Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2025.277

RESEARCH ARTICLE

Mechanistic Insights into the Obesity-Alleviating Properties of Korean Red Ginseng in Canine Adipocyte Cultures Derived from Mesenchymal Stem Cells

Evelyn Saba[1], Usman Rashid[2], Arfan Yousaf[2#], Aayesha Riaz[3], Adeel Sarfraz[4], Waleed Ahsan Khan Tareen[1], Abdul Wahab Akram[5], Yuan Yee Lee[5], Muhammad Moaeen-ud-din[6], Ghazala Naheed[7], Man Hee Rhee[5*] and Mansur Abdullah Sandhu[1*]

[1]Department of Veterinary Biomedical Sciences, Faculty of Veterinary and Animal Sciences, PMAS-Arid Agriculture University, 46300, Rawalpindi, Pakistan. [#]Research Fellow, Shinawatra University, Pathum Thani 12160, Thailand; [2]Department of Clinical Studies, Faculty of Veterinary and Animal Sciences, PMAS-Arid Agriculture University, 46300, Rawalpindi, Pakistan; [3]Department of Microbiology and Parasitology, Faculty of Veterinary and Animal Sciences, PMAS-Arid Agriculture University, 46300, Rawalpindi, Pakistan; [4]Department of Anatomy and Histology, Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, 63100. Bahawalpur, Pakistan; [5]Laboratory of Physiology and Cell Signaling, College of Veterinary Medicine, Kyungpook National University, Daegu 41566, Korea; [6]Department of Animal Breeding and Genetics, Faculty of Veterinary and Animal Sciences, PMAS-Arid Agriculture University, 46300, Rawalpindi, Pakistan; [7]Senior Veterinary Officer, Poultry Research Institute, 46300, Rawalpindi, Pakistan.
*Corresponding author: rheemh@knu.ac.kr (MHR); mansoorsandhu@uaar.edu.pk

ARTICLE HISTORY (25-531)

Received: June 06, 2025
Revised: August 04, 2025
Accepted: August 20, 2025
Published online: October 16, 2025

Key words:
Adipogenesis
cMSC
FABP4
Korean Red Ginseng
PPARγ

ABSTRACT

Obesity is a progressive condition that increases the risk of life-threatening diseases. Herbal medications can provide persistent and long-lasting therapeutic effects in combating obesity without any side effects. Adipose-derived canine mesenchymal stem cells (AD-cMSCs) were harvested from infrapatellar fat pad (IPFP) and differentiated into adipogenic and osteogenic lineages, confirmed by Oil Red O and Alizarin Red S staining. The adipogenesis of cMSCs was performed for 7 and 14 days in three treatment groups with variable doses of KRG administered in pre-induction, induction, and post-induction stages of adipogenesis. MTT assay and cell doubling time were performed on days 3, 6, 9, and 12. FACS was performed to analyze the expression of stem cell and adipogenic markers (CD90, CD105, and FABP4), while q-PCR evaluated adipogenic gene expressions (*PPARγ* and *FABP4*). G1 and G3 showed the most significant response to KRG treatment at day 14 by effectively inhibiting adipogenesis at significant levels of $P<0.001$ and $P<0.05$. As the dose of KRG increased, converted adipocytes exhibited maximal apoptosis and significantly reduced cell viability ($P<0.001$). *PPARγ* and *FABP4* were substantially downregulated at both day 7 and day 14 in response to elevated KRG doses. Collectively, these findings highlight the potent anti-adipogenic effect of KRG on canine adipose-derived mesenchymal stem cells, supporting its potential as an effective therapeutic agent for obesity management. According to our results, the minimum dose of KRG (<5 µg/mL) may be used as an effective treatment for patients with obesity.

To Cite This Article: Saba E, Rashid U, Yousaf A, Riaz A, Sarfraz A, Tareen WAK, Akram AW, Lee YY, din MM, Naheed G, Rhee MH and Sandhu MA 2025. Mechanistic insights into the obesity-alleviating properties of korean red ginseng in canine adipocyte cultures derived from mesenchymal stem cells. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2025.277

INTRODUCTION

Obesity is a globally recognized problem since 5 decades, resulting from imbalanced calorie intake and spending. It is a root cause of hypertension, diabetes, and cardiovascular disease, making it a leading cause of death (Lin *et al*., 2021). Herbal treatments are popular among people to manage obesity (Chandrasekaran *et al.,* 2012). Adipogenesis is a process by which pre-adipocytes transform into mature adipocytes, involving the activation of key genes such as CCAAT/enhancer-binding protein alpha (C/EBPα), Peroxisome Proliferator-Activated Receptor gamma (PPARγ), and Fatty Acid-Binding Protein 4 (FABP4) (Moseti *et al*., 2016). Numerous pathways, for example,

Mitogen-activated protein kinase (MAPK), Phosphatidylinositol-3-kinase/Protein Kinase B (PI3K/AKT), and Janus Kinases/Signal transducers and activators of transcription (JAK/STAT), are crucial in the process of adipogenesis and induction of obesity (Wen *et al*., 2022). The Extracellular Regulating Kinase (ERK) subunit of MAPK pathway is critical for early adipogenesis (Bost *et al*., 2005). Additional MAPK subunits (JNK and p38) augment adipogenesis by enhancing C/EBPα and PPARγ gene expression (Ambele *et al*., 2020). The glucose uptake and glycogen synthesis are activated by the PI3K/AKT pathway, hence assisting adipogenesis (Hemmings *et al*., 2012).

Fatty acids (FAs) are basic units of lipids that are broken down during digestion and absorbed through the intestine. This increases the circulatory levels of Free Fatty Acids (FFAs), contributing fat accumulation in various organs, leading to insulin resistance, and countless health concerns (Boden, 2008). Fatty Acid Binding Protein 4 (FABP4) expression among adipose cells facilitates FA transport, regulates glucose and lipid metabolism to peroxisome proliferator-activated receptor-γ (*PPARγ*) and hormone-sensitive lipase (HSL) (Prentice *et al*., 2019). Amplified FABP4 levels are linked with obesity-related endocrine disruption and cardiovascular problems (Hotamisligil *et al*., 2015). Obesity is associated with increased expression of *PPARγ,* which stimulates adipogenesis and lipogenesis in adipose tissue (Kersten, 2002). Therefore, *PPARγ* and *FABP4* targeting therapy may help reduce obesity (Floresta *et al*., 2022). Mesenchymal stem cells (MSCs) are important in regenerative therapies because they can divide and develop into different cell types (Friedenstein *et al*., 1976; Ramalho-Santos *et al*., 2007). MSCs are non-hematopoietic, multipotent, and have unique superficial markers (Dominici *et al*., 2006). These are derived from many sources such as adipose deposits, bone marrow, amniotic fluid, and umbilical cord (Rashid *et al*., 2021; Sarfraz *et al*., 2021; Saba *et al*., 2024).

