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Stem cell regeneration and differentiation potential of indigenous plants

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ABSTRACT

Stem cell based tissue engineering and regenerative medicine are emerging as most promising approaches for treatment of various ailments including degenerative diseases. Mesenchymal stem cells (MSCs) are most preferred candidates for use in clinical cell based therapies due to their regenerative, immunomodulatory, angiogenic, anti-inflammatory and antiapoptotic capacities. Efficient ways for expansion and differentiation of MSCs are being extensively researched for their utilization in cellular therapies. Use of plant derived extracts and phytochemicals is one novel approach which is being largely explored for cost effective, safe and efficient means of modulating stem cells. Aqueous extracts of eight medicinal plants were investigated for their ability to stimulate rat mesenchymal stem cells.. The potential to stimulate proliferation was estimated using cell viability assay and its ability to differentiate were determind using osteocyte, chondrocyte and adipocyte differentiation assays. The differentiated cells were fixed, stained and quantitated using spectophotometric analysis. The extracts *Boerhavia diffusa*, *Centella asiatica*, *Tridax procumbens*, *Moringa oleifera* flower and *Pureria tuberose* promoted the proliferation to maximum. They also retarded the differentiation of BM MSCs to adipocytes. *Manilkara zapota*, *Pureria tuberose*, *Centella asiatica* and *Moringa oleifera* leaves promoted chondrocyte differentiation and *Moringa oleifera* flower, *Tridax procumbens*, *Boerhavia diffusa* and *Pureria tuberose* promoted osteocyte differentiation. The study could correlate the traditional medicinal use of the plants with specific regenerative activity. It also pointed out application of study plants in economic and safe development of regenerative pharmaceutical medicines.

INTRODUCTION

Stem cell based tissue engineering and regenerative medicine are emerging as most promising approaches for treatment of various ailments including degenerative diseases. Mesenchymal stem cells (MSCs) are most preferred candidates for use in clinical cell based therapies due to their regenerative, immunomodulatory, angiogenic, anti-inflammatory and antiapoptotic capacities. Efficient ways for expansion and differentiation of MSCs are being extensively researched for their utilization in cellular therapies [1]. Use of plant derived extracts and

phytochemicals is one novel approach which is being largely explored for cost effective, safe and efficient means of modulating stem cells [2]. Number of preclinical studies have shown successful application of plant derived products for proliferation and osteogenic as well as chondrogenic differentiation of MSCs [3]. With increasing investigations in this area, it is speculated that botanical products may become a new perspective in stem cell based tissue engineering.

The Indian Traditional Systems (ITM) of Medicine are highly systematized discipline based on well defined codified theories and thousands of years of practice. Ayurveda is one important ITM system, whose therapies are based on restoration of body balance and nourishment of tissues [4]. One of the integral component among the eight major divisions of Ayurveda is 'Rasayana' therapy. It involves range of herbal formulations which are known as tonics of rejuvenation and used for improvement of longevity, memory, intelligence, health, youth, complexion, motor and sensory strengths. They hold potential to decelerate aging process, impart youthfulness or longevity in the individuals in particular by providing protection against oxidative and DNA damage [5]. The nourishing abilities of Rasayana drugs are suggestive of having role in regeneration of cells and tissues. Earlier investigations on Medhya group of Rasayana drugs have shown their influence on proliferation and neuronal differentiation [6]. In present study, we have evaluated the effect of selected Rasayana drugs on proliferation and differentiation of rat mesenchymal stem cells. *Pueraria tuberose* (PT), *Abutilon indicum* (AI), *Boerhavia diffusa* (BD), *Centella asiatica* (CA), *Manilkara zapota* (MZ), *Moringa oleifera* leaf (MOL), *Moringa oleifera* flower (MOF), and *Tridax procumbens* are the herbs selected for present study. The study is intended to determine the potential of these plants in stimulating proliferation and selecting differentiation pathways of stem cells. This study will be of help to gain insights in the growth promoting abilities of study plants which are valuable for pharmacologic and regenerative biology.

MATERIALS AND METHODS

Chemicals and Reagents

Dimethyl Sulphoxide (DMSO), Paraformaldehyde, Oil-Red-O, Alcian Blue, Alizarin Red, Gaunidine HCL, 3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT dye) were procured from Sigma Aldrich India. Fetal Bovine Serum (MSC qualified), EMEM Alfa graded, StemPro Adipocyte differentiation basal media, StemPro chondrocyte differentiation basal media, StemPro osteocyte differentiation basal media were procured from Gibco by life Technologies, Penicillin & Streptomycine, Trypsine-EDTA were obtained from HiMedia BioSciences, Isopropanol, Hydrochloric Acid, Acetic Acid, Ammonium Hydroxide, Diethyl ether procured from SRL.

Preparation of plant extracts

The selected plants were procured from their natural habitat and authenticated from Agharkar research Institute Pune. Approval for use of plants for present study was obtained from Maharashtra State Biodiversity Board. The parts of plants used for the study are specificed in table 1. The plant material were washed, shade dried and powdered. 5gm of powder was extracted with 50ml of water for 48.00 hrs at room temperature. The extracts were filtered, lyophilized and dissolved in Phosphate Buffered Saline (PBS) for further use.

Isolation and Culture of rat bone marrow mesenchymal stem cells

Use of rats for the present study was approved by Institutional Animal Ethics Committee of Bharati Vidyapeeth Deemed to be University. (Ref no:BVDUMC/3305/2016/019/002). The procedure described by Zhang *et al* was used for preparation of primary culture [7]. Briefly, Wistar rat of 4-6 weeks age was anesthetized by diethyl ether and dissected. Tibia and femur bones were isolated. Bone marrow was flushed and collected in centrifuge tubes containing α-MEM supplemented with 10% MSC-FBS and 50 U/ml penicillin and 50µg/ml streptomycin. The tube was centrifuged at 2000 rpm for 10 mins. Pellet was washed twice and finally resuspended in α-MEM supplemented with 10% MSC-FBS and 50 U/ml penicillin and 50µg/ml streptomycin. About $1X10^6$ cells were seeded in

each well of 6 well plates and incubated at 37^0C in 5% CO_2 atmosphere. Media change was given after every third day. Cells after third passage were used for further experiments.

