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Aloe vera gel as a stimulant for mesenchymal stem cells differentiation and a natural therapy for radiation induced liver damage

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ABSTRACT

Aloe vera is a medical plant that has been used, traditionally, in treatment of several dermal disorders. In addition to its role as an anti-cancer, anti-oxidant, anti-diabetic, and anti-hyperlipidemic agent. *Aloe vera* gel extract contains several compounds like minerals, enzymes, hormones and carbohydrates. Therefore, *Aloe vera* as well as its bioactive constituents have been studied to determine its intriguing potential roles in medicinal science. Mesenchymal stem cells (MSCs) are biologically active precursor cells that can self-renew and develop into a variety of cell types. Plant extracts have been used, *in vitro*, to enhance the proliferation and differentiation of MSCs. Therefore, the present study aimed to evaluate the therapeutic effect of lyophilized *Aloe vera* gel together with bone marrow (BM)-MSCs transplantation against radiation induced liver damage (RILD) in X-ray irradiated Sprague dawley male rats. By determining the oxidative stress, antioxidant enzymes, and pro-inflammatory cytokines in liver tissue homogenate, the antioxidant and anti-inflammatory properties of lyophilized *Aloe vera* gel were investigated. The degree of liver damage and NF-κB expression were determined using histological and immunohistochemical staining techniques. The results showed that treatment of irradiated rats with lyophilized *Aloe vera* gel and MSCs transplantation has led to an improvement in liver function and a decrease in fibrotic markers, oxidative stress, and pro-inflammatory cytokines; as well as, a reduction in the pathological alterations in the rats' liver and a reduced NF-κB activation. Lyophilized *Aloe vera* gel provided two important functions; where it stimulated the differentiation of transplanted MSCs and alleviated the radiation induced damages in liver. *Aloe vera*'s antioxidant and anti-inflammatory properties have enhanced liver function, as well as, creating a favorable environment for MSCs development in the liver. MSCs, in combination with lyophilized *Aloe vera* gel, hold promise for regenerative medicine; where, it has a considerable impact on MSCs differentiation.

1. Introduction

The liver is a vital organ that performs a variety of tasks, such as bile production, nutrients' metabolism, waste products' removal, storage of glycogen and synthesis of plasma proteins (Abdel-Misih & Bloomston, 2010). During radiation therapy for cancers in the upper abdominal area, lower part of right lung, esophagus (distal part), or radiation for the whole body or abdomen; the liver is frequently irradiated by accident (Benson et al., 2016; Lawrence et al., 1995). Radiation is sometimes considered as an ultimate option for a variety of solid tumours (Farid, El-Dewak, Safwat, & Diab, 2021), where it is frequently utilized as a final therapy, either alone or in a combination with chemotherapeutic agents (Landis et al., 2015). Also, it has been utilized as a follow-up treatment following primary tumour excision surgery. However, because of the potentially fatal consequence of radiation induced liver diseases (RILD), it is only used in the management of primary or advanced malignant tumours (Lawrence et al., 1995). There are two types of RILD: non-classic and classic. In classic RILP, ascites and hepatomegaly are the characteristic symptoms with an elevation in alkaline phosphatase that appears from two to three months following medication (Cheng et al., 2002). Blockage and damage of the hepatic lobules' central veins, progressive congestion, and necrosis of

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hepatocytes are, also, from its pathological findings (Kim et al., 2007). Non-classic RILD is defined as a dysregulated liver injury with notably high levels of transaminases in serum (five times more than the normal level) that occurred from one week to three months after radiotherapy exposure (Kim et al., 2007; Xu et al., 2006). It is the most common type of RILD in patients with cirrhosis or hepatitis who are treated with contemporary radiation therapy methods (Xu et al., 2006).

Nowadays, there is no specific pharmacological treatment for RILD; hence it is critical to find strategies to reduce damage or to detect toxicity utilizing diagnostic markers in an early stage. According to Symon et al. (2001), amifostine preserves hepatocytes from radiation exposure without impairing tumour control. Feng et al. (2012) investigated the radioprotective effect of amifostine against whole liver radiotherapy; where amifostine elevated the liver resistance. Vitamin E (α-tocopherol) has minimized the risk of RILD by lowering hepatic lipid peroxidation and increasing the antioxidant capacity in liver (Gençel et al., 2010). However, due to a scarcity of clinical trials, the use of such radioprotectors in ordinary medical practice is still experimental (Benson et al., 2016). Liver transplantation that is indicated as the only appropriate treatment option for severe liver disease is restricted by the inflated prices and the scarcity of donors (Kang et al., 2018). Mesenchymal stem cells (MSCs) transplantation gives patients a positive future; where various types of liver problems can be successfully treated by cell based treatment (Viswanathan et al., 2019). The most commonly used cells in liver transplantation were hepatocytes and MSCs. MSCs were used in numerous clinical studies for a variety of ailments during the last few decades (Saeedi et al., 2019). Despite the fact that MSCs have been shown to be effective in a number of diseases in numerous laboratory and clinical experiments, there are currently no rules to control MSCs therapeutic uses (Kim et al., 2015; Yang et al., 2021).

Also, the effectiveness of MSCs for stem cell based therapies is limited by the lack of well-established methods, the challenge of *in vivo* development, and the possibility of tumor growth (Chhabra & Brayman, 2013). MSCs, also, lost their activities and establish senescence as a result of DNA strand break, cytokines secretion and broad traumatic injury (Campisi & d'Adda di Fagagna, 2007). According to Vono et al. (2018), senescence serves two purposes: it shelters cells from oxidative damage and limits oncogene expression; but unfortunately, it slows the tissue repair resulting in age-related damage. According to Jeong and Cho (2016), reactive oxygen species (generated by MSCs) that help in their proliferation and/or differentiation cause DNA damage and hamper MSC function.