Ginseng, specifically Panax ginseng, is a popular herbal remedy in East Asia with a variety of health benefits for diabetes, inflammation, cancer, and reproductive disorders (Oh *et al*., 2019; Ham *et al*., 2019). Korean Red Ginseng (KRG) is made by steaming fresh ginseng roots to increase their medicinal potency and is used to treat inflammation and cardiovascular disease (Qi *et al*., 2011; Baek *et al*., 2012). Despite its potential, there is a paucity of studies examining the effects of KRG on mesenchymal stem cells.

Recently, the occurrence of obesity in dogs' ranges from 25% to 44% in developed nations. It is the third most common condition following periodontal disease and otitis externa. Canine obesity has a profound effect on their health, diminishing their quality of life and increasing susceptibility to cardiovascular issues, arthritis, respiratory complications, and diabetes (Suarez *et al*., 2022). Although the onset of clinical obesity in dogs is influenced by the lifestyle and dietary practices of their owners, improvements can be made by incorporating herbal alternatives into their regular diet. Evidence suggests that notoginseng saponins and selective herbal treatment promote the osteogenic differentiation of rat bone marrow (BM-MSCs) and human osteoblasts (Li *et al*., 2011; Yin *et al*., 2007); however, few studies have investigated the role of selective herbal treatments in MSC differentiation (Wang *et al*., 2016). However, we could not find any studies related to the effects of KRG on adipogenesis and osteogenicity of canine MSCs. This investigation aims to explore the impact of KRG on the differentiation process of canine adipose-derived mesenchymal stem cells (AD-MSCs), specifically focusing on the expression of key adipogenic genes such as *FABP4* and *PPARγ*

MATERIALS AND METHODS

Tissue collection: This study employed canine mesenchymal stem cells (cMSC) that had been previously removed from the infrapatellar fat pad and stored at -80ºC and used in a previous study (Rashid *et al*., 2021). All procedures and protocols for cell management and disposal adhered to the guidelines set forth by the Institutional Ethics Committee guidelines of PMAS Arid Agriculture University, Rawalpindi (PMAS-AAUR/IEC/665).

Sample preparation: Korean Red Ginseng Extract was generously provided the Korean Society of Ginseng with batch number H2006 (2)-1153. The certificate of analysis showed that the concentration of ginsenosides (Rg1+Rb1+Rg3) in the extract was not less than 5.50 mg/g. A full certificate of analysis is available upon request. Even more than that, High-Performance Liquid Chromatography (HPLC) was conducted in accordance with our earlier research (Lee *et al*., 2019) to evaluate the extract's composition, as illustrated in Fig. 1.

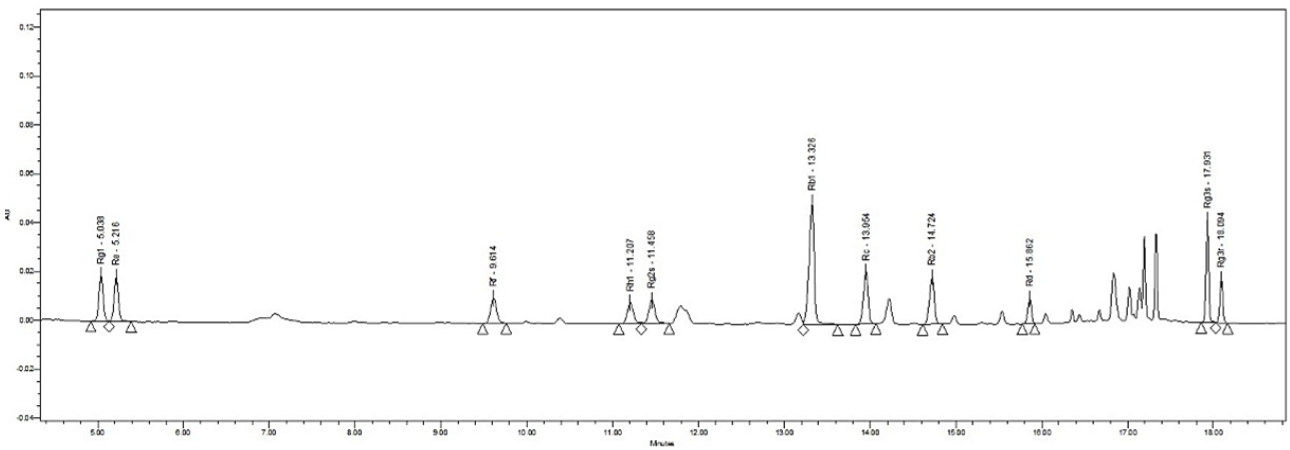

Fig. 1: HPLC chromatogram of Korean Red Ginseng extract.

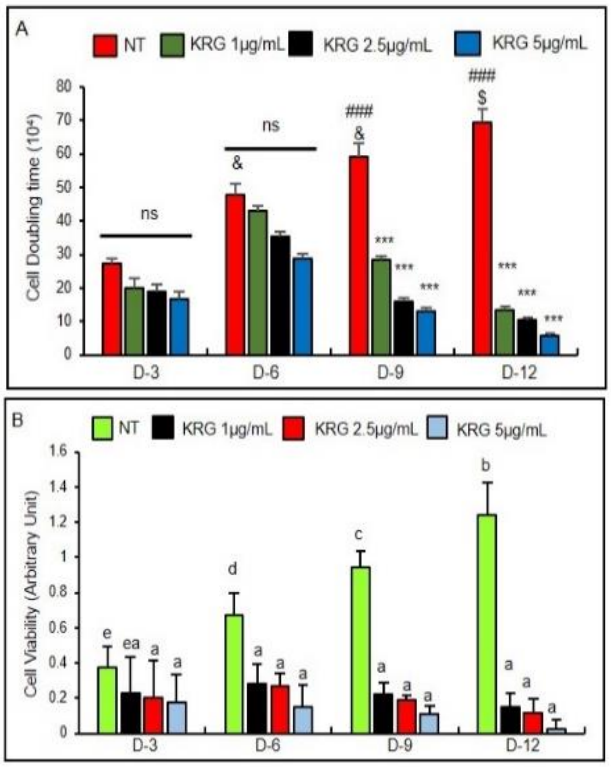

Fig. 2: Effects of KRG on cell number/doubling and cell viability. (A-B) Cell doubling time and viability on days 3, 6, 9, and 12 with and without KRG (1–5 µg/mL). Values in the bar graph are presented as the mean ± least square mean of at least three (n=3) independent experiments for each treatment group.

