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ORIGINAL ARTICLE

Probing Regenerative Potential of *Moringa oleifera* Aqueous Extracts Using *In vitro* Cellular Assays

Evangeline E. Fernandes, Anubha V. Pulwale, Gauri A. Patil, Alpana S. Moghe

Department of Cell and Molecular Biology, Rajiv Gandhi Institute of IT and Biotechnology, Bharati Vidyapeeth Deemed University, Pune, Maharashtra, India

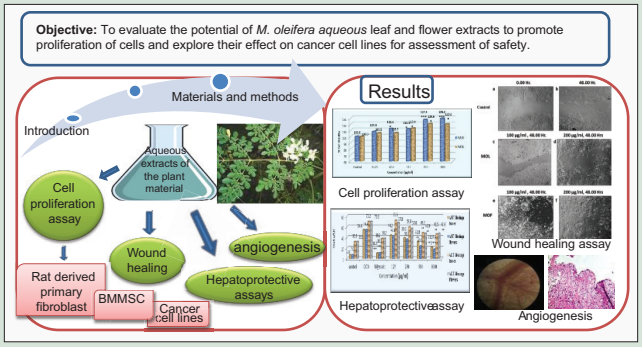
ABSTRACT

Background: Molecules stimulating regeneration and proliferation of cells are of significance in combating ailments caused due to tissue injury, inflammation, and degenerative disorders. *Moringa oleifera* is one of the most valued food plants having the profile of important nutrients and impressive range of medicinal uses. **Objective:** To evaluate the potential of *M. oleifera* aqueous leaf and flower extracts to promote the proliferation of cells and explore their effect on cancer cell lines for assessment of safety. **Materials and Methods:** Aqueous leaf and flower extracts of *M. oleifera* were investigated for effect on rat-derived primary fibroblast, mesenchymal stem cells (MSCs), and cancer cell lines using cell proliferation assay. They were also tested and compared for wound healing, angiogenesis, and hepatoprotective effect using *in vitro* assays. **Results:** Statistically significant increase in the proliferation of primary rat fibroblast, MSCs, and angiogenesis was observed after treatment with aqueous flower extract. The aqueous leaf extract determined a comparatively moderate increment in the proliferation of MSCs and angiogenesis. It however showed prominent cytotoxicity to cancer cell lines and a significant hepatoprotective effect. **Conclusion:** A very clear difference in response of the two extracts to different types of cells was detected in this study. The aqueous flower extract exhibited a higher potential to stimulate cell proliferation while not exerting the same effect on cancer cell lines. The leaf extract on the other hand, had a prominent antitumor and hepatoptotective effects.

Key words: Aqueous, cytotoxicity, fibroblast, *in vitro*, viability

SUMMARY

- *Moringa oleifera* flower extract showed significant ability to promote proliferation of rat fibroblast and mesenchymal stem cells. The extract also had prominent angiogenic and hepatoprotective effects.
- The extract did not influence proliferation of cancer cell lines indicating its safety for human consumption and use in pharmaceuticals.
- The *Moringa oleifera* leaf extract showed relatively less potential to stimulate cells but had prominent cytotoxic effect on cancer cell lines.



Abbreviations Used: ALT: Alanine transaminase, AST: Asparatate amino transferase, ATCC: American type culture collection, BMMSC: Bone marrow mesenchymal stem cells (used in this paper), CAM: Chick chorioallantoic membrane, CCl4: Carbon tetra chloride, DMEM: Dulbecco's modified Eagle medium, DMSO: Dimethyl sulfoxide, EDTA: Ethylene diamine tetraacetic acid, HBL 100: Human breast epithelial cell line, Mcf-7: Human breast adenocarcinoma cell line, αMEM: Minimum Essential Medium Eagle alpha modification, MOF: *Moringa oleifera* aqueous flower extract (used in this paper), MOL: *Moringa oleifera* aqueos leaf extract (Used in this paper), OD: Optical density, PBS: Phosphate buffered saline

Correspondence:
Dr. Alpana S. Moghe,
Department of Cell and Molecular Biology,
Rajiv Gandhi Institute of IT and Biotechnology,
Bharati Vidyapeeth Deemed University,
Pune–Satara Road, Katraj,
Pune - 411 046, Maharashtra, India.
E-mail: alpanasm@gmail.com

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INTRODUCTION

Plants have served as an important source of nutraceuticals which are used by nearly two-third of the world's population for health benefits and prevention of diseases. They also play an equivalent role in modern pharmaceutical biology. Large number of herbs has been utilized for their anticancer properties. They are cytotoxic in nature and induce apoptosis in cancer cells.[1] On the other hand, there are certain herbs having contrasting activity. They promote proliferation of cells.[2] They are of significance in management of ailments such as wounds, burns, infections, bone fractures, hepatic disorders, and ulcers, which are caused due to injury or tissue inflammation. Proliferation and regeneration of cells is also crucial for prevention of degenerative diseases and aging.

