Research Article

Phytochemical Analysis and Growth Suppressing Activity of *Sapindus saponaria* L. Pericarp Extract on Hepatocellular Carcinoma Cells

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ABSTRACT

Sapindus saponaria L. has revealed a number of properties of this plant which includes antioxidant, hepatoprotective, anti-inflammatory, anti- cancer, anxiolytic, fungicidal, spermicidal activity. The present study investigated the anti-cancer activity of *Sapindus saponaria* L. pericarp extract (SSFE) on hepato carcinoma cell lines. The extract and fractions significantly inhibited cell the viability in a time and concentration dependent manner in both the carcinoma cells. Morphological study showed several signs of apoptosis like chromatin condensation, nuclear fragmentation and formation of apoptotic bodies and degraded DNA bands in both the treated hepatoma cells than control. Treated cells exerted significant MMP shift in the mitochondrial transmembrane potential activity involving the release of cytochrome c into the cytosol. The cell cycle phase arrest was observed in the G0/G1 phase change in the EFESS treated cells. These findings suggest that extracts possess cytotoxic and apoptosis activity against both the hepatocellular carcinoma cells. Further mechanistic studies are under process to determine the different pathways attributing to apoptosis.

Keywords: *Sapindus saponaria*, Pericarp, Hepatocellular carcinoma, Mitochondria, Apoptosis.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide, and with a continuously increasing incidence. [1] The dynamic development of innovative therapeutic approaches and molecularly targeted agents using plant derived drugs could provide a chance to study the agents in HCC and gives better prospective for the upcoming future. [2] Recent investigational studies on *S. saponaria* L. has revealed a number of properties of this plant which includes anti-oxidant, hepatoprotective, anti-inflammatory, anti- cancer, anxiolytic, fungicidal, spermicidal etc. [3,4] The ethyl acetate and hexane extract of *S. saponaria* L. also showed inhibitory effect against human skin cancer cell lines (A375.S2, MeWo), lung(A549, NCI-H460), liver cancer cell line (HepG2,PLC/PRF/5) prostate cancer cell lines (PC-3, Du-145, LNCaP)), cervical cancer cell lines (HeLa), bone cancer cell line(MG-63), bladder cell line (T24) , and breast cancer cell lines(MDA-MB-231, Hepa 59T/VGH, NCL, Med).It has been found that ethyl acetate and hexane extract of *S. saponaria* L. shows effective inhibitory activity against all the mentioned cell lines and especially against lung cancer and melanoma cell line. [5] But no detailed work has been done on hepatocellular carcinoma (HepG2 and Huh-7) cell lines so far. Therefore, the present work is an approach to study the anti-cancer activity of the fruit extract of *Sapindus Saponaria* L. and its active fraction on human hepatocellular carcinoma cell lines (HepG2 and Huh-7).

MATERIALS AND METHODS

Collection and Identification of the plant material

Sapindus saponaria L. fruits were collected from Dumka; Jharkhand during August 2017.The specimen was identified at Central National Herbarium, Botanical Survey of India, Shibpur, and Howrah-711103. The specimen identification no. is SM-01.

Extraction, Fractionation and Preparation of test sample

The pericarp of the fruit of *Sapindus saponaria* L. was shade dried and grinded. The sticky paste (200gm) of *Sapindus saponaria* pericarp was soaked in about 500ml of methanol for 7 days at room temperature with occasional shaking. The mixture was filtered by filter paper and was extracted 3 times in rotary evaporator at 35°C for 15 hours and was concentrated under reduced pressure to produce methanol free extract. The sticky methanolic extract was finally obtained as blackish brown residue. It was kept in air container at 4°C and sealed with Para film. The extract was designated as methanolic fruit extract of *S. saponaria* L. (FESS) for the experiments. The methanolic extract of *Sapindus saponaria* L. was further processed for fractionation.10 gm of *Sapindus Saponaria* L. fruit pericarp extracted with methanol was suspended in 200ml of water and equivalent amount ethyl acetate and n-butanol to get EtAc fraction and n-BuOH fraction on the basis of polarity. The fractions are termed as Ethyl acetate fraction of fruit extract of *Sapindus Saponaria* (E-FESS), n-butanol fraction of fruit extract of *Sapindus saponaria*

(B-FESS) and water extract of fruit extract of *Sapindus saponaria* (W-FESS). Stock solution of E-FESS, B-FESS and W-FESS was prepared as 1mg/ml in Phosphate Buffer Solution (PBS), from here the desired amount was taken as required for different experimentation.

Chemicals

DMEM medium with L-glutamine, Fetal Calf serum(FCS), Phosphate buffer saline (PBS), Trypsin (Gibco, USA), Sorafenib Tosylate, Penicillin- Streptomycin, Gentamycin, HEPES, Ethidium bromide, Acridine orange, Propidium iodide,3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide(MTT), Trypan Blue, JC-1, Proteinase K, RNase (SRL), Isoamyl alcohol, phenol, chloroform, Dimethyl sulfoxide (DMSO), Sodium hydroxide, Agarose, Sodium bicarbonate, Ethanol, Methanol etc.

