Original Article

The Effect of Bone Marrow Derived Mesenchymal Stem Cells Versus their Exosomes on Imiquimod-Induced Psoriasis-Like Skin Inflammation in Female Albino Rats: A Histological Study

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ABSTRACT

Introduction: Psoriasis is a chronic skin inflammatory disease. Bone marrow mesenchymal stem cells (BM-MSCs) and their derived exosomes are known for their immunomodulatory properties.

Aim of the Work: To investigate the effect of locally injected BM-MSCs and their derived exosomes in female albino rats subjected to imiquimod (IMQ)-induced psoriasis-like skin inflammation.

Materials and Methods: Fifty albino rats (40 females and 10 males) were used. The female animals were randomly classified into four groups; group I (control group), group II (IMQ group) where the rats received topical IMQ once daily for 5 consecutive days, group III (IMQ + BM-MSCs) where the rats received a dose of 1 million BM-MSCs on the first day only in addition to topical IMQ, and group IV (IMQ + BM-MSCs-derived exosomes) where the rats received purified concentrate of exosomes derived from BM-MSCs in addition to topical IMQ. The 10 male albino rats served as the source of BM-MSCs and their derived exosomes. After 5 days, all animals were sacrificed, and skin specimens were processed for light microscopic studies: H&E and immunohistochemical staining for Proliferating Cell Nuclear Antigen (PCNA) and Y-chromosome identification using Real-time PCR. Morphometric measurements and statistical analysis were done for the mean epidermal thickness, the mean count of PCNA-positive keratinocytes in the epidermis, and the mean area of dermal PCNA-positive reaction.

Results: The general observations and microscopic examination of sections obtained from group II rats revealed psoriasis-like skin inflammatory reactions including acanthosis, parakeratosis, and marked inflammatory infiltrate. There was a statistically significant increase in the epidermal thickness and PCNA-positive reactions in group II compared to other groups. Groups III and IV showed significant improvement, however, group III showed almost normal histological structure.

Conclusion: BM-MSCs and purified exosomes concentrate were shown to significantly ameliorate psoriasis-like inflammatory changes in the skin of animal models.

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Key Words: Exosomes; imiquimod; mesenchymal stem cells; psoriasis.

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INTRODUCTION

Psoriasis is a chronic, non-communicable, immune-mediated inflammatory skin disease involving mainly tissues of skin and joints. Psoriasis affects people of all ages, and in all countries. The reported prevalence of psoriasis in some countries reaches up to 11.43% of the population, making psoriasis a serious global problem with at least 100 million individuals affected worldwide. Epidemiological studies in psoriasis have reported a significantly increased risk of inflammatory co-morbid conditions including psoriatic arthritis, depression, obesity, diabetes, liver disease, metabolic syndrome, and cardiovascular diseases[1].

Imiquimod (IMQ) is a toll-like receptor agonist drug that is mostly used to treat viral skin infections as well as basal cell carcinoma. However, it was noticed that it resulted in psoriasis-like skin changes in treated patients. Furthermore, other studies have proven that IMQ produced psoriasis-like skin inflammatory changes. Thus, IMQ has been used as a standard model in the induction of psoriasis in experimental studies[2].

The main microscopic abnormalities of psoriasis include abnormal epidermal cells differentiation, proliferation, angiogenesis, and increased T-cell infiltration. Psoriasis was found to be associated with the proliferation of activated T helper cells with increased production of pro-inflammatory cytokines[3].

Psoriasis remains one of the diseases without known causes and no standard known treatment. Thus, the management of psoriasis patients is mostly dependent on topical corticosteroids and Vitamin D analogs. However, the search for a complete understanding of the pathogenesis of psoriasis as well as other modalities of treatment is still strongly recommended[4].

Mesenchymal stem cells (MSCs) can be isolated from different tissues including bone marrow, adipose tissue, and umbilical cord. MSCs isolated from bone marrow

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(BM-MSCs) were found to have immune-modulatory properties in syngeneic, allogeneic, and even xenogeneic settings. BM-MSCs play a significant role in the modulation of T cell proliferation and in diseases including Psoriasis and contact dermatitis[5].

However, Stem cells carry the potential risk of transformation with prolonged in *vitro* cultivation. Thus, according to the paracrine hypothesis, the beneficial effects of stem cell therapy are attributed to stimulation of resident cells by secretion of bioactive molecules and release of extracellular vesicles[6].

Recent studies are now focused on cell-free therapy and characterization of the BM-MSCs secretome which includes both soluble factors and extracellular vesicles as exosomes[7]. Exosomes are those extracellular vesicles with a diameter ranging from 30 - 150 nm. Exosomes are released from early endosomes and as they mature into late endosomes/multivesicular bodies, they acquire an increase in their intraluminal vesicles to be released as exosomes upon fusion with the cell membrane[8]. Exosomes secreted by various cells contain proteins, mRNAs, micro RNAs (miRNAs) and enable cell-cell communication, thus a large scale of research is being conducted to investigate its role in various diseases in different tissues[9].

Therefore, this study aimed to investigate the effect of locally injected BM-MSCs and their derived exosomes in female albino rats subjected to imiquimod (IMQ)-induced psoriasis-like skin inflammation.

MATERIALS AND METHODS

Animals

Fifty albino rats (40 females and 10 males) between 8 and 12 weeks of age (average weight 150 - 200 gm) were used in this study. The rats were raised and housed in the Medical Research Center, Ain Shams University (MASRI). The animals were treated in an environmentally controlled room with 12 hours light – 12 hours dark cycles. They were housed in plastic cages with mesh wire covers and given standard food and water. The animals were left for 1 week before any intervention to get acclimatized to the new environment. Animal use was performed in accordance with the guidelines for care, use of laboratory animals and approved by the ethical committee of Ain Shams University.

Experimental Design

The female animals were randomly classified into four groups (10 rats per each group) as follows:

Group (I) – Control group: Ten female albino rats were subdivided into two subgroups:

- Subgroup Ia: Five rats were left without treatment and served as a negative control group.
- Subgroup Ib: Five rats were injected subcutaneously with an equivalent volume of Phosphate Buffer Saline (PBS).

Group (II) – Imiquimod group: Ten female albino rats were subjected to topical imiquimod cream to induce psoriasis-like skin inflammation. The cream was applied once daily (62.5 mg, 5% cream) on a shaved part of the skin of the back (area of 2 x 2 cm) for 5 consecutive days[10].

Group (III) – Imiquimod and BM-MSCs group: Ten female albino rats were subjected to topical imiquimod cream for five days as in group II concomitant with a single subcutaneous injection of 1 million BMSCs in 1 ml PBS[11] on the first day only.

Group (IV) – Imiquimod and BM-MSCs derived exosomes group: Ten female albino rats were subjected to topical imiquimod cream for five days as in group II concomitant with a single subcutaneous injection of purified exosome concentrate collected from the conditioned medium of 1 million BMSCs in 1ml PBS on the first day only.

Sample collection

At the end of the experiment (5 days), rats were sacrificed by decapitation after ether inhalation anesthesia. Skin specimens were excised from the skin of the back. Then, the bodies of the dead animals were disposed of by incinerator.

Source of BM-MSCs and BMSCs derived exosomes

Ten male albino rats were used as a source of mesenchymal stem cells and their derived exosomes. The stem cells were isolated and cultured from the bone marrow of the animals' hind limbs according to Sangeetha *et al*[12].

