/M 51 5.1 0.5
S 134 13.4 1.3
SUB G0 215 21.5 2.2


HEPG2-CTRL
SUB G0
G0/G1
S
G2/M
Count
PE-Texas Red-A
(x 1,000)
Tube: CTRL
Population #Events %Parent %Total
All Events 10,000 #### 100.0
P1 8,175 81.8 81.8
P2 1,013 12.4 10.1
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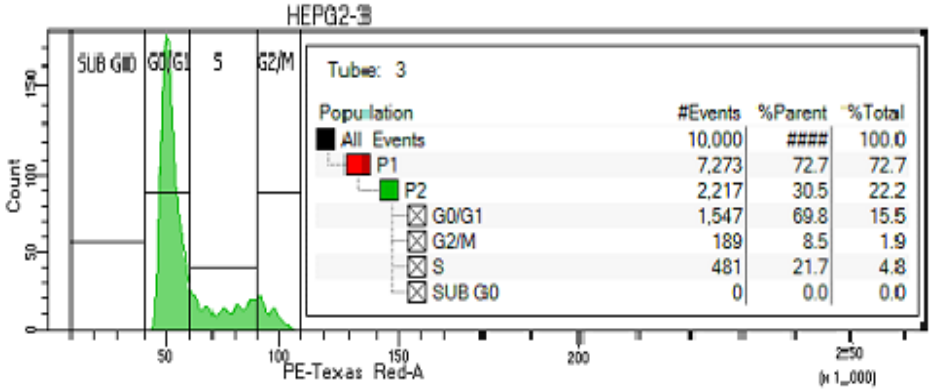
G2/M 63 6.2 0.6
S 489 48.3 4.9
SUB G0 0 0.0 0.0


HEPG2-3
SUB G0
G0/G1
S
G2/M
Count
PE-Texas Red-A
(x 1,000)
Tube: 3
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All Events 10,000 #### 100.0
P1 7,273 72.7 72.7
P2 2,217 30.5 22.2
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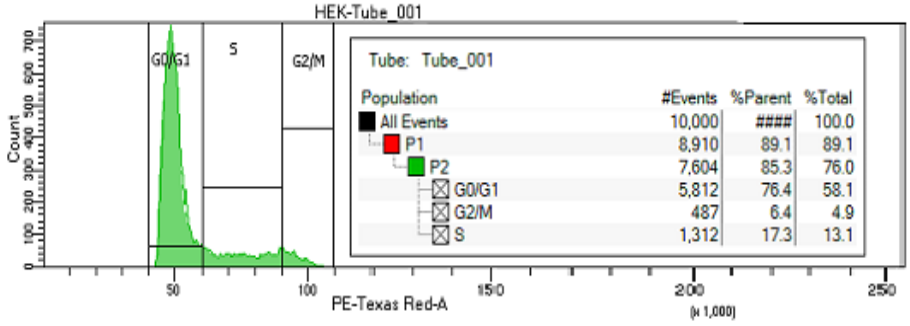
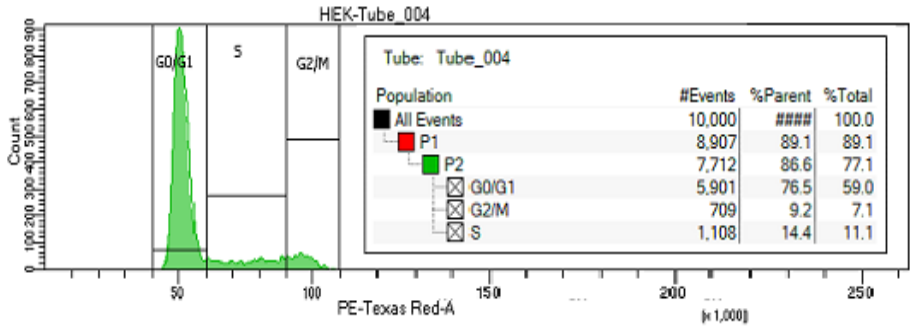
G2/M 189 8.5 1.9
S 481 21.7 4.8
SUB G0 0 0.0 0.0


