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The Role of Fragmented Adipose Mesenchymal Stem Cells in Vitro Therapy for Human Hepatoma Cell Line Growth

Mona M. Atia[*1], Ebtehal T. Sallam[1,2], Hanem S. Abdel-Tawab[1], Alshaimaa A. I. Alghriany[1]

[1] Laboratory of Molecular Cell Biology and Laboratory of histology, Zoology Department, Faculty of Science, Assiut University, 71516, Egypt

[2] Affiliated with Department of Biology, Faculty of Education, Taiz University, Yemen.

[*]Corresponding Author: e-mail**:** monatia@aun.edu.eg

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ABSTRACT

Background: Stem cells (SCs) from adipose tissue are being studied as part of regenerative medicine and used in vitro tissue engineering. **Aim:** The study goal was to highlight the modifications of fragmented adipose mesenchymal stem cells (AD-MSCs) on human hepatoma cell lines (HepG2 and Huh7) by inducing cell death (either apoptosis or necrosis). **Materials and Methods:** This study explains the methods of isolating, identifying, and characterization of fragmented frozen AD-MSCs. Also, investigate the phenotype of AD-MSCs culturing cells obtained in Passage 3 using CD105 & CD90 as positive markers and CD45 as a negative marker. Two Hepatoma cell line (HCC) cell lines were cultured in RPMI 1640/10%. The viability and apoptosis of the HCC cell line were evaluated by MTT and AO/EB methods, respectively. **Results:** Showed that, compared to CD45, the percentages of brown dots in the AD-MSC nucleus and cytoplasm increased for CD105 and CD90. HepG2 and Huh7 cells' growth and apoptosis were affected by the perceived seeding of fragmented AD-MSCs. Also, increasing the fragmented AD-MSC concentration (from 1:15 µl for 24 h to 1:30 µl for 48 h) in vitro decreased HCC cell line viability, in which

Huh7 was more susceptible than HepG2 after 24–48 h of incubation time ($p < 0.001$), as assessed by MTT. Moreover, cell counting by AO/EB assays appeared to initiate cell death, which increased in Huh7 cells compared to HepG2 cells. **Conclusion:** New evidence suggests that fragmented AD-MSCs could be highly efficient in preventing carcinogenesis by inducing apoptosis in HCC cell lines.

INTRODUCTION

Cancer is one of the world's major diseases, risking human health **[1].** Liver cancer is a serious health concern due to its devastating impact on our lives. Currently, HCC (hepatocellular carcinoma) has a high mortality rate is 95 % **[2]**. Due to an imbalance between excessive cell growth and apoptosis, P53, one of the most relevant tumors suppressor genes, is associated with HCC development **[3]**. HepG2 and Huh7 are two immortalized hepatic tumor cell lines commonly used as models for hepatocytes, although they only slightly imitate physiological liver function and tumor tissue in vitro **[4,5].** Since cell lines are similar to primary tissue, they are easy to be used , cheap, and culturable scientific research. Further, the use of such cells in research provides unlimited sources of biomaterials without the ethical implications associated with animal and human tissues **[5].** New targeted Therapeutic strategies are needed to prevent HCC Development in the cirrhotic liver or limit metastasis. **[3].** Regarding MSCs (mesenchymal stem cells), their use in tumor-targeted therapy has been widely considered an ideal method of delivering medication, which can improve therapeutic response and reduce side effects **[6].** Bone marrow mesenchymal stem cells (BM-MSCs) are readily isolated and proliferated in vitro for use in mesodermal cell differentiation **[7].** Since stem cells (SCs) can self-renew and differentiate into multiple cell lines, they are widely used for in vitro tissue engineering **[8].** In contrast to CD34, CD45, and HLA-DR on hematopoietic cell surface, CD44, CD90, and CD105 are found on the cell surface of mesenchymal stem cells (MSCs) **[9]**. Unlike other cells, they grow easily in the culture dish and even exhibit plastic adhesion ability. Furthermore, they possess intrinsic differentiation abilities and secrete plenty of growth factors and cytokines **[10].** Thus, in vitro co-culturing with tumor cells can inhibit tumor cell growth **[11]**. Over the past few years, MSCs have shown suppressive effects on a variety of tumor types through the

regulation of cell signaling, progression of the cell cycle, induction of apoptosis, and the engraftment of immune cells **[12].** New research indicates that MSCs can be genetically engineered to produce antitumor molecules such as interferon (INF) and tumor necrosis factor-related apoptosis inducing ligand (TRAIL) and that they could be used to generate therapeutics such as cytokines, apoptosis inducers, and prodrugs **[3]**. AD-MSCs that expressed TRAIL were active against myeloma in vitro and significantly increased myeloma cell death **[11]**. Our current study illustrates a proposed a target therapy strategy to see if fragmented AD-MSCs may cause cell death of hepatoma cell line (either apoptosis or necrosis) and reducing the viability and proliferation of human HepG2 and Huh7 cell lines in vitro

MATERIALS AND METHODS

2.1.Materials

Acridine orange, Ethidium bromide (AO/EB), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), RPMI-1640, fetal bovine albumin (FBS), collagenase type II Ethylenediaminetetraacetic acid (EDTA) and phosphate buffered saline (PBS) were used in this study and purchased from Sigma-Aldrich (St. Louis, MO, USA). The human HCC cell lines (HepG2 and Huh7) (Nawah-Scientific, Egypt) were cultured in RPMI-1640 growth medium supplemented with 1% antibiotic mix (10.000 u penicillin/ml, and 10.000 u streptomycin/ml) and 10% FBS at 37 °C under an atmosphere of 100% humidity and 5% CO_2.

2.2. Isolation and culture of AD-MSCs

Isolation of AD-MSCs from the fat tissue of healthy male rats were done following the previous study done by Atia et. Al. **[13]** Briefly, the adipose tissue of adult male rats was sliced into pieces, washed in sterile PBS. Following enzymatic digestion, the tissues were treated with collagenase type II (0.25 percent in PBS with 20% FBS) for 45 minutes. During digestion at 37°C, the Falcon tubes (50 mL) were rotated every 10 minutes for 20 minutes, after which the collagenase activity was stopped by adding FBS. After centrifugation (micro, ultra –centrifuge,), the AD-MSCs were reconstituted in 12

mL culture media. A cell strainer (40 mm) was used to filter the suspension before plating it on 10-cm culture dishes. For approximately two weeks, the cells were cultured at 37°C with 5% CO2. Three passages of AD-MSC transplantation were performed.

2.3. Cell Culture procedure.

The Cell lines (HepG2 and Huh7) were handled under aseptic conditions according to the standard method with a 1% anti-biotic mix. Both cell lines were cultured in RPMI 1640/10%, FBS at (37 °C, 5% CO2 in a 100% humid atmosphere. Every 2-3 days, media resupply under the same conditions was carried out. Cells were grown in plate culture and used at the exponential growth phase for experiments.

2. 4. Study design

Human hepatoma cell lines were used in this study. HepG2 and Huh7 cell lines are subdivided into three groups. **G (1):** as a control group for HepG2 or Huh7 cell lines ($2x10^3$cells/well) with DMSO 0.2%. **G (2):** HepG2 or Huh7 cell line ($2x10^3$cells/well) treated after 1 hour with fragmented frozen AD-MSCs ($6x10^4$cells) at a concentration of 1:15 µL. **G (3)**: HepG2 or Huh7 cell lines ($2x10^3$cells/well) treated with fragmented frozen AD-MSCs ($6x10^4$cells) at a concentration of 1:30 µL.