Isolation and Culture of BM-MSCs

Isolation and culture of BM-MSCs were performed in Stem Cells Research Unit, Histology and Cell Biology Department, Faculty of Medicine, Ain Shams University. According to Caplan's method, ten male albino rats of average weight 100 gm were used to collect the bone marrow from both the tibia and femur[13].

Flow cytometry analysis of BM-MSCs

Trypsin harvested BM-MSCs have washed with PBS three times and aliquots of 105 cells were incubated with phycoerythrin-conjugated monoclonal antibodies directed against CD34 and CD44 (CD34: Catalog no. 11-0341-82, CD44: Catalog no. 17-0441-82, Thermo Fisher Scientific, USA) for 20 min on ice before being washed with PBS supplemented with 1% BSA three times and fixed in 1% formaldehyde overnight at 4°C. FAC Scan Flow cytometer (BD Biosciences) was used to analyze the specific surface antigen profile of the cells[14].

Isolation and Characterization of exosomes

Isolation, purification, and characterization of exosomes from conditioned media collected from cultured BM-MSCs were performed according to Lane *et al*[15]. The gold standard of exosome isolation is by the ultracentrifugation method. Characterization of exosomes was performed by

Transmission Electron Microscopy, the gold standard of exosome characterization[16].

Experimental Procedures

At the end of the experiment, the rats of all groups were sacrificed by decapitation after ether inhalation anesthesia. Skin specimens were excised from the skin of the back. Then, the bodies of the dead animals were disposed of by incinerator.

Skin specimens were fixed in 10% formal saline for 7 days and then were furtherly processed to obtain paraffin-embedded tissue blocks for light microscopic studies[17]. Paraffin sections (5 -7 µm thickness) of the skin were cut and used for the following studies:

Light Microscopic Studies - the following histological stains were used

- Harris Hematoxylin and Eosin (H&E) stain.
- Immunohistochemical staining for Proliferating cell nuclear antigen (PCNA)[17]:

Indirect Immunohistochemical staining using Avidin-Biotin immune-peroxidase technique for detection of proliferating cell nuclear antigen (PCNA) (mouse anti-rat monoclonal antibody PC10, catalog no. M0879, Dako, Denmark) was performed according to manufacturer's recommendations. The secondary antibody used was peroxidase-conjugated (goat anti-mouse) where the PCNA reaction appeared as brown nuclear staining. To confirm the specificity of the immunohistochemical staining, the negative control sections were processed by replacing the primary antibody with phosphate buffer saline then all other steps were performed in the same manner. Positive immunoreactivity for PCNA appeared as brown color in the nuclei of cells.

Identification of Y chromosome in skin tissue of female rats using Real-Time PCR

a. Total RNA extraction from formalin-fixed paraffin tissue (FFPT): Total RNA was extracted from FFPT, using the RNeasy FFPE Kit, cat no: 73504 (Qiagen, Hilden, Germany) according to the manufacturer's protocol[18].

b. Reverse Transcription: The reverse transcription step was performed by the QuantiTect Reverse Transcription Kit, cat. No: 205310, (Qiagen, Hilden, Germany) according to the manufacturer's protocol[19].

c. Y chromosome gene amplification analysis: The Y chromosome gene expression level was amplified from mRNA using10x QuantiTect Primer Assay [Rn_Arxes2], 2x QuantiTect SYBR Green PCR Master Mix, Hs_ACTB_1_SG QuantiTect Primer Assay, RNase-free water according to the manufacturer's protocol[20]. The expression levels were normalized to β-actin levels as a reference gene. The relative expression level for the Y chromosome was normalized to internal control (β-actin) and relative to calibrator (negative control sample) and was calculated using the equation 2-ΔΔCt test control according to Livak and Schmittgen[21].

Morphometric Measurements and Statistical Analysis:

Morphometric Study

Light microscope-measured parameters were performed by using an image analyzer Leica Q win V.3 program installed on a computer in the Histology and Cell Biology Department, Faculty of Medicine, Ain Shams University. The computer was connected to a Leica DM2500 microscope (Wetzlar, Germany) to measure:

a. The mean epidermal thickness of H&E-stained sections of the skin (X400).
b. The mean count of PCNA-positive keratinocytes in the epidermis in immunohistochemically stained sections of the skin (X400).
c. The mean area percentage of PCNA-positive reaction in the dermis in immunohistochemically stained sections of the skin (X200).

Measurements were taken from five different slides obtained from each animal. Five randomly selected non-overlapping fields were examined for each slide.

Statistical Analysis

Statistical analysis was performed to evaluate the obtained morphometric measurements. The data were expressed as mean ± standard deviations (SD) and tested for statistically significant differences using IBM SPSS statistics software (version 20 IBM Corporation, New York, USA). The differences in the measured data among different groups were statistically analyzed using a one-way analysis of variance (ANOVA). The probability of chance (*p-value*) is used to determine the significance of data:

- *P-value* > 0.05 is statistically insignificant.
- *P-value* ≤ 0.05 is statistically significant.

RESULTS

Isolation and Culture of BM-MSCs

Examination of the primary culture of BM-MSCs using a phase-contrast microscope showed that most cells appeared rounded in shape on day 1 (Figure 1a). On day 3, most of the attached cells showed processes, where some cells appeared spindle-shaped while others showed a star-shaped appearance (Figure 1b). On day 7 of the culture, microscopic examination showed colonies of attached cells with vesicular nuclei and interdigitating processes (Figure 1c).

Characterization of BM-MSCs

Flow cytometric analysis of cell-surface antigens of rat BM-MSCs showed a positive reaction for CD44 and a negative reaction for CD34 (Figure 2).

Characterization of Exosomes

Examination of the purified exosome samples by Transmission Electron Microscopic studies showed the presence of membrane-bounded vesicles ranging in sizes between 30 – 150 nanometers (Figure 3).

General Observation

All rats were healthy till the end of the experiment without any deaths recorded. Rats of Group I (control group) showed an apparently normal skin of the back all through the experiment (Figure 4a). Group II rats (Imiquimod group) showed psoriasis-like skin inflammation in the form of excessive scaling and redness of the skin (Figures 4b,c). In Groups III and IV, the rats showed slight scaling (Figures 4d,e).

Light Microscopic Studies

H&E-stained sections

In Group I, microscopic examination of the stained sections revealed apparently normal histological structure. The sections showed the epidermis of the skin containing all layers of keratinocytes of thin skin; the strata basale, spinosum, granulosum and corneum. The stratum basale is formed of a single layer of columnar keratinocytes resting on a regular basement membrane. The stratum spinosum layer showed multiple layers of polygonal keratinocytes. The stratum granulosum layer is formed of one or two layers of flattened keratinocytes containing the characteristic basophilic keratohyalin granules. Lastly, the stratum corneum layer formed of multiple layers of acidophilic keratinized cells. The dermis showed apparently normal hair follicles, sebaceous glands, and blood capillaries. The epidermal basement membrane appeared intact throughout the sections and flattened except at the sites of hair follicles (Figures 5a,b,c).

Microscopic examination of sections obtained from Group II rats showed apparently thickened epidermis (acanthosis) with a prominent increase in thickness of the stratum corneum layer (hyperkeratosis) which was confirmed by morphometric measurement and statistical analysis of the mean epidermal thickness showing a significant marked increase in group II compared to other groups (120.17 ± 39.19 µm, $P<0.05$). The examined sections also showed abnormal wide spaces between the keratinocytes. The nuclei of Keratinocytes appeared eccentric and flattened. There were abnormally retained keratohyalin granules in the stratum corneum layer. Also, there was a prominent infiltration of inflammatory cells occupying both layers of the dermis reaching the upper layers of the epidermis as well. The basement membrane showed irregularities with indistinct boundaries between the dermis and the epidermis in some areas. The dermis showed densely packed hair follicles with an apparent increase in their number compared to the control group. The hair follicle sheaths showed an abnormal increase in the layers of the covering epidermal cells with homogenous acidophilic cytoplasm. There were abnormally dilated blood capillaries throughout the sections of the psoriasis group as well (Figures 5d,e,f) (Table 1).