Intake of antioxidant phytochemical-rich natural products becomes a major topic (Farid, Yousry, & Safwat, 2022); where it can be regarded as radioprotective agents that reduce radiation harm (Farid, Haytham, et al., 2021; Patyar & Patyar, 2018). Across centuries, plants have provided unending supply for medication. The way they're made, what they're used for, and how they're used all differ. Dietary supplements (vitamins and minerals) and botanical drugs (chemical extract used for therapy) have all been classified as natural products from plants (Farid et al., 2020; Schmidt et al., 2008). Plant extracts from various parts (roots, barks, flowers, leaves, and seeds) can be applied for a variety of therapeutic applications (Farid, Haridyy, Ashraf, Ahmed, & Safwat, 2022). Extracts from plants contain bioactive ingredients such as polyphenol, flavonoid, and a variety of other constituents and chemicals that are useful in the treatment of both infectious and chronic conditions disorders (Kornicka et al., 2017). Plant phytochemicals have gaining a lot of attention because of their health benefits, necessitating more scientific research (Thangapazham, Sharad, & Maheshwari, 2016). Twenty seven clinical trials on MSCs, from 680, have incorporated nutritional supplements (especially herbal components) according to the National Institutes of Health (Kornicka et al., 2017). Blueberries, matcha, catechins, carnosines, and vit. D have been proven to increase the development of stem cells. Both of oleic and linoleic acids stimulate haemopoietic stem cell growth (Olatunbosun et al., 2012). Supplementing MSCs with plant extract has enhanced their proliferation and differentiation in standard *in vitro* conditions (Lee et al., 2012). Furthermore, extracts have been demonstrated to improve the anticancer efficiency of stem cells (Saud et al., 2019).

Aloe barbadensis miller (*Aloe vera*) is a desert plant that thrives in hot dry areas; and because of its ability to survive in certain severe climates, it has healing and antibacterial properties (Reynolds & Dweck, 1999). Leaves of *Aloe vera* have been used to treat eye disorders as well as spleen and liver enlargements (Chandan et al., 2007). Furthermore, its antioxidant and anti-inflammatory actions have been shown to protect the liver (Can et al., 2004; Lim et al., 2003). *Aloe vera* has been reported to protect the skin from the harmful effects of radiation (Haddad et al., 2013). *Aloe vera* has seventy five therapeutically active elements like minerals, vitamin, enzyme, carbohydrate, lignin, saponin and salicylic acid (Surjushe et al., 2008). *Aloe vera* has long been used to treat radiation; according to a survey by the Radiation Therapy Oncology Group, half of the institutes employed *Aloe* gel as a preventative treatment (Fisher et al., 2000).

Therefore, the present study aimed to evaluate the therapeutic effect of lyophilized *Aloe vera* gel together with bone marrow MSCs transplantation against RILD in X-ray irradiated Sprague dawley male rats. The antioxidant and anti-inflammatory capabilities of *Aloe vera* were examined by measuring the oxidative stress, antioxidant enzymes and pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) in liver tissue homogenate. Histological and immunohistochemical staining techniques were performed to determine the degree of liver injury and NF-κB expression.

2. Materials and methods

2.1. Aloe vera gel powder preparation

Gel powder from *Aloe barbadensis* miller was prepared according to Farid, Tawfik, et al. (2021). Leaves of *Aloe vera* (two years old and fifty cm long) were cleaned by distilled H_2O followed by chlorine (0.5%). The gel was collected from the leaves and frozen for 1 h at −18 °C; and lyophilized to powder. The characterization of gel was performed according to AOAC (2005); and polysaccharides content was measured according to Eberendu et al. (2005) by the colorimetric Congo red method. An expert botanist has identified the plant; and a voucher specimen was deposited at the herbarium of the department of Botany, Faculty of Science; Cairo University, Egypt. The plant name was checked with http://www.theplantlist.org.

2.2. Bone marrow – mesenchymal stem cells (BM-MSCs) preparation

Rats were intraperitoneally (i.p) injected with sodium pentobarbital (50 mg/kg); the femora were harvested aseptically using phosphate buffered saline (PBS). The cells were plated in RPMI-164 media containing glutamine, foetal bovine serum, gentamicin/amphotericin B, and fibroblast growth factor (at 10^6 cells/ml). 10% Fetal bovine serum and 5% pencillin-streptomycin were added to the culture, which were then incubated in 5% CO_2 (5%) at 37 °C until they reached confluence (85–90%). After fourteen days, the cells were detached using 0.25% trypsin and washed numerous times with PBS. The cell surface markers (CD34, CD45, CD90, and CD105) were explored using flow cytometry.

2.3. Procedure of radiation

50 mg/ml sodium pentobarbital was used to anaesthetize the animals before radiation exposure; where each rat were placed in a lying face dawn position on the bench and irradiated by Gierth QP 400 X ray machine (Germany), Faculty of Science, Cairo University. The left lung and mediastinum were protected by a lead block; and the irradiation field was 2cmx3cm. Animals were bombarded with X ray (6 mV) created by a linear accelerator with a dose of 4 Gy at a rate of six Gy per minute; where the distance between the irradiation source and animal's surface

was 1 m. The overall irradiation dose was twelve Gy, which has been separated into three doses of four Gy each. After irradiation, the rats awoke spontaneously. Animals' body weight was measured on the 7th and 14th day post irradiation.