Moringa oleifera is one of the most widely cultivated species of family Moringaceae. It is native to Southeast Asia, Africa, and America. The leaves, flowers, and tender pods of the tree are consumed as vegetable in these countries.[3] The plant contains a profile of important nutrients and phytochemicals. It serves as a rich source of proteins, vitamins, antioxidants, flavonoids, phenolics, and minerals such as calcium, phosphorus, magnesium, potassium, sodium, sulfur, zinc, copper, manganese, iron, and selenium.[4] It is prescribed in the nutritional program of malnourished children and lactating mothers.[5] Traditionally, the plant is used as antispasmodic, stimulant, expectorant, diuretic, antidiabetic, antiparalytic, and for combating viral infections.[6]

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All the parts of this valued plant have medicinal properties. They exhibit antihyperglycemic, antidislipidemic, antioxidant, antihypertensive, immunomodulatory, chemoprotective, radioprotective, diuretic, anti-inflammatory, antipyretic, antiepileptic, antitumor, antiulcer, antispasmodic, antibacterial, and antifungal activities.[7] This impressive broad range of pharmacological attribute is probably due to unique combination of potentially bioactive compounds such as rhamnosyloxy benzyl isothiocyanate and its derivatives, niaziminins, niazinins, β-sitosterol, niacin, phenolic acids, glucosinolate, flavonoids, gallic acid, coumarin, and caffeic acids in *M. oleifera.* Of special importance among these is unique combination of compounds zeatin, quercetin, β-sitosterol, caffeoylquinic acid, and kaempferol obtained in leaves and other parts of this plant.[8,9]

One of the prominent facets of *M. oleifera* is its tissue protective ability. Earlier investigations have revealed the effect of leaf extract in prevention of acetaminophen-induced liver toxicity, chromium induced testicular toxicity, selenite-induced cataractogenesis, gentamicin-induced nephrotoxicity, and isoproterenol-induced cardiotoxicity in rats.[10-14] The ability of *M. oleifera* leaf (MOL) extract to enhance the healing was determined in wound healing, ulcerogenic, and hepatoprotective studies.[15,16] The hepatoprotective and antiinflammatory properties were also demonstrated by flower extract indicating wide distribution of healing component in the plant.[17] The hepatoprotective activity in particular was attributed to the presence of quercetin, β-sitosterol, and kaempferol in leaves and other parts of *Moringa*.[18] β-sitosterol isolated from other plants has also shown to stimulate proliferation and regeneration of cells.[19] These data emphasize a high therapeutic regenerative potential of *M. oleifera* plant and indicate the need for undertaking systematic study of its effect on different populations of cells. In present study, the aqueous extracts of *M. oleifera* leaves and flowers were evaluated and compared for their proliferative potential using *in vitro* cell proliferation, wound healing, angiogenesis, and hepatoprotective assays employing rat derived fibroblast, mesenchymal stem cells (MSCs), primary hepatocytes, and cancer cell lines.

MATERIALS AND METHODS

Chemical reagents

Dulbecco's Modified Eagle's medium (DMEM), HAM's F12 K, α-MEM, trypsin, glutamine, fetal bovine serum (FBS), MSC qualified FBS were obtained from GIBCO by Life Technologies. 3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), carbon tetra chloride (CCl_4) were purchased from Sigma – Aldrich Company. Collagenase, Penicillin and Streptomycin, and L-15 Medium were purchased from Hi Media Laboratories, India.

Collection of plant material and preparation of extracts

The leaves and flowers of *M. oleifera* were collected from a field in Sangli district of Maharashtra and authenticated by a Botanical Survey of India, Pune. The plant material was washed, separated, shed dried, and powdered. Five grams of powder was extracted with 50 ml of water for 48 h at room temperature. The extracts were filtered and freeze dried. Ten milligrams of freeze dried sample was dissolved in 1 ml of sterile phosphate buffered saline (PBS) and further diluted to different concentrations for treatment on cells.

Effect on proliferation of cells

The crude aqueous leaf and flower extracts were tested for effect on rat primary fibroblast cells, bone marrow derived MSCs, HBL 100, and Mcf7 breast cancer cell lines using MTT cell proliferation assay. Primary rat fibroblast cells and bone marrow derived MSCs were isolated and cultured in the laboratory using procedures described below. The Mcf7 and HBL 100 cell lines were procured from the National Animal Science Cell Repository, Pune, and cultured using DMEM and L15 medium, respectively. The media were supplemented with 2 mM glutamine, 50 IU/ml penicillin, 50 μg/ml streptomycin, and 10% FBS.

Isolation and culture of rat fibroblast cells for 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide and wound healing (scratch) assay

A 5-week-old Wistar rat on diethyl ether anesthetization was dissected and lung fragment of ~ 1 cm size was isolated. The tissue was cut in small pieces, washed in PBS and stirred with 10 ml of enzyme solution (0.025% collagenase, 0.25% trypsin, 0.25 mM EDTA in DMEM/F12 medium) at 37°C for 20 min. The solution was filtered and the filtrate was centrifuged at 2000 rpm for 5 min. Cell pellet was washed twice and resuspended in fibroblast medium (DMEM/F12 supplemented with 15% FBS) and counted using trypan blue dye exclusion method. About 5×10^4 and 1×10^5 cells were seeded in 96 and 24 well plates respectively. Cultures were incubated at 37°C in 5% CO_2 atmosphere. Media change was given after 3 days of incubation.[20]