Sections obtained from Group III rats showed that most of the epidermis and keratinocytes appeared nearly similar to the control group. The mean epidermal thickness showed a significant decrease compared to group II (34.88 ± 4.56 µm, $P<0.05$). There was little separation between some keratinocytes that appeared in a few of the examined sections as well as some cells showed a vacuolated appearance. The keratohyalin granules appeared similar to the control group. The basement membrane appeared regular, and the dermis showed mild inflammatory cellular infiltration in some of the examined sections. The histological structure of the hair follicles appeared similar to the control group (Figures 5g,h,i) (Table 1).

Microscopic examination of the sections obtained from Group IV rats showed epidermal thickness and stratum corneum appeared nearly similar to the control group except for some areas where the epidermal thickness was mildly increased (mild acanthosis). The mean epidermal thickness showed a significant decrease compared to group II (52.34 ± 4.15 µm, $P<0.05$). Most Keratinocytes showed normal histological structure however, some areas showed vacuolar changes. The basement membrane appeared regular in most of the sections. The dermis showed hair follicles and sebaceous glands nearly similar to that in the control group. However, some areas showed mild inflammatory cellular infiltration (Figures 5j,k,l) (Table 1).

Immunohistochemically stained sections for PCNA

Group I showed PCNA-positive brownish reaction in the stratum basale of the epidermis of the skin and in the external root sheaths of the dermal hair follicles (Figures 6a,b). However, sections examined from group II showed a prominent increase in PCNA-positive reaction in the stratum basale reaching up to the upper layers of the epidermis which was confirmed by morphometric measurements and the statistical analysis of the mean count of PCNA-positive keratinocytes revealing a significant increase compared to the other groups (107.4 ± 3.77, $P<0.05$). There was also an apparent increase in the reactivity of root sheaths cells of the dermal hair follicles and in the cells occupying the interfollicular tissues. This was also confirmed by morphometric measurements and the statistical analysis of the mean area percentage of PCNA-positive reactions revealing a significant increase compared to the other groups (13 ± 1.11, $P<0.05$) (Figures 6c,d). Sections obtained from groups III and IV showed a significant decrease ($P<0.05$) in the mean count of PCNA positive cells in the epidermis (29.9 ± 1.96) (37.4 ± 2.06) respectively and a significant decrease in the mean area percentage of PCNA-positive reaction in the dermis and around hair follicles (2.08 ± 0.23) (3.88 ± 0.66) respectively compared to group II. (Figures 6e,f and Table 1).

Identification of Y chromosome in skin tissue of female rats using Real-Time PCR

There was an upregulation in the expression levels for the Y-chromosome (8-fold change) when normalized to an internal control sample (β-actin) and the sample obtained from the control group (Figure 7).

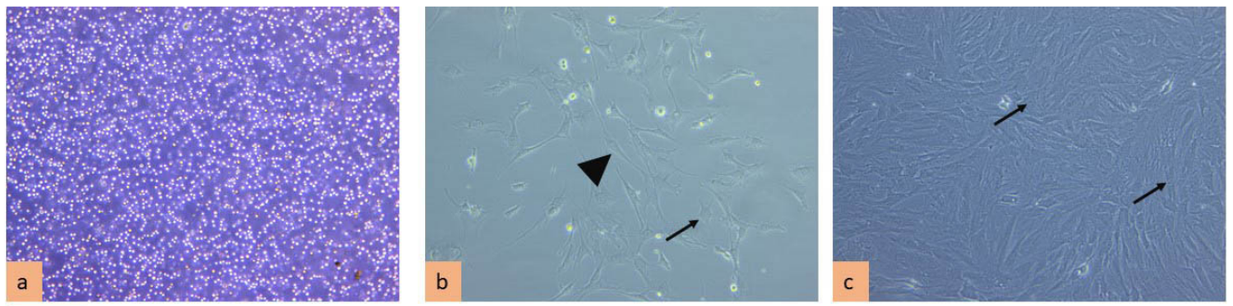

Fig. 1

Fig. 1: Primary culture of BM-MSCs showing: (a) Most cells appeared rounded in shape on day 1. (b) On day 3, most of the attached cells showed processes, where some cells appeared spindle-shaped (▲) while others showed a star-shaped appearance (↑). (c) On day 7 of the culture colonies of attached cells appeared with vesicular nuclei (↑) and interdigitating processes. [Phase contrast, Fig. 1a x100 and Figs. 1b&c x200]

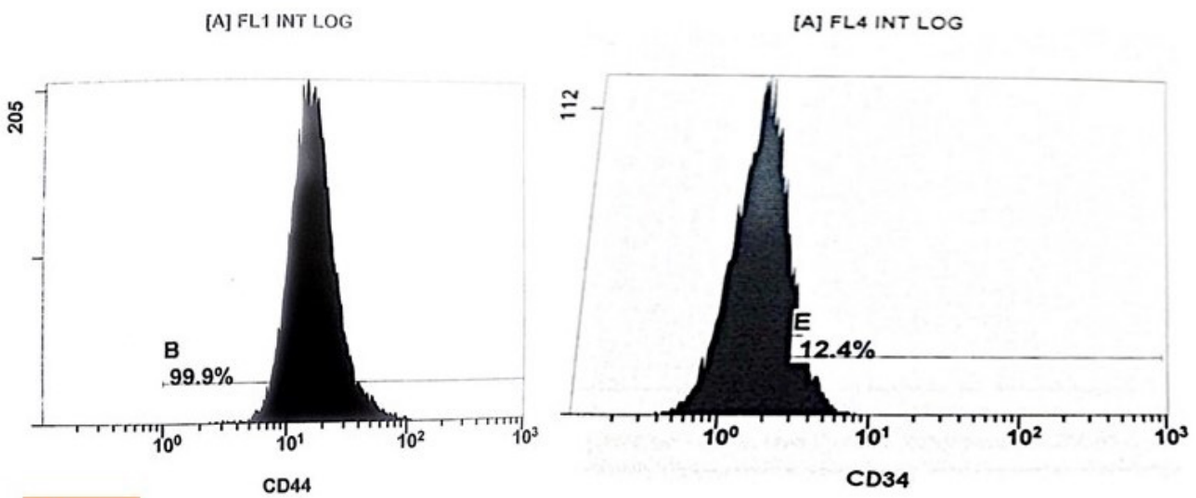

Fig. 2

Fig. 2: Flowcytometric analysis of cell-surface antigens of rat BM-MSCs showing positive reaction for CD44 and negative reaction for CD34.