2.4. Animals and experimental design

Forty two Sprague dawley male rats (eight weeks and 200–220 g weight) were divided into six groups (seven rats/group): control rats (gpI), lyophilized *Aloe vera* gel administrated control rats (gpII), irradiated control rats (gpIII), irradiated rats that received lyophilized *Aloe vera* gel (gpIV), irradiated rats that received MSCs (gpV) and irradiated rats that received lyophilized *Aloe vera* gel and MSCs (gpVI). Animals were maintained, under standard housing conditions, in the animal house of Cairo University. Animals received diet, which contained protein (24%) and fat (4%); and H_2O ad libitum throughout the experimental time. Lyophilized *Aloe vera* gel was administrated, by oral gavage, daily at 250 mg/l (Farid, Tawfik, et al., 2021) for fourteen days starting from the following day after radiation exposure. MSCs (2×10^6 cells/animal) were injected intravenously, once, the following day after radiation exposure (Farid, Haridyy, Ashraf, Ahmed, & Safwat, 2022). At the end of experiment, animals were anesthetized by 50 mg/kg of sodium pentobarbital; blood were collected by cardiac puncture followed by centrifugation for serum separation (Hegazy, Farid, Rabae, & El-Amir, 2015; Madbouly, El Amir, Kader, Rabee, & Farid, 2021). Sera were divided into aliquots and stored at −80 °C. Liver samples were collected from all experimental groups and divided into two parts; one for biochemical analysis and the other for histopathological examination. All experimental procedures were approved by the ethics committees.

2.5. Preparation of liver tissue homogenate

Liver tissue samples (1 g) were homogenized in 10 mmol cold Tris-HCl buffer of pH = 7.4 (4.5 ml); followed by centrifugation for 15 min at 4 °C. Protein content in tissue homogenates was quantified by Lowry method according to Waterborg and Matthews (1984).

2.6. Evaluation of liver damage

The effect of radiation exposure on liver function was determined, by rat ELISA kits, by measuring aspartate aminotransferase (ab263883, abcam, USA; AST), alanine aminotransferase (ab234579, abcam, USA; ALT), alkaline phosphatase (MBS2509314, MyBioSource, USA; ALP), total bilirubin (MBS730053, MyBioSource, USA; TB), and direct bilirubin (MBS9389077, MyBioSource, USA; DB) in serum samples. Serum levels of laminin (ab119573, abcam, USA; LN), hyaluronic acid (MBS727090, MyBioSource, USA; HA), type 3 procollagen (MBS705525, MyBioSource, USA; PCIII), and type 4 collagen (MBS732756, MyBioSource, USA; IV-C) were measured by rat ELISA kits to asses liver fibrosis. Assay procedures were in accordance to the manufacturer's protocols.

2.7. Evaluation of oxidative stress in liver tissue homogenate

Lipid peroxidation in liver was determined by measuring malondialdehyde (MDA; MBS268427, MyBioSource, USA) level; and the antioxidant capabilities were evaluated by measuring glutathione (GSH; MBS265966, MyBioSource, USA), catalase (CAT; MBS726781, MyBioSource, USA) and superoxide dismutase (SOD; MBS036924, MyBioSource, USA) levels in liver tissue homogenates.

2.8. Evaluation of pro-inflammatory cytokines levels in liver tissue homogenate

Tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 were measured, in liver tissue homogenate, by rat ELISA kit; ab236712, ab255730 and ab234570 (abcam, USA) according to the manufacturer's protocols.

2.9. Histological examination

Liver tissue samples were fixed in ten % neutral formalin buffer for a day. The liver segments received a tab water wash after fixation. Using ethanol concentrations of 70, 90, 95, and 100 percent, respectively, tissues were dehydrated. They were then cleaned in xylene and embedded in paraffin wax at 55 °C. Each portion was divided into five sections, each measuring 4 m thick. Hematoxylin and Eosin stain was applied to all sections for a standard histological evaluation (Farid, Moussa, et al., 2022).

2.10. Immunohistocemical examination

Deparaffinized four μm liver sections were rehydrated in graded alcohols, and washed in PBS. Endogenous peroxidase activity was blocked with H_2O_2 (3%), then washed with PBS and blocked with bovine serum albumin (BSA 5%) for 60 min. After 30 min of incubation with the primary antibody [anti-nuclear factor-kappa beta (NF-κB) p65 antibody (ab86299, abcam, USA)], liver sections were washed in PBS. Liver sections were incubated, for an hour, with HRP-rabbit anti-rat IgG secondary antibody (ab6734, abcam, USA). For colour development, chromogen (3, 3-diaminobenzidine, DAB) was utilized; the brown colour reflecting a positive result. After washing, the sections were counterstained with hematoxylin (0.1%).

2.11. Statistical analysis

Data were evaluated with One Way ANOVA test and compared with Tukey test; and results were expressed as mean ± SD. Values were considered significant at $p < 0.05$.

3. Results

3.1. Characterization of lyophilized Aloe vera gel

Aloe vera gel contained a 97.4% moisture and 0.9% fibers; with a pH of 4.8 and 4.5% protein. In addition, it contained 403.2 mg/g dry weight of polysaccharides and 0.02% of aloin.

3.2. MSCs identification

CD90 and CD105 were highly expressed on the surface of the cultured cells, with the absence of CD34 and CD45 markers, indicating that the cultured cells were MSCs without haematopoietic stem cells (HSCs).

3.3. Body weight

Body weight, on the 7th and 14th day post irradiation, of untreated irradiated group (225.4 g and 252.4 g, respectively) was lower than that of the control group (234.6 and 281.5 g, respectively). On the 7th day, no significance difference was observed among irradiated untreated group and irradiated treated groups with lyophilized *Aloe vera* gel or MSCs alone (Table 1). The body weight of the irradiated group, which received the combined treatment (lyophilized *Aloe vera* gel + MSCs), was highly elevated when compared to that of the untreated irradiated group. Moreover, the body weight were similar to those of control group and lyophilized *Aloe vera* gel administrated control group.

