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Modulatory Effect of PRP Versus Propolis as Therapeutic Agents on LPS -Induced Inflamed Human Dental Pulp Stem Cells

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Aim: This project aims to explore the potential modulatory effects of platelets rich plasma (PRP) and propolis on lipopolysaccharide (LPS)-induced inflamed human dental pulp stem cells (HDPSCs) cultures and determining the propolis concentration that exhibited the greatest anti-inflammatory activity.
Materials and Methods: HDPSCs were isolated, cultured and equally split into five groups: Group I: HDPSCs without any additions; Group II: LPS was added to induce inflammation (iHDPSCs); Group III: iHDPSCs treated with PRP; Group IV: iHDPSCs treated with propolis (10 ng); Group V: iHDPSCs treated with propolis (30 ng). Morphological changes in all studied groups were examined microscopically. Inflammatory microenvironment stimulated with LPS were investigated by assessing cell viability, evaluating oxidative damage by measuring the levels of GSH and MDA, evaluating pro-inflammatory cytokines TNF-α, IL-6, PGE-2, IL-4 and IL-10. Quantitative expression of AKT, p38 MAPK and Caspase-3 was also evaluated.
Results: Cell viability of iHDPSCs treated groups was much closer to that of the control group. Potent antioxidant action evidenced by significant increase in levels of GSH and marked decrease of MDA levels in iHDPSCs treated groups. Pro-inflammatory cytokines TNF-α, IL-6 and PGE-2 were evidently downregulated in iHDPSCs treated groups, while highly significant increase in the production of IL-4 and IL-10 was demonstrated. Gene profile of AKT, p38 MAPK and Caspase-3 revealed statistically significant down regulation in iHDPSCs when treated with PRP and propolis.
Conclusion: PRP and propolis play a pivotal role in controlling the inflammatory microenvironment in HDPSCs stimulated with LPS. Higher concentration of propolis exerted much better effects.

Keywords: PRP, Propolis, iHDPSCs, Oxidative damage, Pro-inflammatory cytokines.

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Introduction

Propolis is produced from a variety of plant materials, such as tree buds and bark. It serves two very significant purposes: it blocks the entry hole, seals up holes and fractures in the hive, and covers insects that perish inside the hive and are too big to be removed. [1] Propolis is a resinous substance which gathered by honeybees *(A. mellifera L.)* to give their nests both physical and biochemical protection. It constitutes a mixture of resin (45-55 %), wax and fatty acids (25-35 %), pollen (5 %) and other organic substances and minerals (5 %). [2]

The word propolis comes from the Greek terms pro-, which means before or in front, and Polis, which means town and it refers to the material that bees utilize to build the "town" entrance on their hives. "Bee glue" is another name for it. [3]

The majority of propolis's constituents are phenolic in nature; flavonoids, in particular, are produced by plants in reaction to microbial infections and are known to have potent antibacterial properties against a variety of microbes. Propolis is utilized as an alternate therapy for infections because of its bactericidal and fungicidal qualities. The synergistic effects of flavonoids and aromatic chemicals give propolis its broad spectrum of antimicrobial activity. [4]

Propolis has been used for a long time at least since 300 BC. Its activity has been the subject of increased research in recent years, demonstrating impressive immunomodulatory, antibacterial, antiviral, antifungal, antioxidant, anti-inflammatory and anticancer effects. Propolis has a wide range of biological properties that make it useful in the pharmaceutical and food supplement industries. Because of its amazing medicinal properties and therapeutic benefits, propolis is highly prized in several nations for treating a variety of illnesses in people as well as cosmetic uses. [5,6]

Propolis was separately utilized for medicinal purposes by "ancient Egyptians, Greeks, Persians, Romans, Indians, Mayans, and Australian Aborigines", according to historical documents. Propolis is used medicinally in general to treat a variety of conditions, such as anaemia, respiratory disorders, dental care, dermatology (tissue regeneration, eczema, ulcers, wound healing, especially burn wounds), mycosis, mucous membrane infections and cancer. [7]

Reactive oxygen species (ROS) production and the incapacity of endogenous antioxidants to regulate their synthesis are the hallmarks of oxidative stress. An imbalance between the antioxidant and oxidant systems can lead to tissue and cellular damage, which in turn promotes the development of the inflammatory process. [8] In order to reduce these consequences and improve human health, it is important to look for alternative therapies because oxidative stress and inflammation are linked to a number of disorders. Because natural products have more advantages than synthetic medications and fewer adverse effects, research on them has increased recently.[9,10]

Literature-based data has demonstrated propolis' protective effects in inflammatory illnesses, including diabetes, skin conditions, chronic renal disease, and neuronal degenerative disease. Nevertheless, the exact processes by which propolis carries out these functions remain somewhat contentious and remain incompletely known. [11] Propolis's anti-inflammatory properties are mostly attributed to its suppression of prostaglandin and cyclooxygenase production, scavenging of free radicals, inhibition of nitric oxide synthesis, and decreased release of inflammatory cytokines. Additionally, propolis has been noted for its potential to protect and repair skin, promoting the development and regeneration of skin tissues and enhancing the creation of collagen and cell viability following oxidative stress. [12]

