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The Effects of *Pleurostylia capensis* Crude Extracts on the Chondrogenic Differentiation of Porcine Adipose-Derived Mesenchymal Stem Cells

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ABSTRACT

It is well known that articular cartilage (AC) lacks the ability to repair itself once damage, thereby making it therapeutic treatment challenging. A number of efforts are been made to induce adult stem cells with growth factors or bioactive molecules for their characterisation and mechanisms involved in their chondrogenic differentiation. This study investigated the effect of *Pleurostylia capensis* (*P. capensis*) bark and root extracts on chondrogenic differentiation of porcine adipose-derived mesenchymal stem cells (pADMSCs). The effect of *P. capensis* bark and root extracts at 5, 15, 30 and 50 µg/mL and TGF-β3 (10 ng/mL) as positive control on cellular growth viability and behaviour of pADMSCs was investigated using MTT and xCELLigence assays. The biosynthesis of glycosaminoglycan (GAG) and the expression of chondrogenic markers *SOX 9*, aggrecan (*AGG*), proteoglycan (*Proteo*), collagen type II (*Col II*) and X (*Col X*) of pADMSCs in pellet culture was investigated *in vitro*. The results showed that *P. capensis* bark extracts at 5 and 50 µg/mL stimulated the proliferation of pADMSCs from 24 to 48 h of incubation with cell viability of about 100%, and the root extracts showed cell viability of about 90% with all treatments at 48 h. The amount of GAG synthesised was high with bark extracts at 5 and 15 µg/mL and with root extracts at 15 and 30 µg/mL over both control and TGF-β3 at 21 days. Bark extracts at 30 µg/mL induced the highest expression of *SOX 9*, *Proteo*, *Col II* and *Col X* significant at $p<0.01$ at 14 days. Whereas, root extracts at 15 µg/mL induced the highest expression of *SOX 9* and *AGG* at 14 days. All the cells treated with *P. capensis* bark and root extracts displayed a strong positive stain for Safranin-O and strongly observed Toluidine blue at day 14. Immunohistostaining revealed little positive staining at matrix for COL-10 from both groups of treatments. Nevertheless, *P. canpensis* bark at 30 µg/mL and root extracts at 15 µg/mL is likely to be a future treatment strategy for chondrogenic differentiation of stem cells, and supports the use of this plants extracts as used in indigenous knowledge.

Keywords: *Pleurostylia capensis*, Glycosaminoglycan, Extracellular matrix, Adipose-derived mesenchymal stem cell

INTRODUCTION

Articular Cartilage (AC) injury and deterioration are predominant in athletes, obese and ageing populations and results from chronic joint stress or acute traumatic injuries [1]. Due to the limited healing capacity of AC caused by vascularity and self-repair for cartilage, it is hard to repair without external treatments after injury. Injury to cartilage is a major risk factor for early development osteoarthritis (OA) [2-7]. OA is a joint disease that is commonly defined as the erosion of joint cartilage but in reality, affects multiple tissues of the joint including the ligaments, bone, synovium and meniscus (if the joint involved is the knee) as more recently redefined by the Osteoarthritis Research Society International (OARSI) [8]. Repetitive loading of AC activities can lead to progressive articular cartilage degradation with an accumulation of catabolic enzymes, cytokines, fragmentation of collagen and aggrecan as well as a progressive breakdown of the articular surface [9].

Many surgical (microfracture, abrasion arthroplasty, osteochondral autologous, autologous chondrocyte implantation (ACI), matrix-induced autologous chondrocyte implantation (MACI) and subchondral drilling), cell transplantation (stem cell or chondrocyte implants) and medical treatments have been described with the common goal of improving joint function and halting disease progression [10-12]. These techniques were designed to repair cartilage with the production of hyaline cartilage formation. Despite increased research on the treatment

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techniques available for articular cartilage defects, there is no agreement as to the best option.

Currently, the research focus has shifted to investigating the use of adult mesenchymal stem cells (MSCs) in tissue engineering and regenerative medicine for cartilage repair, because of their self-renewal capacity and their ability to differentiate along multiple lineages including chondrocytes [13,14]. MSCs are used to repair and replace tissues or organs that are damaged for cell-based therapies [15-18]. In general, stem cell behaviors, such as attachment, proliferation, and differentiation into specific lineages is dependent on a multitude of physical, chemical and environmental factors, including substrate topography, extracellular matrix's (ECM), stem cell growth factor/chemical inducer interactions and stem cell-substrate interactions [19].

This study, aimed to investigate the influence of *Pleurostylia capensis* water extracts (bark and root) as plant-based morphogenetic factors on porcine adipose-derived mesenchymal stem cells (pADMSCs). The cellular behavior, proliferation and differentiation of pADMSCs into chondrogenic lineages was studied using the amount of proteoglycan secreted, the deposition of GAG and expression of chondrogenic markers such as *SOX 9*, aggrecan, proteoglycan, collagen type II and X. It was hypothesised that the treatment of pADMSCs with *P. capensis* crude extracts would stimulate the proliferation rate and chondrogenic differentiation of pADMSCs.

MATERIALS AND METHODS

Plant collection and selection

Pleurostylia capensis bark and root materials were selected based on its ethno pharmacological use in the management of osteoarthritis, mode of preparation and administration by traditional healers, and the absence of published literature describing their effects on pADMSCs differentiation into chondrocytes by gene profiling. Medicinal plants were collected at Limpopo province in the Venda region of South Africa during March 2016 (summer season). The plant materials collected were barks and roots. The materials were sent to the botanist at the University of Venda for identification and voucher specimen number was given (MPT0060).