3.4. Liver damage

Radiation exposure led to a disturbance in the function of liver;

Table 1
Body weight in different experimental group on the 7th and 14th day after radiation exposure.

Group	7th day post irradiation	14th day post irradiation
Control group	234.6 ± 6.2	281.5 ± 3.4
***Aloe vera* administrated control group**	236.4 ± 4.9	279.7 ± 9.4
Irradiated untreated control group	225.4 ± 11.4[a]	252.4 ± 8.2[a]
***Aloe vera* administrated irradiated group**	227.2 ± 6.8[a]	261.4 ± 12.6[a,b]
MSCs administrated irradiated group	225.1 ± 5.5[a]	259.3 ± 14.4[a,b]
***Aloe vera*-MSCs administrated irradiated group**	232.1 ± 19.4[b,c,d]	278.6 ± 6.6[b,c,d]

Results were expressed as mean ± SD. [a] indicates significance when compared to control group, [b] indicates significance when compared to irradiated untreated control group, [c] indicates significance when compared to *Aloe vera* administrated irradiated group and [d] indicates significance when compared to MSCs treated irradiated group; p less than 0.05.

where a significant elevation in serum levels of ALT, AST, ALP, TB and DB was observed in irradiated untreated rats when compared to control group or lyophilized *Aloe vera* gel administrated control group (Fig. 1a and b). Moreover, irradiated untreated group showed a significant increase in serum levels of LN, HA, PCIII and IV-C. On the other hand, the co-treatment with lyophilized *Aloe vera* gel and MSCs reduced the serum levels of liver function enzymes (ALT, AST and ALP) together with TB and DB; Also, liver injury markers (LN, HA, PCIII and IV-C) were significantly reduced when compared to untreated irradiated control group and lyophilized *Aloe vera* gel or MSCs irradiated treated (Fig. 1c).

3.5. Oxidative stress in liver tissue homogenate

Irradiated untreated group showed a significant increase in MDA level and a significant decrease in GSH, SOD and CAT levels in rats' liver tissue (Fig. 2a and b). Although, the treatment with lyophilized *Aloe vera* gel only or MSCs only decreased the effect of radiation exposure on oxidative stress and antioxidant enzyme levels, the levels of MDA remained higher than that of control group. The combined treatment significantly modulated the levels of MDA and antioxidant enzymes to be similar to those of control group or lyophilized *Aloe vera* gel administrated control group.

3.6. Levels of pro-inflammatory cytokines in liver tissue homogenate

Compared to control group or lyophilized *Aloe vera* gel administrated control group, levels of TNF-α, IL-1β and IL-6 in liver tissue of untreated irradiated group were highly elevated in a significant way ($p < 0.05$). Administration of lyophilized *Aloe vera* gel only, for 14 consecutive days, slightly decreased the levels of pro-inflammatory cytokines; the same was observed with MSCs transplantation (Fig. 3). However, the combined treatment succeeded in returning the cytokines levels to their normal values in comparison to control group ($p < 0.05$).

3.7. Histopathological and immunhistochemical results

Hematoxylin and eosin staining of liver sections of all experimental groups were shown in Fig. 4. In the control group and lyophilized *Aloe vera* gel administrated control group, hepatocytes were arranged tidily in a radial shape surrounding an intact central vein. Untreated irradiated group showed hepatic cell edema, inflammatory cells infiltration around the central vein and congested blood vessels. Also, vacuolated hepatocytes and congested central vein were noticed in the rats' liver sections of untreated irradiated group or in groups treated with either of MSCs or lyophilized *Aloe vera* gel alone. Moreover, marked positive immunohistochemical reaction for NF-κB was observed in irradiated untreated group, lyophilized *Aloe vera* gel administrated irradiated group and MSCs treated irradiated group (Fig. 5). The co-treatment with MSCs and lyophilized *Aloe vera* gel has alleviated the pathological changes in rats' liver, where, a diminished reaction for NF-κB was observed.

4. Discussion

The goal of this study was to determine if the lyophilized *Aloe vera* gel combined with BM-MSCs transplantation could minimize RILD in X-ray irradiated Sprague dawley male rats. In this study, the lyophilized