Numerous investigations have emphasized the function of platelets rich plasma (PRP) in the release of growth factors and its importance in controlling biological processes linked to chemotaxis and inflammation. Researchers have investigated the impact of PRP on different cell populations involved in wound healing in earlier studies. [13] However, the potential role of PRP on HDPSCs to reduce inflammation and promote angiogenesis has not been thoroughly explored. Our research sought to shed light on this area by comparing the modulatory effects of propolis and PRP on lipopolysaccharide (LPS)-induced inflamed HDPSCs cultures and determining the propolis concentration that exhibited the greatest anti-inflammatory activity.

Materials and Methods

HDPSCs Isolation and Culturing

Twenty sounds impacted wisdoms were extracted from healthy individuals (18-35 years old) at the Oral and Maxillofacial Surgery Clinic, Faculty of Dentistry. In order to lessen the aging effects of HDPSCs, a patient population with a relatively limited age range was selected. Following extraction, the teeth were immediately moved to the tissue culture unit and kept on ice in phosphate buffer saline (PBS) ("Thermo Scientific®, USA").

The research protocol was conducted and approved by the Research Ethics Committee at the Faculty of Dentistry, in accordance with the principles outlined in the Declaration of Helsinki. Prior to their participation, all patients were provided with and signed an informed consent document.

Primary cells were obtained and cultivated from impacted molars that had been extracted, following the methodology described in previous studies. The pulp tissue was extracted from the pulp chamber and root canal of the collected samples using a sterile endodontic barbed broach, and then immediately transferred to a falcon tube containing PBS. [14,15]

Subsequent to the extraction of pulpal tissues, they were exposed to 0.3 mg of collagenase type II enzyme ("Sigma Aldrich, USA") for a duration of two hours at 37°C within a CO_2 incubator. The tissues were agitated at ten-minute intervals until complete digestion was achieved. A single cell suspension was then obtained by passing through a 70-µm cell strainer, and the cells suspended in the plating media were harvested through centrifugation at 60 g for 10 minutes. The cells were then cultured at 37°C in a CO_2 incubator, using RPMI-1640 supplemented with L-glutamine ("BioWhittaker®, Lonza, USA"), 10% fetal bovine serum ("LSP®, USA"), and 1% penicillin/streptomycin ("Biowest®, USA"). The culture media was refreshed every three days until cell confluence reached 80%–90% over three passages. For the promotion of odontogenic differentiation, the plating media was supplemented with 10 mM dexamethasone, 5 mL/mol ß-glycerophosphate, and 50 µg/mL ascorbic acid, all of which were procured from Sigma-Aldrich.

Phenotypical and Immunological Characterization of HDPSCs

An inverted light microscope from Leica®, Germany was employed for the examination of cultured HDPSCs to authenticate the distinctive characteristics and morphology of the stem cells.

Following the guidelines of the International Society of Cell Therapy Criteria for Human Stem Cells, the identification of surface antigens specific to CD105 (“R&D Systems, USA”; cat #, “FAB1320F-025”) and CD45 (“R&D Systems, USA; cat # FAB114A”) was conducted through fluorescence-activated cell sorting analysis, with the exception of hematopoietic endothelial cells. [16,17]

Preparation of Propolis Extract

Methanolic extraction of propolis was performed, 80 g of propolis were combined with 300 ml of methanol (96%). The mixture was centrifuged for 30 minutes at 20°C at 3500 rpm. In order to isolate the insoluble fraction by filtration for use in the experiment and to collect the supernatant. Then, 300 ml of cold phosphate buffer and 30g of propolis were combined for 20 minutes at 37°C. The mixture was then left overnight and centrifuged for 15 minutes at 3000 rpm. The supernatant was filtered and utilized in this study. [18]

Preparation of Platelet Rich Plasma (PRP)

Approximately 10 mL of blood sample was extracted from the participant through venipuncture of the antecubital vein. Subsequently, the tubes underwent centrifugation via a gentle spin, followed by the transfer of the resultant supernatant plasma - which houses platelets - into a separate sterile tube devoid of anticoagulant. The centrifuge tube was promptly subjected to a more vigorous spin (referred to as a hard spin) at a speed of 3,000 rpm for a duration of 10 minutes, with the aim of isolating a platelet concentrate located in the lower one-third section of the tube.

Lipopolysaccharides (LPS)-induced Inflammation in HDPSCs

Based on earlier findings, which did not change the prominent properties of HDPSCs, in order to create an environment that resembled inflammation, isolated cells were given 2 milliliters of basal medium (“Sigma-Aldrich”) containing LPS at a concentration of 1 µg/ml and incubated for 48 hours at 37°C with 5% CO_2. HDPSCs exposed to LPS will hereafter be referred to as iHDPCs. [19,20]

Study Design

At the third passage, HDPSC tissue culture plates with 96 wells were equally split into five groups: Group I: control group consisted of HDPSCs without any additions; Group II: LPS was added to induce inflamed HDPSCs (iHDPSCs); Group III: iHDPSCs treated with PRP; Group IV: iHDPSCs treated with propolis (10 ng); Group V: iHDPSCs treated with propolis (30 ng).