Cell Culture

Cell lines: HepG2, Huh-7 and WRL 68 were obtained from National Centre for Cell Science, Pune, India. The cells were cultured and routinely maintained in DMEM medium supplemented with 10% heat inactivated fetal calf serum, 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. Both Hep-G2 & Huh-7 cells are adherent in nature. During sub culturing of the cells, the adherent property can be diminished by adding 1x Trypsin solution in the cell.

Phytochemical Investigation of Methanolic Extract of *Sapindus saponaria* Pericarp (FESS)

Tests for alkaloids

A small portion of the solvent free chloroform was stirred with alcoholic and water extract separately with few drops of dilute Hcl and filter. The filtrate was tested carefully with various alkaloidal reagents such as Mayer's reagent (cream precipitate), Dragendorff's reagent (orange brown precipitate), Hager's reagent (yellow precipitate), and Wagener's reagent (reddish brown precipitate).

Test for carbohydrates

A small amount (200mg) of alcoholic and water extract was dissolved separately in 5 ml of distilled water and filter. The filtrate was tested carefully with Millon's reagent. Millon's test: 2-3 ml water extract was treated with few drops of alpha-naphthol solution in alcohol and was shaken and then conc. H_2SO_4 was added from sides of the test tube. Violet ring was formed at the junction of two liquids. Fehling's test: A mixture of 1 ml Fehling's A and 1 ml Fehling's B solution was boiled for 1 minute. Equal volume of test solution was added to it and heated in boiling water bath for 5-10 minutes. First a yellow, then brick red precipitation was observed.

Tests for glycosides

A small portion of the extract was hydrolyzed with dilute hydrochloric acid for a few hours in water bath and the hydrolysate was subjected to Liebermann-Burchard's, Legal's and Borntrager's test. Liebermann-Burchard's test: 2 ml extract was mixed with 2 ml chloroform and to that 2 ml acetic anhydride and 2 drop conc. H_2SO_4 from the side of test tube was added. First red, then blue and finally green color appeared. Legal's test: 1 ml pyridine and 1 ml sodium nitroprusside were added to aqueous or alcoholic extract. Pink to red color appeared. Borntrager's test: In 3 ml water extract, dilute H_2SO_4 was added and boiled and filtered. 2ml of 5% ferric chloride solution and equal volume dichloromethane or chloroform was added to the filtrate and mixed well. The organic solvent was separated and to that ammonia was added. Ammoniacal layer turned pink or red.

Test for phenolic compounds and tannins

Treat 2-3 ml water extract or alcoholic extract with few drops of following reagent:

a) 5% $Fecl_3$ solution: deep blue-black color.

b) Lead acetate solution: white ppt.

c) Gelatin solution: white ppt.

d) Bromine water: discoloration of bromine water.

e) Acetic acid solution: red color solution.

f) Potassium dichromate: red ppt.

g) Dil. iodine solution: transient red color.

h) Add 1 drop of NH_4OH with excess 10% $AgNO_3$ solution and heat for 20 minutes in boiling water bath. White precipitate observed then dark silver mirror deposits on wall of test tube.

Tests for proteins and amino acids

2-3 ml water extract or alcoholic extract was treated with few drops of water and the solution was subjected to Millon's Biuret and Ninhydrin test. Millon's Biuret test: 2 ml extract was mixed with 5 ml Millon's reagent. White, Warm precipitation turned brick red. Ninhydrin test: 3 ml extract and 3 drops 5% ninhydrin solution was heated in boiling water bath 10 minutes. Purple or bluish color appeared.

Tests for saponins

1 ml of water extract or alcoholic extract was diluted separately with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. A one-centimeter layer of foam appeared which indicates saponins.

Hemolytic test

The extract was added to one drop of blood and placed on glass slide. Hemolytic zone appeared.

Test for fixed oils: Saponification test

Few drops of 0.5N alcoholic potassium hydroxide was added to a small quantity of various extracts along with drops of phenolphthalein separately and was heated on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

Test for flavonoids: Shinoda test

Few drops of magnesium turnings and concentrated Hcl dropwise was added to the extract, pink scarlet, crimson red or occasionally green to blue colour appeared. Alkaline reagent test: Few drops of sodium hydroxide solution to the test solution, intense yellow colour is formed which turned to colorless on addition of few drops of dilute acid. [6]

Cell Viability Study by Trypan Blue Exclusion Method

Hep-G2, Huh-7 & WRL68 cells ($1x10^5$), 100µl cell suspension per well in a log phase were seeded in 96-well sterile plates and incubated in a humidified atmosphere of 5% CO_2 in air at 37ºC for 24 hours before treatment. The cells were treated with different concentrations (10µg, 25µg, 50µg, 100µg) of FESS for 24, 48 and 72 hours and the untreated cells were considered as control. After treatment for 24, 48, 72 hours in respective time 10µl of Trypan blue dye (1mg/ml) was added to all wells containing cells accordingly. The cell viability studies were done by Trypan blue exclusion method using light microscope. [7] The IC_{50} dose of FESS on the two hepatoma cells was determined. Drug used as standard: Sorafenib Tosylate (2.09µg)