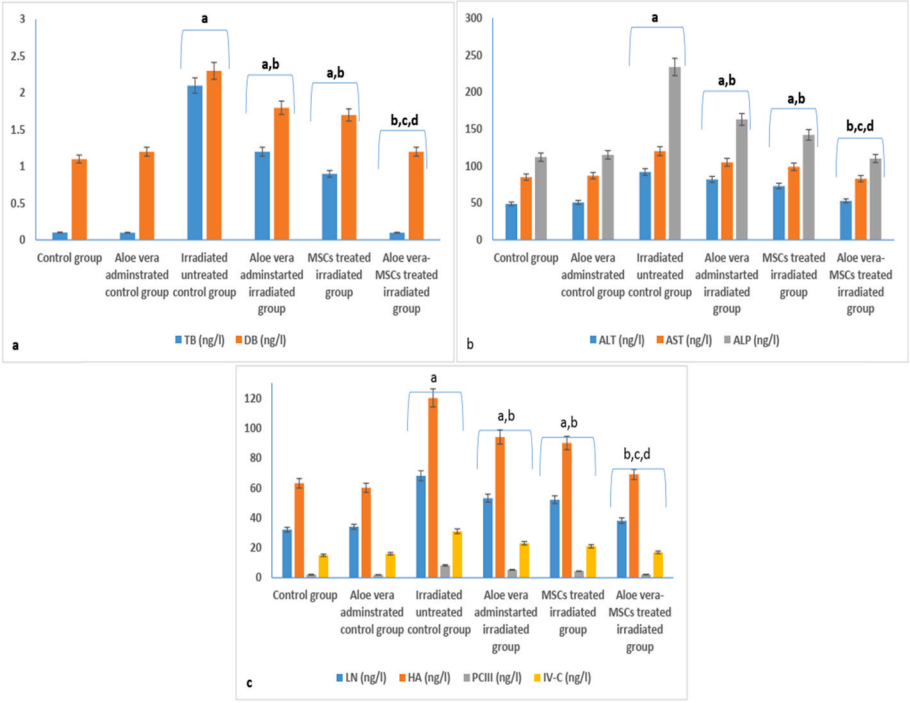

Fig. 1. Assessment of disturbance in liver function (a and b) and liver damage (c). AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, TB: total bilirubin, DB: direct bilirubin, LN: laminin, HA: hyaluronic acid, PCIII: type 3 procollagen and IV-C: type 4 collagen. [a] indicates significance when compared to control group, [b] indicates significance when compared to irradiated untreated control group, [c] indicates significance when compared to *Aloe vera* administrated irradiated group and [d] indicates significance when compared to MSCs treated irradiated group; p less than 0.05.

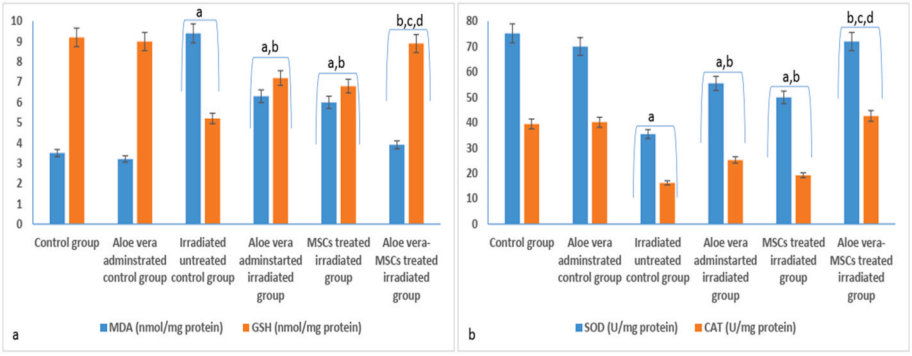

Fig. 2. Evaluation of oxidative stress and antioxidant enzyme levels in liver tissue. MDA: malondialdehyde, GSH: glutathione, CAT: catalase and SOD: superoxide dismutase. [a] indicates significance when compared to control group, [b] indicates significance when compared to irradiated untreated control group, [c] indicates significance when compared to *Aloe vera* administrated irradiated group and [d] indicates significance when compared to MSCs treated irradiated group; *p* less than 0.05.

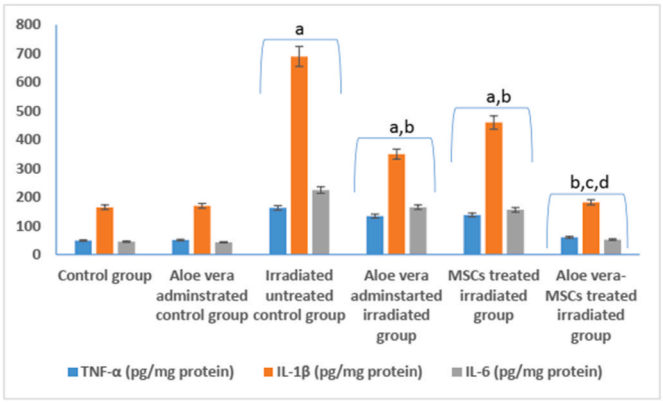

Fig. 3. Pro-inflammatory cytokines level in liver tissue. TNF: tumor necrosis factor and IL: interleukin. [a] indicates significance when compared to control group, [b] indicates significance when compared to irradiated untreated control group, [c] indicates significance when compared to *Aloe vera* administrated irradiated group and [d] indicates significance when compared to MSCs treated irradiated group; *p* less than 0.05.

gel from *Aloe vera* has high water content (97.4%) and 403.2 mg/g dry weight of polysaccharides; in addition to 0.9% fibers, 4.5% protein and 0.02% of aloin. These results were in agreement with Farid, Tawfik, et al. (2021) who reported that *Aloe vera* gel has a 98.55% moisture and the polysaccharide acemannan (389.1 mg/g). Also, the presence of CD90 and CD105 markers on the surface of the cultured cells, as well as the absence of CD34 and CD45 markers, indicated that the cultured cells were MSCs rather than HSCs. The untreated irradiated group showed a decrease in animals' body weight and anti-oxidant enzymes levels; in addition to an elevation in liver function parameters, oxidative stress and pro-inflammatory cytokines. Moreover, liver sections showed vacuolated hepatocytes, edema, inflammatory cells infiltration and congested blood vessels; with marked positive immunohistochemical staining for NF-κB. On the other hand, the body weight of the irradiated group that received the combined treatment (lyophilized *Aloe vera* gel + MSCs) was significantly higher than that of the untreated irradiated group. An improvement in liver function and a reduction in fibrotic markers, oxidative stress and pro-inflammatory cytokines were, also, observed; together with an alleviation in the pathological changes in rats' liver and a diminished reaction for NF-κB.