Morphological Analysis

After 48 hours of incubation, any morphological alterations in the iHDPSCs treated with PRP and different propolis concentrations were examined under an inverted light microscope (“Leica®, Germany”). Three randomly selected photomicrographs of each sample were taken at various magnifications for additional analysis.

Cell Viability Assay

The cytotoxicity of Lipopolysaccharide (LPS) against Human Dental Pulp Stem Cells (HDPSCs) was evaluated using a colorimetric microculture tetrazolium (MTT) assay, which assesses cellular metabolic activity as an indicator of cell viability. Subsequently, the cells were observed continuously using an inverted light microscope, followed by incubation at 37°C with MTT stock solution (100 µl; 2 mg/ml in PBS) added to each well for a duration of 2-4 hours. The conversion of the reagent to formazan, an insoluble crystalline compound displaying a deep purple color, is facilitated by NADPH-dependent oxidoreductase enzymes present in viable cells. The determination of cell viability was based on the absorbance readings of each well, which were recorded using an ELISA plate reader at a wavelength of 540 nm. [21]

Oxidative Status Assessment

Malondialdehyde (MDA) is produced by lipid peroxidation, and its detection was crucial for evaluating oxidative stress in pathophysiological processes. One helpful way to quantify oxidative damage is to measure the end products of lipid peroxidation. As per the manual instructions, BioVision's Lipid Peroxidation Assay Kit ("Catalog # K739-100 Milpitas, CA 95035USA") offers a handy tool for sensitive MDA detection in HDPSCs samples of all examined groups. The MDA concentration was given as (nmol/mg protein).

Reduced glutathione (GSH) is a biomolecule that fulfils a crucial function in the cellular defence mechanism against oxidative stress in various tissues and cells. The utilization of commercially accessible glutathione detection kits, such as BioVision's ("Catalog #K264-100; Milpitas, CA 95035-USA"), offers a distinctive and handy approach for the identification of GSH in samples of HDPSCs across all examined cohorts in accordance with the manual guidelines. The quantification of GSH content was denoted as (µg/mg protein).

Inflammation Markers Levels Measurement

Prior to the inflammatory markers being assayed, the cells were lysed. The cells were reconstituted at a density of 107 cells/mL in new lysis buffer. The protein estimation kit from Genei, Bangalore, was used to assess the protein content in the cell lysates using the Bradford (1976) technique. [22] A reader for Enzyme-Linked Immuno-Sorbent Assays (ELISA) was used to measure each kit. With the use of an ELISA plate reader ("Stat Fax 2200, Awareness Technologies, Florida, USA"), colour absorbance was measured at an optical density range of 490 to 630 nm.

Tumour necrosis factor Alpha (TNF-α) is an inflammatory cytokine was assessed in cell lysates of all samples using ELISA assay kit instruction manual ("SEA133Hu 96 cloud-clone crop, USA").

Interleukin 6 (IL-6) is an inflammatory cytokine was assessed in cell lysates of all samples using ELISA assay kit instruction manual ("SEA079Hu 96 cloud-clone crop, USA"). Prostaglandin (PG) E2 was assessed in cell lysates using competitive ELISA assay kit (Catalog Number: MBS721434, MyBiosource.Com, USA) according to manual instructions.

Interleukin *4 (IL-4)* ELISA kit applies to the in vitro quantitative determination of Human IL-4 in cell lysates Catalog No: E-EL-H010, Elabscience, China) according to manual instructions. *Interleukin 10 (IL-10)* is an anti-inflammatory cytokine was assessed in cell lysates of all samples using Assay Kit instruction manual AVIVA systems Biology ELISA Kit (OKBB00193, USA).

RNA extraction and Real time PCR

RNA extraction was conducted on HDPSCs via the RNA easy Mini Kit (Qiagen) and subsequently assessed for both quantity and quality utilizing the Beckman dual spectrophotometer (USA). To quantify the expression levels of AKT, p38 MAPK, and Caspase-3, 100 µg of total RNA from each sample underwent cDNA synthesis through reverse transcription employing the High-capacity cDNA Reverse Transcriptase kit (“Applied Biosystem, USA”). The resulting cDNA was then amplified utilizing the TaqMan PCR Master Kit (Fermentas) in a 96-well plate on the Step One instrument (“Applied Biosystem, USA”), involving an initial enzyme activation step at 95 ºC for 10 minutes, succeeded by 40 cycles consisting of 15 seconds at 95ºC, 20 seconds at 55 ºC, and 30 seconds at 72 ºC for the amplification phase.