Preparation of extracts

Collected bark and root materials were washed with water to remove soil and then placed in a shade to dry at room temperature for about two weeks. The dried materials were ground to powder form (mechanical blender, ATO MSE mix) and kept in airtight polyethylene bags until needed for extraction purpose. Tiwari et al. [20], describe the extraction method used with minor modification. About 50 g of powdered plant material was dissolved in 500 mL of distilled water. The mixture was shaken vigorously for 24 h at room temperature. The mixture was filtered after which it was frozen for overnight. The frozen materials were dried under freeze dryer to get the crude extracts. The crude extracts were used for various biological assays.

Isolation and culture of stem cells

The porcine adipose-derived mesenchymal stem cells (pADMSCs) was isolated from the stifle (knee) joint of 3 month old porcine that was obtained within 6 h of slaughter from a local abattoir. Isolation of MSCs from adipose tissue was performed as previously described by Khan et al. [21] with minor modification. The fatty tissue was washed twice with PBS. Subsequently, the tissues were minced and digested with 0.15% m/v type II collagenase (Invitrogen, Carlsbad, CA) at 37°C for 45 min. The suspension was centrifuged at 1500 rpm for 10 min. The supernatant was removed, the pellet was washed twice with alpha-minimal essential medium (α-MEM) containing 10% FBS (v/v) and suspended in 1 ml of α-MEM/10%FBS v/v/5 ng/ml FGF-2. The suspension was filtered through 50 µm nylon-mesh strainer to remove fibrous debris. Cells were grown to confluence (80%). The medium was changed for the first time after three days. The cell monolayer was washed with PBS and was detached with 0.05% v/v trypsin-EDTA. Cells were counted and assigned to different assays at passage zero (P0).

Cell culture for proliferation

Cellular proliferation and behavior were performed by plating 100 µL 2 × 10^4 cells/mL in 96 well plates and a special plate E-plate 16 overnight at 37°C with 5% CO_2. After incubation, the media was aspirated and 100 µL *P. capensis* bark and root extracts at 5, 15, 30, 50 and 100 µg/mL was added to each well with three replicates. The dosage was determined based on our previous study [22]. TGF-β3 was used as a positive control at 10 ng/mL as used in previous study [23]. The plates were incubated for 24, 48 and 72 h and at the end of each incubation time an MTT assay and cellular behavior test using the xCELLigence system was performed and an optimal concentration was selected for further analysis.

Cell culture for chondrogenic differentiation

pADMSCs were cultured in a pellet model and investigated for chondrogenic differentiation. For this, 5 × 10^5 of pADMSCs were centrifuged at 1500 rpm for 5 min to make cell pellets in 1.5 mL sterile conical micro tubes with removable screw-type lids. The pellets were then cultured in 0.5 mL 1% FBS/complete α-MEM and *P. capensis* (bark and root extracts) at 5, 15 and 30 µg/mL and 10 mg/mL TGF-β3 at 37°C in a humidified, 5% CO_2 tissue culture incubator. The medium was changed every three days, and pellets were harvested on day 14 and 21. At the end of each culture stage, the cell pellets were assessed biochemically for their glycosaminoglycan (GAG) matrix and DNA contents. This was done histologically for the GAG matrix

using immunohistological staining for cartilage-specific matrix proteins and by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) for the gene expression analysis.

Viability analysis for pADMSCs

The cellular viability was assessed indirectly, by quantifying the cellular conversion of a tetrazolium salt (MTT) into a formazan product. The behaviour of the cell was investigated in time-dependent cells response impedance using the xCELLigence System (RTCA DP Instrument, ACEA Biosciences, Inc.). The CI value at each time point is defined as the (Rt-Rb)/15 where Rt is the cell-electrode impedance of the well with the cells at different time points, and Rb is the background impedance of the well with the media alone. The normalised cell index was calculated by dividing the cell index value at a particular time point by the cell index value at the time of interest.

Biochemical analysis

After 14 and 21 days of pellet culture, pellets were rinsed with 500 µL DPBS to remove any residual medium and then digested in 250 µL proteinase K (1 mg/mL in Tris EDTA buffer) overnight at 56°C. The GAG content was measured spectrophotometrically, using one, 9-dimethyl methylene blue (DMMB) (Sigma-Aldrich); metachromatic cationic dye, which binds to anionic GAG molecules. The degree of metachromaticity is directly proportional to the amount of GAG present in the reaction mixture. GAG was calculated as µg/mL of chondroitin-4-sulfate (CS) equivalents. The DNA content was determined using the CyQuant cell proliferation assay Kit (Invitrogen, ON, Canada) with the supplied bacteriophage λ DNA as a standard.

Gene expression analysis of pADMSCs

Total RNA was isolated from two-time points (14 and 21 days) using NucleoSpin® RNA kit according to the manufacturer's instructions (MACHEREY-NAGEL, Düren, Germany). RNA (100 ng) was reverse transcribed to cDNA by iScript Reverse transcriptase (Bio-Rad Laboratories, Inc.). Reverse-transcription quantitative polymerase chain reaction was performed in a DNA Engine Opticon I Continuous Fluorescence Detection System (Bio-Rad, CA, USA) using hot start iTaq Universal SYBR Green Super mix (Bio-Rad Laboratories, Inc.). Primers sequences were obtained from Inqaba biotech **(Table 1)**. Expression levels of mRNA relative to the control (untreated) culture were calculated using the threshold cycle (ΔCT).

Table 1. Porcine chondrocytes primer sequences both forward and reverse.