Our results were in agreement with Lee and Friedman (2011) who reported that the pathogenesis of RILD was complicated with vasodilatation, elevated synthesis of collagen, and successive increased expression of vital cytokines and growth factors like TNF-α and TGF-β; where

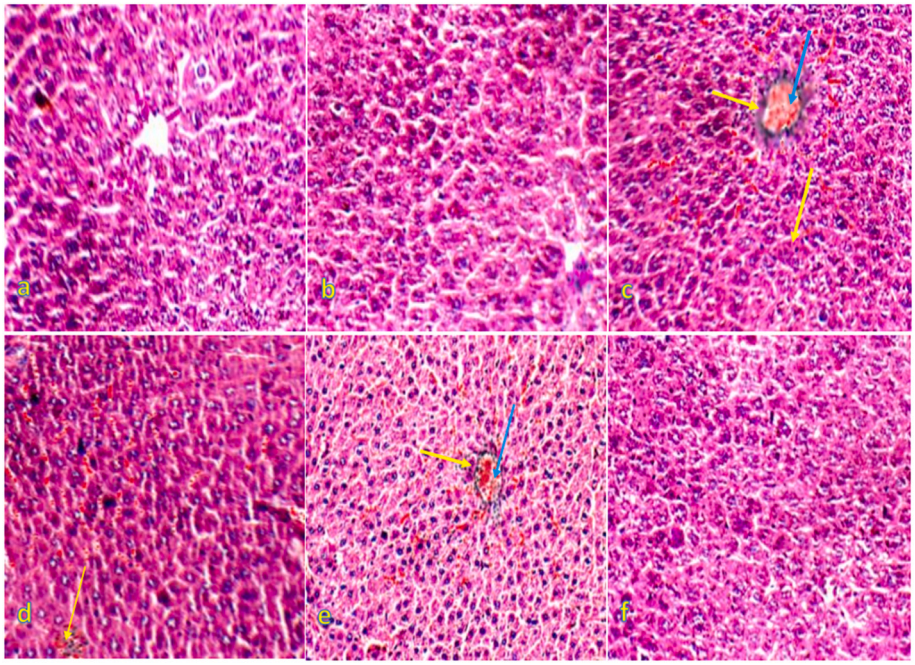

Fig. 4. Hematoxylin and eosin liver section showing normal hepatocytes arranged in strands around the central vein with no edema or inflammatory cells infiltration in control group (a), *Aloe vera* administrated control group (b) and *Aloe vera*-MSCs treated irradiated group (f); congested blood vessels (blue arrow) and inflammatory cells infiltration (yellow arrow) in irradiated untreated control group (c), *Aloe vera* administrated irradiated group (d) and MSCs treated irradiated group (e). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

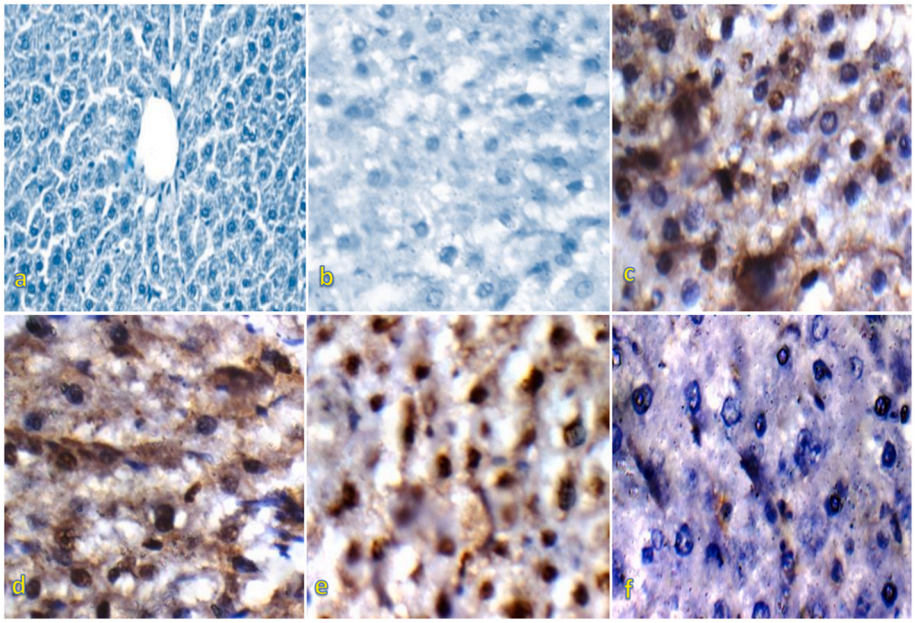

Fig. 5. Immunohistochemical staining for NF-κB in liver tissue showing negative reaction (−) in control group (a) and *Aloe vera* administrated control group (b); marked positive reaction (++++) in irradiated untreated control group (c), *Aloe vera* administrated irradiated group (d) and MSCs treated irradiated group (e); moderate positive reaction (+) in *Aloe vera*-MSCs treated irradiated group (f). Degree of positivity is identified by the degree of brown colour. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