Cell separation and culture technique: In summation, the adipose tissue underwent a thorough washing with DPBS$^{-/-}$ (BioWest, Nuaillé, France) containing 5% penicillin-streptomycin (P-S) (Caisson, Smithfield, UT, USA) and Amphotericin-B (A-B) (Caisson, Smithfield, UT, USA), followed by mincing and enzymatic digestion in LG-DMEM (BioWest, Nuaillé, France) with Collagenase Type-I (Solarbio, BJ, China) (0.1 mg/mL) at 37ºC for 135 minutes. The addition of an equal volume of LG-DMEM with 10% FBS (BioWest, Nuaillé, France) effectively halted the enzymatic reaction. Following this, the mixture underwent filtration through a 100 µm cell strainer and was centrifuged at 548g for 10 minutes. The cell pellet obtained was resuspended in complete LG-DMEM, which included 10% FBS, 1% P-S, and A-B, and subsequently transferred to T-25 tissue culture flasks. The cells were then incubated in a humidified chamber at 37ºC with 5% CO_2, with fresh media being replenished every 48 hours until the cells reached confluence (80% to 90%). Subculturing was performed by detaching the cells using a trypsin-EDTA solution (Caisson, Smithfield, UT, USA) (0.05% and 0.53 mM w/v, respectively) for 10 min. Next, the trypsin activity was neutralized by adding 10% FBS, and the isolated cells were subcultured until passage number two (P-2). The cells were categorized into distinct groups based on the dosage of KRG. Group I included concentrations of 1µg/mL, 2.5µg/mL, and 5µg/mL during all three phases of adipogenesis, namely pre-induction, induction, and post-induction maintenance media. Group II consisted of KRG at 1µg/mL, 2.5µg/mL, and 5µg/mL, applied solely during the adipogenic induction phase. Lastly, Group III was defined by the application of KRG at 1µg/mL, 2.5µg/mL, and 5µg/mL exclusively during the adipogenic post-induction maintenance phase.

cMSCs doubling time: In Passage 3, the doubling time of cells was assessed on days 3, 6, 9, and 12. Specifically, 5000 cells/well were inoculated in a 48-well culture plate, both with and without KRG at concentrations ranging from 1-5 µg/mL. On the designated day, the culture media was removed after performing two washes with DPBS-/-. After the cells were trypsinized, they were collected and resuspended in 1 mL of complete medium, with cell counts performed through a modified Neubauer chamber.

cMSCs MTT assay: The metabolic activity of cMSCs was evaluated through the application of MTT dye on days 3, 6, 9, and 12. A total of 5000 cells per well were cultured in 48-well plates, with or without the addition of KRG (1–5 µg/mL) in complete LG-DMEM. Following the application of 0.25 µg/mL MTT and a 3-hour incubation period, the supernatant was discarded, and the formazan crystals were dissolved in 100 µL of DMSO. The optical density at 630 nm was recorded using a microplate reader (BioTek 800TS, USA).

Immunophenotyping of MSCs: At passage 3, cMSCs were characterized by flow cytometric analysis with and without KRG (1–5 µg/mL) as described elsewhere (Rashid *et al*., 2021). After trypsinization, 5×10^5 cells were resuspended in DPBS$^{-/-}$ and rabbit raised antibodies against CD90, CD105, and FABP4 (1:100) (Elab Science, TX, USA) were incubated at 37°C for 15 minutes. Subsequently, the cells were washed with DPBS$^{-/-}$ and co-incubated with secondary antibody conjugated with Alexa Fluor-488 (1:300) in DPBS$^{-/-}$ at room temperature for 15 minutes, in separate FACS tubes designated for each antigen. After-wards cells were washed again with DPBS$^{-/-}$, and flow cytometry was conducted using a FACScan from BD Biosciences, CA, USA.

Adipogenic and osteogenic differentiation: At P-3, IPFP derived cMSCs at a concentration of 2.5×10^4 cells/well were plated in a 24-well plate to reach confluence and underwent differentiation into adipocytes with adipogenic induction medium (LG-DMEM, 10% FBS, 10 µM rosiglitazone, 0.1 mM IBMX, 0.3 mM dexamethasone, 1% P-S and 5 µg/mL insulin, with various KRG concentrations from 1-5 µg/mL). After a 48-h induction period, the medium was transitioned to an adipogenic maintenance medium (LG-DMEM, 1% Excyte, 1% P-S, and 5 µg/mL insulin, also with and without KRG) for durations of 7 and 14 days, with medium changes occurring every 2 days. After washing the cells with DPBS$^{-/-}$ and fixing them in 4% buffered formalin for 30 min, they were rinsed again with DPBS$^{-/-}$. The application of ORO dye followed, with the cells being incubated in darkness for 30 min. After the rinsing process, the ORO stain was eluted with anhydrous isopropanol, and the absorbance was assessed at 490 nm.
To induce osteogenic differentiation, 2.5×10^4 cells were seeded in each well of a 24-well plate. Following the attainment of 90% confluence, the cells underwent treatment with osteogenic medium, which consisted of αMEM supplemented with 0.75 nM Vitamin D3, 50 µM ascorbate-2-phosphate, 10 mM ß-glycerophosphate, 100