HEK-Tube_001
G0/G1
S
G2/M
Count
PE-Texas Red-A
(x 1,000)
Tube: Tube_001
Population #Events %Parent %Total
All Events 10,000 #### 100.0
P1 8,910 89.1 89.1
P2 7,604 85.3 76.0
G0/G1 5,812 76.4 58.1
G2/M 487 6.4 4.9
S 1,312 17.3 13.1


HEK-Tube_004
G0/G1
S
G2/M
Count
PE-Texas Red-A
(x 1,000)
Tube: Tube_004
Population #Events %Parent %Total
All Events 10,000 #### 100.0
P1 8,907 89.1 89.1
P2 7,712 86.6 77.1
G0/G1 5,901 76.5 59.0
G2/M 709 9.2 7.1
S 1,108 14.4 11.1

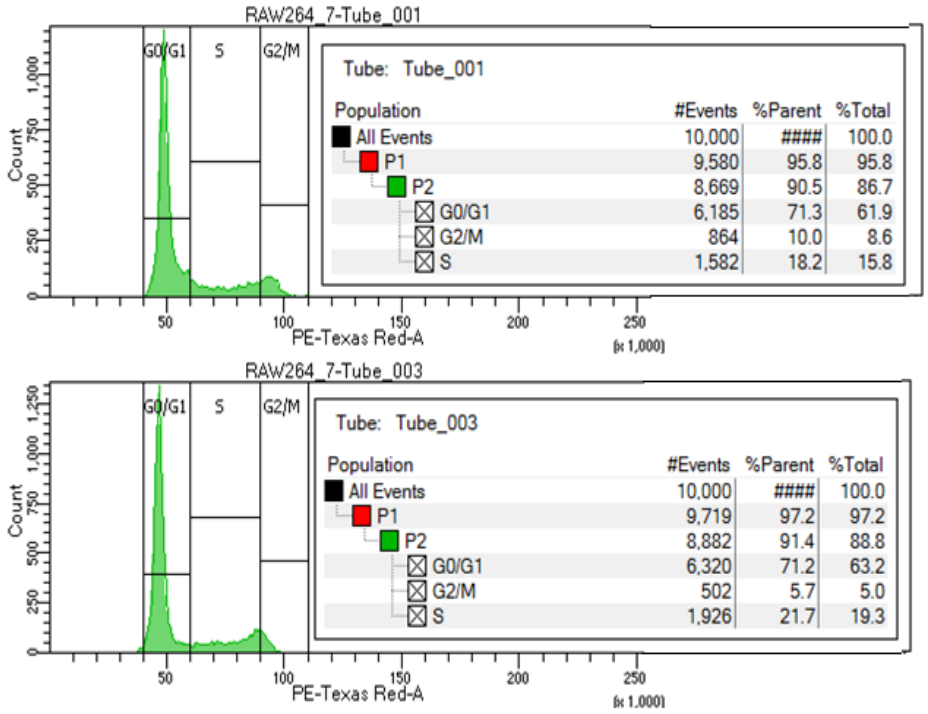


Fig.7: Flow cytometric analysis of cell cycle phase distribution in control (A) and treated (B) of SW480, Huh-7, HepG2, Hek293T and Raw264.7 cells respectively after 18 hrs treatment at desired dose of OSME. Histograms represent various contents of DNA with actual number of cells.