Alterations in the target gene's expression will be standardized in relation to the average critical threshold (CT) values of GAPDH, serving as the housekeeping gene, through the 2-(AACT) method. Both primers specific to the target gene were utilized at a concentration of 1 µM. The primer sequences for AKT, p38 MAPK, Caspase-3, and GAPDH genes were specified as follows:
“AKT, F: CAGTGATGAATCTAATGG,
R: CTGATTTGCTGCTGTCTGAC; p38
MAPK, F:
GGAACGGACATTCGGTCCTT, R:
AGTCCGTCTAAGAAGCACGC;
Caspase 3, F:
GGGAACCAGATCTCTCACC, R:
AAAATGGCGAATCCAATTCC;
GAPDH, F:
TGCACCACCAACTGCTTAGC, R:
CCCCACGGCCATCA”

Statistical Analysis

The data were depicted as the mean ± standard deviation, and the results were replicated a minimum of three times. Employing GraphPad Prism version 7, a one-way analysis of variance (ANOVA) with Tukey's post-hoc test was applied to examine the data across multiple data sets. Significance was established when the P value was below 0.05, indicating statistically noteworthy distinctions between the groups.

Results

HDPSCs Characterization and Morphological Assessment

Immunological characterization was evaluated since HDPSCs were negative to CD45 surface antigen and positive to CD105 surface antigen as shown in Fig. 1 A.

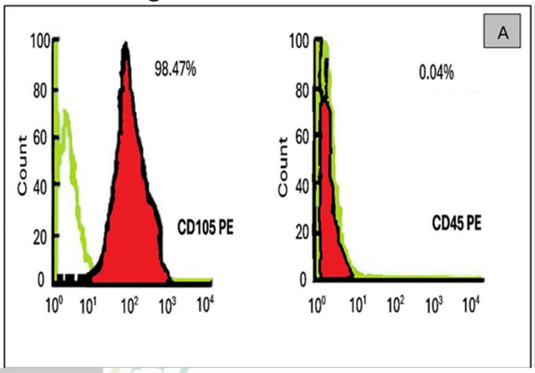


Fig. 1: (A) Immunological characterization of HDPSCs.

HDPSCs were examined under an inverted light microscope to determine their phenotypic characteristics, which included spindle fusiform morphology and plastic adherence. Apparent morphological signs of increased apoptosis were detected in iHDPSCs group. Phenotypically, apoptosis is distinguished by cellular shrinkage, chromatin condensation, fragmentation of DNA, and the disintegration of the cell into small fragments known as apoptotic bodies.

Our microscopic findings are somewhat surprising since, treated groups with PRP and propolis demonstrated a morphological appearance of iHDPSCs much closer to that of the control group, that nearly maintained elongated spindle-shaped typical morphology of mesenchymal stem cells that remained unchanged, after treatment and diminished feature of apoptosis (Fig. 2 a-e).

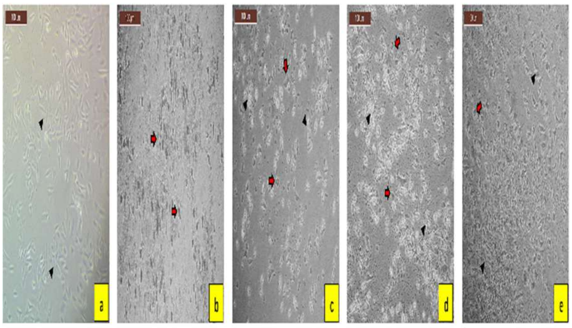


Fig. 2: (a) Control group: the cells were fusiform, adhesive, and highly proliferative. Cells were glistening indicative of their viability (Black arrow heads). No apoptotic cells were present. (b) iHDPSCs group: demonstrating less adhesive and less proliferative cells compared to control group. Cells were dusty indicative of their less viability. High count of apoptotic cells was observed (Red arrows). (c) iHDPSCs + PRP group and (d) iHDPSCs + propolis (10 ng): the cells were fusiform, adhesive, and proliferative. Cells were glistening indicative of their viability (Black arrow heads). Moderate count of apoptotic cells was revealed (Red arrows). (e) iHDPSCs + propolis (30 ng): the cells appeared fusiform, adhesive, and proliferative. Cells were glistening indicative of their viability (Black arrow heads). Low count of apoptotic cells was demonstrated (Red arrow).

Cell Viability Via MTT Assay

In the current work MTT cell proliferation assay was represented as mean±SD (Table 1), it was revealed that the mean percentage of cell viability in control group of HDPSCs (0.693±0.11) was significantly inhibited with addition of LPS in iHDPSCs group (0.0636±0.04). MTT activity had a statistically highly significant increase in group III in which iHDPSCs treated with PRP (0.362±0.07) when compared to the control group ($p < 0.00001$). Moreover, there was a highly significant difference ($p < 0.00001$) between groups IV and V when compared to control group in which iHDPSCs were treated with different concentration of propolis with the highest values of group V (0.152±0.054) and (0.58±0.032), respectively, giving the idea that when the concentration of propolis was increased, the cell viability was significantly increased and reached to that of the control group of HDPSCs (Fig. 1 B).