Isolation and culture of rat bone marrow mesenchymal stem cells (BMMSC)

Rat bone marrow mesenchymal stem cells (BMMSC) primary culture was prepared using procedure described by Zhang *et al.*[21] Briefly, Sprague Dawley rat on diethyl ether anesthetization was dissected. Femur and tibia were removed and bone marrow was flushed out of bones using 10 ml of PBS in a syringe. Bone marrow cells were suspended by aspiration using 1 ml pipette. Cell suspension was centrifuged and the cell pellet was washed twice with α MEM supplemented with 10% qualified FBS. On viable counting, cells were seeded in 25 cm^2 tissue culture flasks and incubated in 5% CO_2 atmosphere. Media change was given after 3 days. 1×10^5 cells of third passage were seeded in 96 well plates.

3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide cell proliferation assay

96 well plates seeded with primary rat fibroblast cells, BMMSC, Mcf7, and HBL 100 cell lines were incubated at 37°C in humidified 5% CO_2 atmosphere for 24 h. The cells were then treated with varying concentrations of leaf (MOL) and flower (MOF) aqueous extracts in triplicate wells and further incubated for 48 h. 10 μl of 5 mg/ml MTT dye was added to all the wells and incubated in dark for 4.0 h. Formazan crystals were dissolved in DMSO (100 μl) and plates were kept in dark for 15 min. The absorbance was measured at 570 nm wavelength. Assays were performed at least twice to confirm the results. The cell survival was measured as absorbance (optical density) of the mean of triplicate wells compared to that of control.

In vitro wound healing (scratch) assay

Rat primary fibroblast cells seeded in 24 well plates were incubated in CO_2 incubator till the culture was confluent (48 h). Media from wells was pipetted out and using a sterile 200 μl tip, an end to end scratch was made in all the wells. Wells were washed twice with PBSA and replaced with fresh media. Varying concentration (50, 100, and 200 μg/ml) of extracts were seeded in duplicates to the test wells. Equal volume of plain

PBS was added to wells which served as controls. Plates were reincubated in 5% CO_2 atmosphere and observed for cell migration and proliferation at 24 h, 48 h, and 72 h time interval.

Chick chorioallantoic membrane assay

The influence of *M. oleifera* extracts on angiogenesis was evaluated using chorioallantoic membrane (CAM) assay.[22] Fertilized white leghorn chicken eggs were procured from a local hatchery. A small window was made in the shell on day 3 of chick embryo development to withdraw 2–3 ml of albumin. The window was resealed with adhesive tape and eggs were returned to the incubator until day 7. A small sterile gel foam piece loaded with 100 µl of aqueous extracts (50 µg, 100 µg, and 200 µg/ml) was implanted on top of the membrane. A similar volume of PBS was added to the eggs which served as control. Windows were sealed with transparent adhesive tape and eggs were incubated at 37°C for 4 days with appropriate humidity and rotation. On 12th day of fertilization, the sealing tape was removed. The egg shell was broken to remove CAM layer from the embryo and spread evenly in a petri plate for observation under microscope.

In vitro hepatoprotective assay using CCl_4 treated primary rat hepatocyte cultures

Primary hepatocytes were isolated from the liver of wistar rats using collagenase perfusion technique described previously.[23] About 5×10^4 and 1×10^5 cells were seeded in each well of 96 and 24 well plates, respectively. The plates were incubated in humidified 5% CO_2 atmosphere for 24 h. The media from plates was then replaced with fresh medium containing 1% of carbon tetra chloride and varying (79 µg/ml –5 mg/ml) concentrations of aqueous MOL and MOF extracts for 1.5 h. Plain medium was added to control wells. Ten microliters of 5 mg/ml MTT dye was added to 96 well plates and MTT assay performed using protocol described earlier. In 24 well plates, the supernatant was collected and evaluated for concentrations of liver specific enzymes aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase, and bilirubin using Pathozyme kit (Pathozyme Diagnostics Pvt. Ltd., India).

Statistical analysis

All experiments were performed in triplicate. The data are presented as mean of the replicates of at least two independent experiments. Significance testing was performed using paired *t*-test to compare the data. $P < 0.05$ was considered to be consistent with statistical significance. *, **, and *** stars in the graph denotes the level <0.1, <0.05, and <0.01 of significance, respectively.

RESULTS

The study describes the effect of aqueous MOL and MOF extracts on primary rat fibroblast, BMMSC, hepatocytes, and cancer cell lines using *in vitro* cell proliferation (MTT), wound healing, angiogenesis, and hepatoprotective assays. Figure 1 describes the effect of varying concentration of the two aqueous extracts on rat primary fibroblast cells. It shows the graph of percent cell viability plotted against the concentrations 79 µg/ml – 5 mg/ml of extracts. The MOL extract did not produce any significant effect on cells up to concentration as high as 5 mg/ml. The MOF extract, however, determined the dose dependent increase in cell viability. About 37% increase in cell viability was noted at concentration 0.313 mg/ml. As high as two-fold increment in viability was observed at concentrations from 1.25 mg/ml ($P < 0.01$).