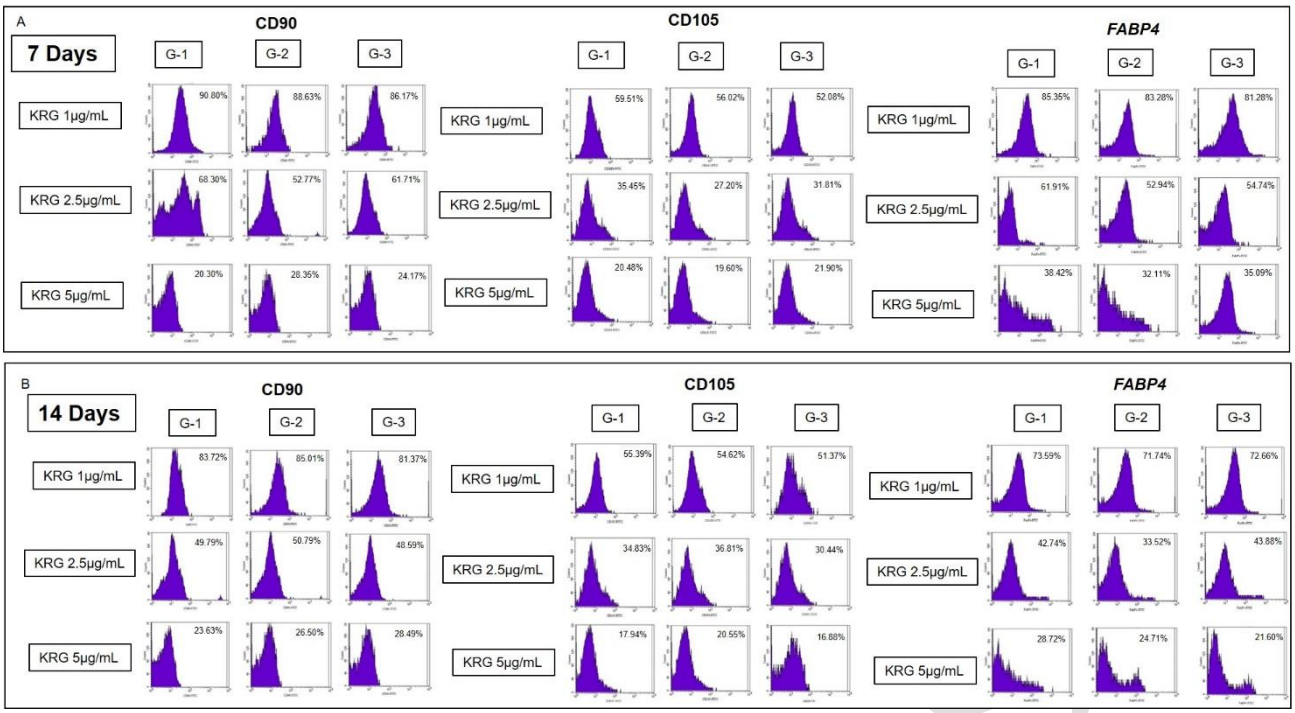

Fig. 3: Flow cytometry (FACS) of CD-90, CD-105, and FABP4 in cMSC. Control and adipogenic converted MSCs expressions on day 7 (A) and day 14 (B) of adipogenesis with and without KRG (1–5 µg/mL).

nM dexamethasone, 1% P-S, A-B, and FBS 10%. Following 21 days, the degree of extracellular mineralization was assessed through Alizarin Red S (ARS) staining. Initially, cells were rinsed with $DPBS^{-/-}$ and subsequently fixed in 4% buffered formalin (30 min), and subsequently stained with a 40 mM ARS solution for 45 min in the dark.

Gene expression studies: For gene expression analysis, 1×10^5 cells were seeded into T-25 cell culture flasks. The detailed protocol and primer sequences were based on our previous study (Rashid *et al*., 2021). Reactions were carried out in triplicate while preserving the GAPDH housekeeping gene, and the relative expression was evaluated through the $2^{-\Delta\Delta CT}$ technique.

Statistical analysis: Statistical analysis of the data was conducted using a two-way analysis of variance (ANOVA), followed by the Holm–Sidak post-hoc test, utilizing SigmaPlot 12.0 (Systat Software Inc., San Jose, CA, USA). The findings are expressed as mean ± least squares mean (LSM), with a $p < 0.05$ believed statistically significant.

RESULTS

Influence of KRG on cell population and survival: Cell counts exhibited an initial increase over time; however, following KRG treatment, a dose-dependent decline was observed. On day 9, the treated cohort experienced a notable decrease in cell numbers, with the most pronounced reduction occurring at the highest concentration of 5 µg/mL. By day 12, all treatment groups demonstrated significant reductions in cell counts when compared to the untreated cohort as shown in Fig 2A. A cellular viability assay corroborated this pattern, revealing a dose-dependent decline in viability that commenced on day 3 across all concentrations (ranging from 1 µg/mL to 5 µg/mL), with significant reductions recorded on days 3, 6, 9, and 12 as the KRG concentrations escalated as shown in Fig 2B.

Immunophenotypic Analysis of IPFP-MSCs:
On day 7 (Fig. 3A) and day 14 (Fig. 3B), stem cell markers, such as CD-90 and CD-105, and the adipogenic marker FABP4 expressions were found to be highest in cells treated with 1 µg/mL of KRG and lowest in cells treated with 5 µg/mL of KRG in all three treatment groups. This suggests that KRG treatment resulted in a dose-dependent reduction in the number of cells expressing stem cell and adipogenic markers.

Bi-lineage differentiation capability of cMSCs into Osteocytes and Adipocytes: In the lineage differentiation capacity of cMSCs, the osteogenic transformation was validated through hydroxyapatite deposition observed after 21 days (Fig. 4A). The successful hydroxyapatite deposition serves as an indicator of this transformation. Additionally, cMSCs underwent differentiation into adipocytes over periods of 7 and 14 days (Fig. 4B–C). Notable decreases in ORO uptake were recorded in Group 1 when comparing the induced group with KRG concentrations of 5 µg/mL and 2.5 µg/mL ($P<0.001$), as well as between KRG 1 µg/mL and KRG 5 µg/mL ($P<0.05$), as detailed in Table 1. Group 2 exhibited a comparable trend on both days 7 and 14. In Group 3, significant differences were observed between the induced and KRG 5 µg/mL groups, as well as between the non-induced and induced groups ($P<0.001$), with further significant differences noted between KRG 2.5 µg/mL and KRG 1 µg/mL compared to KRG 5 µg/mL ($P<0.05$). Collectively, ORO uptake was markedly inhibited on days 7 and 14 during the adipogenic differentiation process, particularly in Group 3 at the KRG concentration of 5 µg/mL.

Gene expression analysis via RT-PCR: RT-PCR analysis validated the expression of CD-90 and CD-105 in cultured MSCs, demonstrating positive expression in both non-induced and adipose-induced stem cells (Fig. 5A). Additionally, *PPARγ* and *FABP4* expression across all three experimental groups revealed that both genes were expressed in differentiated adipocytes by day 7, with a notable reduction observed at the highest KRG dosage across all groups (Fig. 5B & D). Comparable trends were noted on day 14 (Fig. 5C & E).