Cell proliferation (MTT) assay

$5.0X10^4$ cells of BM-MSCs were seeded in 96 well plates and incubated at 37^0C in 5% CO_2 atmosphere for 24.00 hrs. Various concentrations (15.6 µg/ml -1000µg/ml) of the extracts were inoculated in triplicates and the plates were incubated for next 72.00 hrs. After incubation period, 10µl of 5mg/ml MTT dye was added to each well and incubated in dark condition for 4.0 hrs followed by addition of 100µl of DMSO and 25µl of glycine buffer. After incubation for 15 mins the absorbance was measured at 570 nm in Biorad PR 4100 plate reader and plotted against the concentration [8].

Adipogenic differentiation

Cell suspension containing 2.5 X 10^5 cells of passage three BM-MSC culture were seeded in 24 well plates and incubated at 37^0C in 5% CO_2 atmosphere for 24.00 hrs. The cells were then switched to an adipocyte differentiation medium. Various concentrations of plant extracts (125µg/ml-1000 µg/ml) were added in triplicates except in cell control wells and further incubated up to 7 days. After incubation cells were fixed with 4% paraformaldehyde, washed and stained with 0.3% Oil Red-O solution. The cells were observed and photographed using ZEISS Axiocam Microscope Camera. Oil Red O was eluted by adding 100% isopropanol and incubated for 3.0 hrs. Absorbance was measured at 500 nm using 100% isopropanol as blank. The absorbance was plotted against the various concentration of extracts and compared [9].

Chondrogenic differentiation

2.5 X 10^5 cells of passage three BM-MSC culture were seeded in 24 well plates and incubated at 37^0C in 5% CO_2 atmosphere for 24.00 hrs. The cells were then switched to a chondrocyte differentiation medium. Various concentrations of plant extracts (125µg/ml-1000 µg/ml) were added in triplicates except in cell control wells and further incubated up to 14 days. After incubation the cells were fixed with 4% paraformaldehyde, washed and stained with 1% Alcian Blue solution. Quantification of aggrecans formation was performed by eluting Alcian Blue in 8M guanidine HCL solution and incubating over night, at 2-8°C. Absorbance (O.D.) was read at 600 nm using 8M guanidine HCL as blank. Graphs of optical density versus concentration of extracts were plotted and compared [10].

Osteogenic differentiation

2.5 X 10^5 cells of passage three BM-MSC culture were seeded in 24 well plates and incubated at 37^0C in 5% CO_2 atmosphere for 24.00 hrs. The cells were then switched to a osteocyte differentiation medium. Various concentrations of plant extracts (125µg/ml-1000 µg/ml) were inoculated in triplicates except the control wells. Plates were incubated up to 21 days. After incubation cells were fixed with 4% paraformaldehyde, washed and stained with 2% Alizarin Red solution. Quantification was done by eluting Alizarin Red stain using 10% acetic acid followed by three freeze and thaw cycles. Absorbance was read at 405 nm and plotted against the various concentration of extracts [11].

Statistical analysis

The experiments were performed in triplicates. The data are presented as mean of the replicates. Significance testing was performed using paired *t*-test. *P* value less than 0.05 are considered for statistical significance. *, ** and *** stars in the graphs indicate significance <0.05, <0.01 and <0.001 respectively.

RESULTS

Cell proliferation assay

Investigations of seven TIM herbs *Pueraria tuberose* (PT), *Abutilon indicum* (AI), *Boerhavia diffusa* (BD), *Centella asiatica* (CA), *Manilkara zapota* (MZ), *Moringa oleifera* leaf (MOL), *Moringa oleifera* flower (MOF), and *Tridax procumbens* (TP)for effect on proliferation and differentiation of rat mesenchymal stem cells are presented in this paper. The aqueous extracts of study plants were treated with bone marrow derived rat mesenchymal stem cells for 72.00 hrs and tested for cell viability using cell proliferation (MTT dye based) assay. Fig i (a) and Fig ii (b) determines the percent cell viability of BM MSCs after treatment with various

concentrations of test extracts. All the extracts determined dose dependent increase in percent cell viability. The extracts CA, BD, TP, MOF and PT determined about 39.2 %, 19.9 %, 16.3% , 15.7% and 13% increase in cell viability at 62.5 µg/ml concentration respectively (P<0.01). Highest increments among all eight extracts were shown by TP, PT, CA and BD (94.3%, 86%, 79.3% and 75.9% respectively) at 1000 µg/ml concentration. The extracts MZ and MOF determined significant (48% and 47% respectively) increment in cell viability at highest concentration tested. AI and MOL had less influence on MSCs and showed effect only at 1000 µg/ml concentration 17% & 15% respectively) (Fig i (a) & Fig ii (b).)

Adipocyte differentiation assay

Fig ii (a), ii (b) and ii (c) denotes the effect of test extracts on differentiation of rat BMMSC to adipocytes after treatment with test samples in adipocyte induction medium for 7 days followed by fixing and staining with oil - red - o stain. Fig ii (a) i to iiii shows the photographs of undifferentiated control, differentiated control and drug treated cells respectively after fixing and staining with oil-red-o stain. None of the extracts promoted differentiation of MSCs to adipocytes. The MOF, BD, PT, CA, TP and AI determined significant reduction in adipocyte differentiation from 125 µg/ml concentration (52.1%, 43.2%, 37.8%, 36.1%, 33.6% and 25% respectively) and showed 67.2%, 54.1%, 47.4%, 43.19%, 57.1% and 42.2% reduction at highest concentration of test extracts respectively (Fig ii b) (P< 0.001). MOF and TP thus determined maximum reduction in adipocyte differentiation potential. (Fig ii c). MOL and MZ on the other hand did not show much effect on same at 1000µg/ml concentration in the present study. (p<0.05)

Chondrocyte differentiation assay

Fig iii (a) shows the alcian blue stained undifferentiated cell control, differentiated cell control and drug treated differentiated BM MSCs respectively (Fig iii a i – iiii) after incubation with chondrocyte differentiation medium for 14 days. Fig iii b and c denotes the effect shown by extracts on chondrocyte differentiation of MSCs. Most of the extracts showed increment in chondrocyte differentiation. MZ, PT, CA have shown more than 25% increase in chondrocyte differentiation at 250 µg/ml concentration (P <0.01).