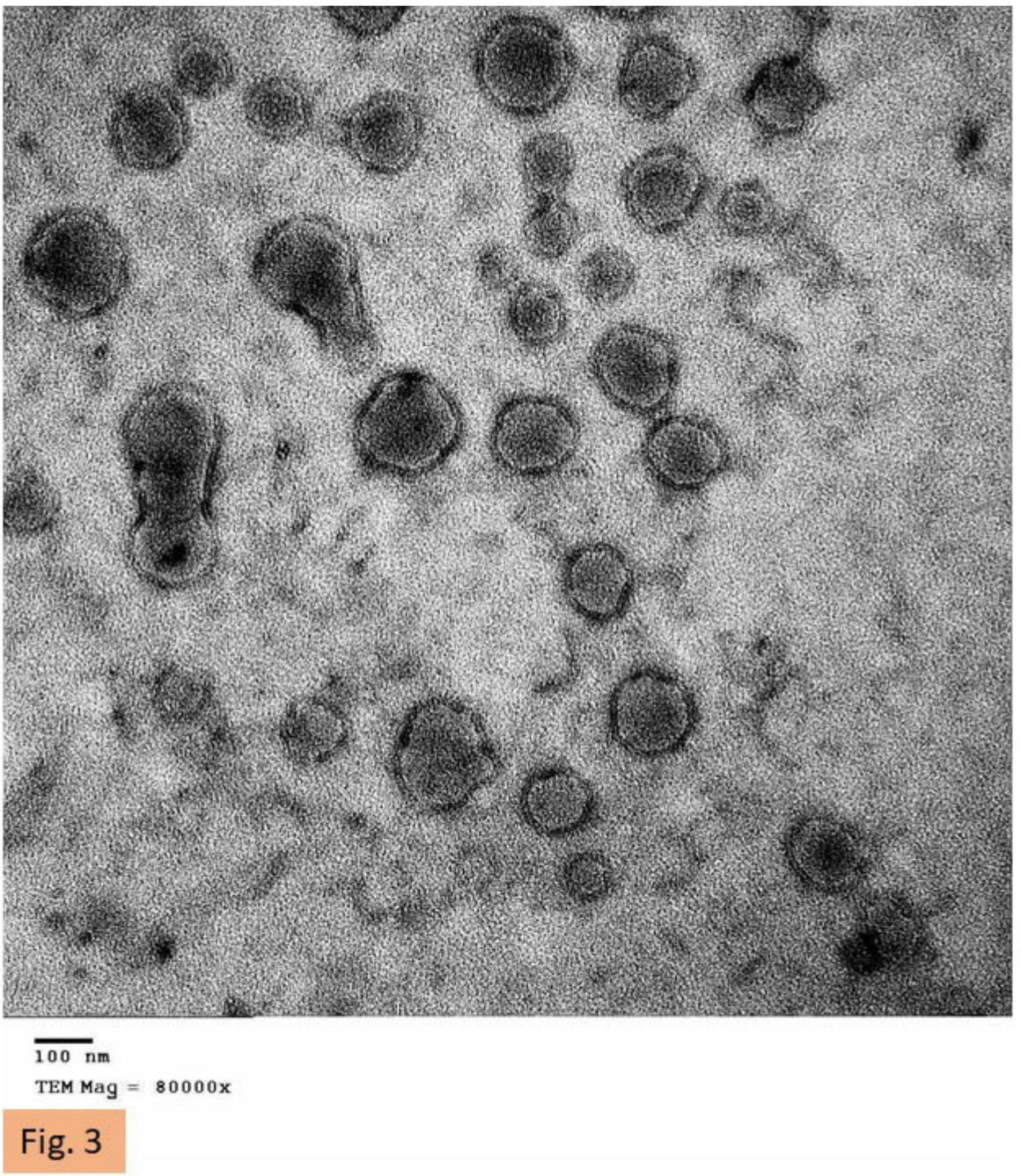

Fig.3: Photomicrograph of purified exosome by TEM showing the presence of membrane-bounded vesicles ranging in sizes between 30 – 150 nm (TEM, x80,000)

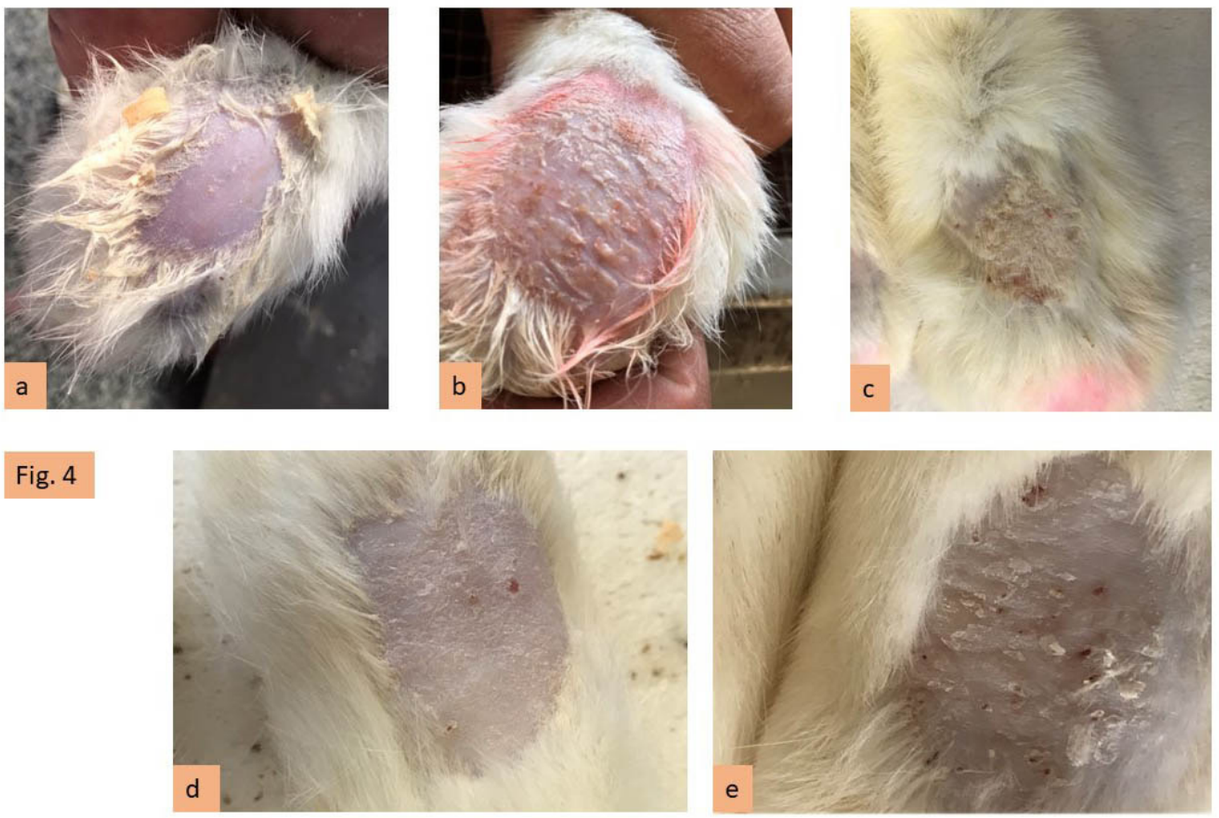

Fig. 4

Fig. 4: Macroscopic picture of the rats' skin of the back after 5 days. (a) Group I shows apparently normal back skin. (b & c) Group II shows Psoriasis-like skin inflammation in the form of excessive scaling and redness. (d & e) Group III and Group IV show slight scaling.