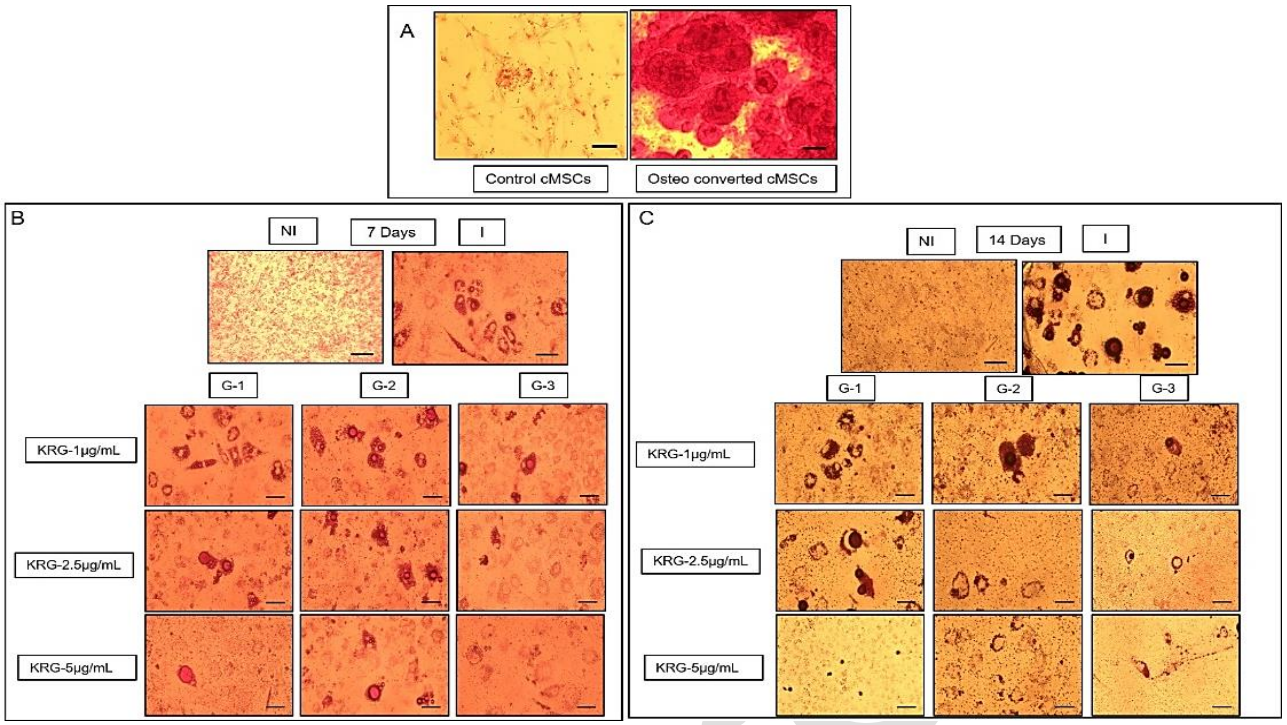

Fig. 4: Bi-lineage differentiation of MSCs into osteocytes and adipocytes. (A) Conversion of MSCs into osteocytes after 21 days of osteogenic induction (Alizarin Red S stain). (B-C) Adipogenic conversion of MSCs into adipocytes after 7 and 14 days with and without KRG (1–5 µg/mL) (Oil Red O stain). The photographs were captured using the microscope at a magnification of 10X.

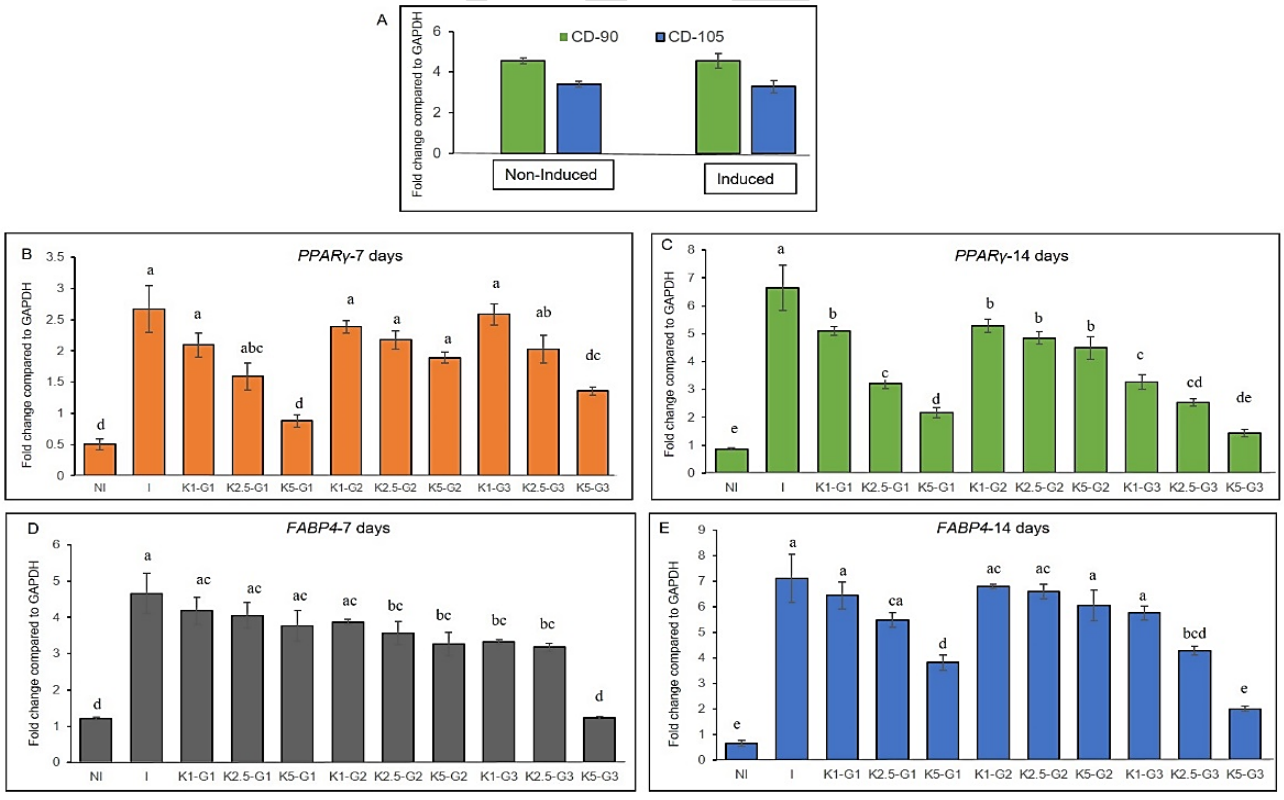

Fig. 5: Gene expression levels of CD-90, CD-105, *PPARγ* and *FABP4*. The changes in stem cell marker expression CD-90 and CD-105 (A), *PPARγ* on days 7 and 14 (B-C) and *FABP4* on days 7 and 14 (D-E) were evaluated using real-time PCR. SYBR Green PCR reagent was used to extend the cDNA obtained from MSCs with and without adipogenic induction. The bar graph values present the mean ± least square mean of at least three (n=3) independent experiments for each treatment group.