Maximum chondrogenic activity among all the extracts are determined by MZ, PT, CA, TP and MOL (100.3%, 80.44%, 73.3%, 53% and 39.3% respectively) at highest concentration tested. (p>0.01)

Osteocyte differentiation assay

Fig iv a i – iii shows the undifferentiated control, differentiated control and drug treated BMMSCs grown in osteocyte differentiation medium. For 21 days and subsequently stained with Alizarin red. The third picture (Fig iv a iii) shows the increment in stained cells on treatment with herb extracts. Each concentration of the extract was tested in triplicate. Quantitation was done by eluting alizarin stain by 10% acetic acid and 10% ammonium hydroxide after three freeze thaw cycles. The mean of the absorbance of optical density at 405 nm of triplicate wells were plotted. Fig iv b and c shows MOF to have a prominant osteocyte induction activity from 125 µg/ml onwords. (P <0.01). The extracts BD, CA and TP also determind 54.8%, 64.1% and 25.6% effect at 250 µg/ml concentration respectively. However CA did not show the same effect at higher concentration. TP, MOF, BD and AI on the other hand determind 42.5%, 55.7 %, 104.1% and 26% increment at 1000µg/ml concentration respectively. The plants PT, MOL and MZ did not show significant effect on osteogenesis of cells.

To summarize, the plants BD, CA, PT, MOF and TP determined significant potential to stimulate proliferation of rat BMMSC in present study. The same plants also exhibited highest ability to retard adipocyte differentiation. MZ demonstrated maximum ability to promote chondrocyte differentiation. Plants PT, CA, TP and MOL also possessed significant chondrogenic potential. Plants BD, MOF and TP could promote osteogenic differentiation. The results are summarized in Table 2. The extracts showing more than 50% increase in proliferation and/or differentiation in a dose dependent manner are highlighted.

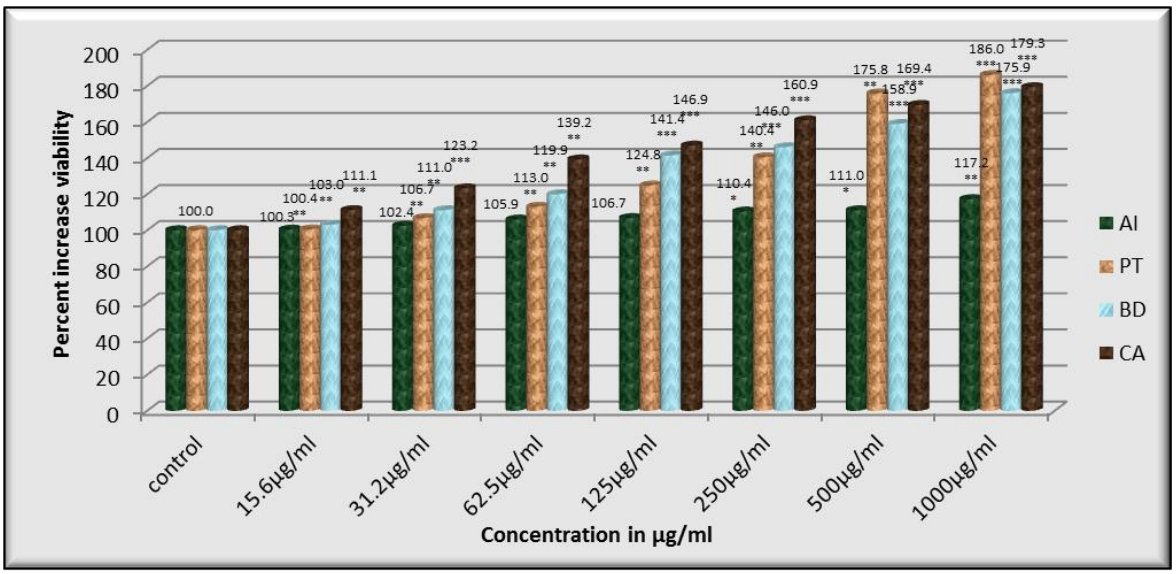


Fig i (a): Effect of *Abutilon indicum* (AI), *Pueraria tuberose* (PT), *Boerhavia diffusa* (BD), *Centella asiatica* (CA) extracts on viability of mesenchymal stem cells deriverd from rat bone marrow.

Fig i (a): Effect of various concentrations (15.6µg/ml-1000µg/ml) of aqueous *Abutilon indicum* (AI), *Pueraria tuberose* (PT), *Boerhavia diffusa* (BD), *Centella asiatica* (CA) extracts on viability of rat bone marrow derived mesenchymal stem cells (BMMSCs) evaluated using MTT assay. The percent viability values at various concentrations of extracts were calculated by comparing with control group.

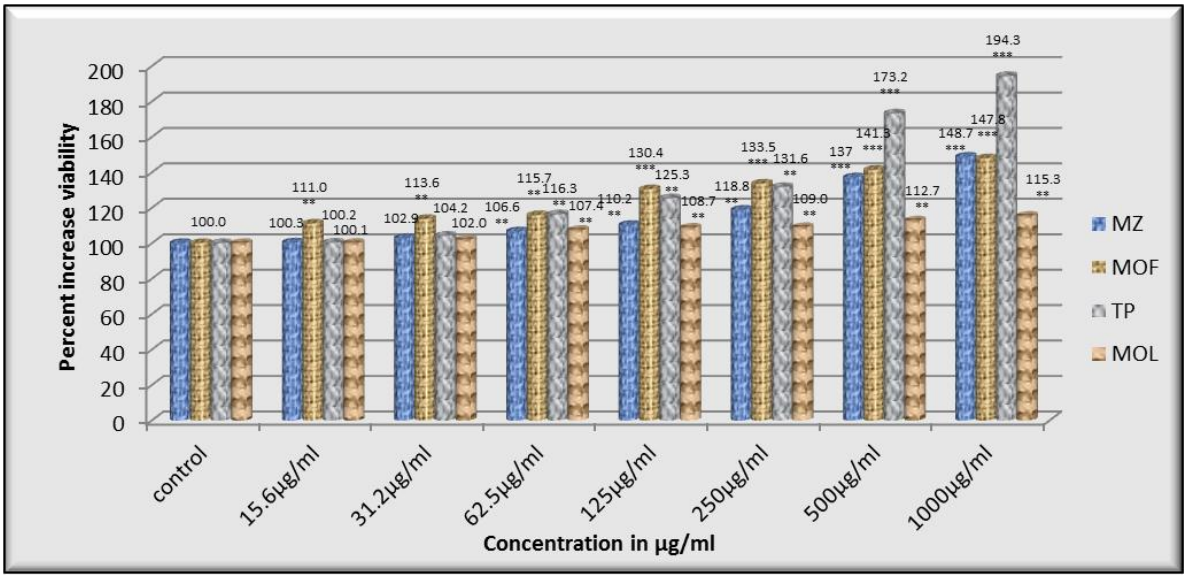
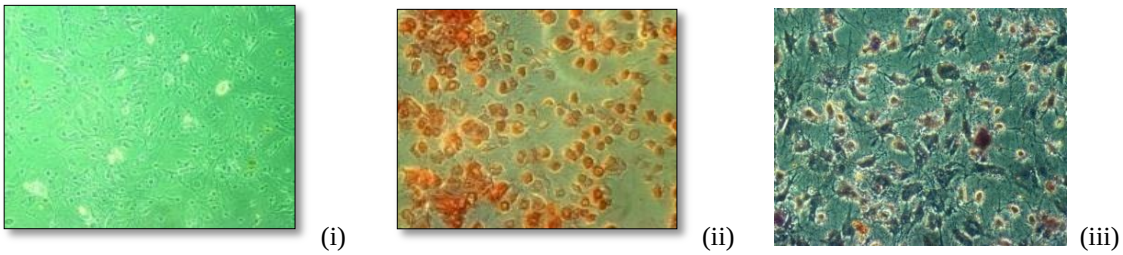