2.5. Cell cultures of fragmented AD-MSCs, HepG2 and Huh7 cells preparation

When AD-MSCs reach to P3 of growth, cell culture flasks transferred from CO2 incubator to -80 C for freezing for two hours. Cell cultures were hit carfely and thawed at room temperature. All contents of cell flasks were collected into centrifuge tubes and centrifuged at 10000 rpm for 10 min. pellets of AD-MSCs were collected into 1.5 ml eppendorf tubes and kept in -80 C for further use. Then pellet resuspended in 15 and 30 µL of RPMI 1640/10%, and FBS. The incubation of different concentrations of fragmented frozen AD-MSCs on HepG2 and Huh7 cells were seeded in 96-well plates at a density of ($2x10^3$cells/well) **[14].**

2.6. Immunocytochemistry (ICC)

Samples were treated in 4% paraformaldehyde for 20min. The cells were washed three times for five minutes each with PBS. They were lysed in PBS with fresh 0.2% Triton X100 for 5 min until being washed three times for 5 min each. Following the protocol, a secondary antibody HRP Anti-Polyvalent (AEC) stain was also used. Additionally, non-specific background staining was minimized by incubating the slides for 10 min in a blocking buffer and then rinsing them twice with PBS. The slides were then incubated for one hour at room temperature with primary antibodies against CD105, CD90 (2:100), and CD45 (1:100) until they were washed four times for 5 minutes each with PBS buffer. A 10-minute incubation at room temperature with the Ultra-Tek anti-polyvalent stain was followed by four rinses. A few minutes later, the cells were counterstained and cover slipped after adding DAB chromogen to the DAB substrate mix as previously described **[13,15].**

2.7. Cell viability assay

In vitro, MTT were used to detect the effect of AD-MSCs on cell viability, survival, and growth in a variety of human HCC cell lines. as previously described **[16].** $2x10^3$cells/well of Hep G2 and Huh7 cells were seeded in 96-well plate and cultured for 1-2 days. Following incubation with 15 µL and 30 µL fragmented AD-MSCs, instead of the 200 µl growth medium were used, supplemented with 50 µl of MTT (2 mg/ml) for 3 hours. In 200 µl of DMSO the crystals formed from the formazan reaction were dissolved and measured the absorbance. A microplate reader (Bio Tek ELISA Plate Reader (Bio Tek Instruments, Inc., Highiand Park, Winooski, VT 05404-0998, USA) was used to measure absorbance at 570 nm wavelength after incubation. Cell viability is calculated as a percentage of control cells exposed only to DMSO.

2.8. Dual fluorescent dye for AO/EB

The dual fluorescent dye Acridine orange/Ethidium bromide (AO/EB) assay was performed to visualize apoptosis which emit green and orange fluorescence when they bind to DNA in a cell suspension of 2.0×10^6 cells/ml following other research group **[17].** In 12 wells plate, HCC were incubated with fragmented AD-MSCs in suitable

concentrations for 24 h. In summary, treated cells were washed in 200 μL of warm 1X PBS, resuspended in 150 μL of AO/EB dye for 5 minutes, rinsed in 400 μL of 1X PBS, centrifuged (micro, ultra –centrifuge) at 277 x g for 2 minutes at room temperature, resuspended in PBS, and examined under a fluorescence microscope. A 20X objective lens of NA 0.40 with total 200X magnification in Olympus BX41 fluorescence microscope with a 480/30 nm excitation filter and a 535/40 nm emission filter was used for examining samples mounted with moviol/DABCO. Toup Tek ToupView, Version: x86, Compatible with Windows XP/Vista/7/8/10, China, was used to capture the images and differentiate between living and apoptotic cells with bright green indicating intact nucleus. Apoptotic cells recognized as proapoptotic if chromatin is dense green and late apoptotic if chromatin is orange, and necrotic if the nucleus is red .

2.9. Statistical analysis

The statically analysis for cell viability (apoptosis or necrosis) were analyzed using Prism version 8.4.3 (686) (Graph Pad Software, San Diego, Inc., USA). A two-way ANOVA with repeated measures was conducted, followed by the Student t-test (Student-Newman-Keuls test T-test) multiple comparison tests to assess the differences between the three classes, with significance determined at $P < 0.05$. The data were expressed as mean ± SE from at least three separate experiments.

RESULTS

3.1. Characterization of AD-MSCs.

AD-MSCs were observed to be circular and suspended since day 0 of culture **Fig.1 (a**). The cells formed thin attaching spindles another day after differentiation **Fig.1 (b**). In passage one (P1), some AD-MSCs appeared spindly **Fig.1 (c**); in passage two (P2), smaller colonies formed **Fig.1 (d**); and in passage three (P3) fibroblastic cells appeared **Fig.1 (e**). The immunocytochemistry of AD-MSCs at P3 for CD105 is illustrated in **Fig. 2 (a & b**) and CD90 **Fig. 2 (c & d**) showed a strong positive detection as brown dots in nuclei and cytoplasm. In contrast, CD45 were appeared negative reaction **Fig. 2 (e & f**).