The result of similar assay performed to test 31.25 µg/ml to 500 µg/ml concentrations of two extracts on rat bone marrow derived mesenchymal stem cells (BMMSC) is given in Figure 2. The MOL extract in contrast to the response on primary fibroblast, determined a moderate 20% increment in cell viability on BMMSC cells. The MOF extract showed greater effect on BMMSC cells ($P < 0.01$). Statistically significant increase in cell viability and thus number was noted from 62.5 µg/ml and about 29% increase in cell viability was observed at 500 µg/ml concentration of MOF extract. The increment in proliferation of BMMSC shown by treatment of 125, 250 and 500 µg/ml of MOF extract after specific (24, 48, and 72 h) time interval can be observed in the photographs given in Figure 3a-l. The percent increase in cell number and viability is indicative of the extent of stimulation of cells by the *Moringa* extracts. The MOF extract thus demonstrated comparatively higher potential to stimulate proliferation of bone marrow derived mesenchymal stem cells. The effect of *Moringa* extracts on stem cells have not been investigated earlier. However, studies on β-sitosterol have shown pronounced effect on neural stem cells proliferation.[19] β-sitosterol is one of the important constituent of *M. oleifera* plant which may have been responsible for the observed effect on BMMSC in this study.

The effect of extracts on cell lines Mcf7 and HBL 100 are denoted in Figures 4 and 5, respectively. Mcf7 is a breast cancer cell line while HBL 100 is human breast epithelial cell line isolated from normal young female. The cell line was later detected to contain tandemly integrated

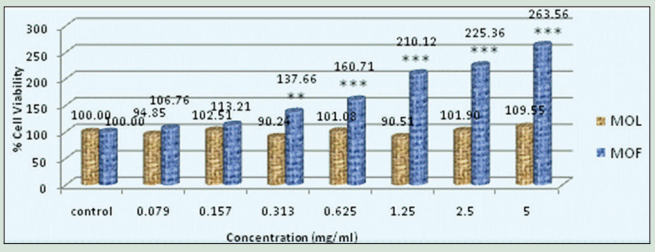

Figure 1: Effect of various concentrations (0.079–5 mg/ml) of aqueous *Moringa oleifera* leaf and flower extracts on viability of rat derived primary fibroblast cells using 3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide assay. The percent viability values at various concentrations of extracts were calculated by comparing with control group. 163% increase in cell viability was observed at 5 mg/ml *Moringa oleifera* flower extract. *, **, and *** stars indicate significance <0.1, <0.05 and <0.01 respectively

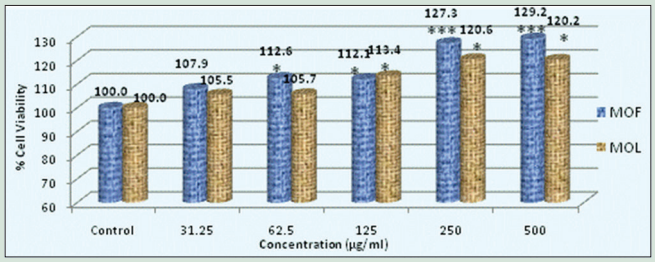

Figure 2: Effect of various concentrations (31.25–500 µg/ml) of aqueous *Moringa oleifera* leaf and flower extracts on viability of rat bone marrow derived mesenchymal stem cells (BMMSC) evaluated using 3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide assay. The percent viability values at various concentrations of extracts were calculated by comparing with control group. 29% and 20% increase in cell viability was observed at 500 µg/ml *Moringa oleifera* flower and leaf extract respectively. *, **, and *** stars indicate significance <0.1, <0.05 and <0.01 respectively

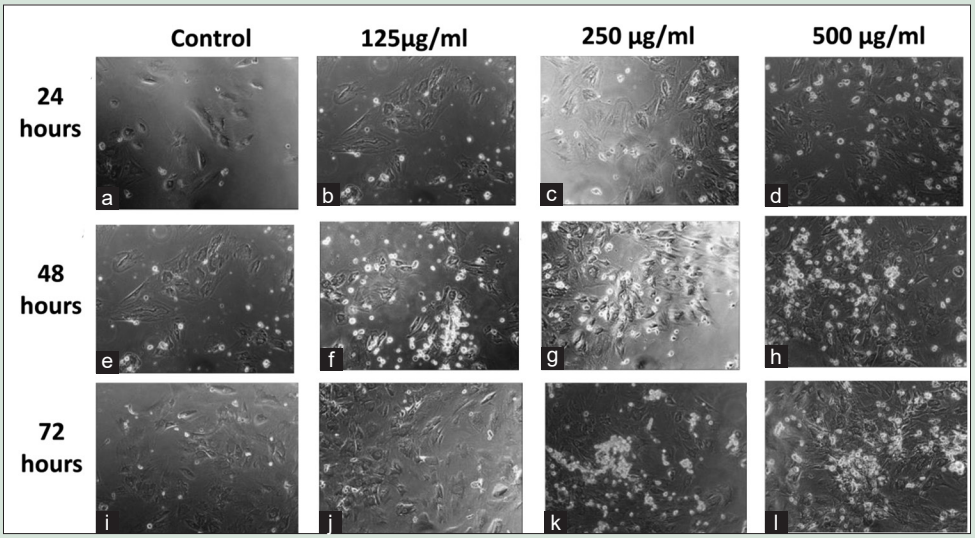

Figure 3: Effect of *Moringa oleifera* aqueous flower extract on proliferation of BMMSC (a-l) shows the effect of 125, 250 and 500 µg/ml of *Moringa oleifera* aqueous flower extract on proliferation of rat bone marrow derived mesenchymal stem cells observed after 24, 48 and 72 h time interval