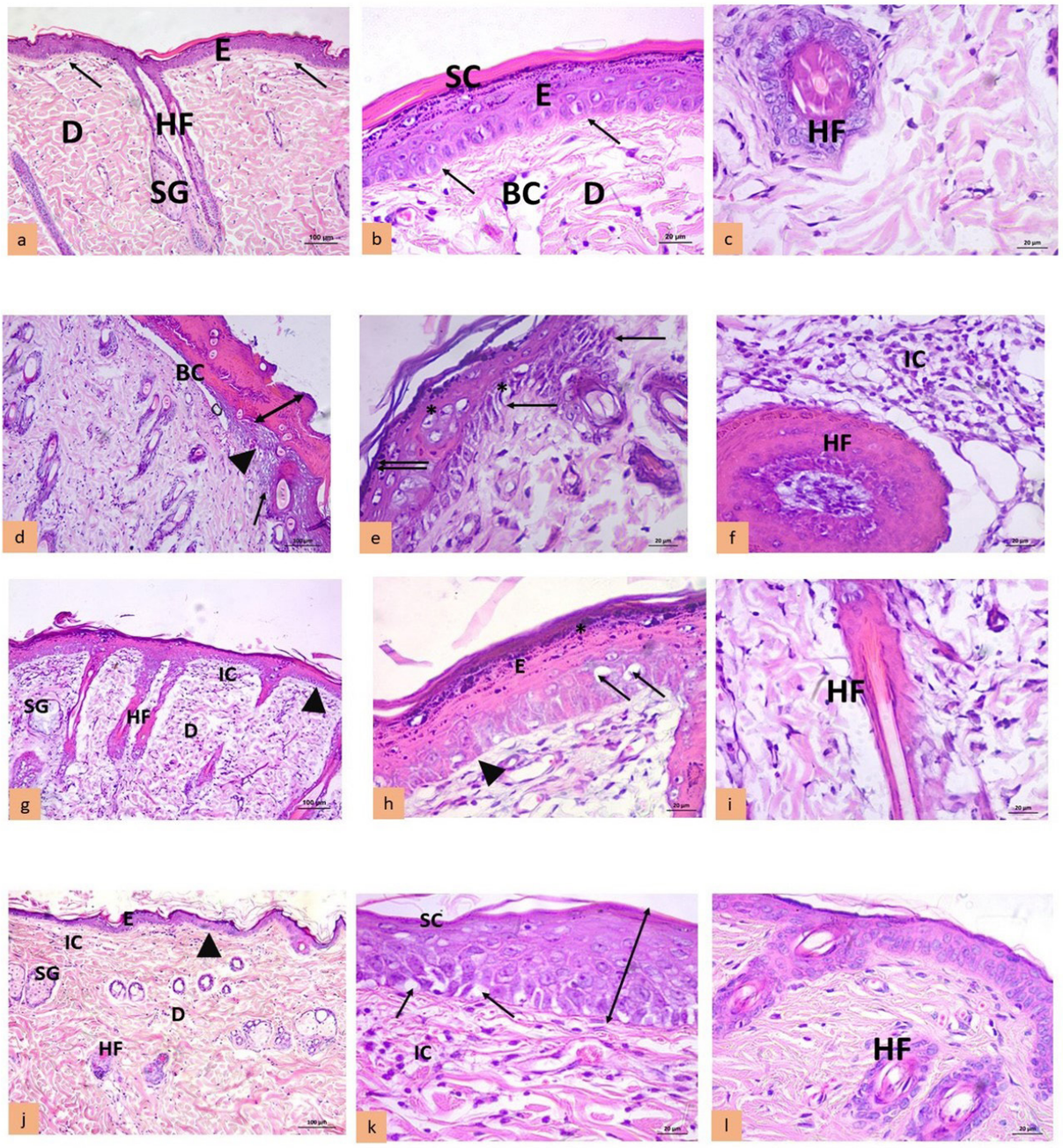

Fig. 5

Fig. 5: Photomicrographs of H&E-stained sections of the skin: (a, b & c) Group I shows the epidermis (E), stratum corneum layer (SC), regular basement membrane (↑) and dermis (D) containing hair follicles (HF), sebaceous glands (SG) and blood capillaries (BC). (d, e & f) Group II shows a marked increase in the thickness of the epidermis (▲) and stratum corneum layer (↕). Most of the keratinocytes showed eccentric and flattened nuclei as well as widening and separation between them (*). There were abnormally retained keratohyalin granules in the stratum corneum layer (↑↑). The basement membrane appeared irregular with indistinct boundaries between the dermis and the epidermis in some areas (↑). The dermis showed inflammatory cell infiltrate (IC) and densely packed hair follicles with an apparent increase in the layers of the covering epidermal cells showing homogenous acidophilic cytoplasm (HF) as well as dilated blood capillaries (BC). (g, h & i) Group III shows most of the epidermis (E) and dermis (D) are nearly similar to the control group. However, few keratinocytes show vacuolated appearance (↑). The keratohyalin granules appeared similar to the control group (*). The dermis shows hair follicles (HF) and sebaceous glands (SG) with mild inflammatory cell infiltrate (IC). The basement is regular in most of the examined sections (▲). (j, k & l) Group IV shows epidermis (E) and stratum corneum (SC) appeared nearly similar to the control group except focal areas showing an increase in the epidermal thickness (↕). Some areas showed vacuolated keratinocytes (↑). The basement membrane appeared regular in most of the sections (▲). The dermis (D) showed hair follicles (HF) and sebaceous glands (SG) nearly similar to that in the control group. However, some areas showed inflammatory cells infiltration (IC). [H&E, Figs. 5a,d,g&j x100, Figs. 5b,c,e,f,h,i,k,&l x400].