Cytotoxicity Study by MTT Assay

Hep-G2 and Huh-7 ($1x10^5$) cells (100µl of cell suspension per well) were seeded in 96-well plates and incubated inside a CO_2 incubator for 24 hours before treatment. The cells were treated with W-FESS, B-FESS & E-FESS fractions in doses of 10µg, 25µg, 50µg, 100µg for a period of 24 hours at 37ºC in a humidified atmosphere containing 5% CO_2 in air, untreated cells served as control. After 24, 48 & 72 hours of treatment, 20 µl of MTT (3mg/ml) was added to each well. The plate was then allowed to incubate for 3-4 hours at 37°C in CO_2 incubator. 100 µl of DMSO was added to each well to dissolve the formazan crystal formed. The O.D values were recorded at 570 nm by microplate manager (Reader type: Model 680 XR Bio-Rad laboratories Inc).[8]

Morphological Study by Fluorescence Microscope

HepG2 and Huh-7 hepatoma cells ($1x10^6$) were treated with different IC_{50} doses of EFESS for 24 hours and observed using a fluorescence microscope for determining morphological changes. After 24 hours, the untreated control cells and EFESS treated cells were harvested separately and washed with PBS and then stained with Acridine orange and ethidium bromide in a ratio of 1:1. The cells were then immediately mounted on slides and observed under a fluorescence microscope at a magnification of 60X for the morphological determination of the cells undergoing apoptosis.

Morphological Study by Confocal Microscope

HepG2 and Huh7 hepatoma cells ($1x10^6$) were treated with different IC_{50} doses of EFESS respectively for 24 hours and observed using a confocal microscope for determining nuclear changes. After 24 hours, the untreated control cells and EFESS treated cells were harvested separately and washed with PBS and then stained with 10µg/ml of Propidium iodide. The cells were then immediately mounted on slides and observed under a confocal microscope at a magnification of 60X for the nuclei determination of the cells undergoing apoptosis.

DNA Fragmentation Study by Agarose Gel Electrophoresis

HepG2, Huh-7 and WRL 68 cells ($1x\ 10^6$) were treated with IC_{50} dose of EFESS. After trypsinization, cells were harvested and washed twice with PBS. The cells were resuspended in 500µl of lysis buffer [1(M) Tris Hcl, 5(M) NaCl, 20% Triton X], 10µg/ml of Proteinase - K was added and kept for incubation at 55ºC for 30 minutes and then at 37°C overnight. After incubation was done by following the general phenol-chloroform extraction procedure and kept at -20°C overnight. After centrifugation, DNA precipitates were washed with 70% ethanol, dried and evaporated at room temperature and suspended in TE buffer at 4ºC overnight. In order to detect the DNA fragments the isolated DNA samples were electrophoresed at 20V in 1% agarose gel and stained with ethidium bromide. DNA fragmentation was observed in UV transilluminator and Chemi-Doc ™ MP Imaging System, Bio-Rad.

Cell Cycle Arrest by Flow Cytometric Analysis

In order to study the stage of cell cycle arrest in a flow cytometer, HepG2 and Huh-7 cells ($1x\ 10^6$) were treated with IC_{50} dose of EFESS was taken as standard drug for 18 hours. Cells were washed with PBS, fixed with chilled methanol kept at 4°C for 90 minutes. Cells were pelleted down, dissolved in cold PBS, treated with RNase for 30 min at 37 °C and stained with Propidium iodide and transferred to FACS tube. Cell cycle phase distribution was determined on FACS (Becton Dickinson FACS Fortessa 4 laser cytometer), fluorescence detector equipped with 488 nm argon laser light source and 623 nm band pass filter (linear scale) using BD FACS Diva software (Becton Dickinson).

Detection of Mitochondrial Membrane Potential (Δψm) using JC-1

HepG2 and Huh-7 ($1x10^6$) cells were treated with EFESS with IC_{50} dose for 18 h to assay the mitochondrial membrane potential activity of cell in a flow cytometry.

Cells were washed with PBS, pelleted down and dissolved in prewarmed PBS. 200 µM JC-1 stain was added and the samples were incubated at 37 °C for 15 min. Shift in the mitochondrial membrane potential was determined by FACS (Becton Dickinson FACS Fortessa 4 laser Cytometer), fluorescence detector equipped with 488 nm argon laser light source and 623 nm band pass filter (linear scale) with the help of BD FACS Diva software (Becton Dickinson).

Statistical Analysis

Student's t test was used for statistical analysis and $p<0.005$ was considered significant.

The percentage of cell growth was calculated by the following formula:

% Cell inhibition= 100x (O.D of control – O.D of the treated)/ O.D of control

Where O.D refers to Optical density

The percentage cell viability was calculated by the formula:

Viable cells (%) = (Total number of viable cells per ml/ Total number of cells per ml) x100.