all of that were essential components in the repair response for liver damage. Damage in DNA, elevation in free radicles like reactive oxygen species are initial consequences of radiation exposure that lead to hepatocytes death and inflammatory cell response (Robbins & Zhao, 2004). Despite the fact that hepatocytes are resistant to radiation more than non-parenchymal tissue; the released TNF-α from kupffer cells makes hepatocytes vulnerable to irradiation that leads to apoptosis and increase the hepatocellular mortality (Christiansen et al., 2004). They added that irradiation of hepatocytes' culture did not cause cytotoxicity, whereas irradiated kupffer cells released TNF-α into the growth media. Moreover, the secreted TNF-α has induced hepatocytes death leading to liver damage. Irradiated liver is characterized with total central vein obliteration by RBCs entrapped in a collagen fibers network leading to vascular congestion, reduced oxygen delivery, loss of hepatic architecture and finally liver fibrosis (Kim & Jung, 2017). Significant sinus congestion with bleeding, central vein dilatation, deteriorated vacuolated hepatocytes and activated kupffer cells were detected 36 h after abdominal irradiation of rats with 8 Gy (Ozyurt et al., 2014). TNF-α and TGF-β, which are crucial regulators in repair responses to liver injury, are a few of the main growth factors and cytokines that are sequentially activated as part of the pathophysiology of RILD (Lee & Friedman, 2011). The liver is damaged by radiation either immediately or over time. Hepatocellular apoptosis and acute inflammatory reactions are among the irradiation's early side effects, which also include DNA damage, oxidative stress, and reactive oxygen species generation (Robbins & Zhao, 2004). Reactive oxygen species are created after radiation exposure and are capable of activating TGF-β (Barcellos-Hoff & Dix, 1996). In the radiation-induced adaptive response, NF-κB, an active transcription factor, was increased following irradiation (Ahmed & Li, 2008). Radiation induces a number of NF-κB-regulated cytokines, such as TNF-α, which increase cells' susceptibility to radiation (Cheng et al., 2015). TNF-α stimulates NF-κB via receptor activation and controls the expression of many immune and inflammatory response genes (Blonska et al., 2004; Cheng et al., 2015). TNF-α becomes an apoptotic agent after inducing an acute phase response in hepatocytes naturally (Tello et al., 2008). TNF-α is involved in a variety of activities, such as cell survival, inflammation, and immunology (Farid, Hany, et al., 2022; Shater et al., 2022), and it may be a crucial first step in the development of RILD and liver fibrosis (Cheng et al., 2015).

MSCs have been differentiated into several types of cells like adipocyte, osteoblast, chondroblast, and hepatocyte like cell (HLC) (Wang et al., 2018); this property apparently opened a door to medical science. MSCs (obtained from several tissues like bone marrow, adipose tissues and umbilical cord) have previously been demonstrated to develop into HLC *in vitro* (Coronado et al., 2019; Mou et al., 2013; Shi et al., 2020). MSCs have been shown in clinicopathological experiments to dramatically increase liver cell regeneration in a variety of liver disorders. Also, MSCs increased regeneration of liver in mice following an eighty percent hepatectomy, according to Despeyroux et al. (2018). Chen et al. (2017) found that transplanted menstrual blood MSCs have been attracted to damaged liver areas in CCl4 induced liver fibrosis in mice studies; and added that few cells have been developed into HLCs. Shi et al. (2017) found that following intraportal vein injection of three million per kg of human derived BM-MSCs in D galactosamine induced pig model, human derived hepatocytes accounted for five percent of the entire pig's hepatocytes. After intravenous injection, von Bahr et al. (2012) found that slightly expanded BM-MSCs had restricted long term survival and no abnormal organogenesis. This suggests that MSCs can enhance regeneration of liver primarily via pathways other than HLC differentiation. MSCs have been shown to boost liver cell proliferation and preventing the death of hepatocytes (Yang et al., 2021).

Despite the fact that MSCs treatments have been demonstrated to be effective in the treatment of liver problems, investigators have been looking for ways to improve the potency of MSCs application. MSCs acquire replicative senescence *in vitro*, regardless of growth technique, with clear consequences for therapeutic benefits. Baxter et al. (2004) discovered that even low expansion techniques cause MSCs to age rapidly, which could affect their phenotypic and signaling pathways capacity. Progressive reductions in cytokine levels during long term MSCs culture could be associated with a depression in their proliferation and differentiation potential; also, because of MSCs stability and high anti-inflammatory capabilities, the scientists proposed that MSCs at early stages were more appropriate for treatment than those at late stages (Choi et al., 2010). Therefore improving the selection criteria for MSCs donors and low-passage MSCs can improve the efficacy of their transplantation. One of the strategies for improving the therapeutic efficiency of MSCs *in vitro* or *in vivo* is pretreatment prior to administration; where the phenotype of MSCs is influenced by the culture circumstances. According to Kojima et al. (2019), BM-MSCs cultivated under hypoxic settings had better medicinal value in mice with liver cirrhosis than those cultivated in standard condition. Pretreatment of adipose MSCs with melatonin has improved their therapeutic effects in

acute liver damage (Fang et al., 2018). Furthermore, the impact of MSCs cultivation on genetic instability and tumorigenesis has been established by researchers (Dahl et al., 2008). Another strategy for improving MSCs transplantation efficiency is the utilization of plant extract; where *in vivo* and *in vitro*, medicinal plants have attracted a great awareness as promoters for MSCs proliferation (Potu et al., 2009). Kornicka et al. (2017) reported that plant extracts have promoted the proliferation of adult stem cells while inhibiting tumour growth. Moreover, the MSCs' ability to proliferate is regulated by the stimulant's concentration (Yu et al., 2016). The use of citrus extract (1–100 μg/ml) boosted the growth and differentiation of human bone marrow MSCs; however, the use of higher concentration (200 μg/ml) decreased their growth (Peng-Zhang et al., 2009). 50 μg/ml of naringin promoted MSCs growth in rat, while 100 μg/ml concentration inhibited the rate of proliferation (Yu et al., 2016). Furthermore, utilizing 0.1–10 g/ml concentrations of Laminaria japonica (fucoidan) brown algae extracts promotes the growth of human-derived MSCs (Kim et al., 2015). Sal B, a salvianolic acid derived from Salvia miltiorrhiza, was found to have no observable effect on the viability of MSCs at a variety of doses, indicating that Sal B had no cytotoxicity to MSCs (Xu et al., 2014). Another study looking at flavonoids from Herba epimedii revealed that as the concentration increased, the Herba considerably decreased the proliferation of MSCs while having the opposite effect on their osteogenesis (Zhang et al., 2010). In a separate investigation, Li et al. (2010) discovered that the ethanol extract of the fruit of Ligustrum lucidum inhibited the growth of human bone marrow-derived mesenchymal stem cells in a dose-dependent manner and displayed a cytotoxic effect at a concentration of around 200 μg/ml (Li et al., 2010).