Names	Forward	Reverse
GAPDH	TCC CTG CTT CTA CTG GTG CT	TGA GCT TGA CAA AGT GGT CG
Collagen type II	TGT TCT GAG AGG TCT TCC TGG CAA	CAG GAG CTC CAG CTT CAC CA
Collagen type X	GCC AAC CAG GGA GTA ACA GG	TGG GTC ATA GTG CTG TTG CC
SOX 9	TCC TCC TTG GAA TCA GAA AGC CTG	TTA GTT GAA CAG TGT GCC CAG CTC
Aggrecan	AGC CTG AGG AGC CCT TTA CAT TTG	ACA CTG CTC GTA GCC TGC TTC
Proteoglycan	TGG AGA AAC AAC ACA CAG GTT AGG	GTG AGT CTG ATG GGC GAA TAC

Histology of pad MScs chondrogenesis

After 14 and 21 days of pad MScs being treated with plants extracts in the micro mass pellet, the media was removed from the cells pellet, fixed overnight in 10% formalin, dehydrated and embedded in paraffin wax. Section of 5 µm thickness was cut and stained with 0.01% (w/v) Safranin-O and 1% (w/v) Toluidine blue to reveal the GAG matrix deposition [24].

Immunohistological staining of pad MScs chondrogenesis

The sections were deparaffinised, rehydrated, then pre-treated with proteinase K, endogenous peroxidase activity block with 3% hydrogen peroxide in PBS and incubated with primary antibody anti-collagen type X (COL-10) ab49945 (Abcam) and incubated for 1 h at room temperature. A One-Step Polymer-HRP reagent (Biogenex Super Sensitive One-Step Polymer-HRP Detection System) was applied to the section and it was incubated for 15 min. A 200 µL volume of DAB substrate solution (Biogenex Super Sensitive One-Step Polymer-HRP Detection System) was added to each section. The slides were counterstain with Mayer's hematoxylin and mounted with paramount.

STATISTICAL ANALYSIS

The experiments were performed using three biological replicates (N=9), with a minimum of three technical replicates for each experimental time point. The statistical analysis was performed using Graph-Pad Prism 5; a one-way ANOVA followed by Bonferroni's/Dennett multiple comparison tests. For this ***$p<0.001$, **$p<0.01$, *$p<0.05$ was considered significant.

RESULTS

Cellular proliferation and behavior

Cell viability and cell cytotoxicity were used to evaluate whether *P. capensis* extracts affected the cellular growth of pad MScs cultured *in vitro* and to select optimal concentrations that supported pad MScs growth better at about 80%. Basic toxicology, the LD50 value is defined as the statistically derived dose that, when chemical or substances are administered in a cell of test, is expected to cause death in 50% of the treated cells in a given period [25]. Isolated pad MScs showed typical fibroblastic morphology on days 1, 4 and 7 after treatment with *P. capensis* extracts inoculation and grew in whorl-like and myoblast formation. The viability of pad MScs cells was assessed using MTT assays based on the cellular conversion of tetrazolium salts into Formosan products. The results showed cell viability above 100% at 48 h with bark extracts at 5 and 50 µg/mL, while 15 and 30 µg/mL showed viability at about 80%. At 72 h, the bark extracts showed cell viability of 67% with all treatment **(Figure 1)**. TGF-β3 at 10 ng/mL showed cellular viability at about 90% at 48 h, the proliferation rate was declined by 12% at 72 h. The root extracts at 48 h showed cellular viability of about 90% and at 72 h, the viability was decreased by 18% with all the treatments **(Figure 1)**. These results suggest that the plant materials were not toxic to the cells as all treatments possessed inhibition concentration (IC) of about 33%. Cellular behavior of pad MScs after treatment with different concentrations of *P. capensis* bark and root extracts was monitored with the xCELLigence system measuring CI at a given time **(Figure 2)**. The CI was proportional to the number of adherent cells remaining on the E-plate at 24 h. As shown in **Figure 2**, TGF-β3 showed an increase in CI until 120 h compared to the untreated cells. The bark extracts at 5, 15 and 30 µg/mL showed increased in CI until 72 h, these results correspond with MTT results shown in **Figure 1**. Root extracts showed a trend increased in CI until 120 h with 5, 15, 30 and 50 µg/mL. This result indicates that the cells were proliferating, as the graph does not show CI below zero as indication of toxicity **(Figure 2)**. There was a reduction in CI at 50 and 100µg/mL of bark extracts and 100 µg/mL of root extracts.

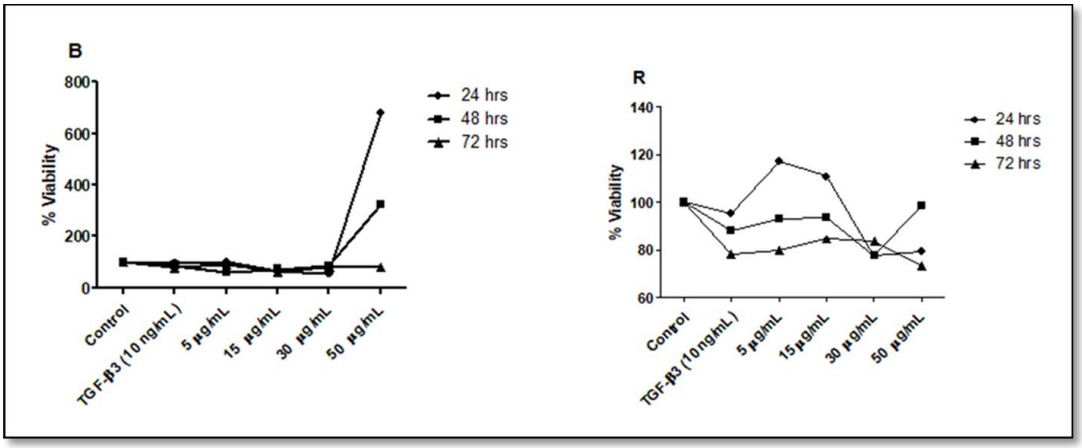

Figure 1. Cell proliferation of pad MScs treated with *P. capensis* (bark and root) extracts at different concentrations and TGF-β3 as positive control (n=6) for 24, 48 and 72 h. Data all presented as mean ± standard deviation.