RESULTS

Phytochemical Investigation of Methanolic Extract of *Sapindus saponaria* Pericarp (FESS)

The fruit (pericarp) extract of *Sapindus saponaria* L. (FESS) were subjected to preliminary phytochemical study for detection of alkaloids, carbohydrates, glycosides, phenolic compounds and tannins, flavonoids, proteins and amino acids, saponins and lipids/fat. The result for the phytochemical screening in presented in the table below:

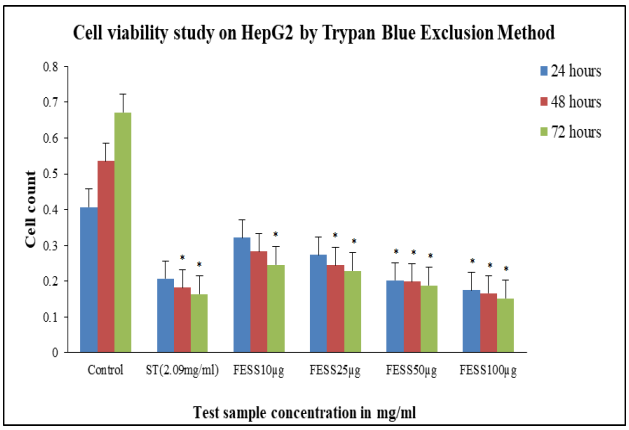
Table 1: General observation table for phytochemical screening.

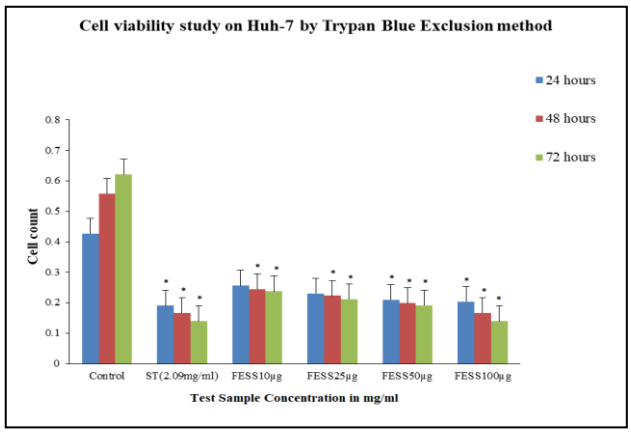
Phytochemical Test	Observation
Test for Alkaloids	-
Test for Carbohydrate	+
Test for Glycosides	-
Test for Phenolic compounds and tannins	-
Test for Proteins and amino acid	+
Test for Saponins	+
Test for Flavonoids	+
Test for sterols and steroids	+
Test for Fixed oils	+
Test for Terpenoids	+

The sign '+' indicates presence of the constituent and '-'indicates the absence of constituents.

Cell Viability Study by Trypan Blue Exclusion Method

The cell viability study performed with the freshly prepared FESS (1mg/ml of stock solution) by Trypan blue exclusion method. FESS at the above mentioned concentrations significantly inhibited in a concentration and time dependent manner in both the Hep-G2 and Huh -7 cells compared to that of control cells after 24, 48 and 72 hour of treatment whereas in normal liver cell i.e. WRL 68, insignificant inhibition was observed after FESS treatment. After 24hrs, the IC_{50} value of FESS on HepG2 was found **33µg** and **38 µg** for Huh-7 cells.





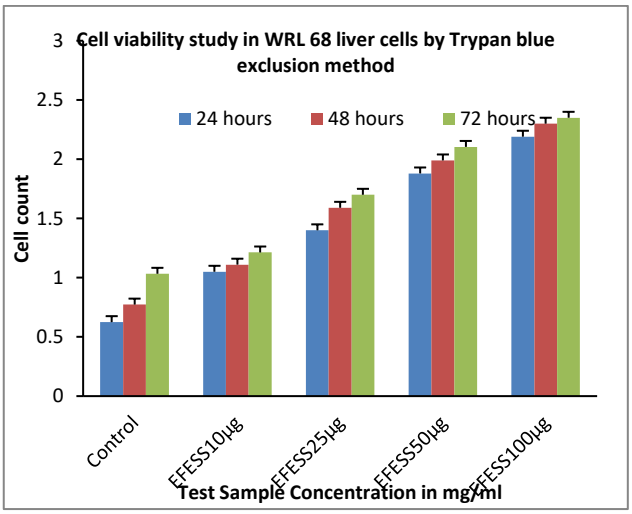
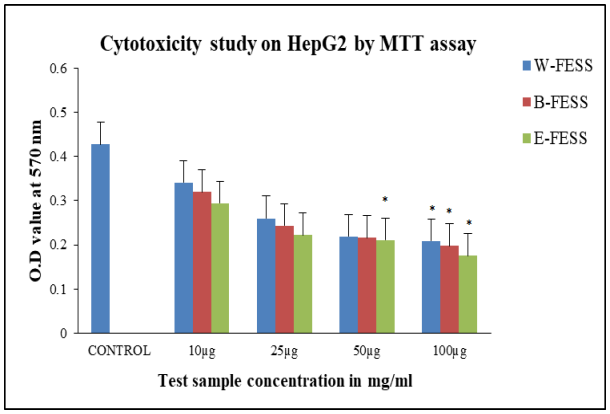


Figure 1: Histogram shows effect of FESS on cell growth inhibition on HepG2, Huh-7 & WRL-68 cell lines after 24, 48 and 72hrs of treatment by measuring cell count. The cell count is compared to the untreated control cells.

Reduction in number of viable cells was observed in a time and concentration dependent manner whereas in WRL68 cell showed gradually increase in number of cells. Data are mean ± S.E. M. * denotes significant decrease in cell count from control values p<0.05.