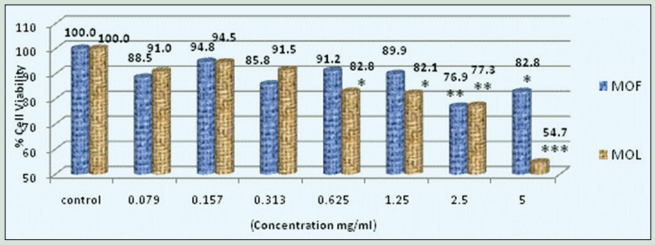

Figure 4: Effect of varying concentrations (0.079–5 mg/ml) of aqueous *Moringa oleifera* leaf and flower extracts on proliferation and viability of Mcf7 cell line evaluated using 3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide assay. The percent viability values at various concentrations of extracts were calculated by comparing with control group. 5 mg/ml concentration of *Moringa oleifera* leaf and flower extracts show 45.3% and 17.2% reduction in cell viability respectively

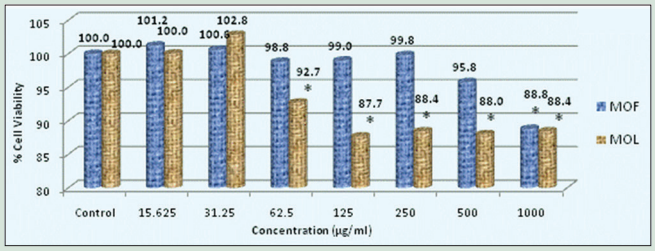

Figure 5: Effect of varying concentrations (15.6–1000 µg/ml) of aqueous *Moringa oleifera* leaf and flower extracts on viability of HBL 100 cell line evaluated using 3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide assay. The percent viability values of various concentrations of extracts were calculated by comparing with control group. *Moringa oleifera* flower and leaf extracts show 11.5% reduction in cell viability

SV40 viral genome and is recommended to not been considered as normal.[24] The extracts did not determine any increment in percent cell viability on both cell lines. While MOF extract determined less effect on cells, the MOL extract showed significant cytotoxicity from 0.625 mg/ml concentration. About 46% reduction in cell viability was shown by MOL extract at higher concentration (5 mg/ml) on Mcf7 cell line. The flower extract in comparison determined only 18% reduction in cell viability. Relatively less cytotoxic effect [12%; Figure 5] was observed on HBL 100 cell line. This may be due to nontumor origin of this cell line.

In vitro wound healing (scratch) assay

The selected extracts were assessed and compared for wound healing effect using *in vitro* scratch test. An end to end scratch was made in confluent cultures of rat primary fibroblast cells grown in 24 well plates and treated with 50, 100, and 200 µg/ml of two extracts in duplicates. The incubated plates were observed for cell migration and proliferation at 24.00 h interval. The images were captured using camera attachment of the microscope and compared visually. The images reflected a dose dependent effect of two extracts on healing of scratch. The effect of extracts on migration and proliferation of cells were best identified after 48 h of incubation as the scratch closed completely at 72.0 h in all treated as well as control wells. Figure 6a-f shows the effect of 100 µg/ml and 200 µg/ml of two extracts on rat fibroblast scratched cells after 48 h. They reflect a difference in healing of scratch by MOL and MOF extracts. The 200 µg/ml concentration of MOF extract promoted migration of fibroblast to cover the scratch almost completely in 48 h of incubation. Whereas, the MOL extract treated and control cells took longer time (72.00 h) for the same. The response given by two extracts in this assay is similar to the one seen in fibroblast cell proliferation assay.

Chorioallantoic membrane assay

The CAM is a chick extraembryonic membrane having dense capillary network. It is widely used to study the process of angiogenesis.[22] Figure 7a-e shows the images of CAM treated with 100 µg/ml and 200 µg/ml of MOL and MOF extracts for 4 days. Figure 7b and c shows considerable increase in blood vessels formation on CAM by 100 and 200 µg/ml of MOL extract as compared to the control. Further prominent increase in formation of blood vessels was shown by 100 µg and 200 µg/ml MOF treated CAM [Figure 7d and e]. It shows both MOL and MOF extracts to promote angiogenesis. The MOF extract exhibited higher ability to stimulate formation of blood vessels and serve as