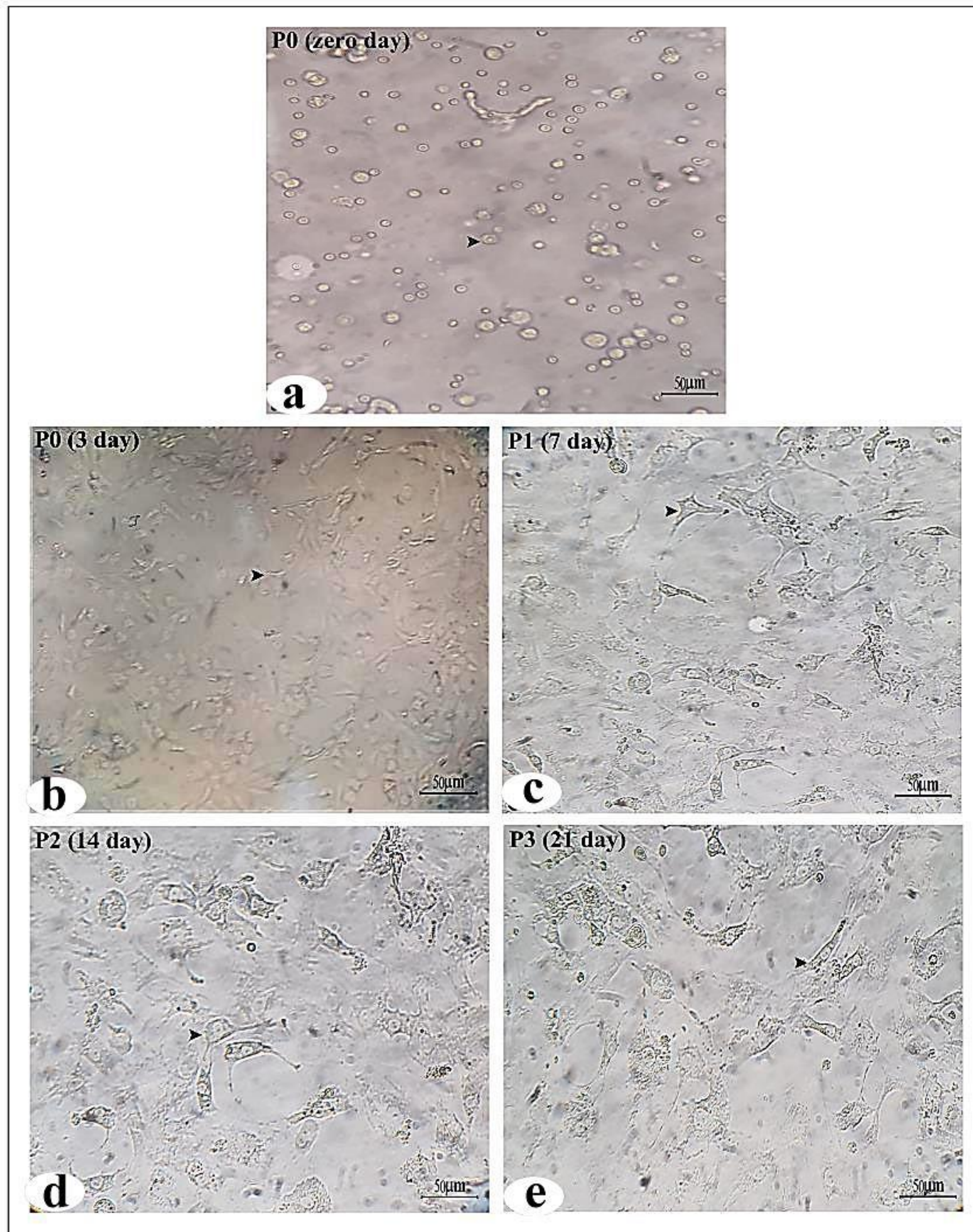


Fig. 1. Photomicrographs of AD-MSC recognition utilizing phase contrast microscopy at various times and passages. **(a)** Day zero were showed AD-MSCs circular and suspended; **(b)** P0 at 3 days, The cells formed thin attaching spindles; **(c)** P1, some AD-MSCs appeared spindly; **(d)** P2, smaller colonies formed; **(e)** P3, fibroblastic cells appeared and arrows head detect an AD-MSC.

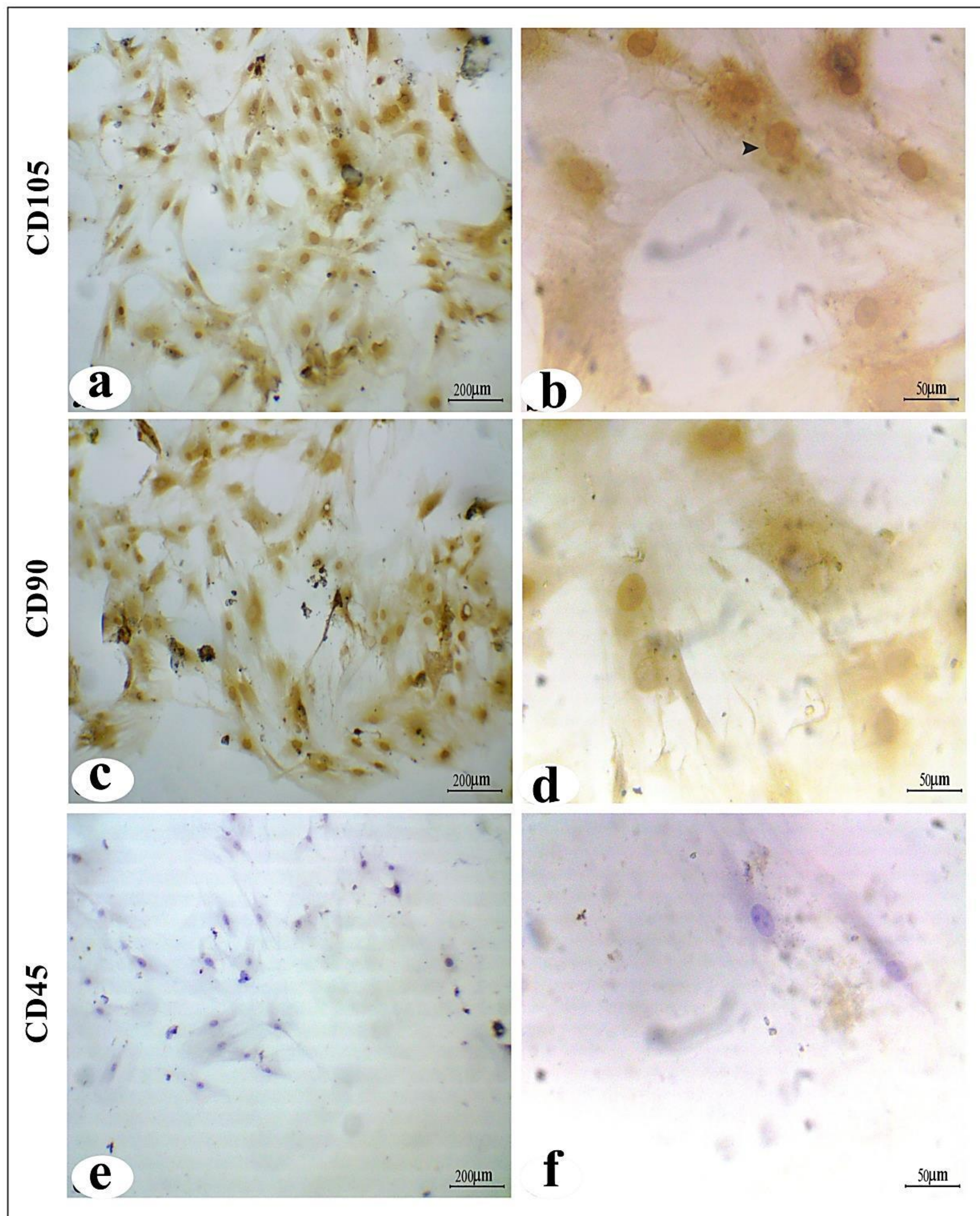


Fig. 2. Photomicrographs of Immunocytochemistry staining of cell surface markers of AD-MSCs: There is high positive expression for CD105 **(a &b),** CD90, **(c &d),** and negative expression for CD45 **(e &f).**

3.2. Cultural morphology of AD-MSC, HepG2 and Huh-7 cells

The cell culture morphology of AD-MSC resembled an attached fibroblastic appearance on the 3rd passage **Fig.3 (a**). HepG2 cells have a high proliferation rate and an epithelial-like morphology **Fig.3 (b**). A Huh-7 (mutation in the p53 gene) is an immortal epithelial-like tumorigenic cell line adhere to flasks or plates **Fig.3 (c**). Phase-contrast photomicrography was performed using an inverted phase-contrast microscope.