Cytotoxicity Study

In cytotoxicity study by MTT assay, EFESS showed significant reduction in the O.D values than W-FESS and B-FESS of treated cells after 24 hours compared to the control cells. The observations showed inhibition of cell proliferation in a time and concentration dependent manner. The IC_{50} value of EFESS calculated after MTT assay is **16 µg** and **20µg** for HepG2 cells and Huh-7 cells respectively.



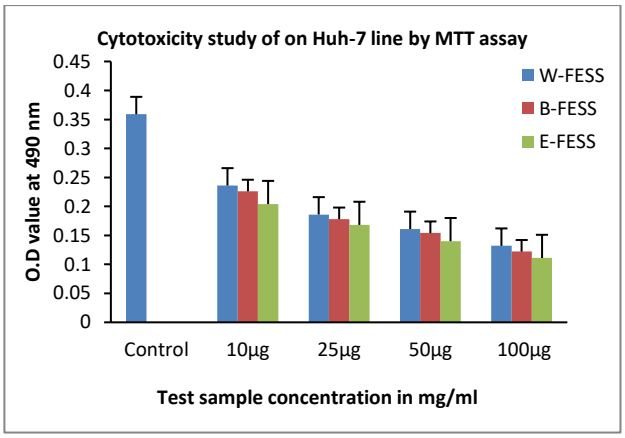


Figure 2: Histogram shows the effect of EFESS in comparison to W-FESS & B-FESS on HepG2 & Huh-7 cell lines after 24hrs. Reduction in the O.D at 570 nm is observed in a time and dose dependent manner. Data are S.E.M ± mean. * denotes significant decrease in O.D value from the control values p<0.05.

Morphological study by fluorescence microscopy

Fluorescence microscopic observations of IC $_{50}$ dose of EFESS treated HepG2 and Huh-7 cells stained with ethidium bromide and acridine orange, revealed the presence of apoptotic cells (both early and late) as compared to the untreated control cells. Arrays of nuclear changes were observed including chromatin condensation and apoptotic body formation that are the indication of occurrence of apoptosis.

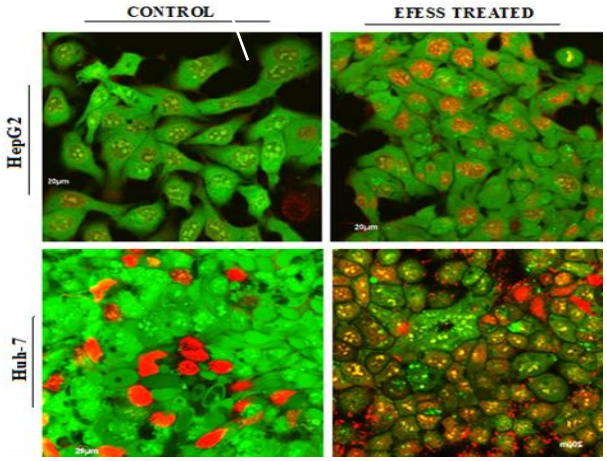


Figure 3: Represents untreated control and EFESS treated HepG2 and Huh-7cells. Both the cell lines were stained with Acridine orange & Ethidium bromide. Both the treated cells showed apoptosis indicated as by arrow.

Morphological study by Confocal Microscope

Confocal microscopic observations of IC $_{50}$ dose of EFESS treated HepG2 and huh-7 cells stained with propidium iodide, revealed the nuclear changes in cells as compared to the untreated control cells. Arrays of nuclear fragmentation were observed including chromatin condensation and apoptotic body formation that are the indication of occurrence of apoptosis.

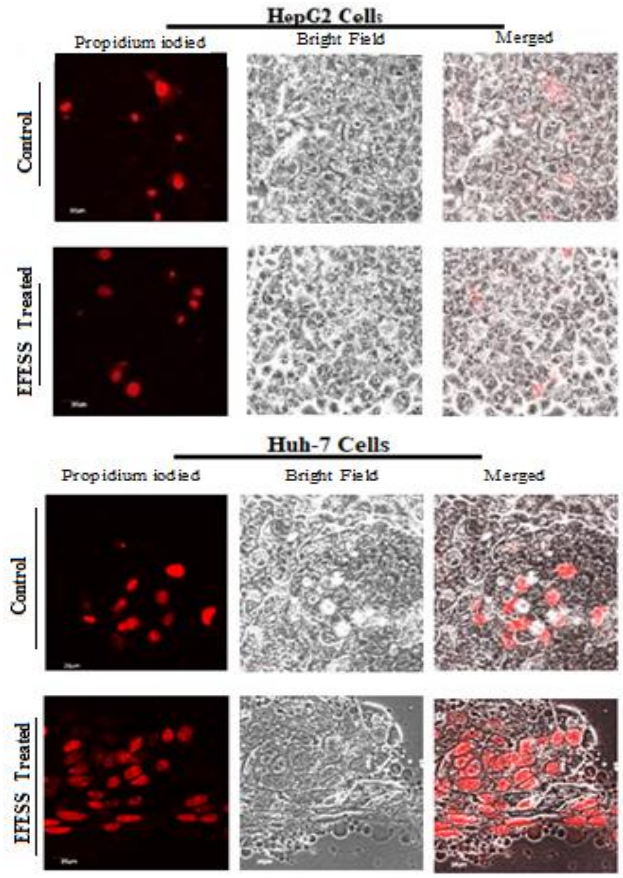


Figure 4: Confocal microscopic images of HepG2 and Huh-7 cell lines after 24 hours of treatment with EFESS.