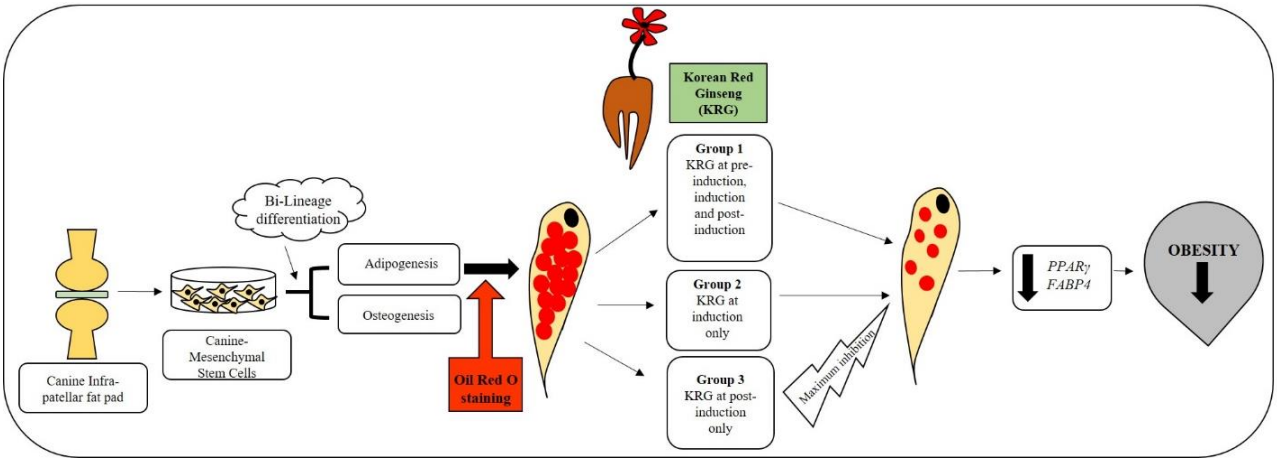

Table 1: ORO quantification in Groups 1-3. Data were normalized using the ORO accumulation in the non-induced group and expressed as the mean ± LSM. In Group-1. ^{A,B} indicate P<0.001 significance between the overall means after 7 and 14 days of adipogenesis. ^{a,b} show significance *P*<0.05 among induced, non-induced, and KRG 5 µg/mL, 2.5 µg/mL, and 1 µg/mL groups. $^{\#}$ represents significance at p = 0.12 between KRG 5 µg/mL and 1 µg/mL groups. For Group-2, ^{a,b} show significance *P*<0.05 among induced, non-induced, and KRG 5 µg/mL, 2.5 µg/mL, and 1 µg/mL groups. $^{\#}$ represents significance at *P*<0.05 between KRG 5 µg/mL and 1 µg/mL groups. For Group-3, ^{A,B} indicate *P*<0.001 significance between the overall means after 7 and 14 days of adipogenesis. ^{a,b} show significance between induced, non-induced, and KRG 5 µg/mL and 2.5 µg/mL groups. ^{c} represents significance, p = 0.032, between the KRG 5 µg/mL and 1 µg/mL groups.

	Treatment	7 day	14 day	Overall means
Group 1	Non-Induced	0.411	0.629	0.520^{b}
	Induced	0.687	1.206	0.946^{a}
	KRG 1µg/mL	0.326	0.939	0.633^{b}
	KRG 2.5µg/mL	0.214	0.631	0.423^{b}
	KRG 5µg/mL	0.151	0.423	0.287$^{b\#}$
	Overall Mean	**0.358^{A}**	**0.766^{B}**	-
Group 2	Non-Induced	0.269	0.622	0.459^{b}
	Induced	0.579	1.144	0.862^{a}
	KRG 1µg/mL	0.340	0.804	0.572^{b}
	KRG 2.5µg/mL	0.213	0.687	0.450^{b}
	KRG 5µg/mL	0.141	0.494	0.317$^{b\#}$
	Overall Mean	**0.314^{A}**	**0.750^{B}**	-
Group 3	Non-Induced	0.318	0.514	0.416^{b}
	Induced	0.723	1.226	0.974^{a}
	KRG 1µg/mL	0.369	0.976	0.673^{ac}
	KRG 2.5µg/mL	0.301	0.725	0.513^{b}
	KRG 5µg/mL	0.180	0.457	0.318^{b}
	Overall Mean	**0.378^{A}**	**0.779^{B}**	-

DISCUSSION

The name 'Ginseng' is derived from a Greek word, '*Panax*', which translates to 'cure for all', highlighting its widespread medicinal benefits. Although KRG has been explored for its potential in treating obesity (Zhang *et al*., 2017), its effect on cMSCs is still insufficient. This study aimed to evaluate the effects of different doses of KRG on adipogenesis in canine MSCs, which can serve as a suitable model for human obesity (Stachowiak *et al*., 2016). Our results specified that elevated KRG dosage markedly reduced cellular proliferation and viability, likely leading to apoptosis in adipose-derived MSCs. This observation implies that KRG supplementation may eliminate precursor cells essential for adipocyte development. The results of experiment are consistent with earlier studies that demonstrated the ability of Chinese herbal medicines to suppress adipogenesis in human MSCs via diminished *PPARγ* regulation and antioxidant pathways (Di Giacomo *et al*., 2015).

The expression of cell surface markers is vital for comprehending the functionality of MSCs. Stem cells are characterized by the presence of specific markers; otherwise, the processes of extraction and isolation require optimization (Rashid *et al*., 2023). The CD-90 and CD-105 were used to verify MSC expression. CD-90 plays a critical role in mediating cell interactions that govern growth and tissue regeneration (Sauzay *et al*., 2019). Meanwhile, CD-105 serves as a co-receptor in the TGF-β signalling pathway, endorsing the proliferation of various cell types, particularly endothelial cells (Lebrin *et al*., 2004). The cells demonstrated maximal expression of both markers; however, a reduction in the number of positively stained cells with increasing dosages of KRG, suggesting an inhibitory effect of KRG on cell proliferation.

Data from ORO staining showed that adipogenically differentiated cells reached peak fat accumulation at day 14 with a cell diameter of 21.1 µm, whereas the cell diameter observed at day 7 was 18.4 µm (Rapid Analysis of Human Adipose- Derived Stem Cells and 3T3-L1 Differentiation Toward Adipocytes Using the Scepter™ 2.0 Cell Counter., 2012). Nevertheless, the cellular uptake of ORO staining was minimal at the highest KRG concentration in group 3, suggesting that the reduced cell number resulted in decreased adipogenesis and reduced fat droplet formation. Previous studies have similarly shown that epigallocatechin gallate and Euglena can reduce ORO staining in MSCs by inhibiting the levels of *PPARγ* and *C/EBP* (Chani *et al*., 2016; Sugimoto *et al*., 2018). Furthermore, herbal supplements have been shown to produce similar effects in MSCs derived from human bone marrow (Kim *et al*., 2020).

The supplementation of KRG was found to inhibit cell proliferation and the retention of ORO staining, which led to an exploration of the mechanisms involved. Our study focused on the expression of genes associated with the adipogenic pathway, with a particular emphasis on *PPARγ,* an early transcription factor in adipogenesis that is activated by fatty acids and prostaglandins. Activation of *PPARγ* enables the expression of C/EBPs, resulting in the development of a differentiated adipocyte phenotype (Wu *et al*., 2020). By quantitative PCR analysis, we observed that *PPARγ* expression was most significantly suppressed in group 3 treated with KRG during the maintenance period, especially on day 14. This observation is consistent with previous studies showing that ginsenosides function

as antagonists of *PPARγ*, thereby reducing hepatic fat accumulation and inhibiting lipogenesis in various experimental models (Zhang *et al*., 2014). Furthermore, investigations into whole ginseng extract and "sun ginseng" have demonstrated their anti-obesity properties through the down regulation of *PPARγ* and associated lipogenic genes (Shin *et al*., 2018).