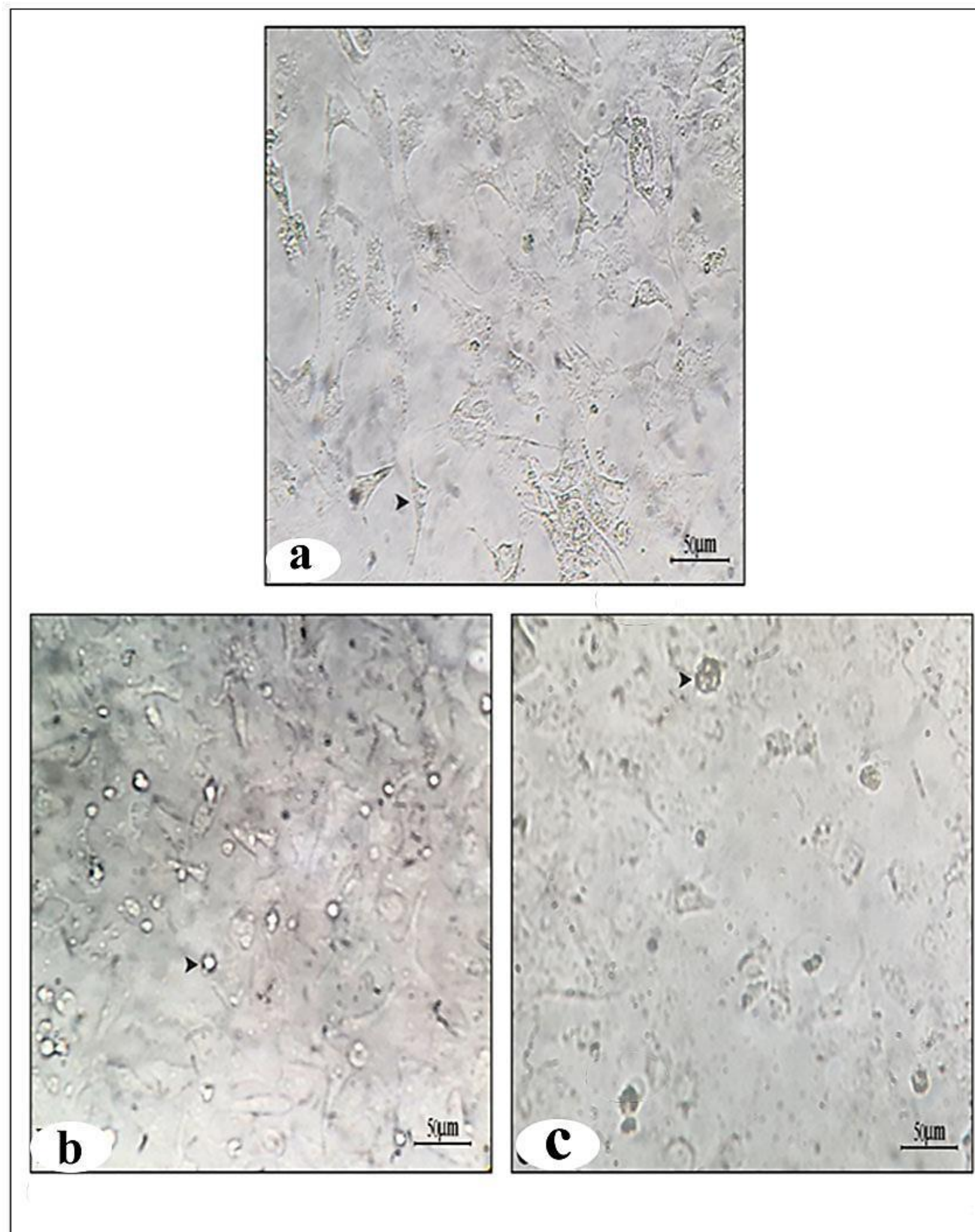


Fig.3. Photomicrographs of inverted phase-contrast microscopically image showed cell culture morphology of **(a)** AD-MSC in the 3rd passage, **(b)** HepG2 cell line 2 days after culturing **(c)** Huh7 cell line 2 days after culturing.

3.3 AD-MSCs suppress cell line viability

Different concentrations; 1:15 µl and 1:30 µl of fragmented AD-MSCs were applied to a panel of HCC cell lines taken from human hepatoma to study its effect on cell viability for 2 different durations; 24 &48h. An MTT assay was applied to demonstrate the changes in cellular viability with dark purple coloration indicating high viability and light coloration indicating low viability **Fig. (4).** There was a significant time and concentration dependent decrease in the percentage of viable cells ($p < 0.001$) of HepG2 and Huh-7 cell lines incubated with fragmented AD-MSCs against the control group and the HepG2 cells was less sensitive to fragmented AD-MSCs than the Huh7 cell line **Fig.5 (a&b).**

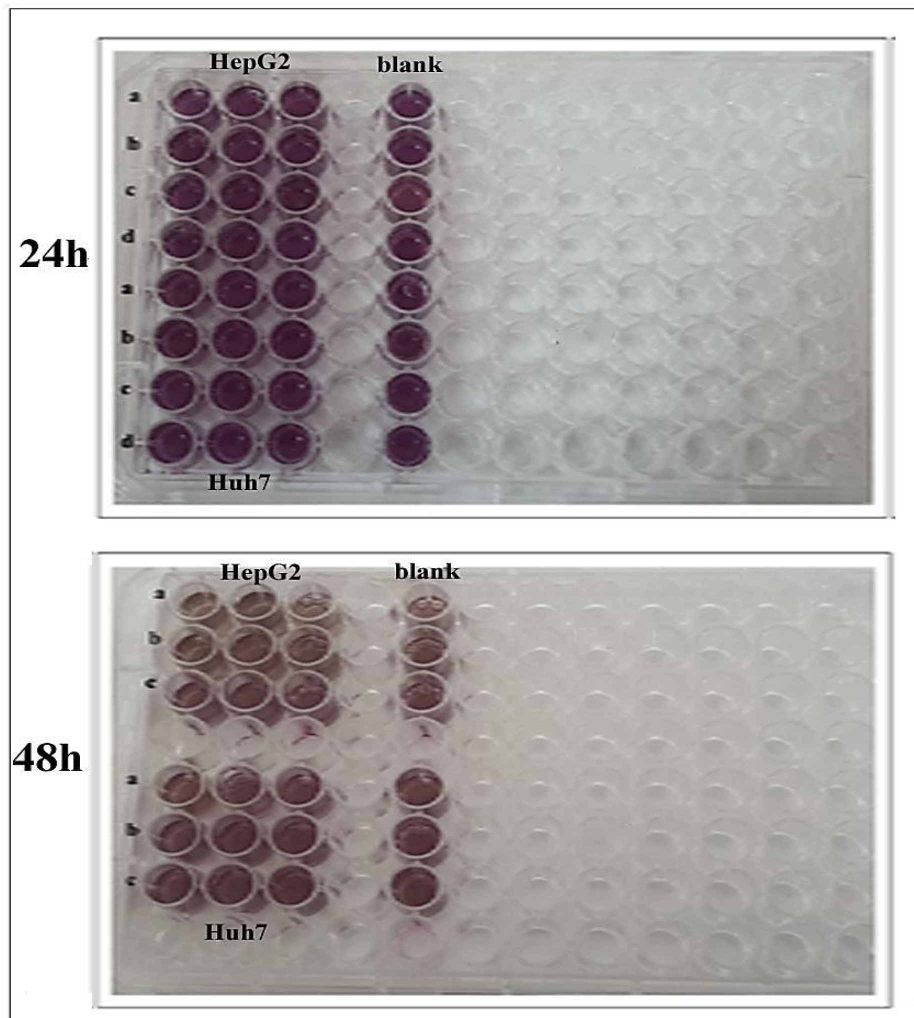


Fig.4: Microliter plate after MTT test. Increase amount of viability cells with increase of dark purple color indicates high viability **(a)** Controls Hepg2 &Huh7control wells **(b)** HepG2&Huh7 cell line with fragmented AD-MSCs at 1:15 µl **(c &d)** HepG2&Huh7 cell line with fragmented AD-MSCs at1:30 µl and each an incubation for 24 h and 48 h.