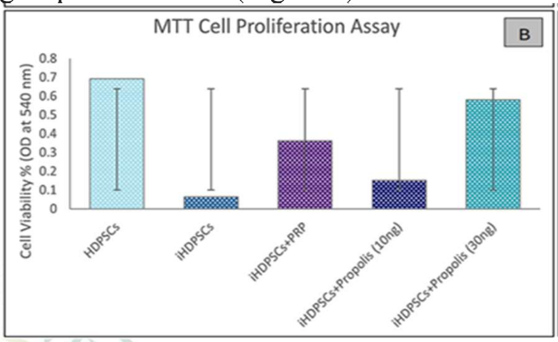


Fig. 1: (B) MTT test for cell proliferation with the mean percentage of cell viability.

Table 1: Mean values and measures of variability (SD) in MTT assay using One-way Anova test and Tukey's HSD calculations of significant differences for all studied groups.

Mean ± SD of MTT Cell Proliferation Assay in All Studied Groups					
	HDPSCs	**iHDPSCs**	**iHDPSCs+ PRP**	**iHDPSCs+ Propolis (10ng)**	**iHDPSCs+ Propolis (30ng)**
	0.69±0.11	0.063±0.049	0.36±0.076	0.18±0.05	0.58±0.03
f-ratio value	F = 113.640				
p-value	< 0.00001				
Post hoc Tukey's Significant Differences					
Pairwise comparison		**HSD.05 = 0.1029** **HSD.01 = 0.1274**	**Q.05 = 4.1534 Q.01 = 5.1439**		
GI: GII	$M_1 = 0.69$ $M_2 = 0.06$	0.63	Q = 25.40 (p < 0.00000)		
GI: GIII	$M_1 = 0.69$ $M_3 = 0.36$	0.33	Q = 13.33 (p < 0.00000)		
GI: GIV	$M_1 = 0.69$ $M_4 = 0.18$	0.51	Q = 20.79 (p < 0.00000)		
GI: GV	$M_1 = 0.69$ $M_5 = 0.58$	0.11	Q = 4.56 (p < 0.02632)		
GII: GIII	$M_2 = 0.06$ $M_3 = 0.36$	0.30	Q = 12.07 (p < 0.00000)		
GII: GIV	$M_2 = 0.06$ $M_4 = 0.18$	0.11	Q = 4.61 (p < 0.02418)		
GII: GV	$M_2 = 0.06$ $M_5 = 0.58$	0.52	Q = 20.84 (p < 0.00000)		
GIII: GIV	$M_3 = 0.36$ $M_4 = 0.18$	0.18	Q = 7.45 (p < 0.00017)		
GIII: GV	$M_3 = 0.36$ $M_5 = 0.58$	0.22	Q = 8.77 (p < 0.00002)		
GIV: GV	$M_4 = 0.18$ $M_5 = 0.58$	0.40	Q = 16.23 (p < 0.00000)		
G: Group, SD: Standard Deviation , M: Mean , *The mean difference is significant at the 0.05 level					

Oxidative Status Assessment

Regarding to measurement of oxidative damage, the levels of GSH and MDA are summarized in **table 2**.

It was observed that levels of GSH were significantly decreased in iHDPSCs group (0.475±0.138) when compared to the control group (1.86±0.183) and it demonstrate significant elevation ($p < 0.00001$) in other studied groups to reach much closer to the level of control HDPSCs in iHDPSCs group that treated with 30 ng propolis (1.7±0.14). Moreover, highly significant changes in MDA levels between studied groups were observed ($p < 0.00001$) where marked elevation in iHDPSCs (1.52±0.05) was revealed in comparison to the control group (0.45±0.07). MDA expression was downregulated in treated groups to reach to nearly to normal level in iHDPSCs group that treated with 30 ng propolis (0.60±0.04) when compared to control group (Fig. 1 C).

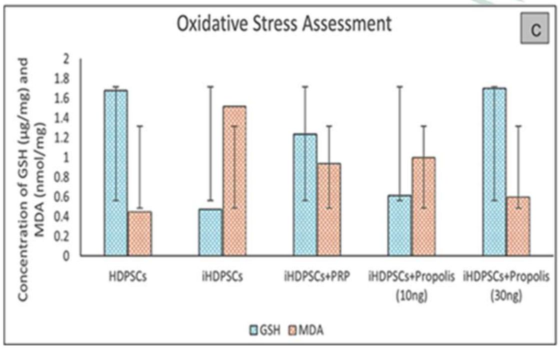
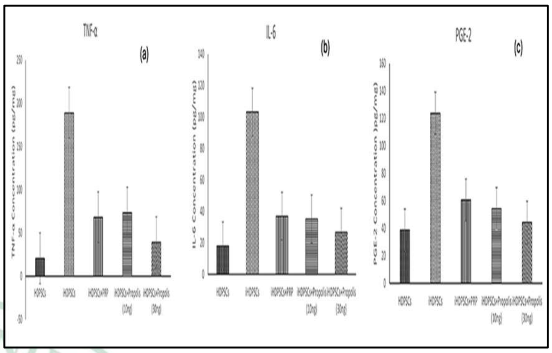


Fig. 1 (C): Mean percentage concentrations of GSH and MDA in all tested groups. GraphPad Prism version 7.0's one-way ANOVA test found significant differences ($p < 0.00001$).