The expression of *FABP4* was inhibited by the peak concentration of KRG in group 3 on the 14th day of adipogenesis, which is consistent with research indicating that fermented ginseng extracts diminish lipid metabolism by down regulating *FABP4* and *PPARγ* in high-fat diet mice (Li *et al*., 2018). Furthermore, white ginseng has been shown to possess anti-hypercholesterolemic properties in rats through the suppression of *FABP4* (Nalbant *et al*., 2020). Other substances, such as hesperetin (Subash-Babu *et al*., 2015), dihydrotestosterone (Gupta *et al*., 2008), and muscadine grape seed oil (Zhao *et al*., 2015), have also been found to inhibit adipogenesis by decreasing *FABP4* expression. The findings from PCR were corroborated by FACS analysis, which revealed a comparable inhibitory effect with escalating doses of KRG.

We achieved some favorable outcomes with KRG; however, the constraints of our study primarily involved the absence of experimentation using an *in vivo* canine model of obesity, and the application of dosages below 1µg/mL for the *in vitro* studies, which were limited by time, funding, and ethical considerations. These limitations will be addressed in the future to establish a more robust framework for obesity improvement at the stem cell level.

Conclusions: These findings indicated that KRG exhibited toxicity towards adipocytes at a concentration of 5 µg/mL. Conversely, 1 µg/mL, KRG appeared to be relatively safe, showcasing its anti-adipogenic and anti-obesity properties. Nonetheless, there remains an opportunity for further investigation at lower concentrations than those examined in this study. Given our current findings, we hereby support the potential usage of KRG in both humans and companion animals experiencing direct or indirect obesity and associated complications.

Ethical approval and consent to participate: All experimental protocols followed the Institutional Animal Ethics Committee guidelines of PMAS Arid Agriculture University, Rawalpindi (PMAS-AAUR/IEC/665).

Data Statement: The main manuscript file contains all the necessary data.

Competing interests: All the authors have no competing interests to declare.

Acknowledgments: This work was supported by the Higher Education Commission (HEC) of Pakistan under the Start-up Grant Project (SRGP-2600) and the Young Researcher project granted by the Korean Society of Ginseng (GS 302-207) to Dr. Evelyn Saba.

Author contributions: Conceptualization and methodology were done by ES and MAS, was designed by ES, MAS, and UR. The software was implemented by AWA and YYL, validation was done by AS and WAKT, formal analysis was performed by MAS and MHR, the investigation was conducted by ES, UR, AY, AR, MMD and GN, resources were managed by ES, MAS, and MHR, data management was conducted by MS and ES, writing—original draft preparation was performed by ES, AWA, and UR. Writing, review and editing were done by MAS and MHR; project administration and funding acquisition were done by ES, MAS, and MHR. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Akram A, Shin JH, Batmunkh U, *et al*., 2025. Ginsenoside Rg5 inhibits platelet aggregation by regulating GPVI signaling pathways and ferric chloride-induced thrombosis. J Ginseng Res 49:460-469.

Ambele MA, Dhanraj P, Giles R, *et al.,* 2020. Adipogenesis: Acomplex interplay of multiple molecular determinants and pathways. Int J Mol Sci 16:4283.

Baek SH, Bae ON and Park JH, 2012. Recent methodology in ginseng analysis. J Ginseng Res 36:119-134.

Boden G, 2008. Obesity and free fatty acids. Endocrinol Metab Clin North Am 37:635-646.

Bost F, Aouadi M, Caron L*., et al*., 2005. The extracellular signal-regulated kinase isoform ERK1 is specifically required for in vitro and in vivo adipogenesis. Diabetes 54:402-411.

Chandrasekaran CV, Vijayalakshmi MA, Prakash K, *et al.,* 2012. Review Article: Herbal Approach for Obesity Management. Am J Plant Sci 3:1003-1014.

Chani B, Puri V, Sobti RC, *et al*., 2016. Epigallocatechin Gallate Inhibits Mouse Mesenchymal Stem Cell Differentiation to Adipogenic Lineage. J Stem Cells Regen Med 12:16-24.

Di Giacomo C, Vanella L, Sorrenti V, *et al*., 2015. Effects of Tithonia diversifolia (Hemsl.) A. Gray extract on adipocyte differentiation of human mesenchymal stem cells. PLoS One 10: e0122320.

Dominici M, Le Blanc K, Mueller I, *et al*., 2006. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8:315-317.

Floresta G, Patamia V, Zagni C, *et al.,* 2022. Adipocyte fatty acid binding protein 4 (FABP4) inhibitors. An update from 2017 to early 2022. Eur J Med Chem 240:114604.

Friedenstein AJ, Gorskaja JF and Kulagina NN, 1976. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol 4:267-274.

Gupta V, Bhasin S, Guo W, *et al*., 2008. Effects of dihydrotestosterone on differentiation and proliferation of human mesenchymal stem cells and preadipocytes. Mol Cell Endocrinol 296:32-40.

Ham SW, Kim JK, Jeon HY, *et al*., 2019. Korean Red ginseng extract inhibits glioblastoma propagation by blocking the Wnt signaling pathway. J Ethnopharmacol 236:393-400.

Hemmings BA and Restuccia DF, 2012. PI3K-PKB/Akt pathway. Cold Spring Harb Perspect Biol 4: a011189.

Hotamisligil GS and Bernlohr DA, 2015. Metabolic functions of FABPs--mechanisms and therapeutic implications. Nat Rev Endocrinol 11: 592-605.

Kersten S, 2002. Peroxisome proliferator activated receptors and obesity. Eur J Pharmacol 440: 223-234.

Kim DH, Kim DH, Heck BE, *et al*., 2020. A natural supplement formula reduces anti-oxidative stress and enhances osteo-chondrogenic differentiation potential in mesenchymal stem cells. J Clin Biochem Nutr 66:206-212.

Kim JH, 2018. Pharmacological and medical applications of Panax ginseng and ginsenosides: a review for use in cardiovascular diseases. J Ginseng Res 42:264-269.

Lebrin F, Goumans MJ, Jonker L, *et al.,* 2004. Endoglin promotes endothelial cell proliferation and TGF-beta/ALK1 signal transduction. EMBO J 23:4018-4028.

Lee YY, Saba E, Irfan M, *et al*., 2019. The anti-inflammatory and anti-nociceptive effects of Korean black ginseng. Phytomedicine 54:169-181.