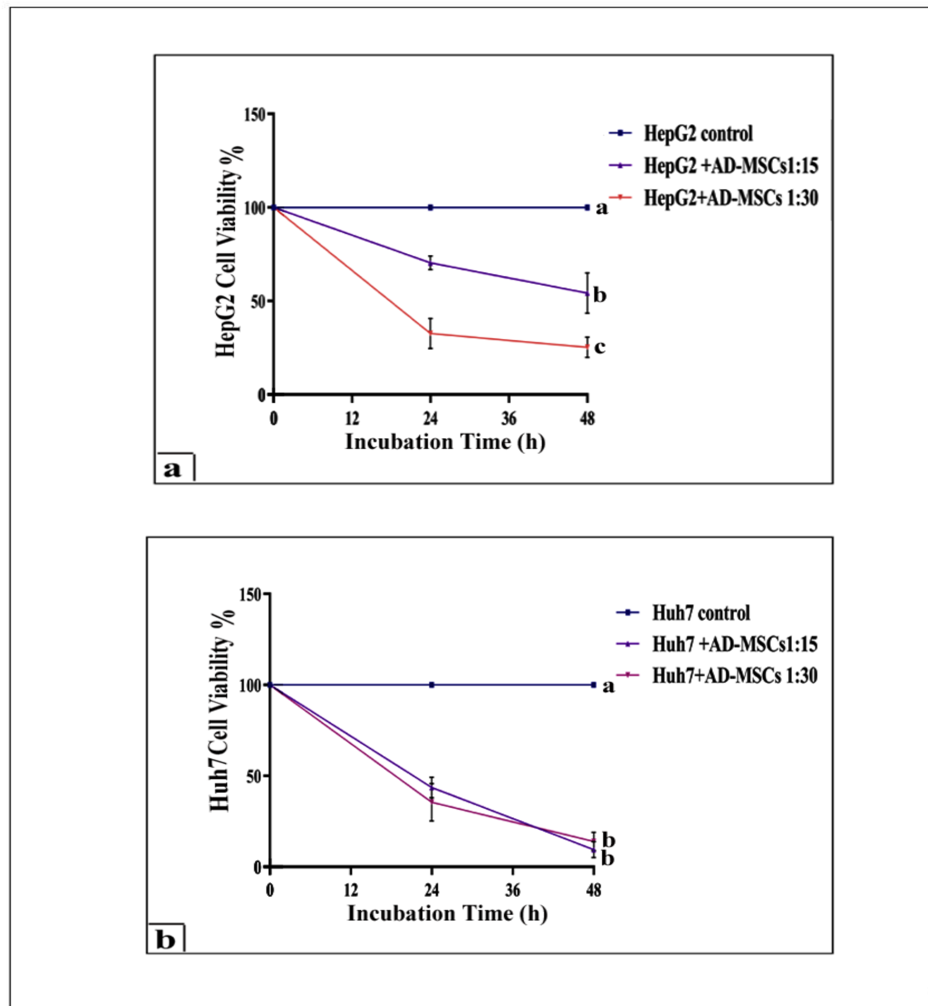
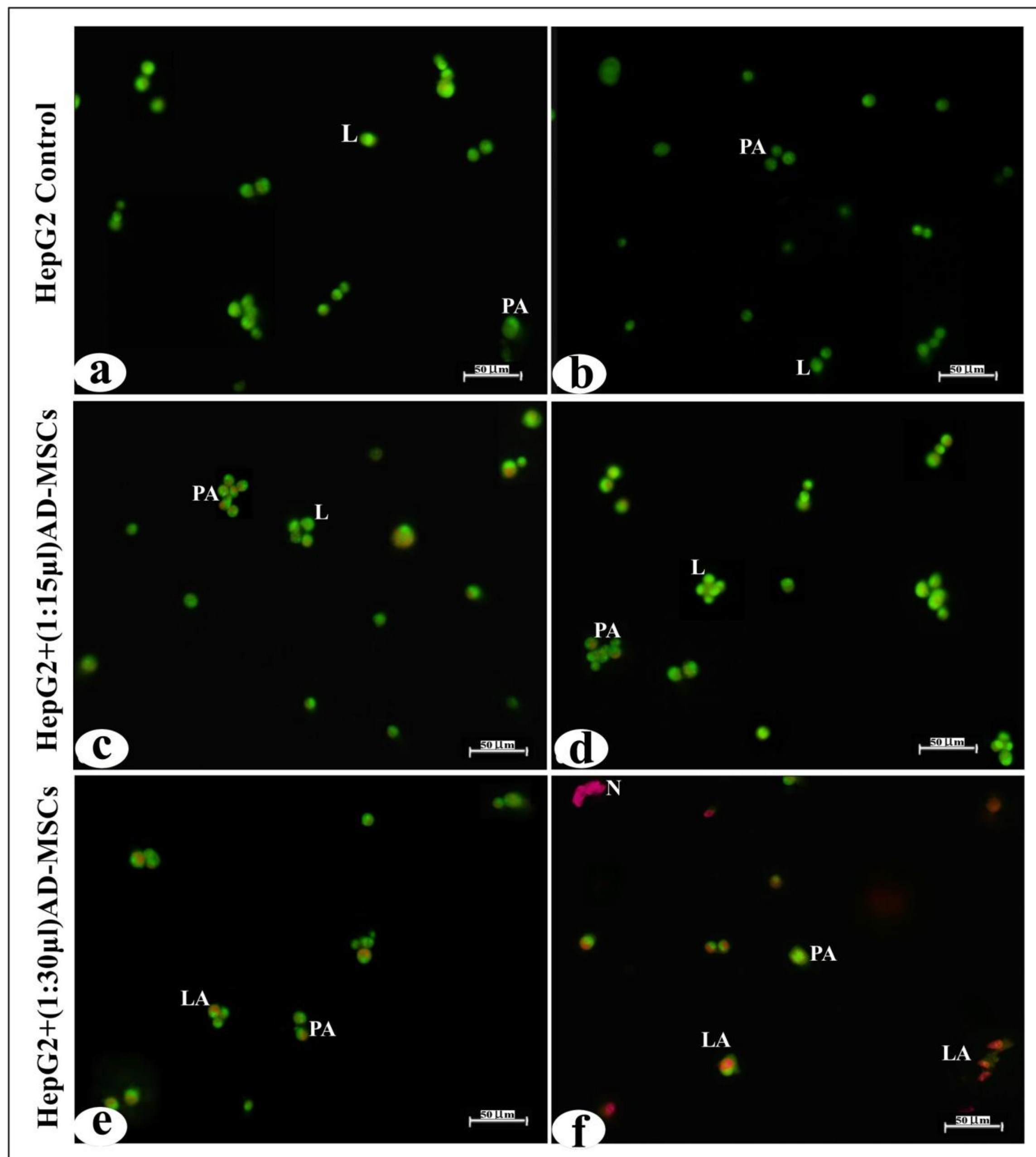


Fig.5 : Fragmented AD-MSCs (1:15 µl and 1:30 µl). Show decreased the viability of HepG2 and Huh-7 hepatic cancer cells measured by MTT assay. Data represented the mean ± SE after 24 and 48 h of treatment. The values in the column with unlike superscript letters were significantly different ($p < 0.001$).

3.4 Apoptosis assay by AO / EB fluorescent staining

The AD-MSCs were tested for their anti-carcinogenic potential using the dual fluorescent dye method AO / EB staining **Figs. (6-8)**. Cells staining were variable from green fluorescence to orange fluorescence corresponding to living to apoptosis or necrosis, respectively. The control group (HepG2 and Huh7 cells) were shown no cell death; this is evident by the full green and homogenate nucleus **Figs. 6, 7 (a & b) and Fig.8 (a & b)** respectively. Fragmented AD-MSCs were used in the screening at a concentration of 1:15 µl for 24 h, which showed a fragmented nucleus with dense green or orange chromatin colors appearing, which indicates the initiation early and late of cell death in HepG2 and Huh7cells **Figs. 6 , 7 (c & d) and Fig.8 (a & b)** respectively. The AD-MSCs at 1:30 µl for 24 h was increased the death percentage, and very little green fluorescence was detected in HepG2 and Huh7 cells **Figs. 6,7 (e & f) and Fig.8 (a & b)** respectively. the incubation of fragmented AD-MSCs with Huh7 cells at 1:15 µl or 1:30 µl were effective in death more than HepG2 cells **.**



Figs.6. Photomicrographs of fluorescence microscope images of Hep G2 cells stained with AO/EB dual fluorescent dyes. **(a& b)** represent the control group, **(c&d)** represent Hep G2 cells + 1:15 µl AD-MSCs while **(e&f)** represent Hep G2 cells + 1:30 µl AD-MSCs . Note; **L**: living cell; **PA**: Proapoptotic; **LA**: late apoptosis; **N**: necrosis.