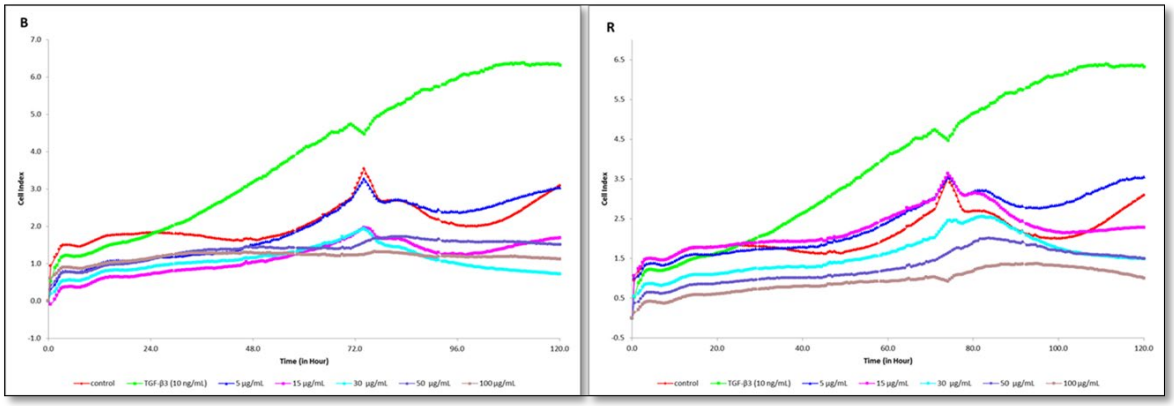

Figure 2. Real-time monitoring of cell proliferation of pad MScs cells treated with *P. capensis* bark and root extracts at different concentrations (5, 15, 30 and 50 mg/mL) and TGF-β3 (10 ng/mL) as positive control were seeded in the E-plate and cell index was continuously monitored for 120 h.

Biochemical analysis

Biochemical analysis was performed to assess the synthesis of glycosaminoglycan chondroitin sulphate (CS), DNA content and GAG/DNA ratio at 14 and 21 days of pad MScs chondrogenic differentiation. *P. capensis* bark at 5 and 15 µg/mL treatment showed a higher synthesis of GAG matrix when compared to control **(Figure 3)**. We have observed a decrease in GAG matrix synthesis by *P. capensis* root at 5 µg/mL at day 21 of treatment. However, root extracts at 15 and 30 µg/mL showed a higher synthesis of GAG matrix when compared to both control and TGF-β3, which is a positive control as shown in **Figure 4**. DNA content was lower than control in all the treatment groups at 14 and 21 days. GAG/DNA (µg/ng) ratio shows a slight increase of above 0.5 at 21 days with all treatments as shown in **Figure 3**. *Pleurostylia capensis* root extract at 5 µg/mL showed a decrease in DNA content from day 14 to 21 **(Figure 4)**. There was a slight decrease in the synthesis of GAG by *P. capensis* root extract from 14 to 21 days.