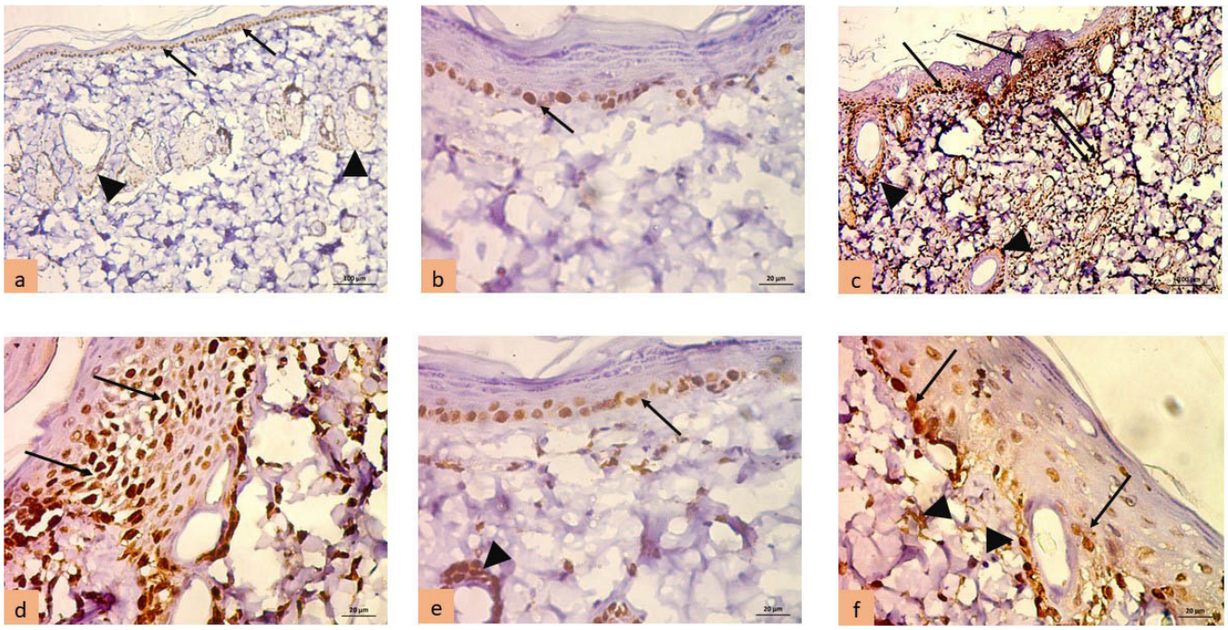

Fig. 6

Fig. 6: Photomicrographs of immunohistochemically stained sections of skin for PCNA: (a & b) Group I shows PCNA-positive reaction in the stratum basale of the epidermis of the skin (↑) and in the external root sheaths of the dermal hair follicles (▲). (c & d) Group II shows numerous cells with PCNA-positive reaction in the stratum basale reaching up to the upper layers of the epidermis (↑) and apparent increase in the reactivity of root sheaths cells of the dermal hair follicles (▲) and in the cells occupying the interfollicular tissues (↑↑). (e) Groups III PCNA positive reaction in the cells of stratum basale (↑) and around hair follicles (▲). (f) Group IV some areas show PCNA-positive reaction in some keratinocytes (↑) and in the dermis and around hair follicles (▲). [Immunoperoxidase staining, Figs. 6a&c x100 and Figs. 6b,d,e,f x400]

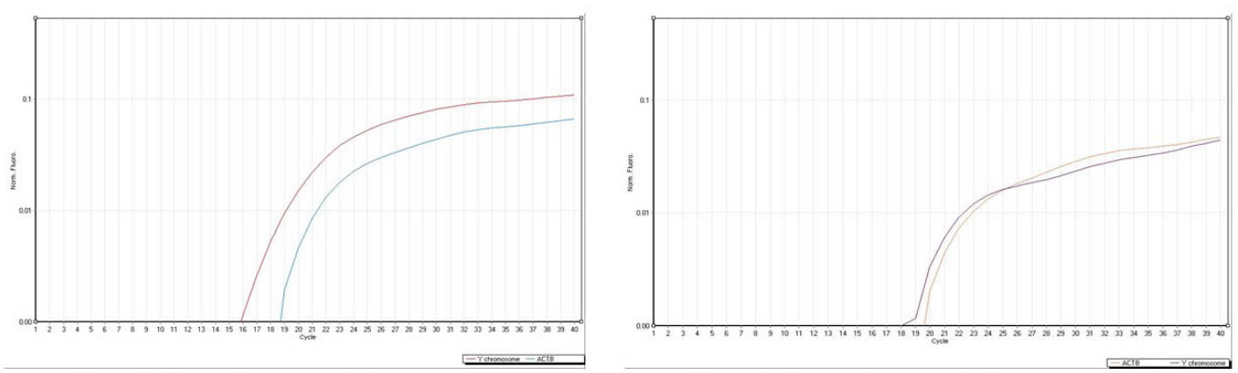

Fig. 7

Fig. 7: Showing an 8-fold change (upregulation) in the amplification plot illustrating expression of Y-chromosome genome between group I and group III.

Table 1: Mean values of the epidermal thickness, count of PCNA +ve keratinocytes, area percentage of PCNA +ve reaction in the dermis

	Group I Control	Group II Imiquimod	Group III Imiquimod + MSCs	Group IV Imiquimod + Exosomes
Mean epidermal thickness length (µm)	15.75 ± 1.98	120.17 ± 39.19*	34.88 ± 4.56▲	52.34 ± 4.15▲♦
Mean count of PCNA-positive keratinocytes	30.2 ± 1.68	107.4 ± 3.77*	29.9 ± 1.96	37.4 ± 2.06▲°
Mean area % of PCNA-positive reaction in the dermis	1.85 ± 0.48	13 ± 1.11*	2.08 ± 0.23	3.88 ± 0.66▲°

Statistical significance ($p<0.05$):

- * Significant difference compared to other groups.
- ▲ Significant difference compared to the control group.
- ° Significant difference compared to group III.
- ♦ Non-Significant difference compared to group III.

DISCUSSION

In recent years, a lot of research has been conducted to understand the pathogenesis of psoriasis and explore new modalities of treatment aiming at ameliorating disease comorbidities and enhancing the patient's quality of life[22].

In the present study, imiquimod was used to induce psoriasis-like skin inflammatory changes. Imiquimod has become one of the most commonly used drugs to initiate and induce psoriatic skin changes in the skin of animal models[23]. It has been proven to be convenient, easily applied on shaved skin areas, relatively inexpensive, and can produce reliable results in short time periods[3].

The general observations and microscopic examination of H&E-stained sections of group II rats have shown results that are consistent with the psoriasis-like inflammatory changes of the skin. General observations have shown inflammatory changes in the form of excessive scaling and redness of the skin known as erythema. These findings were consistent with Lee *et al*[10]. Microscopic examination revealed inflammatory reactions and abnormal epidermal and dermal changes. There was a statistically significant increase ($P < 0.05$) in the thickness of the epidermis (acanthosis) with an increased number of layers in each stratum especially the stratum corneum (hyperkeratosis) compared to other groups. These findings were concomitant with Burger *et al*[24]. These changes can be attributed to the imbalance that results in normal epidermal cell proliferation and differentiation that can be greatly affected in psoriasis. Moreover, it can be attributed to the abnormal increase in activated T lymphocytes with the subsequent abundance of proinflammatory cytokines pooling in the affected areas resulting in an abnormal increase in epidermal cell proliferation[25]. This explanation can be further supported by the immunohistochemically stained sections for PCNA revealing a significant increase ($P < 0.05$), as compared to other groups, in the mean count of PCNA-positive nuclear brownish reaction in the epidermal keratinocytes, even reaching up to the upper layers of the epidermis, and in the mean area percentage of PCNA-positive nuclear brownish reaction in the dermis denoting increased proliferative activity. Other studies have also mentioned that these findings represent a typical finding of psoriatic skin changes and are considered the cause of excessive scaling of the skin[3].

The presence of the vacuolated appearance of keratinocytes has been reported in previous studies following imiquimod application on the skin of animal models[26]. Some studies have suggested that these changes represent an important initial step for the induction of psoriasis-like inflammatory changes resulting from the fact that these necrotic changes result in the production of damage-associated molecular patterns thus triggering an immune response[27]. These studies have also reported the occurrence of abundant keratohyalin granules as well as separation and widening between keratinocytes attributed to the ongoing inflammatory reaction with abnormal proliferation and differentiation of keratinocytes. In addition, other studies have reported that the widening and separation between keratinocytes resulted from the reduction of desmosome junctions and proteins resulting in defective cell-cell adhesion[3]. These studies have also confirmed the occurrence of basement membrane irregularities along the dermo-epidermal border which was consistent with our results. This was attributed to the abundant infiltration of lymphocytes and neutrophils in the affected areas with the subsequent release of proteolytic enzymes that disrupt collagen type IV and laminins which are essential components in the structure of the basement membrane.

Microscopic examination has also revealed prominent infiltration of inflammatory cells in the dermis and epidermis of the affected areas. These findings were in accordance with Kim *et al*[28]. They reported in their study the presence of T lymphocytes, Dendritic Cells (DC), and Macrophages in the affected psoriatic plaques. In addition, mast cells were also found to be infiltrating psoriatic plaques which are attracted to the site of inflammation by the proinflammatory cytokines released by inhabitant keratinocytes according to Abdelnoor and Al-Akl[29]. It has also been shown that imiquimod resulted in an abnormal regulation in the IL-17/IL-23 axis with subsequent upregulation of IL-17 and IL-23 which resembles the imbalance occurring in psoriasis patients[30]. In addition, DC have been shown to release IL-23 resulting in the stimulation of T helper-17 cells to produce significant amounts of proinflammatory cytokines including IL-17 and IL-22 leading to hyperproliferation of keratinocytes[31]. Furthermore, Alghandour *et al*[3] reported in their study increased production of pro-inflammatory factors like

antimicrobial peptides that further augment the psoriatic inflammatory process, an important component of these peptides is the S100 proteins.