DISCUSSION

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Mutations in genes can also cause cancer by accelerating cell division rates or inhibiting normal controls on the system, such as cell cycle arrest or programmed cell death. A significant part of drug discovery in the last forty years has been focused on agents to prevent or treat cancer. Colorectal cancer is the third most common cancer in the world.[15] Hepatocellular carcinoma which is a type of primary liver cancer is the fifth most common cancer in the world and third most common cause of cancer mortality.[16] Drug discovery from medicinal plants has played a very important role in the treatment of cancer.[17] Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization.[18] *Ocimum sanctum* has been used traditionally in the treatment of various ailments. The present study is to investigate the anti-neoplastic activity of methanol extract of *Ocimum sanctum* (OSME) against three types of colorectal carcinoma, one type of gastric adenocarcinoma and two types of hepatocellular carcinoma by using

SW480, HCT116, HT29, AGS, Huh-7 and HepG2 cell lines respectively. We also investigated the toxic effect of OSME on normal human embryonic kidney cell line HEK293T and murine macrophage RAW264.7 cell line. The cytotoxic activities of OSME were supported by the observations in MTT assays. OSME inhibited the metabolic activities of all the carcinoma cells in a concentration and time dependent manner. Anti-neoplastic effect of OSME was investigated by morphological studies like fluorescence microscope. The process of apoptosis is characterized by several morphological changes such as cell shrinkage, membrane blebbing, chromatin condensation, nuclear fragmentation and formation of apoptotic bodies. Fluorescence microscopic images clearly showed nuclear disintegration of OSME treated hepatocellular carcinoma cells compared with that of the untreated control cells when stained with acridine orange and ethidium bromide. The untreated control cells showed bright green fluorescence with intact membrane. On the contrary, OSME treated cells showed more intense orange-red fluorescence and reduced green fluorescence. Further evidence in support of the anti-cancer activity of OSME was obtained from the gel patterns of agarose gel electrophoresis. OSME treated carcinoma cells showed degraded DNA bands in the form of ladders, a typical indication of apoptosis, whereas the untreated control cells showed intact DNA bands when observed in UV transilluminator. The mitochondrial membrane potential assay by JC-1dye showed markedly increase in apoptotic cells in OSME treated carcinoma cells than control cells. The depolarization led to a transmembrane shift from red to green fluorescence leading to release of cytochrome C. Experiments also showed increased number of cells in the early and late apoptotic stage after treatment with OSME implying the fact that apoptosis was triggered by the treatment with OSME in carcinoma cells. Cell cycle analysis revealed that treatment with OSME arrested the cell populations in the sub G0/G1 phase of the cell cycle. In the present study we also investigate the toxic effect of *Ocimum sanctum* methanolic extract (OSME) on normal human embryonic kidney cell lines (HEK293T) and murine macrophage cell line (RAW 264.7) cell lines. OSME insignificantly inhibited the cell growth in both the normal cells. Further toxic effect of OSME was investigated in mitochondrial membrane potential study showed insignificant increase in apoptotic cells which have healthy mitochondrial membrane potential. Experiments also showed insignificant increased number of cells in the early and late apoptotic stage after treatment with OSME implying the fact that apoptosis was not triggered by the treatment with OSME in both the normal cells. Cell cycle analysis revealed that treatment with OSME did not change the cell populations as compared to control cells of cell cycle.

CONCLUSION

It has been concluded that OSME possesses anti-neoplastic effect against colorectal cancer (SW480, HCT116 and HT29) and hepatocellular carcinoma cell (Huh-7 and HepG2) cells and cytotoxic effect on gastric cancer cells(AGS), but it shows insignificant inhibition and non- apoptotic activity against normal human embryonic kidney and murine macrophage cells (HEK293T and RAW264.7). Therefore, we can conclude that OSME specifically shows activity towards cancer cells. Indian mythology attaches a great significance to plant *Ocimum sanctum*(Tulsi) by recognizing it as a holy herb, because this holy herb possesses medicinal effect to mankind from ancient time. It was experimentally proved that the plant *Ocimum sanctum* possesses lots of medicinal value. It can be considered as a potent anti-cancer agent for treatment of colorectal cancer, gastric and hepatocellular carcinoma with less side effects. Further mechanistic study should be done and identify the active compound/compounds of *Ocimum sanctum* plant which are responsible for anti-neoplastic/anticancer activity on cancer cells.