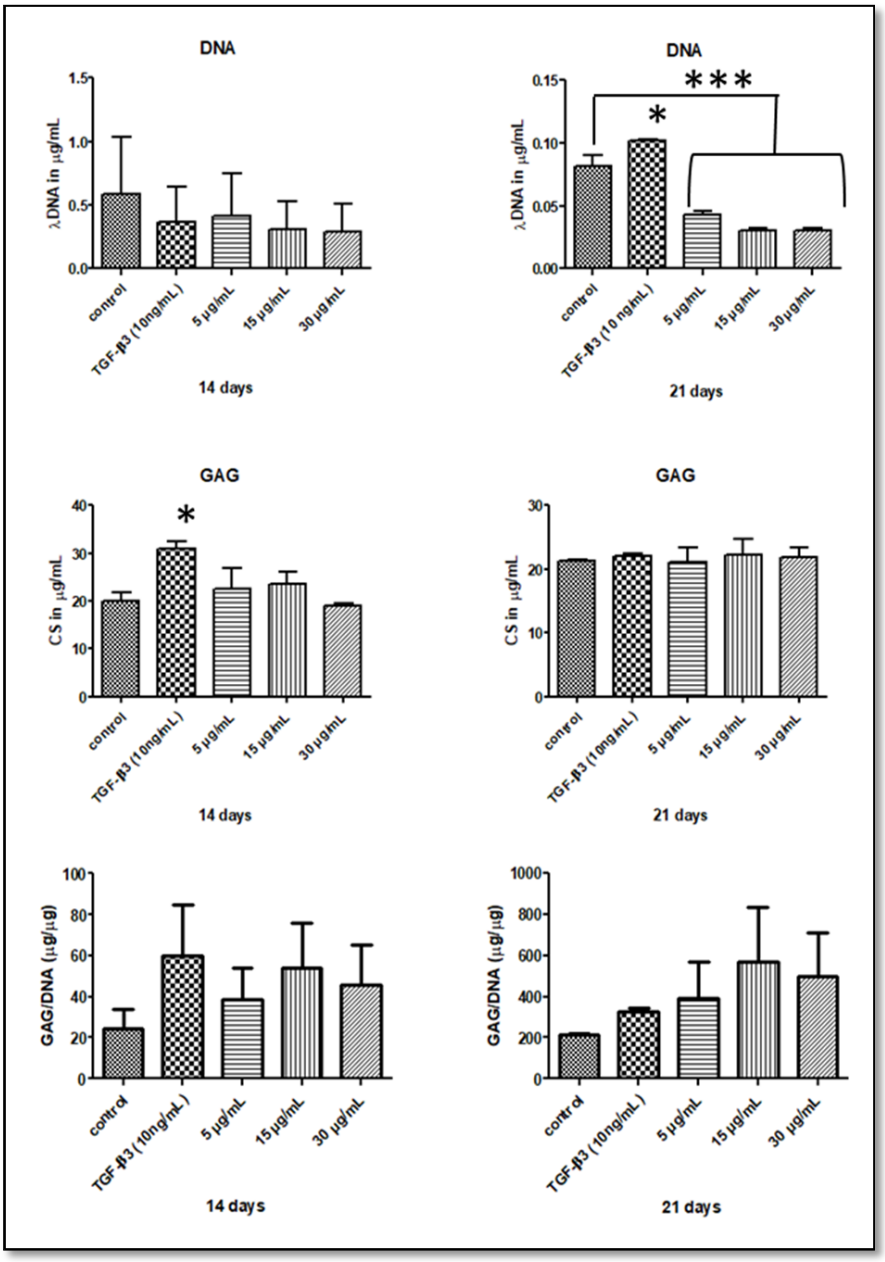

Figure 3. Biochemical analysis of pad MScs for chondrogenesis after stimulation with bark extracts (n=9) at day 14 and 21. DNA, GAG and GAG content was normalized to DNA content and expressed as µg/µg. Values represent mean ± SEM with ***p<0.0001 and *p<0.05 compared to control.

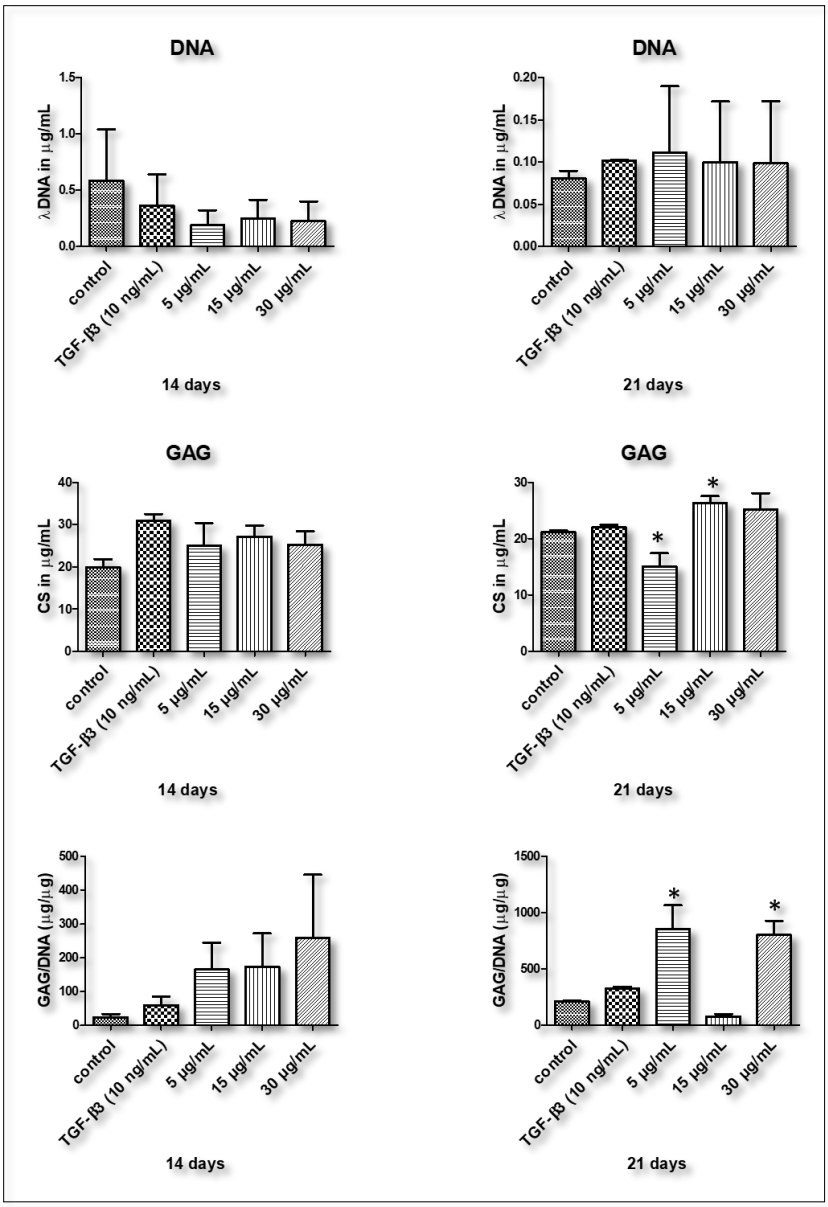

Figure 4. Biochemical analysis of pad MScs for chondrogenesis after stimulation with root extracts (n=9) at day 14 and 21. DNA, GAG and GAG content was normalized to DNA content and expressed as µg/µg. Values represent mean ± SEM with *p<0.05 compared to control.