Both the treated cells showed apoptosis indicated by arrow.

Agarose gel electrophoresis study

After Agarose gel electrophoresis, the DNA samples isolated from the untreated control HepG2 and Huh-7 cells showed intact DNA bands whereas the DNA samples from both the cells treated with EFESS showed fragmented DNA bands in the form of ladders. So, the observations confirmed that the treatment with EFESS, caused apoptosis in both HepG2 & Huh-7 but there was no fragmented DNA in WRL68 cell lines.

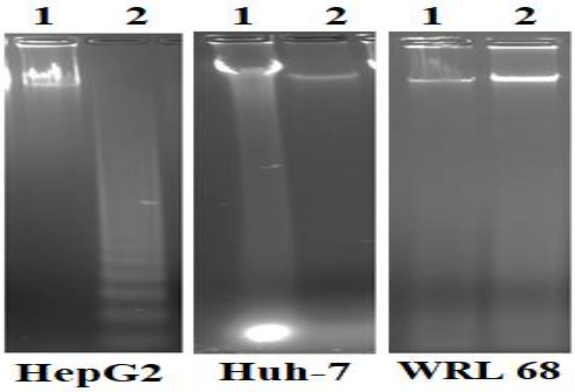
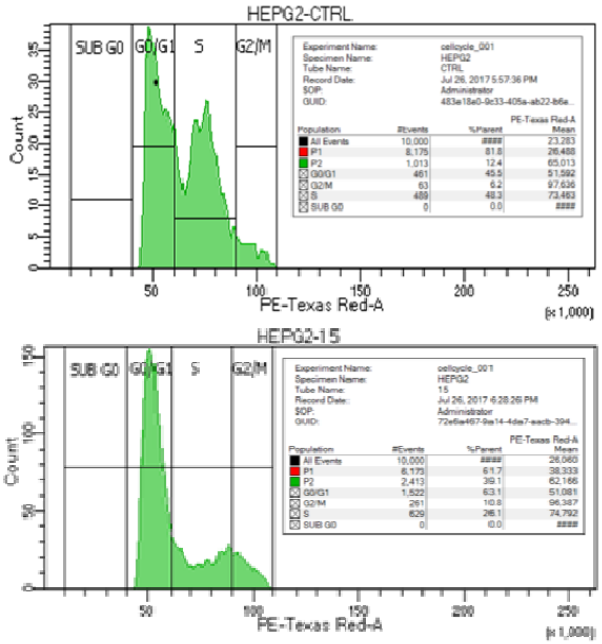


Figure 5: DNA fragmentation by Agarose gel electrophoresis assay in HepG2 and Huh-7 cells. Lane 1 represents HepG2, Huh-7 and Wrl-68 control cells, lane 2 represents EFESS treated HepG2, Huh-7 & Wrl-6 cells respectively.

Study of Cell Cycle Arrest by Flow Cytometric Analysis

Flow cytometric analysis showed that after 24 hours' treatment of HepG2 & Huh-7 with EFESS at IC_{50} dose, the DNA content increased in G1 phase. The results indicated that drug treatment arrested the cell cycle of both the cell lines at G0/G1 phase.



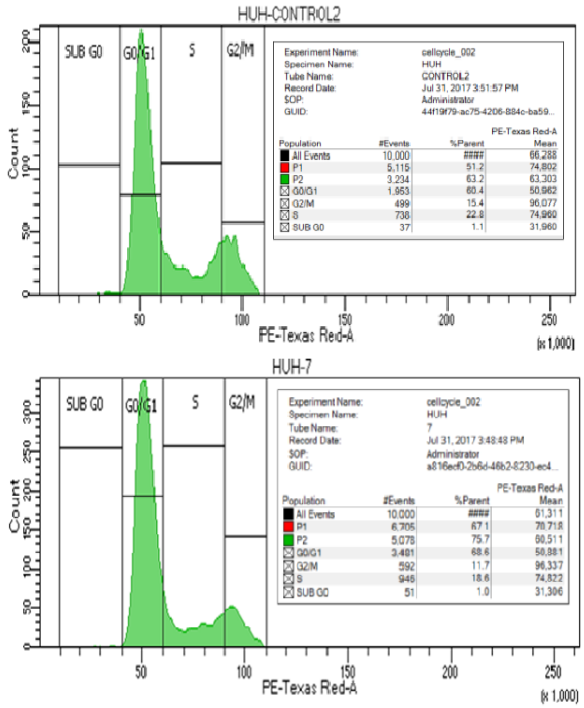
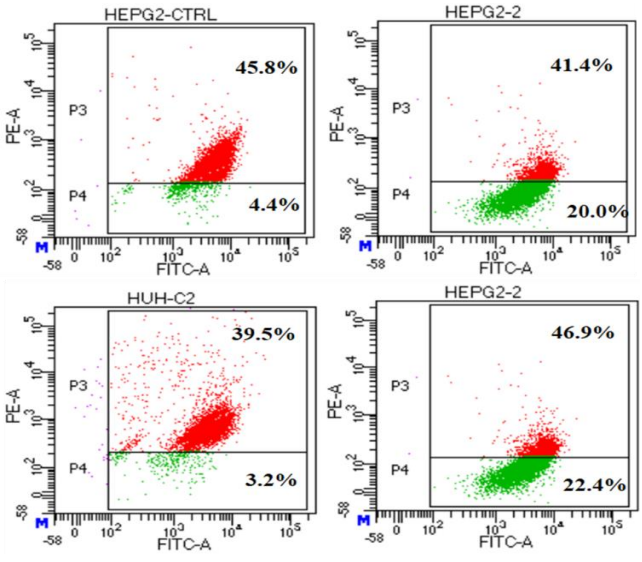