Inflammation Markers Levels Measurement

The levels of TNF-α, IL-6 and PGE-2 in the iHDPSCs group were evidently increased when compared to the control group. Treatment with RPR and propolis resulted in a decrease in these proinflammatory cytokines when compared with the iHDPSCs group (Fig. 3 a-c).



Furthermore, a highly significant decrease in the production of IL-4 and IL-10 were also demonstrated in iHDPSCs group in a comparison with the control one, while treated groups revealed marked elevation in their concentration with highest level in iHDPSCs group that treated with 30 ng propolis (Fig. 3 d & e), **(Table 3).**

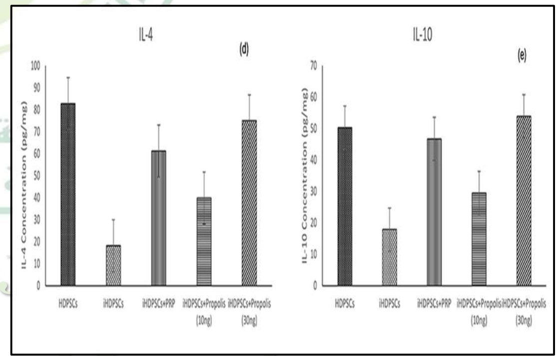
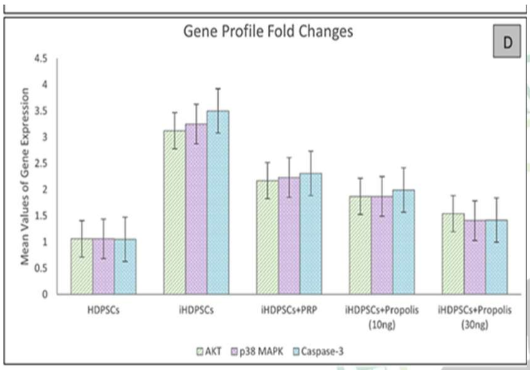


Fig. 3 Inflammatory markers assessment using ELISA kit. Levels of TNF-α, IL-6 and PGE-2 demonstrating significant decrease in treated groups when compared to iHDPSCs group (a-c). Treatment with PRP and propolis revealed significant increase in IL-4 and IL-10 in comparison to iHDPSCs group (d & e), ($p < 0.00001$).

Fold Changes in Gene Expression

The gene profile of AKT, p38 MAPK and Caspase-3 relevant to the cytotoxic and apoptotic effects was represented as mean values of expression

(Table 3, Fig. 1 D). It was observed that these genes showed highly statistically significant fold increase ($p < 0.0001$) in iHDPSCs when compared to control group. In the meantime, they revealed statistically significant down regulation ($p < 0.0001$) when treated with PRP and propolis especially after treatment with a 30ng concentration of propolis.



(Fig. 1 D):Mean values of gene profile fold changes in all studied groups. Error bars represent standard deviations.

Discussion

The potential application of propolis in the pharmaceutical industry has sparked attention in last years, leading to increased investigation into the substance's anti-inflammatory, antioxidant and antitumoral qualities. Propolis has been shown to contain hundreds of different chemical components. Flavonoids, phenolics, and other aromatic chemicals such as quercetin, pinocembrin, caffeic acid, caffeic acid phenyl ester, or pinastrobin are the primary families of substances found in propolis. According to more recent scientific investigations, flavonoids are well-known plant chemicals that include antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory properties, which give them their therapeutic benefits. [23]

In the present study, as expected PRP and propolis affected the cell viability, which was observed in our microscopic findings under an inverted light microscope and confirmed by MTT assay. Our results revealed that morphological appearance and mean percentages of cell viability of iHDPSCs treated with PRP and propolis were much closer to that of the control group. These findings were in agreement with the study of Elkhenany et al. [24] who observed that propolis increases the proliferation rate of bone marrow mesenchymal stem cell. Also, our observations came in the same context with Kale et al. [25] who reported that significant cell proliferation percentage of stem cells from human exfoliated deciduous teeth (SHED) treated with propolis. In addition, our results revealed that when the concentration of propolis was increased, the cell viability was significantly increased and reached to that of the control group of HDPSCs and this observation was supported by the study of Prakashan et al. [26] who found that increasing propolis concentration promotes cell proliferation and viability.

Numerous investigations have emphasized PRP's function in growth factor release and its importance in controlling biological processes associated to chemotaxis and inflammation. PRP has been contemplated for the management of pain and inflammation in ailments such as osteoarthritis, sports injuries, and surgeries such as sinus lift surgery. [27] The effect of PRP was investigated by Bindal et al. [20] for their pro-angiogenic effect on HDPSCs and they reported that viability of iHDPSCs was significantly increased by treating with PRP and this was compatible with current results.