Gene expression analysis

To characterise the ECM generated in pellets, the expression of chondrogenic markers was assessed by qRT-PCR on day 14 and 21. The expression of SOX 9, Proteo, *Col II* and *Col X* was significantly up-regulated in 30 µg/mL bark extract compared to the negative control and TGF-β3 at day 14 (**Figure 5**, p<0.01), while the expression of these genes at day 21 was significantly down regulated. However, mRNA expression of *SOX 9* and *Col X* was up regulated by a five-fold increase in 30 µg/mL root extracts at day 14 (**Figure 6**, p<0.001). Root extracts at 15 µg/mL showed an increase in the expression of AGG, Preteo and *Col II* at day 14 of the pellet culture compared to control **(Figure 6)**. The root extracts at 30 µg/mL significantly up-regulated *Col X* and SOX 9 at day 14. TGF-β3 showed up-regulation of AGG at day 14 and *Col II* at both day 14 and 21. AGG was not expressed by bark at 5, 15 and 30 µg/mL and roots extracts at 5 and 30 µg/mL on day 14. At 21 days, TGF-β3 and root extracts at 5 µg/mL showed no expression of AGG marker. The relative gene expression of *Col X* remained stable with no significant change with bark and root at 30 µg/mL until 21 days. There was an up-regulation of proteoglycan by day 21 with a one-fold increase for 5 µg/mL bark and root extract at 14 days.

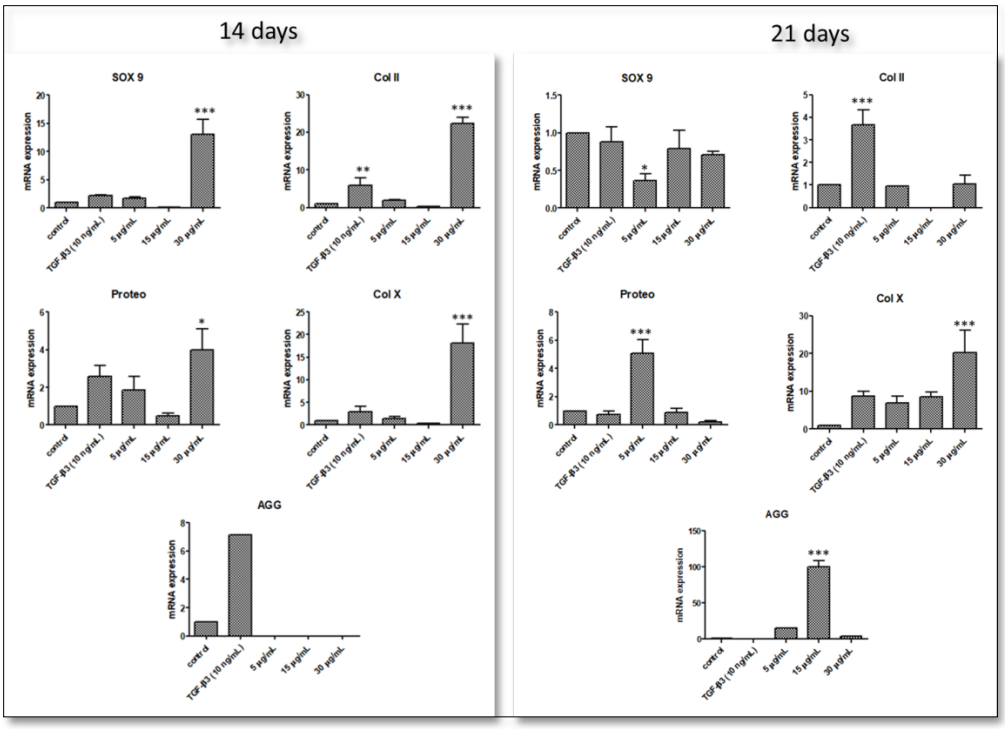

Figure 1. Real-time PCR analysis of cDNA of pad MScs treated with *P. capensis* bark extracts at day 14 and 21 (n=6). Superscript letters represent significant difference between treatments groups and untreated cells (* indicates means that had a $p<0.05$, ** indicates means that $p<0.01$ and *** indicates means that $p<0.001$in comparison to the control). All data presented as mean ± standard deviation.