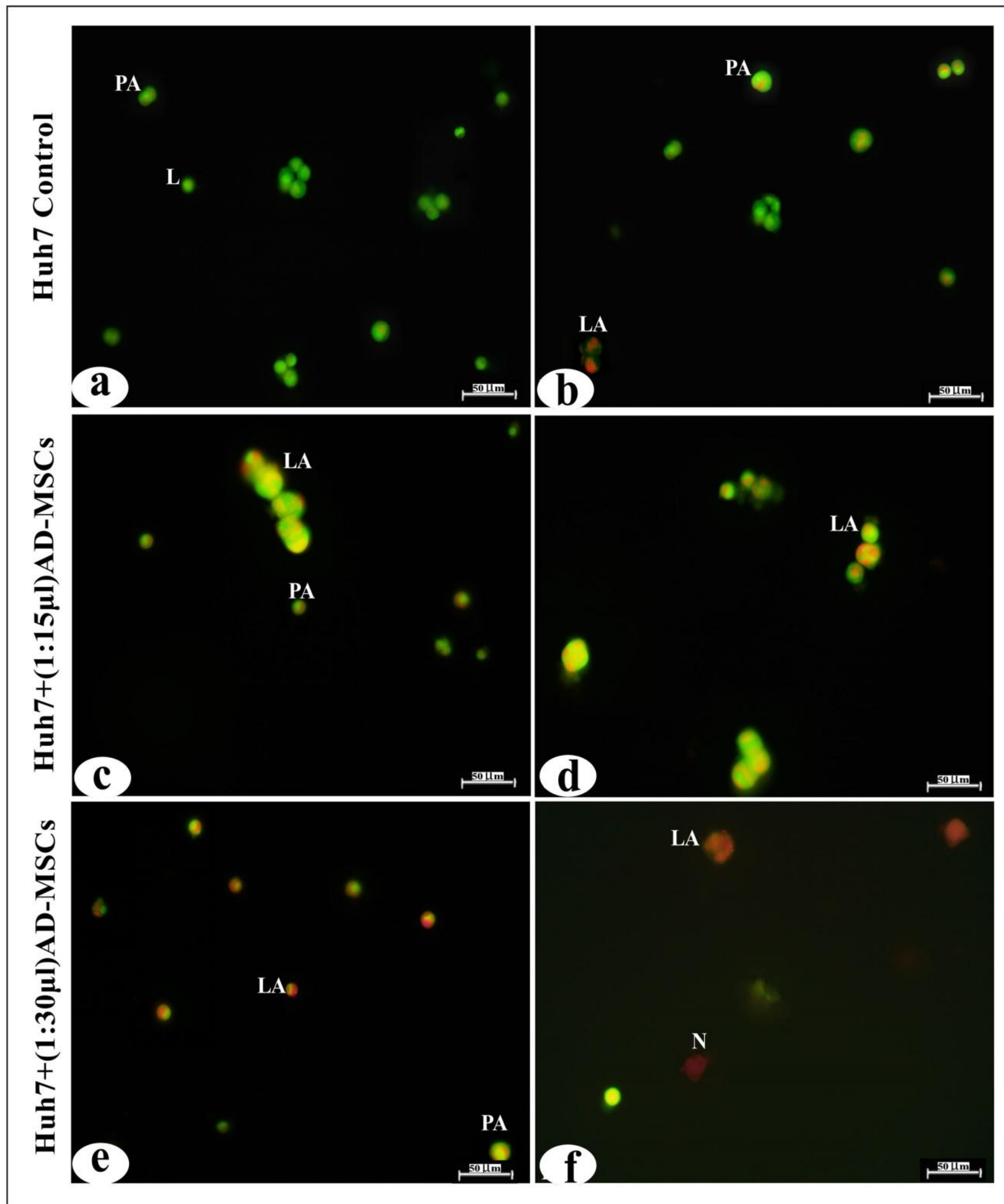


Fig. 7. Photomicrographs of fluorescence microscope images of Huh7 cells stained with AO/EB dual fluorescent dyes. **(a& b)** represent the control group, **(c&d)** represent Huh7 cells + 1:15 µl AD-MSCs while **(e&f)** represent Huh7 cells + 1:30 µl AD-MSCs . Note; **L**: living cell; **PA**: Proapoptotic; **LA**: late apoptosis; **N**: necrosis.

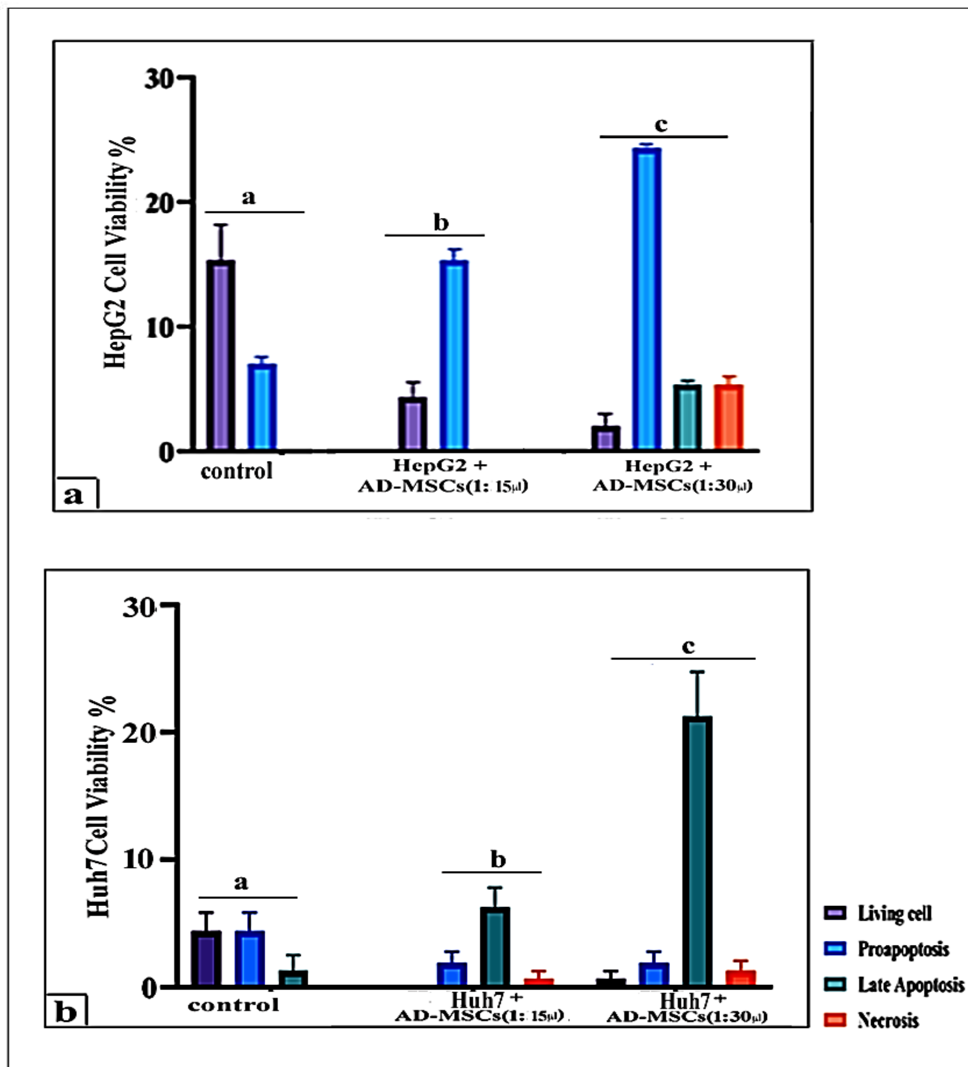


Fig. 8 (a &b). AO/EB dual fluorescent dye Graphs of HepG2 and Huh7were used to determine the control group, HepG2 or Huh7 cells + 1:15 µl fragmented AD-MSCs and HepG2 or Huh7 cells + 1:30 µl fragmented AD-MSCs for 24 hrs. Note; L: living cell; **PA**: Proapoptotic; **LA**: late apoptosis; **N**: necrosis. Data expressed as mean ± SE from at least three separate experiments. Values in the same column with unlike superscript letters are significantly different at $P < 0.05$.