Aloe barbadensis miller is the botanical name for *Aloe vera*; which is a shrubby succulent green plant that belongs to the Asphodelaceae (Liliaceae) family (Surjushe et al., 2008). The leaf is fleshy and triangular in shape with serrated edges; and it is composed of 3 layers (Sato et al., 1990). The exterior layer, known as the rind, protects the leaf and produces necessary proteins and carbohydrates. The interior clear gel (99% water) is made up of glucomannan, peptides, lipid, sterol, and minerals. The intermediate layer, known as latex, is a bitter yellow sap that contains glycosides and anthraquinone (Ro et al., 2000; Roberts & Travis, 1995).

Because of polysaccharides' remarkable and exceptional bioactivities, such as their anti-bacterial (Luiz et al., 2017), anti-cancer (Nazeam et al., 2017), anti-viral (Xie et al., 2016), and anti-oxidant activities (Chen et al., 2016); they are widely employed in a variety of medical services and treatments. Additionally, polysaccharides were some of the Earth's natural biopolymers (Marhefka & Kameneva, 2016) that are employed extensively as biomaterials for tissue engineering (Rahman et al., 2017), medication delivery (Sinha & Kumria, 2001), and wound healing (Jettanacheawchankit et al., 2009). One of the few naturally occurring plants with a high concentration of polysaccharides is *Aloe vera* (Eshun & He, 2004). The large variety of polysaccharides found in aloe gel have been documented to act as immune system stimulants. The majority have been identified as pectin, mannan (acetylated), and glucomannan, all of which have various molecular weights (Pugh et al., 2001). *Aloe* leaves have been demonstrated in numerous studies to possess a wide range of properties such as immunomodulation, anti-bacterial, anti-viral, anti-cancer, and anti-inflammatory characteristics. These properties are all related to the existence of polysaccharides (Harlev et al., 2012). Acemannan, a -(1,4)-acetylated soluble polymannose, is the primary bioactive polysaccharide of aloe vera, and it is extracted from the gel (Sierra-Garcia et al., 2014). In the past several years, acemannan has also been documented to have numerous biological and pharmacological uses in the sectors of medicine and industry, including the treatment of tumour, metabolic disorders, cardiovascular problems, and dental diseases (Bhalang et al., 2013; Djeraba & Quere, 2000). Current acemannan research has concentrated on dental care and wound repair. Using US Food and Drug Administration-approved methods, acemannan has been used to treat wounds and alveolar osteitis (Lobo et al., 2010). According to our results, lyophilized *Aloe vera* gel contained 403.2 mg/g dry weight of polysaccharides; which modulate the immune response, reduced radiation induced damage and inflammation in irradiated rats. This in turn created a suitable environment for MSCs differentiation leading to liver repair.

Aloe vera gel has been shown to protect the skin from the harmful effects of radiation. Appling *Aloe vera* gel led to the release of metallothionein, an antioxidant protein that scavenged OH^- radicals and elevated SOD and Gpx. It prevented the synthesis and secretion of anti-inflammatory cytokines like IL-10 produced from keratinocytes in the skin and thus inhibited ultraviolet induced inhibition of delayed-type hypersensitivity (Byeon et al., 1988). The cyclooxygenase's pathway was, also, inhibited by the gel leading to a reduction in dinoprostone (known as prostaglandin E2) production from arachidonic acid. Furthermore, C glucosyl chromone, a new anti-inflammatory molecule, has been recently isolated from *Aloe vera* gel (Hutter et al., 1996). The gel has inhibited the production of leukotrienes and histamine, from mast cells, by preventing the influx of Ca (Ro et al., 2000). Surjushe et al. (2008) reported that the gel increased the release of IL-1β and TNF-α from macrophages of mice implanted with carcinoma cells, and added that these pro-inflammatory cytokines have led to the cancerous cells' necrosis. Moreover, Hart et al. (1990) showed that *Aloe vera* gel contained many low molecular weight compounds that stop activated neutrophil from releasing reactive oxygen species. Ahluwalia et al. (2016) discovered that *Aloe vera* gel extract has reduced the expression of CD3 and CD325 on T lymphocytes. Furthermore, it inhibited the proliferation of T lymphocytes and reduced the levels of secreted cytokines (IFN-γ, IL-2 and IL-17) in a dosage pattern. In addition, the anti-inflammatory actions of *Aloe vera* gel have been studied in a mouse model of acetaminophen induced hepatitis; where, *Aloe vera* decreased the levels of MDA, IL-12, and IL-18 in liver, as well as ALT, and enhanced GSH content (Werawatganon et al., 2014). Treatment of whole body gamma irradiated mice with *Aloe vera* has significantly boosted metallothionein induction and prevented lipid peroxidation (Nada et al., 2013); where, it has activated the antioxidant enzymes that were important for oxidative stress management and enhancement of the immunity (Ozsoy et al., 2009). Administration of *Aloe vera* extract has elevated hepatic antioxidant enzymes in mouse that in turn decreased hepatic MDA level (Singh et al., 2000). Rajasekaran et al. (2005) and Lim et al. (2003) demonstrated that *Aloe vera* extract has a protective function against oxidative stress-stimulated cell damage and induced synthesis of proteins.

In conclusion, lyophilized *Aloe vera* gel has played two significant roles; the first was a stimulant effect for MSCs differentiation and the second was a therapeutic effect for RILD. The antioxidant and anti-inflammatory consequences of lyophilized *Aloe vera* gel administration have improved the liver function and reduced radiation-induced damages; in addition to the creation of a suitable environment for MSCs differentiation in liver.

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Declaration of competing interest

None to declare.

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