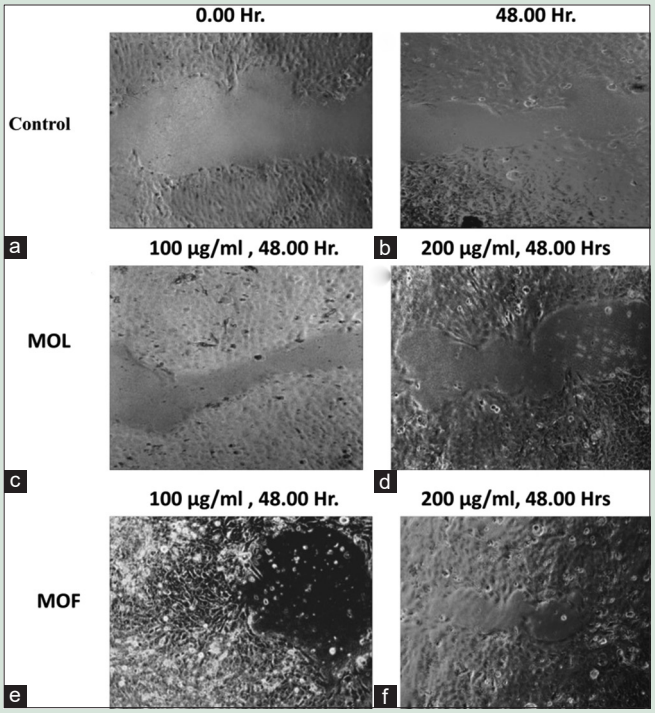

Figure 6: Effect of *Moringa oleifera* aqueous extracts on proliferation of cells using wound healing assay (a-f): Microscopic images showing the effect of *Moringa oleifera* leaf and flower extracts on migration and proliferation of rat primary fibroblast cells tested in wound scratch assay. (a and b) Images of scratch in control well at 0.00 and 48.00 h respectively. (c and d) Images of scratch treated with 100 and 200 µg/ml of *Moringa oleifera* leaf extract and (e and f): Shows images of scratch treated with 100 and 200 µg/ml of *Moringa oleifera* flower extract respectively for 48 h

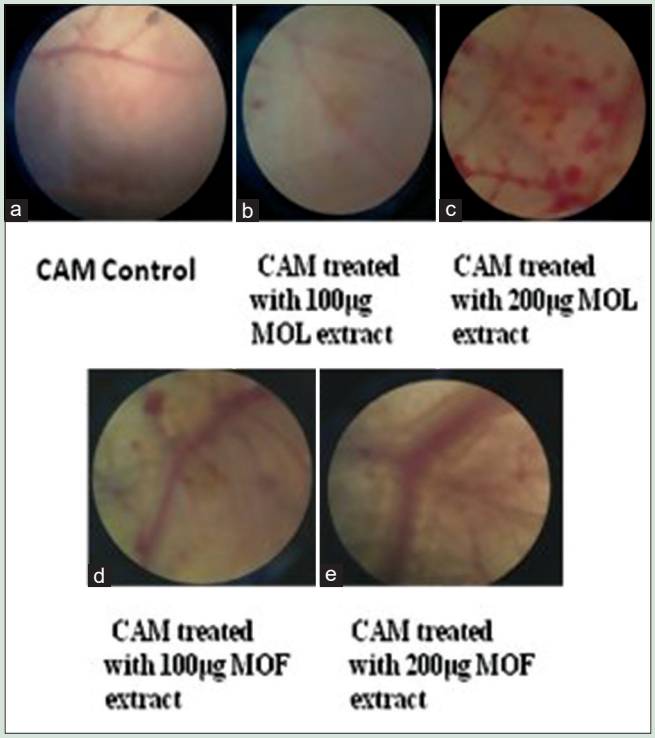

Figure 7: Effect of *Moringa oleifera* aqueous extracts on angiogenesis using chorioallantoic membrane assay (a-e): Images of chick chorioallantoic membrane after treatment with 100 and 200 µg/ml of *Moringa oleifera* aqueous leaf and flower extracts in chorioallantoic membrane assay. (a) shows the image of control, untreated chorioallantoic membrane, (b and c) shows images of chorioallantoic membrane treated with 100 and 200 µg/ml of *Moringa oleifera* leaf extract respectively and (d and e) shows the images of chorioallantoic membrane after treatment with 100 and 200 µg/ml of *Moringa oleifera* flower extract respectively

proangiogenic compound. The proangiogenic effect of MOF was further confirmed by fixing the 12th day MOF treated CAM with 10% formalin and sectioning. Figure 8a-c shows the images of the hematoxylin and eosin stained sections of CAM treated with 100 and 200 µg/ml of MOF extract. The PBS treated control CAM shows normal large and small blood vessels with normal endothelial lining. The MOF (100 and 200 µg/ml) treated CAM show many smaller vessels and abundant blood vessel sprouting which is indicative of proangiogenic effect.

In vitro hepatoprotective study using rat primary hepatocytes

The crude aqueous MOL and MOF extracts were compared for hepatoprotective effect using *in vitro* rat primary hepatocytes. The ability of extracts to promote recovery of carbon tetra-chloride treated rat primary hepatocytes is shown in Figure 9. Primary rat hepatocytes were exposed to 1% CCl_4 with varying concentrations (79 µg/ml – 5 mg/ml) of MOL and MOF extracts and assessed by MTT assay. On CCl_4 exposure for 1.5 h., cell viability was reduced to only 7.8%. Addition of MOF and MOL extracts progressively increased the viability to 81% and 78%, respectively. Effect of MOL and MOF extracts on activity of liver specific enzymes AST and ALT in CCl_4 treated primary hepatocytes are given in Figure 10. Primary hepatocytes treated with varying concentrations of MO extracts (125 µg/ml – 1000 µg/ml) and the standard drug Silymarin (200 µg/ml) showed significant restoration of altered biochemical parameters. A very prominent hepatoprotective effect was thus determined by both extracts in this study.