In the current work, regarding to measurement of GSH and MDA as oxidative damage parameters it was observed that PRP and propolis exerted a potent antioxidant action evidenced by the ability to significantly increase levels of

GSH in iHDPSCs treated group. Moreover, highly significant changes in MDA levels between studied groups were observed where marked decrease in iHDPSCs treated with propolis was revealed.

The results of our study, [28] were consistent with the previous research conducted by scholars, which examined the impact of Iranian propolis on the severity of postoperative peritoneal adhesion in a rat model. It was noted by the researchers that the administration of propolis led to a decrease in MDA levels, whereas there was a significant elevation in GSH levels.

It has been documented that propolis exhibited a notable impact on individuals diagnosed with type 2 diabetes mellitus in a randomized controlled trial following the ingestion of Chinese propolis. An increase in antioxidant parameters such as serum GSH was notably evident, aligning precisely with the present observations. These findings are consistent with a prior investigation on endotoxemic experimental rats, which highlighted the effectiveness of propolis pretreatment in mitigating the decline of renal GSH content and its associated enzymes. [29, 30] According to most recent study that conducted by Tognoloni et al. [31] who demonstrated that PRP reduces protein and lipid oxidative damage and protects tenocytes in tendinopathies from oxidative stress induced cell death. This study supported our findings regarding PRP effect on oxidative damage parameter.

The concentrations of proinflammatory cytokines TNF-α, IL-6, and PGE-2 exhibited a notable decrease in the iHDPSCs group that underwent intervention with PRP and propolis in the present investigation. Conversely, a significant increase in the production of IL-4 and IL-10 was noted in the treated iHDPSCs group. Additionally, our results were in line with prior studies highlighting the anti-inflammatory characteristics of propolis on raw 264.7 cell lines, thereby shedding light on its antioxidative, anti-inflammatory, and antiallergic capabilities in monocytes. [32, 33]

Moreover, a prior investigation that proposed a novel chemotherapeutic strategy involving the concurrent administration of doxorubicin and propolis to enhance monocyte functions resonated with our observations. [34] Similarly, the enhancement of innate immunity through the modulation of cell marker expression, cytokine production, and intracellular pathways in human monocytes as demonstrated by Conte et al. [35] aligns with the outcomes of the current research. Latest study by Jhang et al. [36] explored the use of PRP injections for urological disorders, evaluating the influence of cytokine concentrations in PRP on the treatment outcomes of patients with recurrent urinary tract infections and interstitial cystitis, mirroring the findings of the present study

Regarding the gene profile of AKT, p38 MAPK and Caspase-3 relevant to the cytotoxic and apoptotic effects, it was observed that these genes revealed statistically significant down regulation in iHDPSCs treated with PRP and propolis. These observations were supported by previous study [37] and another study which examined the effect of propolis in improvement of caspase-3 expression in hepatocytes in rats exposed with CCl4. [38] Effect of PRP as antiapoptotic agents which is evidenced by downregulation of caspase-3 expression in methotrexate-induced nephrotoxicity in experimental rats came in same context of the current findings. [39]

Conclusion

The current results depicted the role of propolis and PRP as an antioxidant, anti-inflammatory and anti-apoptotic agents' qualities. Higher concentration of propolis exerted much better effects.

Declarations

Conflict of Interest

Authors confirm the absence of any conflicts of interest.

Funding

This particular investigation did not obtain any financial support from funding organizations or the not-for-profit sector.

Ethics approval

The protocol for the study garnered approval from the Research Ethics Committee at the Faculty of Dentistry, October 6 University, Giza, Egypt, under the designation (RECO6U/30-2023). The study adhered to the principles outlined in the Helsinki Declaration.

Consent to Participate

All participants in the study provided written informed consent for the collection of extracted wisdom, encompassing the entirety of the procedures.

Authors' Contribution

RAA created the project, searching the data and participated in writing the manuscript. NFH interpreted the morphological investigation, analysed, and correlated the results and contributed to writing the literature. NNA doing the gene analysis, participating in writing the manuscript and searching the data. All authors have reviewed and approved the final manuscript.

Data Availability

The paper contains all the data supporting this study's findings

Table 2. Mean values and measures of variability (SD) in Oxidative assessment using One-way Anova test and Tukey's HSD calculations of significant differences for all studied groups.