Li XD, Wang JS, Chang B, *et al*., 2011. Panax notoginseng saponins promotes proliferation and osteogenic differentiation of rat bone marrow stromal cells. J Ethnopharmacol 134:268-274.

Li Z, Kim HJ, Park MS, *et al.,* 2018. Effects of fermented ginseng root and ginseng berry on obesity and lipid metabolism in mice fed a high-fat diet. J Ginseng Res 42:312-319.

Li Z, Wang Y, Xu Q, *et al.,* 2023. Ginseng and health outcomes: an umbrella review. Front Pharmacol 14:1069268.

Lin X and Li H, 2021. Obesity: Epidemiology, Pathophysiology, and Therapeutics. Front Endocrinol (Lausanne) 12: 706978.

Moseti D, Regassa A and Kim WK, 2016. Molecular Regulation of Adipogenesis and Potential Anti-Adipogenic Bioactive Molecules. Int J Mol Sci 17:124.

Nalbant A, Bilgili A, Hanedan B, *et al.,* 2020. Effects of Tribulus terrestris, Avena sativa and white ginseng on adiponectin, leptin, resistin, fatty Acid binding protein 4, homocysteine and paraoxonase-1 levels in hypercholesterolemic rats. CUPMAP 3:135-142.

Oh J, Yoon HJ, Jang JH, *et al.,* 2019. The standardized Korean Red Ginseng extract and its ingredient ginsenoside Rg3 inhibit manifestation of breast cancer stem cell-like properties through modulation of self-renewal signaling. J Ginseng Res 43:421-430.

Prentice KJ, Saksi J and Hotamisligil GS, 2019. Adipokine FABP4 integrates energy stores and counterregulatory metabolic responses. J Lipid Res 60:734-740.

Qi L.W, Wang CZ and Yuan CS, 2011. Isolation and analysis of ginseng: advances and challenges. Nat Prod Rep 28:467-495.

Ramalho-Santos M and Willenbring H, 2007. On the origin of the term "stem cell". Cell Stem Cell 1:35-38.

Rapid Analysis of Human Adipose- Derived Stem Cells and 3T3-L1 Differentiation Toward Adipocytes Using the Scepter™ 2.0 Cell Counter. 2012. Biotechniques 53:109-111.

Rashid U, Saba E, Yousaf A, *et al.,* 2023. Autologous Platelet Lysate Is an Alternative to Fetal Bovine Serum for Canine Adipose-Derived Mesenchymal Stem Cell Culture and Differentiation. Animals (Basel) 13:2655.

Rashid U, Sandhu MA, Yaqoob M, *et al.,* 2021. Critical bone gap repair using autologous adipose derived canine mesenchymal stem cell graft. Pak Vet J 2021:513-518.

Rashid U, Yousaf A, Yaqoob M, *et al.,* 2021. Characterization and differentiation potential of mesenchymal stem cells isolated from multiple canine adipose tissue sources. BMC Vet Res 17:388.

Saba E, Sandhu MA and Pelagalli A, 2024. Canine Mesenchymal stromal cell exosomes: State of the art characterisation, functional analysis and application in various diseases. Vet Sci 11:187.

Sarfraz A, Qureshi AS, Sandhu MA, *et al*., 2021. Isolation and Characterization of Fetal Adnexa-Derived Mesenchymal Stem Cells from Nili-Ravi Buffalo (Bubalus bubalis). Pak Vet J 41:524-530.

Sauzay C, Voutetakis K, Chatziioannou A, *et al*., 2019. CD90/Thy-1, a Cancer-Associated Cell Surface Signaling Molecule. Front Cell Dev Biol 7:66.

Shin SS and Yoon M, 2018. Korean red ginseng (Panax ginseng) inhibits obesity and improves lipid metabolism in high fat diet-fed castrated mice. J Ethnopharmacol 210:80-87.

Stachowiak M, Szczerbal I and Switonski M, 2016. Genetics of Adiposity in Large Animal Models for Human Obesity-Studies on Pigs and Dogs. Prog Mol Biol Transl Sci 140:233-270.

Suarez L, Bautista-Castano I, Romera CP, *et al.,* 2022. Is Dog Owner Obesity a Risk Factor for Canine Obesity? A "One-Health" Study on Human–Animal Interaction in a Region with a High Prevalence of Obesity. Vet Sci 9:243.

Subash-Babu P and Alshatwi AA, 2015. Hesperetin inhibit adipocyte differentiation and enhance Bax- and p21-mediated adipolysis in human mesenchymal stem cell adipogenesis. J Biochem Mol Toxicol 29:99-108.

Sugimoto R, Ishibashi-Ohgo N, Atsuji K, *et al*., 2018. Euglena extract suppresses adipocyte-differentiation in human adipose-derived stem cells. PLoS One 13:e0192404.

Wang Y, Huang X, Tang Y, *et al.,* 2016. Effects of panax notoginseng saponins on the osteogenic differentiation of rabbit bone mesenchymal stem cells through TGF-beta1 signaling pathway. BMC Complement Altern Med 16:319.

Wen X, Zhang B, Wu B, *et al.,* 2022. Signaling pathways in obesity: mechanisms and therapeutic interventions. Signal Transduct Target Ther 7:298.

Wu H, Li X and Shen C, 2020. Peroxisome Proliferator-Activated Receptor γ in White and Brown Adipocyte Regulation and Differentiation. Physiol Res 69:759-773.

Yin XX, Chen ZQ, Liu ZJ, *et al*., 2007. Icariine stimulates proliferation and differentiation of human osteoblasts by increasing production of bone morphogenetic protein 2. Chin Med J (Engl) 120:204-210.

Zhang L and Si H, 2014. American ginseng and its bioactive compounds inhibit preadipocyte differentiation by suppressing peroxisome proliferator-activated receptor gamma in 3T3-L1 cells and human primary preadipocytes (641.11). FASEB J 28:641.611.

Zhang L, Virgous C and Si H, 2017. Ginseng and obesity: observations and understanding in cultured cells, animals and humans. J Nutr Biochem 44:1-10.

Zhang P, Dai KR, Yan SG, *et al.,* 2009. Effects of naringin on the proliferation and osteogenic differentiation of human bone mesenchymal stem cell. Eur J Pharmacol 607:1-5.

Zhang Y, Yu L, Cai W, *et al.,* 2014. Protopanaxatriol, a novel PPAR-gamma antagonist from Panax ginseng, alleviates steatosis in mice. Sci Rep 4:7375.

Zhao L, Yagiz Y, Xu C, *et al*., 2015. Muscadine grape seed oil as a novel source of tocotrienols to reduce adipogenesis and adipocyte inflammation. Food Funct 6:2293-2302.