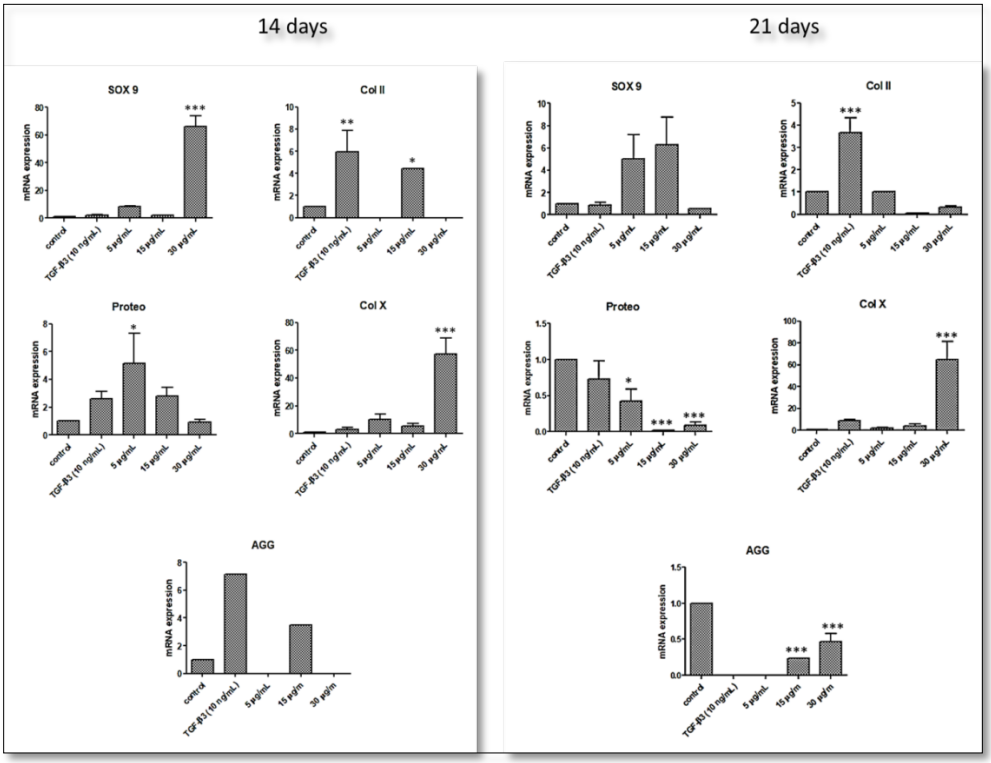

Figure 2. Real-time PCR for analysing the genes expression of *P. capensis* root extracts cultured for 14 and 21 days. Data presented as mean ± SD, with significant differences of * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ with respect to the control.

Histology and immunohistochemistry analysis

After two and three weeks of pellet culture in chondrogenic medium supplemented with *P. capensis* bark and root extracts at 15 and 30 µg/mL, pellets were embedded, cut and stained with Safranin-O and Toluidine blue for GAG deposition (pink/red and blue staining). Positive staining was identified in all treatment groups with higher intensity on day 14 **(Figure 7)**. Toluidine blue stain showed strong staining in 15, 30 bark extract and 15 µg/mL root extract. Weaker blue staining was observed in cells cultured in 30 µg/mL root extract by 14 days, compared to the control groups. At 15 µg/mL bark extract, Safranin-O positive proteoglycan was more intense. Staining for Collagen Type X as a marker for chondrocytes hypertrophy is illustrated in **Figure 8**. Pellet from both groups of cells showed little positive staining at matrix of COL-10 on day 14. As shown in **Figure 6**, bark extracts at 30 µg/mL showed no staining of COL-10 and pellet from root extracts at 30 µg/mL showed per cellular staining at day 21, these were caused by pellet size that were very small.

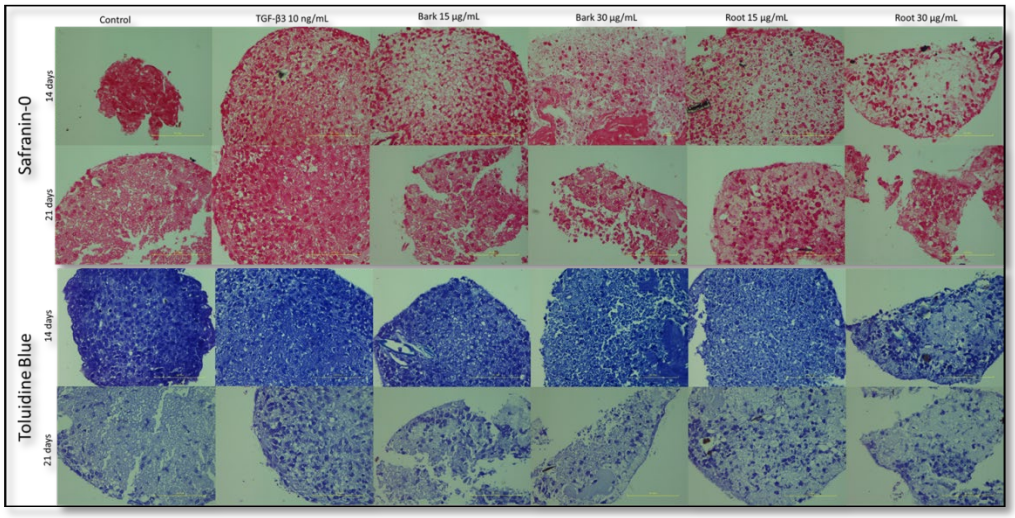

Figure 3. Histology of pad MScs pellet cells. safranin-O and toluidine blue staining after chondrogenic induction with *P. capensis* extracts at day 14 and 21. Magnification (40x) and scale bar=100 µm.

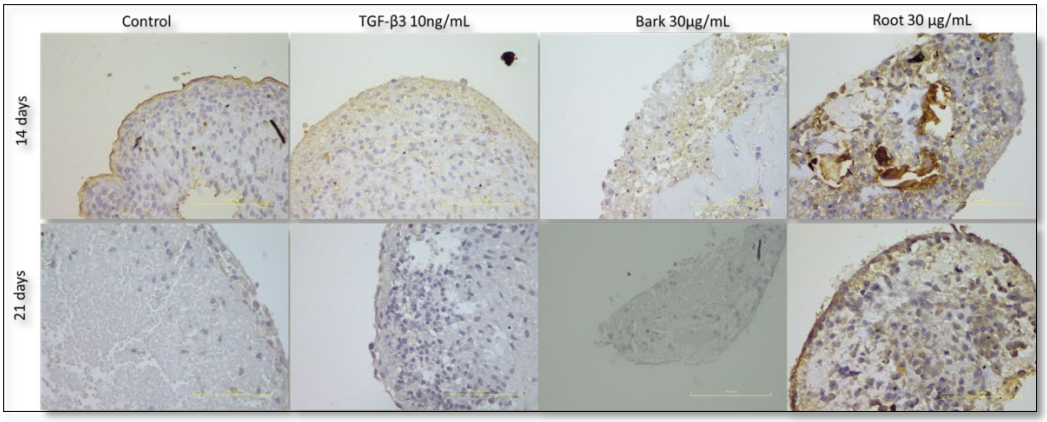

Figure 8. Immunohistostaining with anti-collagen type X (COL-10) (ab49945) in pad MScs treated with *P. capensis* extract (30 µg/mL) on days 14 and 21 and magnification (40x) and scale bar=100 µM.