DISCUSSION

This research aims for assessment the effect of fragmented AD-MSCs on HCC cell line growth in vitro, using HepG2 and Huh7 as hepatoma models, which were detected by MTT and AO/EB. Prior studies have noted that the best alternative to bone marrow-derived MSCs is AD-MSCs, which are easier to obtain in large quantities, can be expanded indefinitely without phenotypic or genetic changes, and proliferate more rapidly than BM-MSCs **[18].**

Due to their production of growth factors and cytokines, AD-MSCs and HepG2 can interact via the cell culture insert **[19,20].** The most notable finding was that immunocytochemistry of AD-MSCs at P3 for CD105 and CD90, in contrast to CD45, indicates the presence of AD-MSCs. The expression of AD-MSC surface markers is in line with those of other similar studies **[13,21,22].** Experiments have shown that isolated hMSCs between P2- P3 will produce adequate cells for analysis. These previous studies are in agreement with our findings on CD90 and CD105 characterization of adipose mesenchymal stem cells [**23,24**]. Isolated and characterized AD-MSCs from male rat adipose tissue that appeared to have fibroblastic morphology have been previously reported [**13,25**].

The most interesting finding was that the effects of fragmented frozen AD-MSCs on HCC cell line viability this agrees with the report of [**22**]. Moreover, this finding is consistent with that of **Xiaoli Zhang,2021[26]** who found that MSCs can homing to TME (tumor microenvironment) in in vitro and animal models studies. Highly tumorigenic HCC cells increase MSC migration capacity, making them an ideal carrier for targeted liver cancer therapy **[27,28].** Interestingly SKOV-3 and A2780 ovarian cancer cells were inhibited by exosomes extracted from human AD MSC-derived conditioned medium. Exosome treatment also resulted in a reduction in cancer cell viability and wound healing **[25,29]**.

Another important finding discovered the dual fluorescent dye AO/EB assay was performed to visualize apoptosis induced by fragmented AD-MSCs, which emit green and orange fluorescence. This result may be explained by the fact that Apoptotic cells

with intact membranes may have been penetrated by AO, which turns green when bound to DNA. An orange-red fluorescence is produced only when DNA fragments or apoptotic bodies are bound to EB this finding was also reported by **[17,30]**. AO/EB dual fluorescent dye assays revealed to increase the initiation of cell death This observation may support the hypothesis that fragmented AD-MSCs could induce cell death by reducing the in vitro cell viability and proliferation of HepG2 and Huh7 cells based on a concentration-and time-dependent manner.

A possible explanation for this might be that AD-MSCs derived Exosomes enhanced pro-apoptotic signaling molecules Bax, caspase 3 and caspase 9 while significantly reducing anti-apoptotic bcl-2 protein **[23].** In addition, these findings follow those of **Tang (2016) [22]**, who found that umbilical cord derived mesenchymal stem Cells (UC-MSCs) effectively inhibit HepG2 cell proliferation. UC-MSCs also promote apoptosis by reducing the expression of genes such as AFP, Bcl-2, and Survivin, all of which contribute to apoptotic signaling pathways. Bone marrow MSC and Their condition medium (CM) has been demonstrated to have anti-tumor cells **[31].** The activation of the BAX gene causes the mitochondrial membrane to become more permeable, resulting in the release of the apoptotic factor cytochrome c, which induces cancer cell death **[32].** A closer look at AD-MSC in vitro HCC therapy could lead to more effective treatments with the ultimate level of safety.

CONCLUSION

In the light of this study, the fragmented AD-MSCs have cytotoxic effect on viability and growth of HepG2 and Huh7cell lines by inducing cell death (apoptosis or necrosis). to the extent of our knowledge , There is few studies previously done in this field. This study will open the door for more experimental researches to develop new strategies in cancer management.

REFERENCES

[1] Liu, He, Y.Zhang, D.Cai, Y.Zhang, C.Zhang, P.Zhu, H.Xu, N.Liang, S., (2017): In vitro anticancer effects of two novel phenanthroindolizidine alkaloid compounds on human colon and liver cancer cells. *Mol Med Rep*, **Vol 16**(No 3) p.2595-2603.

[2] Torre LA, Siegel RL, Ward EM, Jemal A. (2016): Global cancer incidence and mortality rates and trends - an update. *Cancer Epidemiol Biomarkers Prev*. **Vol 25** p.16–27.

[3]Serhal, R.Saliba, N.Hilal, G.Moussa, M.Hassan, G. S.El Atat, O.Alaaeddine, N., (2019): Effect of adipose-derived mesenchymal stem cells on hepatocellular carcinoma: In vitro inhibition of carcinogenesis. *World J Gastroenterol*, **Vol 25**(No 5) p. 567-583.

[4] Verrier, E. R.Colpitts, C. C.Schuster, C.Zeisel, M. B.Baumert, T. F., (2016): Cell Culture Models for the Investigation of Hepatitis B and D Virus Infection. *Viruses*, **Vol 8**(No 9) p. 261.

[5] Arzumanian, V.A. and O.I. Kiseleva, (2021): The Curious Case of the HepG2 Cell Line: 40 Years of Expertise. *Int. J. Mol. Sci.*, **22**(23). p. 13135.

[6] Yin, Z.Jiang, K.Li, R.Dong, C.Wang, L.Multipotent mesenchymal stromal cells play critical roles in hepatocellular carcinoma initiation, progression and therapy. *Molecular Cancer*, **Vol 17**(No 1) (2018) p. 178.

[7] Byun, Chun Sung,Hwang, Soonjae,Woo, Sung-Hun,Kim, Moon Young,Lee, Jin Suk,Lee, Jong In,Kong, Jee Hyun,Bae, Keum Seok,Park, Il Hwan,Kim, Sung Hoon,Eom, Young Woo., (2020): Adipose Tissue-Derived Mesenchymal Stem Cells Suppress Growth of Huh7 Hepatocellular Carcinoma Cells via Interferon (IFN)-β-Mediated JAK/STAT1 Pathway in vitro. *International journal of medical sciences*, **Vol 17**(No 5) p. 609-619.

[8] Gibler, P.,Gimble, J.,Hamel, K.,Rogers, E.,Henderson, M.,Wu, X., (2021): Human Adipose-Derived Stromal/Stem Cell Culture and Analysis Methods for Adipose Tissue Modeling In Vitro: A Systematic Review.*Cells*, **Vol 10**(No 6) p. 1378 .

[9] Chinnadurai, R.Porter, A. P.Patel, M.Lipat, A. J.Forsberg, M. H.Rajan, D.Hematti, P.