Histological examination of group II sections also revealed abundant keratohyalin granules in the stratum corneum of the epidermal layers. These results were in accordance with Cardoso *et al*[32]. The authors referred in their study that abnormal retention of nuclei and keratohyalin granules in stratum corneum can be explained by the defective keratinization process and the failure of cleavage of profilaggrin into filaggrin resulting in abnormal accumulation of keratohyalin granules in the upper epidermal cells. The present study has also shown abnormal hyperplastic changes in the dermal follicles as well as sebaceous glands being densely packed together. These findings are mostly due to the effect of topical imiquimod application and the time period of applying the drug on the shaved areas of the skin in relation to the hair cycle. Amberg *et al*[33] concluded that applying imiquimod in different time periods of the hair cycles induced various changes that seemed to be paradoxical as it induced hair cycle entry in some cases while surprisingly led to precocious anagen induction in other cases. The apparently paradoxical effect of imiquimod was attributed to the type of macrophage resident in the applied areas where proinflammatory M1 macrophages resulted in hair cycle entry due to the production of inflammatory cytokines while M2 macrophages, also known as resident macrophages, produce immunomodulatory cytokines resulting in precocious anagen induction.

In group III, aside from imiquimod, the rats were injected with a dose of one million bone marrow-derived mesenchymal stem cells (BM-MSCs) on the first day. The female rats were injected with BM-MSCs from male animals which were visualized by Real-Time PCR analysis of the specimens to confirm homing of stem cells. The analysis showed positive detection of the Y chromosome genome in the amplification plot confirming the homing of the injected stem cells. The general observations and microscopic examination of the stained sections revealed significantly reduced inflammatory changes. The structure of the epidermis and dermis appeared histologically nearly similar to the control group. These results were consistent with Owczarczyk-Saczonek *et al*[4] and Parganelli[34]. The authors mentioned that BM-MSCs possess immunomodulatory properties that have proven to be of value as potential therapeutic agents in autoimmune diseases. Recent studies have demonstrated the immunomodulatory effects of BM-MSCs as potential therapeutic agents in autoimmune diseases including Psoriasis. The results have shown that BM-MSCs secrete various cytokines that shift T lymphocyte populations from effector T cells to regulatory T cells. These cytokines include transforming growth factor-beta (TGF-β), hepatocyte growth factor (HGF), prostaglandin E2 (PGE2), nitric oxide (NO), and indoleamine 2,3-dioxygenase (IDO). In addition, cell-cell contact was involved in the downregulation of activating Natural Killer (NK) cells receptors resulting in immunomodulation of NK cells. Furthermore, the results have also shown that BM-MSCs, through the action of various paracrine factors like IL-6, PGE2, growth-regulated oncogene (GRO)-γ as well as cell-cell contact through Jagged-2, have suppressed the maturation of Dendritic cells (DC) and lead to the maturation of regulatory DC[35].

However, recent studies have recommended that MSCs therapy carries serious potential risks including graft-versus-host diseases, localized cutaneous reactions, lack of efficacy, and neoplastic proliferation which varies according to the method of administration and the type of MSCs used. Thus, an assessment of the potential risks and benefits should be done before considering MSCs a potential therapeutic choice[34]. Therefore, in the current study, we included a fourth group of rats that received imiquimod as in group II with the additional single dose of purified exosomes. The general observations and microscopic examination of the stained sections revealed reduced inflammatory changes similar to group III despite the presence of some areas showing mild inflammatory infiltrate with the vacuolated appearance of some areas of keratinocytes. The results were consistent with recent studies that confirmed the ameliorative effect of exosomes in enhancing psoriatic inflammatory changes[36]. The authors attributed the results to the fact that exosomes are considered a major part of the paracrine effect of MSCs inducing the same immunomodulatory effects. However, the differences in the efficacy of the applied exosomes, as in group IV, from the efficacy of the directly injected BM-MSCs, as in group III, can be attributed to several factors. Recent studies have mentioned that the immunomodulatory effect of BM-MSCs results not just from its paracrine effect, but also from the behavior of BM-MSCs in areas showing inflammatory reactions. BM-MSCs were shown to increase the secretion of its paracrine factors in tissues hosting increased pro-inflammatory cytokines[9].

CONCLUSION & RECOMMENDATION

The present work demonstrates that BM-MSCs and purified exosomes concentrate significantly ameliorate psoriasis-like inflammatory changes in the skin of animal models. However, there was a slight improvement in the BM-MSCs group. It is recommended that further studies are needed to assess increased concentrations of the applied exosomes and to assess the exosome yield of BM-MSCs cultured in *vitro* in cultured media containing pro-inflammatory cytokines.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Griffiths, C. E. M., van der Walt, J. M., Ashcroft, D. M., Flohr, C., Naldi, L., Nijsten, T., & Augustin, M. (2017). The global state of psoriasis disease epidemiology: a workshop report. Br J Dermatol, 177(1), e4-e7. doi: 10.1111/bjd.15610

2. OuYang, Q., Pan, Y., Luo, H., Xuan, C., Liu, J., & Liu, J. (2016). MAD ointment ameliorates Imiquimodinduced psoriasiform dermatitis by inhibiting the IL-23/IL-17 axis in mice. Int Immunopharmacol, 39, 369-376. doi: 10.1016/j.intimp.2016.08.013

3. Alghandour, Sarah, Sabry, Marwa, & Abdel Mohsen, Manal. (2019). Comparative Histological Study on the Preventive Role of Acarbose and Calcipotriol/ Betamethasone in Mouse Model of Psoriasis-Like Skin Inflammation Induced by Imiquimod. Egyptian Journal of Histology, 0(0), 0-0. doi: 10.21608/ejh.2019.13868.1132

4. Owczarczyk-Saczonek, A., Krajewska-Włodarczyk, M., Kruszewska, A., Placek, W., Maksymowicz, W., & Wojtkiewicz, J. (2017). Stem Cells as Potential Candidates for Psoriasis Cell-Replacement Therapy. Int J Mol Sci, 18(10). doi: 10.3390/ijms18102182

5. Nemeth, K., & Mezey, E. (2015). Bone marrow stromal cells as immunomodulators. A primer for dermatologists. J Dermatol Sci, 77(1), 11-20. doi: 10.1016/j.jdermsci.2014.10.004

6. Gomzikova, Marina O., & Rizvanov, Albert A. (2017). Current Trends in Regenerative Medicine: From Cell to Cell-Free Therapy. BioNanoScience, 7(1), 240-245. doi: 10.1007/s12668-016-0348-0

7. Rad, Fariba, Ghorbani, Mohammad, Mohammadi Roushandeh, Amaneh, & Habibi Roudkenar, Mehryar. (2019). Mesenchymal stem cell-based therapy for autoimmune diseases: emerging roles of extracellular vesicles. Molecular Biology Reports, 46(1), 1533-1549. doi: 10.1007/s11033-019-04588-y

8. Doyle, Laura M., & Wang, Michael Zhuo. (2019). Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. Cells, 8(7), 727. doi: 10.3390/cells8070727

9. Alvites, Rui, Branquinho, Mariana, Sousa, Ana C., Lopes, Bruna, Sousa, Patrícia, & Maurício, Ana Colette. (2022). Mesenchymal Stem/Stromal Cells and Their Paracrine Activity—Immunomodulation Mechanisms and How to Influence the Therapeutic Potential. Pharmaceutics, 14(2), 381.