DISCUSSION

M. oleifera is a perennial angiosperm plant belonging to Moringaceae family. All the parts of this plant have been attributed high nutritive, prophylactic, and medicinal value. The leaves are been extensively investigated for their therapeutic potential and mechanism of action against various clinical symptoms. The flowers however are less studied and very few scientific findings are reported. The flowers are used in tea, curries, and salads in certain areas and are of interest for medicinal applications in folk community. The present study evaluated and compared the potential of *Moringa* leaf and flower extracts to promote proliferation and regeneration of cells. The study also explored their effect on proliferation of cancer cells to assess safety for use in healing and regeneration.

The result of cell proliferation assay in this study showed that *Moringa* flower extract had a high potential to promote proliferation of rat primary fibroblast and BMMSC cells. It determined a dose dependent increase in cell viability and about 37% increase at 313 µg/ml concentration on primary fibroblast and 29% increase at 500 µg/ml concentration on bone marrow derived cells. As high as two fold increase in fibroblast cells was noted at 1.25 mg/ml concentration of flower extract. It however showed no significant influence on cell viability of Mcf7 and HBL 100 cancer cell lines. The leaf extract, on the other hand, determined a relatively lower influence on viability of fibroblast and BMMSC cells but showed a prominent 46% toxicity to Mcf7 cell line at higher concentration.

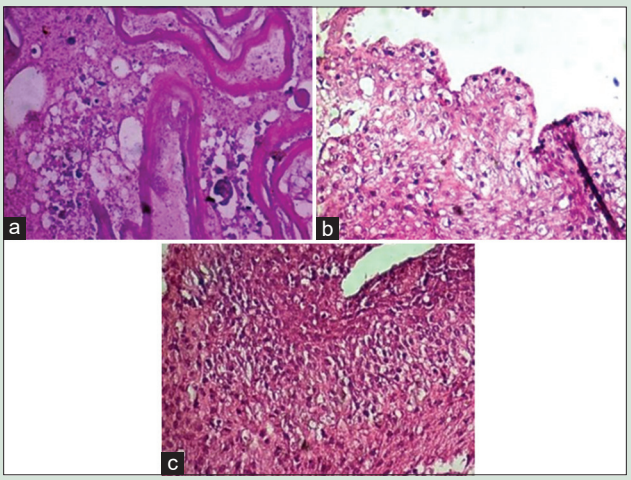

Figure 8: (a-c) denotes the images of chorioallantoic membrane after treatment with phosphate buffered saline (control), 100 and 200 μg/ml of aqueous *Moringa oleifera* aqueous flower extract. The images are obtained after fixing, sectioning and staining of chorioallantoic membrane layer subsequent to *Moringa oleifera* flower treatment. (b and c) shows increase in microvessel formation after treatment with *Moringa oleifera* flower extract

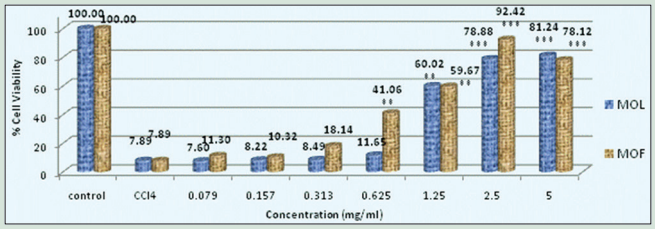

Figure 9: Effect of varying concentrations of *Moringa oleifera* leaf and flower extracts on CCL4 treated primary rat hepatocytes. *, **, and *** stars indicate <0.1, <0.05 and <0.01 significance respectively

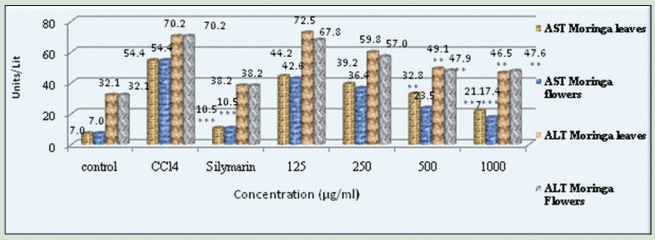

Figure 10: Effect of *Moringa oleifera* extracts on aspartate aminotransferase and alanine transaminase levels in CCl4 treated primary rat hepatocytes: Effect of varying concentrations of *Moringa oleifera* leaf and flower extracts (125–1000 μg/ml) and standard drug sylimarin (200 μg/ml) on activity of enzymes aspartate aminotransferase and alanine transaminase in CCL4 induced primary rat hepatocytes

The results thus reflected a clear difference in the response of two extracts on the proliferation of four different cell types used in this study. While the MOL extract produced no effect on normal rat primary fibroblast cells and moderate increase in cell viability of rat BMMSC cells, it exhibited a very significant level of toxicity to Mcf7 breast cancer cell line. The MOF extract, on the contrary, demonstrated a pronounced increase in percent cell viability of normal fibroblast and BMMSC cells while not being effective in producing toxicity to cancer cell lines. Most importantly, the MOF extract showed stimulation of normal fibroblast and BMMSC cell proliferation but did not show the same effect on abnormal cells of cancer cell lines. This observation provides yet another evidence for safety of *M. oleifera* for human consumption and for consideration of its use in cell stimulatory drugs.