Capitini, C. M.Bruker, C., (2021): Hepatocellular Carcinoma Cells Are Protected from Immunolysis by Mesenchymal Stromal Cells Through Indoleamine 2,3 Dioxygenase. *Front Cell Dev Biol*, **Vol 9**p. 715905.

[10] Pittenger, M. F.,Discher, D. E.,Péault, B. M.,Phinney, D. G., (2019): Mesenchymal stem cell perspective: cell biology to clinical progress. *NPJ Regenerative medicine*, **Vol 4(No1)** p. 22.(1-15)

[11] Javan, M.R., A. Khosrojerdi, and S.M. Moazzeni, (2019): New Insights Into Implementation of Mesenchymal Stem Cells in Cancer Therapy: Prospects for Anti-angiogenesis Treatment. *Front Oncol*, **Vol 9** p. 840.

[12] Wu, L.,Tang, Q.,Yin, X.,Yan, D.,Tang, M.,Xin, J.,Pan, Q.,Ma, C.,Yan, S. (2019): The Therapeutic Potential of Adipose Tissue-Derived Mesenchymal Stem Cells to Enhance Radiotherapy Effects on Hepatocellular Carcinoma. *Front Cell Dev Biol*, **Vol 7**p. 267.

[13] Atia, M.M. and A.A.I. Alghriany, (2021): Adipose-derived mesenchymal stem cells rescue rat hippocampal cells from aluminum oxide nanoparticle-induced apoptosis via regulation of P53, Aβ, SOX2, OCT4, and CYP2E1. *Toxicol Rep*, **Vol 8**p. 1156-1168.

[14] Gad1, M.S., B.A. Elfeky, and A.M. Nofal1, (2014): Stem Cells and Endometrial Hyperplasia *Journal Of Biochemistry, Microbiology And Biotechnology* ,**Vol 2**(No 2) p. 70-75.

[15] Marchenko, S. and L. Flanagan, (2007): Immunocytochemistry: human neural stem cells. J Vis Exp, **Vol** (No 7) p. 267.

[16] Abdel Shakor, Abo Bakr, Atia, Mona, Ismail, Ismail Ahmed, Alshehri, Ali, El-Refaey, Hesham,Kwiatkowska, Katarzyna,Sobota, Andrzej, (2014): Curcumin induces apoptosis of multidrug-resistant human leukemia HL60 cells by complex pathways leading to ceramide accumulation.*Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, **Vol 1841**(No 12) p. 1672-1682.

[17] Renvoizé, C.,Biola, A.,Pallardy, M.,Bréard, J., (1998): Apoptosis: Identification of dying cells. *Cell Biology and Toxicology*, **Vol 14**(No 2) p. 111-120.

[18] Puglisi, M. A.,Saulnier, N.,Piscaglia, A. C.,Tondi, P.,Agnes, S.,Gasbarrini, A., (2011): Adipose tissue-derived mesenchymal stem cells and hepatic differentiation: old concepts and future perspectives. *Eur Rev Med Pharmacol Sci*, **Vol 15**(No 4) p. 355-64.

[19] Liau, L. L.,Makpol, S.,Azurah, A. G. N.,Chua, K. H., (2018): Human adipose-derived mesenchymal stem cells promote recovery of injured HepG2 cell line and show sign of early hepatogenic differentiation. *Cytotechnology*. , **Vol 70**(No 4) p. 1221-1233.

[20] Ling Ling Liau,Makpol Suzana,Abdul Ghani Nur Azurah,Kien Hui Chua, (2016): Hydrogen Peroxide Induces Acute Injury and Up-regulates Inflammatory Gene Expression in Hepatocytes: An in vitro Model. *Sains Malaysiana*, **Vol 45**(No 3) p. 451–458.

[21] Carter-Arnold, J. L.,Neilsen, N. L.,Amelse, L. L.,Odoi, A.,Dhar, M. S., (2014): In vitro analysis of equine, bone marrow-derived mesenchymal stem cells demonstrates differences within age- and gender-matched horses. *Equine Vet J*, **Vol 46**(No 5) p. 589-95.

[22] Tang, Y. M.,Bao, W. M.,Yang, J. H.,Ma, L. K.,Yang, J.,Xu, Y.,Yang, L. H.,Sha, F.,Xu, Z. Y.,Wu, H. M.,Zhou, W.,Li, Y.,Li, Y. H., (2016): Umbilical cord-derived mesenchymal stem cells inhibit growth and promote apoptosis of HepG2 cells. *Mol Med Rep*, **Vol 14**(No 3) p. 2717-24.

[23] Baghaei, K.,Hashemi, S. M.,Tokhanbigli, S.,Asadi Rad, A.,Assadzadeh-Aghdaei, H.,Sharifian, A.,Zali, M. R. (2017): Isolation, differentiation, and characterization of mesenchymal stem cells from human bone marrow. *Gastroenterol Hepatol Bed Bench*, **Vol 10**(No 3) p. 208-213.

[24] Liu, Q. Q.,Zhang, F. F.,Wang, F.,Qiu, J. H.,Luo, C. H.,Zhu, G. Y.,Liu, Y. F., (2015): TIPE2 Inhibits Lung Cancer Growth Attributing to Promotion of Apoptosis by Regulating Some Apoptotic Molecules Expression. *PLoS One*, **Vol 10**(No 5) p. e0126176.

[25] Janockova, J.,Slovinska, L.,Harvanova, D.,Spakova, T.,Rosocha, J., (2021): New therapeutic approaches of mesenchymal stem cells-derived exosomes. *J Biomed Sci*, **Vol 28**(No1) p. 39.

[26]Zheng,Qingyuan,Zhang,Shuijun,Guo,Wen-Zhi,Li,Xiao-Kang, (2021): TheUnique Immunomodulatory Properties of MSC-Derived Exosomes in Organ Transplantation. *Frontiers in Immunology*,**Vol 12** p. 659621.

[27] Endaya, B.,Guan, S. P.,Newman, J. P.,Huynh, H.,Sia, K. C.,Chong, S. T.,Kok, C. Y. L.,Chung, A. Y. F.,Liu, B. B.,Hui, K. M.,Lam, P. Y. P., (2017): Human mesenchymal stem cells preferentially migrate toward highly oncogenic human hepatocellular carcinoma cells with activated EpCAM signaling. *Oncotarget*, **Vol 8**(No 33) p. 54629-54639.

[28] Zhang, X. and N. Li, (2022): The role of mesenchymal stem cells in the occurrence, development, and therapy of hepatocellular carcinoma. *Cancer Medicine*, **Vol 11**(No 4) p. 931-943

[29] Reza, Ammt,Choi, Y. J.,Yasuda, H.,Kim, J. H., (2016): Human adipose mesenchymal stem cell-derived exosomal-miRNAs are critical factors for inducing anti-proliferation signalling to A2780 and SKOV-3 ovarian cancer cells. *Sci Rep*, **Vol 6**p. 38498.

[30] Liu, K.Liu, P. C.,Liu, R.,Wu, X., (2015): Dual AO/EB staining to detect apoptosis in osteosarcoma cells compared with flow cytometry. *Med Sci Monit Basic Res*, **Vol 21** p. 15-20.

[31] Attar-Schneider, O., Zismanov, V., Drucker, L., Gottfried. M. (2016): Secretome of human bone marrow mesenchymal stem cells: an emerging player in lung cancer progression and mechanisms of translation initiation. *Tumour Biol*, **Vol 37(No)** p.4755-4765.

[32] Mokh. A. A. , Abdelhady D. H., Ghazy E. W., Aboumosalem H., Goda W, M., (2019): Sesame oil mitigates initiation stage of diethylnitrosamine hepatocarcinogenesis in rats. *Slov Vet Res*, **Vol 56** p. 487–98.