Fig i (b): Effect of *Manilkara zapota* (MZ), *Moringa oleifera* flower (MOF), *Tridax procumbens*(TP), *Moringa oleifera leaf* (MOL) extracts on viability of mesenchymal stem cells deriverd from rat bone marrow

Fig i (b): Effect of various concentrations (15.6µg/ml-1000µg/ml) of *Manilkara zapota* (MZ), *Moringa oleifera* flower (MOF), *Tridax procumbens*(TP), *Moringa oleifera leaf* (MOL) extracts on viability of rat bone marrow derived mesenchymal stem cells (BMMSCs) evaluated using MTT assay. The values on each bar denotes percent viability of extract.



(i) (ii) (iii)

Fig ii (a): Effect of selected plant extracts on adipocyte differentiation of rat bone marrow derived mesenchymal stem cells.

Fig ii (a) i-iii: Microscopic images of mesenchymal stem cells derived from rat bone marrow after adipocyte differentiation. The microscopic images were taken after treating rat BMMSCs with selected plant extracts in adipocyte differentiation. Fig ii (a) i-shows the image of untreated cell control of rat BMMSCs Fig ii (a) ii- shows the image of treated cell control of rat BMMSCs .Fig ii(a) iii- shows the image of rat BMMSCs treated with selected plant extracts during adipocyte differentiation respectively.

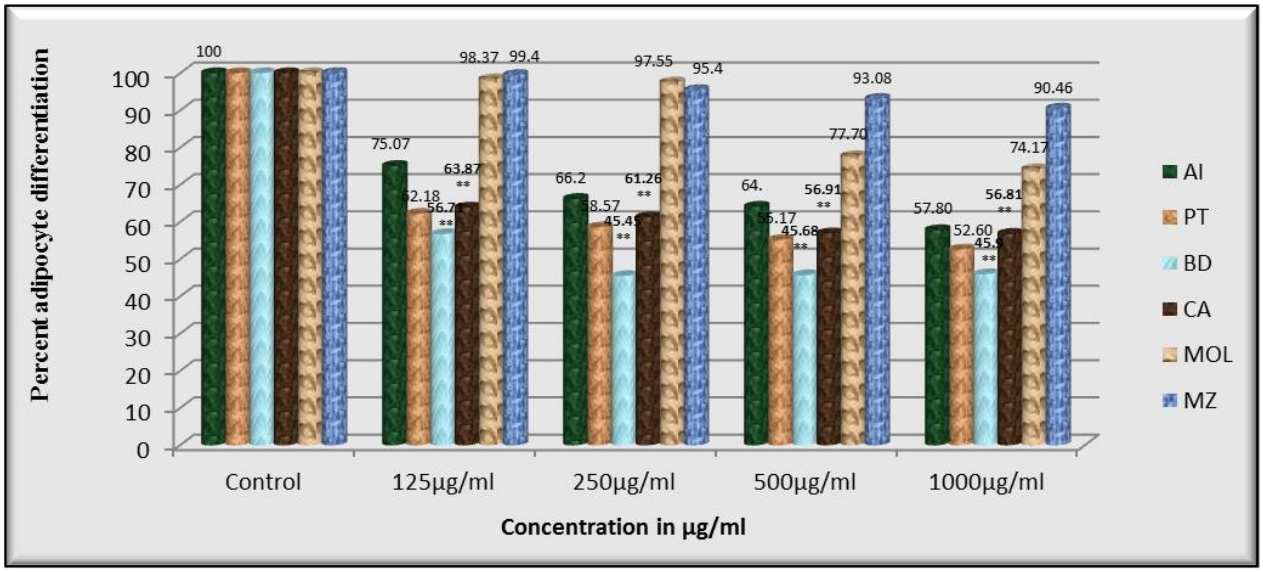


Fig ii (b): Effect of *Abutilon indicum* (AI), *Pueraria tuberose* (PT), *Boerhavia diffusa* (BD), *Centella asiatica* (CA), *Moringa oleifera* leaf (MOL), *Manilkara zapota* (MZ) extracts on adipocyte differentiation of rat bone marrow Mesenchymal stem cells.

Fig ii (b): Effect of various concentrations (125µg/ml-1000µg/ml) of aqueous *Abutilon indicum* (AI), *Pueraria tuberose* (PT), *Boerhavia diffusa* (BD), *Centella asiatica* (CA), *Moringa oleifera* leaf (MOL), *Manilkara zapota* (MZ) extracts on adipocyte differentiation of rat bone marrow Mesenchymal stem cells. The percent adipocyte differentiation of various concentrations of extracts was calculated by comparing with control group.