Figure 6: Flow cytometric analysis of cell cycle phase distribution in control and treated of HepG2 & Huh-7cells respectively after 18 hrs treatment at IC_{50} dose of EFESS. Histograms represent various contents of DNA with actual number of cells (x-axis denotes fluorescence intensity of PE- Texas red and y-axis denotes count).

Detection of mitochondrial membrane potential by JC-1

Depolarization in mitochondrial membrane potential was observed by staining treated and untreated cells with JC1 dye. The depolarization led to a transmembrane shift from red to green fluorescence leading to the release of Cytochrome C. A significant transmembrane shift of 4.4 to 20 (in HepG2) and 3.2 to 22.4 (for Huh-7) in the mitochondrial membrane potential from red to green fluorescence was observed when HepG2 and Huh-7 cells were treated with the IC_{50} dose of EFESS for 18 hours respectively.



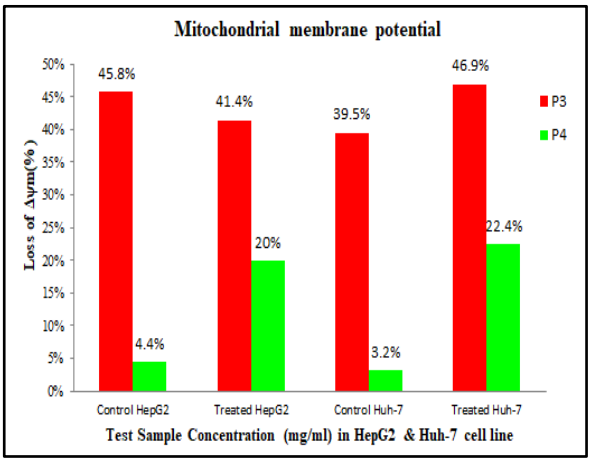


Figure 7: Flow cytometric analysis of mitochondrial membrane potential on HepG2 and Huh-7 cells in both controls and EFESS IC_{50} dose treated cells respectively after 18 hours' treatment. A significant shift of membrane potential from red to green fluorescence was observed in HepG2 and Huh-7 cells respectively.

DISCUSSION

Cancer chemoprevention, by the use of natural, dietary or synthetic agents that can reverse, suppress or prevent carcinogenic progression, has become an appealing strategy to combat the dogma associated with increasing cases of cancers worldwide. [9] Induction of apoptosis is one the most important marker of chemo preventive plant derived agents. Hepatocellular carcinoma (HCC) is the most frequent form of primary liver tumor. HCC is characterized by its remarkable chemo resistance and few therapeutic options are, therefore, available when surgical resection or liver transplantation cannot be proposed. [10] Like many other tumors, HCC cells seem to be incapable of inducing their own deaths through apoptotic pathway, mutant p53 can be frequently accumulated at a fairly high level in HCC, which probably provide malignant cells with the ability to counteract the effect of multiple anticancer drugs. Many HCC cells also show to be less sensitive to apoptotic signals induced by death receptor ligands. [11] Recently focus has been directed towards molecular targeting of herbal compounds to identify the mechanism(s) of action of these newly discovered bioactive compounds. Herbal medicine has become a popular complementary and alternative strategy for cancer; doubts concerning interference with the action of conventional chemotherapeutic drugs have been raised recently. [12]