	Mean ± SD of Oxidative Stress Assessment in All Studied Groups					
GSH		HDPSCs	iHDPSCs	iHDPSCs+PRP	iHDPSCs+Propolis (10ng)	iHDPSCs+Propolis (30ng)
		1.68±0.18	0.475±0.13	1.237±0.0477	0.781±0.07	1.68±0.14
	f-ratio	F = 134.652				
	p-value	< 0.00001				
		Post hoc Tukey's Significant Differences				

GSH	**Pairwise comparison**		**$HSD_{.05}$ = 0.1512 $HSD_{.01}$ = 0.1873**	**$Q_{.05}$ = 4.1534 $Q_{.01}$ = 5.1439**
	GI: GII	$M_1 = 1.68$; $M_2 = 0.48$	1.20	Q = 25.93 (p < 0.00000)
	GI: GIII	$M_1 = 1.68$; $M_3 = 1.24$	0.44	Q = 9.52 (p < 0.00000)
	GI: GIV	$M_1 = 1.68$; $M_4 = 0.80$	0.88	Q = 18.88 (p < 0.00000)
	GI: GV	$M_1 = 1.68$; $M_5 = 1.70$	0.02	Q = 0.43 (p < 0.99800)
	GII: GIII	$M_2 = 0.48$; $M_3 = 1.24$	0.76	Q = 16.41 (p < 0.00000)
	GII: GIV	$M_2 = 0.48$; $M_4 = 0.80$	0.33	Q = 7.05 (p < 0.00034)
	GII: GV	$M_2 = 0.48$; $M_5 = 1.70$	1.22	Q = 26.36 (p < 0.00000)
	GIII: GIV	$M_3 = 1.24$; $M_4 = 0.80$	0.43	Q = 9.35 (p < 0.00001)
	GIII: GV	$M_3 = 1.24$; $M_5 = 1.70$	0.46	Q = 9.95 (p < 0.00000)
	GIV: GV	$M_4 = 0.80$; $M_5 = 1.70$	0.90	Q = 19.31 (p < 0.00000)

MDA		HDPSCs	iHDPSCs	iHDPSCs+PRP	iHDPSCs+Propolis (10ng)	iHDPSCs+Propolis (30ng)
		0.45±0.07	1.528±0.052	0.938±0.174	0.94±0.089	0.60±0.048
	f-ratio	F = 152.491				
	p-value	< 0.00001				
	Post hoc Tukey's Significant Differences					

MDA	**Pairwise comparison**		**$HSD_{.05}$ = 0.1029 $HSD_{.01}$ = 0.1274**	**$Q_{.05}$ = 4.1534 $Q_{.01}$ = 5.1439**
	GI: GII	$M_1 = 0.45$; $M_2 = 1.53$	1.07	Q = 29.51 (p < 0.00000)
	GI: GIII	$M_1 = 0.45$; $M_3 = 0.94$	0.48	Q = 13.29 (p < 0.00000)
	GI: GIV	$M_1 = 0.45$; $M_4 = 1.28$	0.82	Q = 22.59 (p < 0.00000)
	GI: GV	$M_1 = 0.45$; $M_5 = 0.60$	0.15	Q = 4.04 (p < 0.05960)
	GII: GIII	$M_2 = 1.53$; $M_3 = 0.94$	0.59	Q = 16.21 (p < 0.00000)
	GII: GIV	$M_2 = 1.53$; $M_4 = 1.28$	0.25	Q = 6.92 (p < 0.00043)
	GII: GV	$M_2 = 1.53$; $M_5 = 0.60$	0.93	Q = 25.47 (p < 0.00000)
	GIII: GIV	$M_3 = 0.94$; $M_4 = 1.28$	0.34	Q = 9.29 (p < 0.00001)
	GIII: GV	$M_3 = 0.94$; $M_5 = 0.60$	0.34	Q = 9.26 (p < 0.00001)
	GIV: GV	$M_4 = 1.28$; $M_5 = 0.60$	0.68	Q = 18.55 (p < 0.00000)

Table 3. Mean values with measures of variability (SE) of inflammatory markers and mean values with measures of variability (SD) of analysed genes expression using One-way ANOVA test for all studied groups.

	Mean ± SE of Inflammatory markers						Mean ± SD of Gene Profile Fold Changes		
	TNF-α	**IL-6**	**PGE-2**	**IL-4**	**IL-10**		**AKT**	**p38 MAPK**	**Caspase-3**
HDPSCs	20.76±5.83	18.1±3.61	38.73±3.60	82.76±5.5	50.26±13.47	P < 0.00001	1.06±0.09	1.06±0.06	1.05±0.09
iHDPSCs	189.06±23.07	103.13±19.60	123.7±10.06	18.16±2.95	17.86±4.01		3.12±0.14	3.25±0.16	3.50±0.12
iHDPSCs+PRP	68.23±8.93	37 ±3.27	60.63 ±2.10	61.2 ±6.97	46.7±9.72		2.17±0.10	2.23±0.10	2.31±0.03
iHDPSCs+Propolis (10ng)	73.73±9.80	35.24±4.94	54.40±10.82	39.89±5.26	29.5±6.79		1.87±0.27	1.87±0.35	1.99±0.39
iHDPSCs+Propolis (30ng)	39.33±8.08	26.83±5.08	44.46±8.97	74.96±8.58	53.9±7.51		1.54±0.14	1.41±0.07	1.42±0.04

SD: Standard Error, SD: Standard Deviation, *The mean difference is significant at the 0.05 level

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