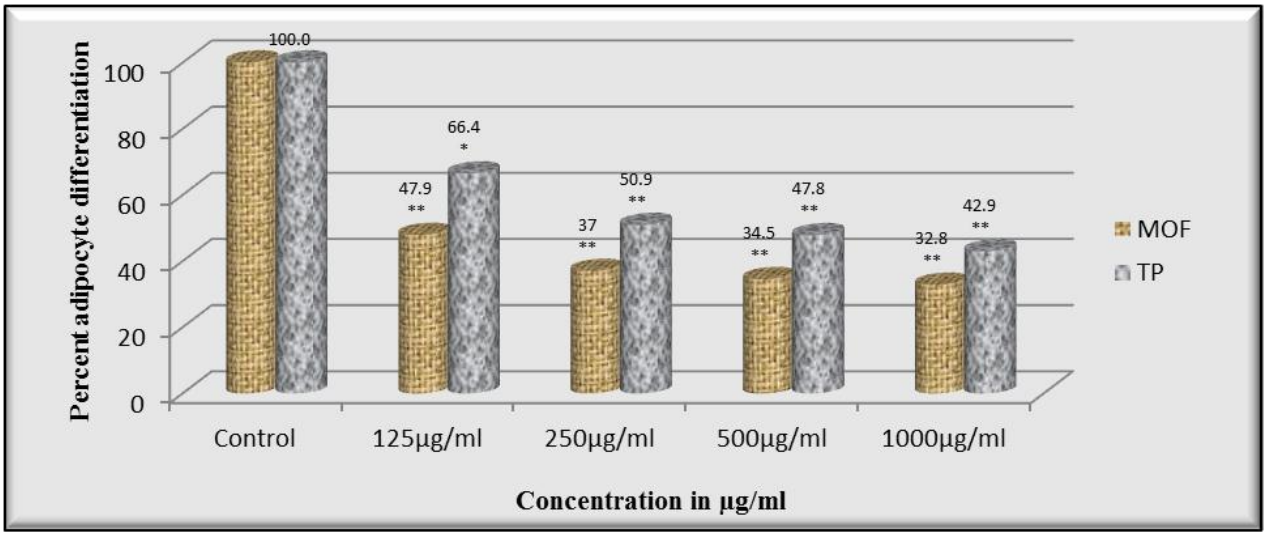


Fig ii (c): Effect of *Moringa oleifera* flower (MOF), *Tridax procumbens* (TP) extracts on adipocyte differentiation of rat bone marrow mesenchymal stem cells.

Fig ii (c): Effect of various concentrations (125µg/ml-1000µg/ml) of aqueous *Moringa oleifera* flower (MOF), *Tridax procumbens* (TP) extracts on adipocyte differentiation of rat bone marrow mesenchymal stem cells. The percent adipocyte differentiation of various concentrations of extracts was calculated by comparing with control group.

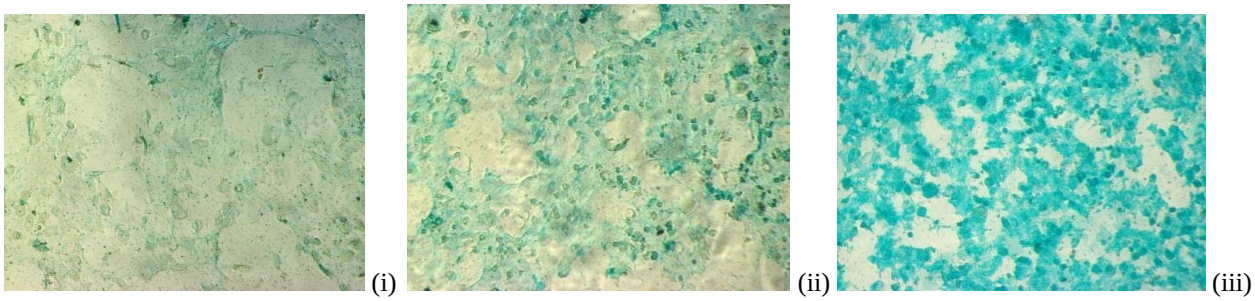


Fig iii (a): Effect of selected plant extracts on chondrocyte differentiation of rat bone marrow derived mesenchymal stem cells.

Fig iii (a) i-iii: Microscopic images of mesenchymal stem cells derived from rat bone marrow after chondrocyte differentiation. The microscopic images were taken after treating rat BMMSCs with selected plant extrats in chondrocyte differentiation. Fig iii(a) i-shows the image of untreated cell control of rat BMMSCs Fig iii (a) ii- shows the image of treated cell control of rat BMMSCs Fig iii (a) iii- shows the image of rat BMMSCs treated with selected plant extracts during chondrocyte differentiation respectively.

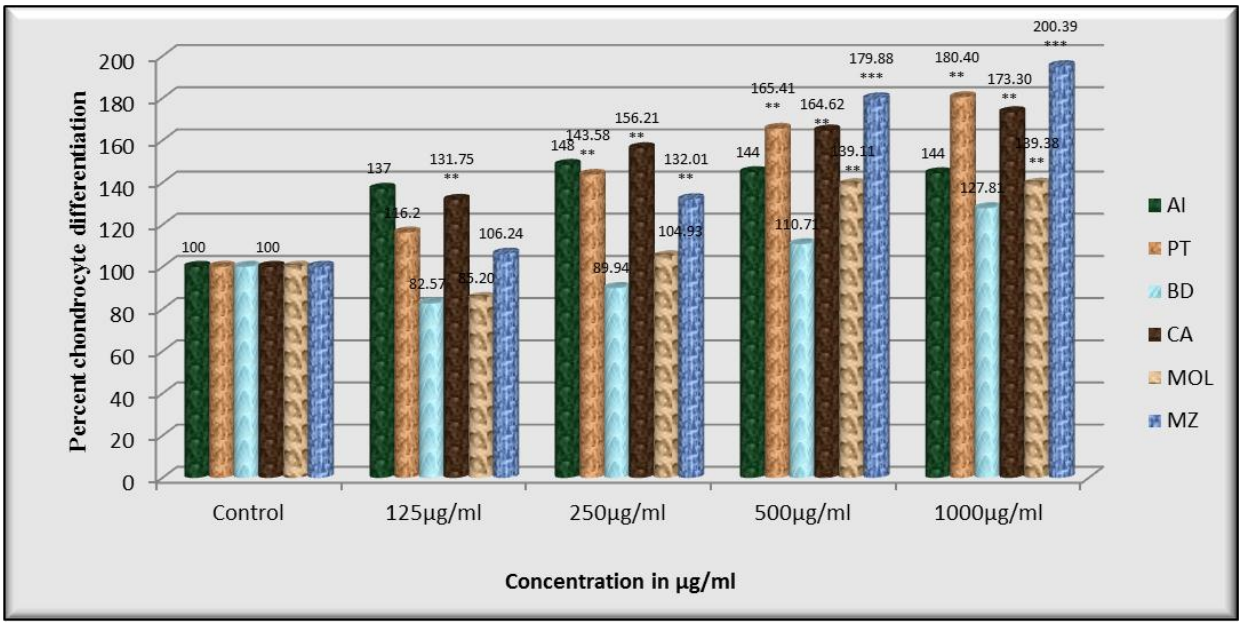


Fig iii (b) : Effect of *Abutilon indicum* (AI), *Pueraria tuberose (PT), Boerhavia diffusa* (BD), *Centella asiatica* (CA), *Moringa oleifera* leaf (MOL), *Manilkara zapota* (MZ) extracts on chondrocyte differentiation of rat bone marrow Mesenchymal stem cells.