ACKNOWLEDGEMENT

The authors of this paper are very much thankful to Council of Scientific and Industrial Research, Indian Institute of Chemical Biology (CSIR-IICB), Kolkata for providing the funding to perform the research work.

Conflict of Interests The authors declare that they have no conflict of interests.

REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell, 2011; 144: 646–674.
2. World Cancer Research Fund and American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective. Washington, DC; American Institute for Cancer Research, 2007.
3. Jemal A, Thun M J, Ries L A. Howe HL. Weir HK. Center MM. Ward E, Wu XC, Eheman C, Anderson R, Ajani UA, Kohler B, Edwards BK. Annual report to the nation on the status of cancer, 1975-2005, featuring trends in lung cancer, tobacco use, and tobacco control. J Natl Cancer Inst, 2008; 100(23): 1672-1694.
4. Parkin D.M., Bray F.I., Devesa S.S. Cancer burden in the year 2000. The global picture. Eur J Cancer, 2001; 37(8): S4–66.

5. Parkin DM. International variation. *Oncogene*, 2004; 23: 6329–6340.
6. Stewart BW, Kleihues P. International Agency Research on Cancer, WHO. World Cancer Report. IARC Publication, 2003.
7. Kumar V, Abbas A, Aster J, Robbins C. Pathologic Basis of Diseases. Elsevier, 2014; 30(4): 272-278.
8. Salem R, Lewandowski R J. Chemo embolization and radio embolization for hepatocellular carcinoma. Clin. Gastroenterology Hepatol, 2013; 11: 604.
9. Kirtikar KR, Basu BD. *Ocimum sanctum* in Indian Medicinal studies on Triterpenes. Ph.D. Thesis, University of Madras Plants. Allahabad: Basu, LM, 1933; 3: 1965.
10. Nadkarni AK. Nadkarni's Indian Materia Medica. Bombay: Popular Book Depot, 1954; 868.
11. Singh TJ, Gupta PD, Khan SY, Misra KC. Preliminary pharmacological investigation of *Ocimum sanctum*. Ind. J Pharmacy, 1970; 32: 93-4.
12. Balta SK, Santkumari G. The anti-fertility effect of *Ocimum sanctum* and *Hibiscus rosasinensis*. Indian Journal of Medical Research, 1971; 59: 777-81.
13. K. Karthikeyan, P. Gunasekaran, N. Ramamurthy and S. Govindasamy. Anti-cancer activity of *Ocimum sanctum*. Pharmaceutical Biology, 1999; 37(4): 285-290.
14. Venkataraman Magesh, Jang-Choon Lee, Kwang Seok Ahn, Hyo-Jung Lee, Hyo Jeong Lee, Eun-Ok Lee, Bum Sang Shim, Hee Jae Jung, Jin Sung Kim, Dae Keun Kim, Seung-Hoon Choi, Kyoo-Seok Ahn and Sung-Hoon Kim. *Ocimum sanctum* Induces Apoptosis in A549 Lung Cancer Cells and Suppresses the *in Vivo* Growth of Lewis Lung Carcinoma Cells. Phytotherapy Res, 2009; 23: 1385–1391.
15. Singh N., Verma P., Pandey B. R., Bhalla M. Therapeutic Potential of *Ocimum sanctum* in Prevention and Treatment of Cancer and Exposure to Radiation. International Journal of Pharmaceutical Sciences and Drug Research, 2012; 4(2): 97-104.
16. Jordan T., Gurg K. Colorectal carcinoma: epidemiology and molecular carcinogen. Lancet, 2007; 132(7): 2557-2576.
17. Serag H., Rudolph K. Hepatocellular carcinoma: epidemiology and molecular carcinogen. Lancet, 2007; 132(7): 2557-2576.
18. Wang H.K., Plant-derived anticancer agents currently in clinical use or in clinical trials. The Investigational Drugs, 1998; 1: 92-102.
19. Elsevier Ltd., Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study. 2015, Lancet, 2015; 388(10053): 1545–1602.