DISCUSSION

In this study, the effect of plant-based morphogenetic protein stimulators of *P. capensis* extracts (bark and root) on chondrogenic differentiation of pad MScs after treatment for 21 days was investigated. This work forms part of a broad research project aimed at finding alternative plant-based induction of stem cells for AC repair. AC is a tissue that is avascular, a neural and a lymphatic, with limited ability to regenerate once damaged, because of a lack of blood supply to facilitate cells and factors that promote healing [26-29]. It was found that *P. capensis* root extracts were good inducers of stem cell proliferation, with about 90% of cell viability **(Figure 1)** at 48 h of treatments. Similar observation was noted with TGF-β3 at 10 ng/mL used as positive control. Whereas, bark extract at 5 and 50 µg/ml showed cell viability of about 100% and 15 and 30 µg/ml with about 80% at 48 h. These results suggest the possibility of using

medicinal plants as a source of inducers for stem cell proliferation. The cellular behavior of pad MScs induced with *P. capensis* bark and root extracts was monitored using the xCELLigence system based on the number of cell adherent to the embedded electrode E-plate as CI value. *P. capensis* bark and root extracts were found to be nontoxic to the cells, with a Cell index value above one. The results observed with the MTT assay corresponded with those of the xCELLigence system, with root extracts being the most effective compared to bark extracts. The results indicated the biological status of the cells, including their cellular behavior in response to treatments.

Cellular nucleic acid content is a reasonable indicator of cell number since the levels of DNA and RNA in cells are highly regulated. Although the DNA levels of individual cells change over time, the net nucleic acid content per cell in a non-synchronous culture typically remains relatively constant [30,31]. The metabolic activity may, however, be changed by different conditions or chemical treatments which can cause considerable variation in the results from these assays [32]. The GAG results showed root extracts at 15 and 30 µg/mL resulted in a significant 76% increase in GAG production over control and TGF-β3 at 10 ng/mL **(Figures 3 and 4)**. However, bark extracts showed 30% GAG synthesis increased at 5, 15 and 30 µg/mL compared to control cells. TGF-β3 results were similar to the one from Hoben et al. [33] with 60% increase in GAG synthesis compared to control. The results showed change in the physiological levels of the cartilage GAG which is thought to be important to chondrogenesis and to normal skeleton formation [34].

The expression of cartilage-specific genes, including SOX 9, Col II, Col X, AGG and Proteo was analysed for chondrogenic differentiation in pad MScs treated with TGF-β3 and *P. capensis* bark and root extracts. The results showed high expression of SOX 9, Col II, Col X and Proteo by bark 30 at 14 days of treatment over control and TGF-β3. However, root extracts showed high expression of SOX 9, Proteo, *Col X* at 14 days. At day 21, *P. capensis* bark and root extracts showed decrease in the expression of Col II marker compared to TGF-β3, while bark at 30 µg/mL expressed Col II over root extracts at all concentrations. Hyaline cartilage contains at least three tissue-specific collagens, types II, IX and XI, with collagen types II representing 95% of ECM and forming fibril interconnected with proteoglycan aggregate [35-38]. The results showed *SOX 9* a transcription factor expressed more by root extracts at 5 and 15 µg/mL compared to both control and TGF-β3 at 21 days **(Figure 6)**. SOX 9 is expressed in the developed cartilage from the skeletogenic progenitor stage and remains expressed in the chondrocytes until hypertrophy throughout adult hood in AC [39-41]. The deposition of GAG matrix was more intense at day 14 when staining with Safranin-O AND toluidine blue. The intensity of the stain change at day 21 with all the treatments. The localization of COL-10 was done to determine if the differentiation undergoes hypertrophic. It was interesting to note that *Col X*, the key factor associated with hypertrophy expression, remained the same at 30 µg/mL on days 14 and 21 **(Figures 5 and 6)**. Correspondingly, immunohistostaining of 30 µg/mL root extract revealed strong positive staining of COL-10 **(Figures 7 and 8)**.

CONCLUSION

To the best of the researchers understanding, this study introduced the proliferative and chondrogenic effect of *P. capensis* bark and root crude extracts in adipose tissue-derived MScs. In conclusion, root extracts were more potent than bark extracts as a potential source of bioactivity for chondrogenesis of pad MScs. The root extracts showed virtuous cellular behavior, proliferation and differentiation of pad MScs into chondrogenic lineages. The root extracts demonstrated increased in GAG production with minor different from bark extracts. Furthermore, chondrogenic differentiation of pad MScs with *P. capensis* root extracts at 15 and 30 µg/mL upregulated AGG, SOX 9 and Col X the markers for chondrogenesis. *P. capensis* root at 30 µg/mL showed localization of hypertrophic marker COL-1O. These findings suggest that root at 30 µg/ml has been probable mineralization zones of hyaline cartilage with formation of bone like structure. *P. capensis* bark extract at 30 µg/mL showed expression of Col II, *Proteo* and SOX 9 at an early stage of chondrogenic differentiation of pad MScs. This study extends the knowledge of how pad MScs respond to *P. capensis* bark, root crude extracts during chondrogenic differentiation and support the use of this plant in indigenous settings for the treatment of diseases. Further studies need to be carried out to extend on the understanding of mechanism in which this plant use and which compounds are responsible for bone and chondrogenic differentiation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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