Fig iii (b): Effect of various concentrations (125µg/ml-1000µg/ml) of aqueous *Abutilon indicum* (AI), *Pueraria tuberose* (PT), *Boerhavia diffusa* (BD), *Centella asiatica* (CA), *Moringa oleifera* leaf (MOL), *Manilkara zapota* (MZ) extracts on chondrocyte differentiation of rat bone marrow Mesenchymal stem cells. The percent chondrocyte differentiation of various concentrations of extracts was calculated by comparing with control group.

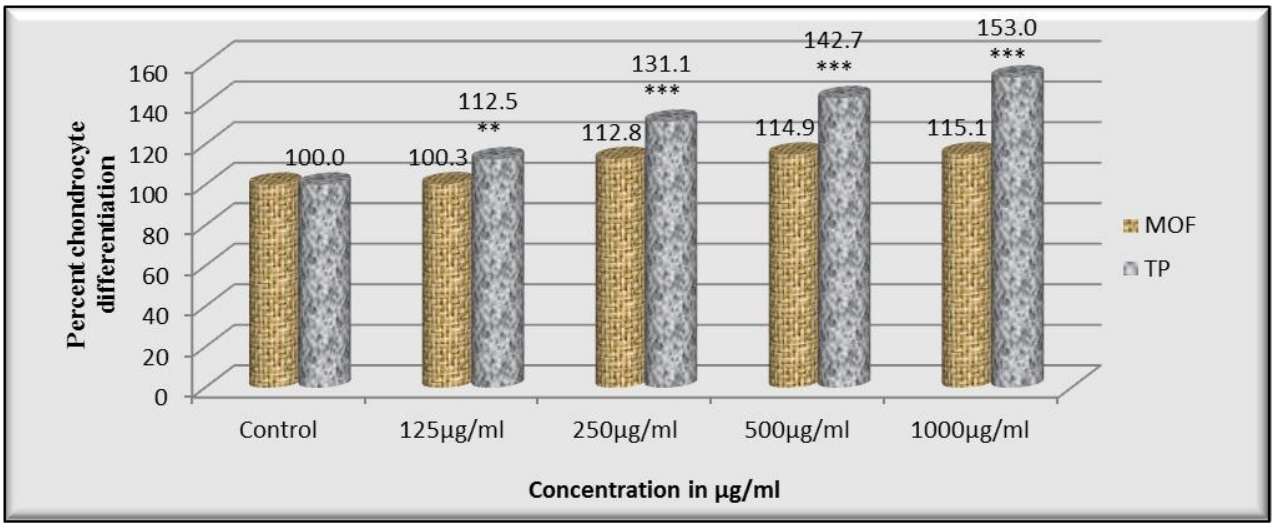
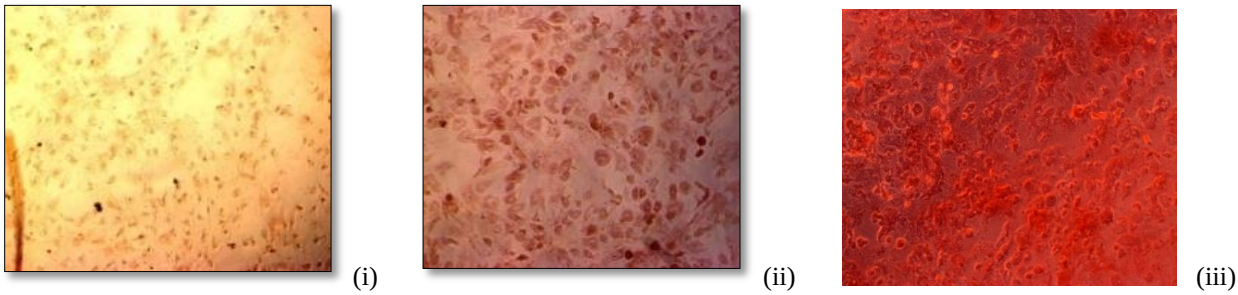


Fig iii (c): Effect of *Moringa oleifera* flower (MOF), *Tridax procumbens* (TP) on chondrocyte differentiation of rat bone marrow mesenchymal stem cells.

Fig iii (c): Effect of various concentrations (125µg/ml-1000µg/ml) of aqueous *Moringa oleifera* flower (MOF), *Tridax procumbens* (TP) extracts on chondrocyte differentiation of rat bone marrow mesenchymal stem cells. The percent chondrocyte differentiation of various concentrations of extracts was calculated by comparing with control group.



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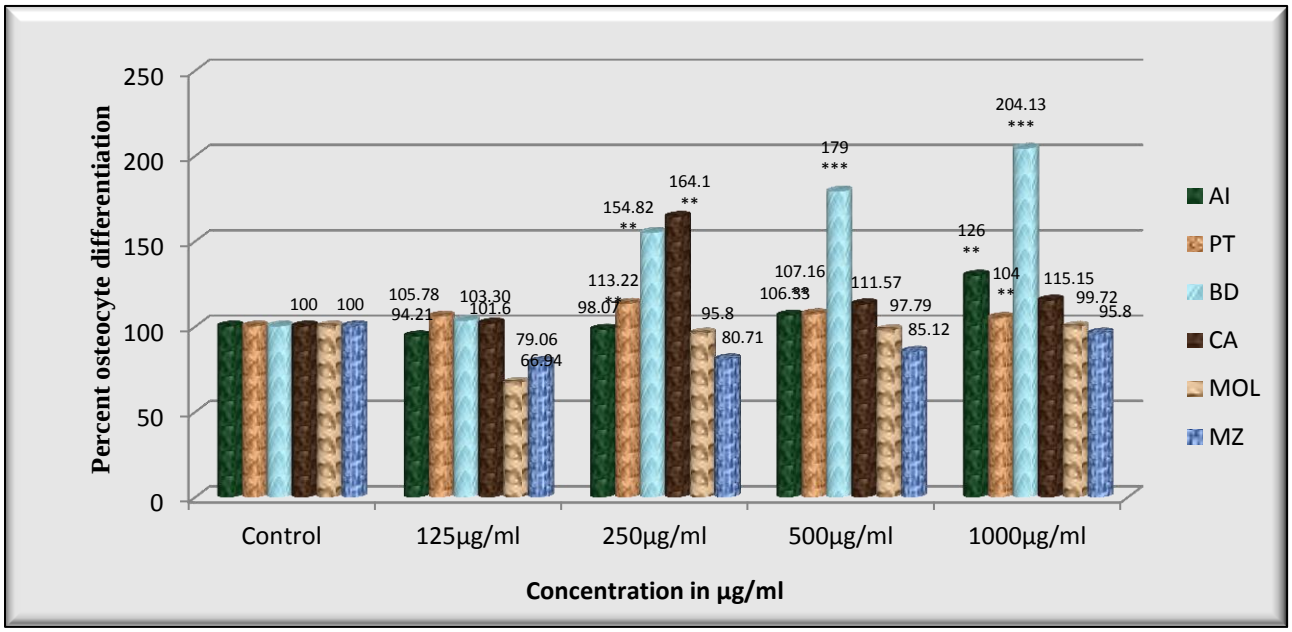