The results of the leaf extract are in agreement with those reported earlier by Muhammad *et al.* who determined the effect of MOL extract on viability of human dermal fibroblast cells. The bioactive compounds vicenin-2, quercetin, and kaempferol isolated from methanolic fraction of leaf extract were correlated with enhanced proliferation and wound healing.[15] To the best of our knowledge, similar investigations on flower extract have not been reported. Earlier reports on *Moringa* flower have defined its antioxidant and antiinflammatory activities. The study identified existence of antioxidant compounds ascorbic acids, carotenoids, tannins, alkaloids, glycosides, flavanoids kaempferol, quercetin, phenolic compounds quinic acid, 4-p coumaroyl quinic acid, and a unique combination of fatty acids including heneicosanoic acid, capric acid, and behenic acid in flower extract. The major flavanoids quercetin and kaempferol were correlated with elevated antiinflammatory activity.[17] An exceptional combination of major phenolic compounds, flavanoids, vitamins, and essential fatty acids may probably be responsible for the enhanced proliferative effect of flower extract observed in the present study.

The results of leaf extract on cancer cell lines also supports the earlier findings of Tiloke *et al.* and Jung *et al.* They determined antitumor potential of *M. oleifera* aqueous leaf extract.[25,26] The novel bioactive glycosidic compounds niazirin, niaziridine, and O-Ethyl-4-(α-L rhamnosyloxy benzyl carbamate) and its isothyocynate derivative isolated from leaves, pods and bark of *M. oleifera* were proposed to be the potent antiproliferative agents.[27] Absence of these glycosidic compounds in aqueous *Moringa* flowers extracts may be the basis for its less pronounced effect on cancer cells.

The effect of leaf and flower extracts on migration and proliferation of fibroblast cells using wound scratch assay also determined the higher potential of flower extract to close the scratch. Wound healing occurs as a cellular response to injury and involves activation of fibroblast, endothelial cells, and macrophages. Fibroblast proliferation is involved in the restoration of structure and function at the wound site. Therefore, therapeutic bioactive agents that are able to stimulate fibroblast growth and proliferation are able to improve or promote wound healing. Increase in wound closure rate by the administration of leaf extract to rats has been reported earlier.[28] The flower extract in our study demonstrated the greater effect in enhancing the proliferation of rat fibroblast cells compared to the crude aqueous leaf extract.

Angiogenesis is the physiological process of forming new blood vessels from the preexisting vasculature. It is a normal and vital process in growth and development, as well as in wound healing and formation of granulation tissue. Stimulation of angiogenesis can be therapeutic in wound healing, ischemic heart disease and peripheral arterial disease.[22] In present study, the *in vitro* CAM assay revealed both MOL and MOF extracts to be proangiogenic although, MOF extract exhibited enhanced ability to promote angiogenesis. It is important to investigate the effect of *M. oleifera* extracts or compounds derived from them on angiogenesis before considering as a potential candidate for antitumor drug.[26]

A comparison of aqueous leaf and flower extract for activity against CCL_4 induced primary rat hepatocytes was attempted in the present study. No statistically significant variation in values of biochemical parameters were seen indicating both aqueous extracts to possess similar hepatoprotection potential. This was in accordance with previously reported data of aqueous *Moringa* leaf extracts in primary hepatocytes and of flower extract on acetaminophen induced liver damage in rats.[10]

CONCLUSION

This study evaluated and compared MOL and MOF aqueous extracts for influence on proliferation of cells using *in vitro* assays. A very prominent ability of flower extract to promote proliferation of rat fibroblast, MSCs, and angiogenesis was identified. The same effect on abnormal cancer cells was however not seen. The leaf extract determined comparatively lesser ability to stimulate cell proliferation but had prominent cytotoxic effect on cancer cell lines. The study thus pointed out an extraordinary ability MOFs to stimulate proliferation and healing of cells which could be explored for development of drugs. Variations in pharmacological activities of leaf and flower relates to the combination of bioactive compounds present in the two extracts. Future studies will be needed to determine the bioactive compounds participating in proliferation and regeneration process.

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Conflicts of interest

There are no conflicts of interest.

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Alpana S. Moghe

ABOUT AUTHOR

Dr. Alpana S. Moghe, is Associate Professor in Rajiv Gandhi Institute of IT & Biotechnology, Bharati Vidyapeeth Deemed University, Pune. Her area of research includes screening of natural products and compounds for efficacy & genotoxicity studies. Ms. Evangeline Fernandes, Anubha Pulwale and Gauri Patil are students pursuing Master's and Ph.D. studies in the Institute