The present work reveals that the methanolic fruit extract of *Sapindus saponaria* L. possess potent anti-proliferative activity against hepatocellular carcinoma cell lines HepG2 and Huh-7. Further FESS was fractionated into three fractions: n butanol fraction (B-FESS), Ethyl acetate fraction (F- FESS) and water fraction (W-FESS). Among the three fractions of FESS, E- FESS showed very significant inhibitory effect against cell proliferation in comparison to that of other two fractions. The cytotoxicity study was also performed in HepG2 and Huh-7 cells and it was found that EFESS showed significant decrease in O.D value of treated cells in a concentration dependent manner which indicates anti-proliferative activity. The MTT assay was also done on normal liver cell line and it showed insignificant cell toxicity. Apoptosis is characterized by a series of typical morphological features, such as shrinkage of cell, fragmentation into membrane bound apoptotic bodies and rapid phagocytosis by neibhouring cells. [13] Further the morphological study of HepG2 and Huh-7 cell treated with EFESS was also conducted by fluorescence microscopy and confocal microscopy. The fluorescence microscopic images by Acridine orange (AO) and ethidium bromide (EtBr) denoted that the two hepatoma cells treated with EFESS showed cell shrinkage, membrane blabbing, nuclear fragmentation and condensation of chromatin which are the hallmark of apoptosis. [14] Apoptogenic activity of EFESS was further evidenced from the confocal microscopic images of the treated HepG2 and Huh-7 cells when compared with that of the untreated control cells. After EFESS treatment, both the cells showed several signs of apoptosis like chromatin condensation, nuclear fragmentation and formation of apoptotic bodies whereas the untreated control cells were with intact nuclei. Internucleosomal fragmentation of genomic DNA has been the biochemical hallmark of apoptosis for many years. [15] Further in support of the apoptotic activity, agarose gel electrophoresis was done and the observations showed that EFESS showed ladder like degraded DNA band in the gel pattern whereas the untreated control cells showed intact DNA band when observed in U.V transilluminator and Chemi Doc™ Imaging System, Bio-Rad. Cell cycle analysis revealed that treatment with EFESS arrested the HepG2 and Huh7 cell populations in the G0/G1 phase of cell cycle. Mitochondrial membrane potential was measured using JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimi-dazolylcarbocyanine iodide), a cell-permeable, cationic carbocyaninedye that exists in a monomeric form that on entering the cytoplasm emits a green fluorescence. Subsequently, on entering the mitochondria it forms J aggregates and emits a red fluorescence. The ratio of red/green fluorescence represents loss of mitochondrial potential. JC-1 staining of HepG2 and Huh-7 cells treated with EFESS shows a significant shift in the transmembrane potential from red to green fluorescence. Hence, the observation suggests a change in the transmembrane potential, thereby reflects the occurrence of apoptosis. From all the above performed experiment it can be confirmed that FESS and its active fraction (EFESS) possesses anti-cancer effect on both the hepatocellular carcinoma, HepG2 and Huh-7 cell lines.

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REFERENCES

1. Chedid MF, Kruel CR, Pinto MA, Grezzana-Filho TJ, Leipnitz I, Kruel CD, Scaffaro LA, Chedid AD, Hepatocellular carcinoma: diagnosis and operative management, ABCD, 30, 2017, 272-8.
2. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, De Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M., Sorafenib in advanced hepatocellular carcinoma. New England journal of medicine. 24, 2008, 378-90.
3. Wahab AS, Selim MA., Lipids and flavonoids of Sapindus saponaria, Fitoterapia, 56, 1985, 167-8.
4. Goyal S., Medicinal plants of the genus Sapindus (Sapindaceae)-A review of their botany, phytochemistry, biological activity and traditional uses, Journal of Drug Delivery and Therapeutics, 14, 2014, 7-20.
5. Chen CY, Kuo PL, Chen YH, Huang JC, Ho ML, Lin RJ, Chang JS, Wang HM., Tyrosinase inhibition, free radical scavenging, antimicroorganism and anticancer proliferation activities of Sapindus mukorossi extracts, Journal of the Taiwan Institute of Chemical Engineers, 41, 2010, 129-35.
6. Kokate CK, Purohit AP, Gokhale SB., Pharmacognosy, 47th edition, 2, Nirali Prakashan, Pune, 2005, 6-19.
7. Strober W. Trypan blue exclusion test of cell viability, Current protocols in immunology, 111, 2015, A3-B.
8. Gerlier D, Thomasset N., Use of MTT colorimetric assay to measure cell activation, Journal of immunological methods, 94, 1986, 57-63.
9. Batra P, Sharma AK., Anti-cancer potential of flavonoids: recent trends and future perspectives, 3 Biotech, 3, 2013, 439-59.
10. Godin C, Louandre C, Bodeau S, Diouf M, Saidak Z, Conte MA, Chauffert B, Barbare JC, Barget N, Trinchet JC, Ganne N., Biomarkers of apoptosis and necrosis in patients with hepatocellular carcinoma treated with sorafenib, Anticancer research, 35, 2015, 1803-8.
11. Yuan Y, Zhang YS, Qu XJ. The Molecular Alterations Regarding Apoptosis in Hepatocellular Carcinoma Cells at a Glance, Inventi Impact: Molecular Pharmacology, 2, 2011, 1-5.
12. Abdel-Hamid NM, Nazmy MH, Mahmoud AW, Fawzy MA, Youssof M., A survey on herbal management of hepatocellular carcinoma, World journal of hepatology, 3, 2011, 175.
13. Saraste A, Pulkki K., Morphologic and biochemical hallmarks of apoptosis, Cardiovascular research, 45, 2000, 528-37.
14. Kerr JF, Wyllie AH, Currie AR., Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics, British journal of cancer, 26, 1972, 239-57.
15. Wyllie AH., Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation, Nature, 284, 1980, 555-6.
16. Koff JL, Ramachandiran S, Bernal-Mizrachi L., A time to kill: targeting apoptosis in cancer, International journal of molecular sciences, 16, 2015, 2942-55.

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