Fig iv (b): Effect of *Abutilon indicum* (AI), *Pueraria tuberose* (PT), *Boerhavia diffusa* (BD), *Centella asiatica* (CA), *Moringa oleifera* leaf (MOL), *Manilkara zapota* (MZ) extracts on osteocyte differentiation of rat bone marrow Mesenchymal stem cells.

Fig iv (b): Effect of various concentrations (125µg/ml-1000µg/ml) of aqueous *Abutilon indicum* (AI), *Pueraria tuberose* (PT), *Boerhavia diffusa* (BD), *Centella asiatica* (CA), *Moringa oleifera* leaf (MOL), *Manilkara zapota* (MZ) extracts on osteocyte differentiation of rat bone marrow Mesenchymal stem cells. The percent osteocyte differentiation of various concentrations of extracts was calculated by comparing with control group.

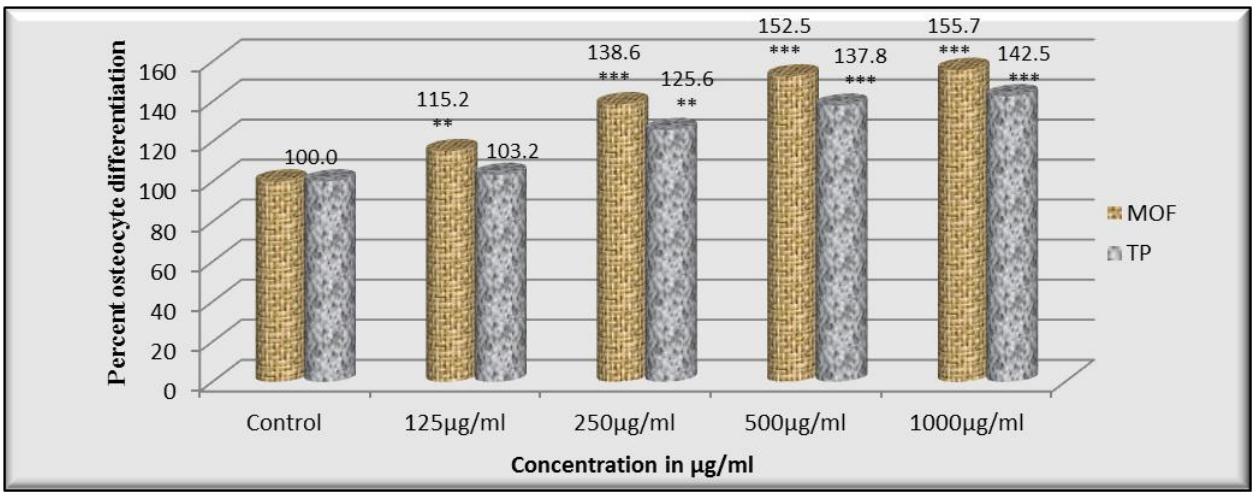

Fig iv (c): Effect of *Moringa oleifera* flower (MOF), *Tridax procumbens* (TP) on osteocyte differentiation of rat bone marrow mesenchymal stem cells.

Fig iv (c): Effect of various concentrations (125µg/ml-1000µg/ml) of aqueous *Moringa oleifera* flower (MOF), *Tridax procumbens* (TP) extracts on osteocyte differentiation of rat bone marrow mesenchymal stem cells. The percent osteocyte differentiation of various concentrations of extracts was calculated by comparing with control group.

Table 1: The plants selected for present study are listed in table 1 given below.

Sr. no	Botanical name	Common name	Part used	Abbreviations used in present study
1	*Pueraria tuberose*	Vidarikand	Root	PT
2	*Abutilon indicum*	Aatibala	Leaves	AI
3	*Boerhavia diffusa*	Punarnava	Leaves	BD
4	*Centella asiatica*	Mandukparni	Leaves	CA
5	*Manilkara zapota*	Chickoo	Leaves	MZ
6	*Moringa oleifera*	Drumstick	Leaves and flower	MOL and MOF
7	*Tridax procumbens*	Dagadi pala	Leaves	TP

Table 1 gives the list of plants and their parts used for present study.

Table 2: Comparison of proliferation and differentiation activities shown by selected plants in present study.

	PT	AI	BD	CA	MZ	MOL	MOF	TP
Increase in cell proliferation	✓		✓	✓				✓
Decrease in adipocyte differentiation	✓	✓	✓	✓			✓	✓
Increase in chondrocyte differentiation	✓			✓	✓			✓
Increase in Osteocyte differentiation			✓	✓			✓	

Table 2 shows the increment in proliferation and /or differentiation shown by study extracts on rat BM MSCs. The plants showing more than 50% increment in proliferation and/or differentiation are ticked. For adipocyte differentiation, the tick denotes decrease in differentiation caused by respective extracts.

DISCUSSION

The plants *Pueraria tuberose*, *Boerhavia diffusa* and *Centella asiatica* are used for medicinal purpose since ancient times in India. They are important constituent of various formulations in Ayurvedic System of Medicine. Most importantly, they belong to a group of drugs called 'Rasayana' which is used for rejuvenation and increasing vigor, vitality and longevity in Ayurvedic Medicinal System. *Moringa oleifera*, *Tridax procumbens* and *Manilkara zapota* are also important constituents of Ayurvedic formulations having more or less similar properties. Pharmacological studies on these plants have revealed profile of bioactive compounds and promising anti-inflammatory, hepatoprotective, neuroprotective, cardioprotective, anti ulcer and wound healing properties. These activities are suggestive of their ability to activate pathways that promote proliferation and restoration of cellular function. Their long established medicinal use is also suggestive of potential to stimulate stem cells of the body. The present study is conducted to evaluated their effect on rat derived mesenchymal stem cells.

BD, PT, CA, TP and MOF determined 19.9%, 13%, 39.2 %, 16.3 % and 15.7% increment in MSC proliferation at 62.5 µg/ml concentration ($P < 0.01$) which increased in dose dependent manner upto almost 90%. AI, PT, CA, BD, TP and MOF are also the plants showing antiadipocyte property. *Boerhavia diffusa* (BD) is considered as a drug that can 'bring back to life' once again [12]. It is employed for treatment of internal inflammation of all kinds, liver, gall bladder and kidney disorders. It is used to gain strength, for treatment of obesity and maintenance of lipid and cholesterol levels. It is also an important medicine in Nepal, Srilanka, Unani, Chinese and Tibetian Medicinal Systems [13]. *Pureria tuberose* (PT) is used as tonic to strengthen body, boost immunity and memory. It revitalizes whole body and increase muscle bulk. It is used for treatment of skin diseases to rejuvenate skin and for healing of chronic wounds [14].

Centella asiatica (CA) is referred as longevity herb and in particular used for strengthening of blood vessels, treat skin diseases, leprosy, psoriasis and eczema. It improves longevity, immunity and intelligence [15]. In present study, CA has shown 79.3% increase in ability to promote MSC proliferation at higher concentration. It has shown 73% and 64 % increase in chondrocyte and osteocyte differentiation. *Abutilon indicum* (AI) is another rasayan drug which is used for healing of ulcers and wounds have determined AI to increase wound contraction rate, skin breaking strength and dry granuloma weight. It showed significant decrease in epithelization period in rats. It has shown significant inhibition in serum cholesterol and triglycerides levels [16]. About 42.2 % decrease in adipocyte differentiation shown by AI in present study supports these findings. About 44 % and 26 % increase in chondrocyte and osteocyte differentiation is explanatory for the role of AI in healing of ulcer, inflammation or wounds.

The leaf extracts of *Manilkara zapota* (MZ) has given 48% increment in cell viability and 100 % in chondrocyte differentiation while not being significant in osteocyte and adipocyte differentiation. High chondrogenic potential of MZ may be a basis for its traditional use for treatment of muscle spams, weakness and pain [17]. *Tridax procumbens* (TP) have presented about 94 % increase in cell viability, 53 % in chondrocyte differentiation. It showed remarkable 57.1% reduction in adipocyte differentiation and 42 % increment in osteocyte differentiation. In addition to the use of TP in number of traditional formulations, is a popular folk medicine for wound healing. It has shown promotion of wound healing in normal as well as immunocompromised rats in dead space wound healing model. It increased lysyl oxidase, protein and nucleic acid content in the granulation tissue [18]. The results of present study suggest TP to be of value for regenerative and pharmacological application.

Moringa oleifera (MO) is highly investigated and utilized plant for its impressive nutritive and medicinal value. It exhibits broad range of pharmacological activities which are attributed to its unique combination of bioactive compounds. One of the promising feature of MO is its tissue

protective activity. Earlier studies have revealed the effect of leaf extract to possess antitumor, heptotoprotective, neuroprotective, cardioprotective, anti-inflammatory and nephroprotective activities [19]. In present study leaf as well as flower extracts were investigated. The aqueous extract of leaf determind significant increment (39.3%) only in differentiation to chondrocytes. The flower extract on the other hand determined significant (47%) effect on cell proliferation and around 55 % increase in osteocyte differentiation. It also determined a prominent 68% reduction in adipocyte differentiation. These results underlines a unique property of MO flowers. The MO flowers are less investigated except that they are known to have anti-inflammatory effect. In our earlier studies, MO flower determind significant cell regenerative ability [20]**.** The flowers today are more used in culinaries than for pharmacological purposes.

Number of similar studies are available in literature, where medicinal herbs have earlier been investigated for proliferative and differntiative potential. *Gingko biloba* [21]. *Morinda citrifolia, Fructus cnidii, Zanthoxylum schinifolium*, Naringen from *Rhizoma drynaria*, Fucoidan polysaccharide from brown seaweed, Icarin, flavanol glycoside of Epimedium, flavanoids of Herba epimeddi, Ferula hermanis of Herba epimedii, salvionolic acid from salvia miltiorrhiza, and resveratrol isolated from various species have shown strong osteogenic ability. Many of them also promoted cell proliferation. However few herbs and its derived bioactive molecules particularly resveratrol, *Herba epimedii, Ligustrum lucidum* exerted cytotoxic effect at high concentration. [22]. The plants BD, PT and MOF of present study showing high osteogenic potential did not show any cytotoxic effect upto highest concentration (1000 µg/ml) tested. They in fact promoted the proliferation of cells suggesting their utilization as stimulatory factors to enhance cell proliferation and differentiation or as vital component of biomaterial in cellular therapy.

To summarize, the present study investigated traditional Indian medicinal herbs for effect on proliferation and differentiation of rat bone marrow derived mesenchymal stem cells. Adult mesenchymal stem cells were isolated from rat bone marrow and cells of third to fifth passage were used. Cells treated with aqueous extracts for 72.00 hrs. were compared for cell viability using MTT dye based cell proliferation assay. The treated cells were incubated with adipocyte, chondrocyte and osteocyte differentiation media to test the ability of test extracts to promote differentiation. The selected plants determined very significant proliferative potential. *Centella asiatica*, *Boerhavia diffusa* and *Pureria tuberose* had highest ability to promote proliferation. These and *Moringa oleifera* flower, *Tridax procumbens* and *Abutilon indicum* demonstrated very promising retardation of adipogenesis. *Manilkara zapota, Pureria tuberose, Centella asiatica, Tridax procumbens* had chondrogenic potential and *Boerhavia diffusa*, *Moringa oleifera* flower, *Tridax procumbens* had maximum osteogenic potential. The study highlight the importance of study plants in regenerative biology and suggest further exploration of mechanism of action for these.

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