10. Lee, Y. S., Sah, S. K., Lee, J. H., Seo, K. W., Kang, K. S., & Kim, T. Y. (2017). Human umbilical cord bloodderived mesenchymal stem cells ameliorate psoriasislike skin inflammation in mice. Biochem Biophys Rep, 9, 281-288. doi: 10.1016/j.bbrep.2016.10.002

11. Liu, Zhen, Hu, Guo-Dong, Luo, Xiao-Bo, Yin, Bin, Shu, Bin, Guan, Jing-Zhi, & Jia, Chi-Yu. (2017). Potential of bone marrow mesenchymal stem cells in rejuvenation of the aged skin of rats. Biomedical reports, 6(3), 279-284. doi: 10.3892/br.2017.842

12. Sangeetha, Palakkara, MaitiS., K., Mohan, Divya S., Shivaraju, S., Raguvaran, R., Rafee, Malik Abu, Pds, Raguvanshi. (2017). Mesenchymal stem cells derived from rat bone marrow (rbm msc): techniques for isolation, expansion and differentiation. Journal of Stem Cell Research & Therapeutics, 2017;3(3):272-277. doi: 10.15406/jsrt.2017.03.00101

13. Lennon, D. P., & Caplan, A. I. (2006). Isolation of human marrow-derived mesenchymal stem cells. Exp Hematol, 34(11), 1604-1605. doi: 10.1016/j.exphem.2006.07.014

14. Baher, Walaa, AboZeid, Asmaa, & Abd Al-Khalek, Hadwa. (2019). The Possible Protective and Therapeutic Effects of Mesenchymal Stem Cells Compared to Vitamin C in Gentamicin Induced Acute Kidney Injury in adult Rats. A Histological Study. Egyptian Journal of Histology, 42(4), 783-797. doi: 10.21608/ejh.2019.11680.1111

15. Lane, R. E., Korbie, D., Trau, M., & Hill, M. M. (2017). Purification Protocols for Extracellular Vesicles. Methods Mol Biol, 1660, 111-130. doi: 10.1007/978-1-4939-7253-1_10

16. Chuo, Steven Ting-Yu, Chien, Jasper Che-Yung, & Lai, Charles Pin-Kuang. (2018). Imaging extracellular vesicles: current and emerging methods. Journal of Biomedical Science, 25(1), 91. doi: 10.1186/s12929-018-0494-5

17. Survarna SK, Layton C and Bancroft JD (2013). bancroft's theory and practice of histological techniques, 7th edition, Churchill Livingstone, El Sevier 2013: 224, 408.

18. Qiagen (Producer). (2021). RNeasy® FFPE Handbook. Retrieved from https://www.qiagen.com/us/resources/download.aspx?id=14e7cf6e-521a-4cf7-8cbc-bf9f6fa33e24&lang=en

19. Qiagen (Producer). (2009). QuantiTect® Reverse Transcription Handbook. Retrieved from https://www.qiagen.com/us/resources/download.aspx?id=f0de5533-3dd1-4835-8820-1f5c088dd800&lang=en

20. Qiagen (Producer). (2011). QuantiTect® Primer Assay Handbook. Retrieved from https://www.qiagen.com/cn/resources/download.aspx?id=882a8baa-29df-4182-a2a5-17083f4dbe11&lang=en

21. Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods, 25(4), 402-408. doi: 10.1006/meth.2001.1262

22. Rendon, Adriana, & Schäkel, Knut. (2019). Psoriasis Pathogenesis and Treatment. International journal of molecular sciences, 20(6), 1475. doi: 10.3390/ijms20061475

23. van der Fits, L., Mourits, S., Voerman, J. S., Kant, M., Boon, L., Laman, J. D., Lubberts, E. (2009). Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. J Immunol, 182(9), 5836-5845. doi: 10.4049/jimmunol.0802999

24. Bürger, C., Shirsath, N., Lang, V., Diehl, S., Kaufmann, R., Weigert, A., Wolf, P. (2017). Blocking mTOR Signalling with Rapamycin Ameliorates Imiquimodinduced Psoriasis in Mice. Acta Derm Venereol, 97(9), 1087-1094. doi: 10.2340/00015555-2724

25. Lin, Z. M., Ma, M., Li, H., Qi, Q., Liu, Y. T., Yan, Y. X., Zuo, J. P. (2018). Topical administration of reversible SAHH inhibitor ameliorates imiquimod-induced psoriasis-like skin lesions in mice via suppression of TNF-α/IFN-γ-induced inflammatory response in keratinocytes and T cell-derived IL-17. Pharmacol Res, 129, 443-452. doi: 10.1016/j.phrs.2017.11.012

26. Okasha, E. F., Bayomy, N. A., & Abdelaziz, E. Z. (2018). Effect of Topical Application of Black Seed Oil on Imiquimod-Induced Psoriasis-like Lesions in the Thin Skin of Adult Male Albino Rats. Anat Rec (Hoboken), 301(1), 166-174. doi: 10.1002/ar.23690

27. Alrefai, Hani, Muhammad, Khalid, Rudolf, Ronald, Pham, Duong Anh Thuy, Klein-Hessling, Stefan, Patra, Amiya K., Serfling, Edgar. (2016). NFATc1 supports imiquimod-induced skin inflammation by suppressing IL-10 synthesis in B cells. Nature Communications, 7(1), 11724. doi: 10.1038/ncomms11724

28. Kim, C. H., Yoo, J. K., Jeon, S. H., Lim, C. Y., Lee, J. H., Koo, D. B., & Park, M. Y. (2019). Antipsoriatic effect of myeloid-derived suppressor cells on imiquimod-induced skin inflammation in mice. Scand J Immunol, 89(3), e12742. doi: 10.1111/sji.12742

29. Abdelnoor, Alexander, & Akl, Nayla. (2013). Factors involved in the pathogenesis of psoriasis. Advanced Studies in Medical Sciences, 1, 75-94. doi: 10.12988/asms.2013.13008

30. Suzuki, T., Tatsuno, K., Ito, T., Sakabe, J. I., Funakoshi, A., & Tokura, Y. (2016). Distinctive downmodulation of plasmacytoid dendritic cell functions by vitamin D3 analogue calcipotriol. J Dermatol Sci, 84(1), 71-79. doi: 10.1016/j.jdermsci.2016.06.003

31. Nakajima, K., & Sano, S. (2018). Mouse models of psoriasis and their relevance. J Dermatol, 45(3), 252-263. doi: 10.1111/1346-8138.14112

32. Cardoso, José Carlos, Veraitch, Ophelia, Gianotti, Raffaele, Ferrara, Gerardo, Tomasini, Carlo F., Singh, Manuraj, Stefanato, Catherine M. (2017). 'Hints' in the horn: diagnostic clues in the stratum corneum. Journal of Cutaneous Pathology, 44(3), 256-278. doi: https://doi.org/10.1111/cup.12839

33. Amberg, N., Holcmann, M., Stulnig, G., & Sibilia, M. (2016). Effects of Imiquimod on Hair Follicle Stem Cells and Hair Cycle Progression. J Invest Dermatol, 136(11), 2140-2149. doi: 10.1016/j.jid.2016.06.613

34. Paganelli, A., Tarentini, E., Benassi, L., Kaleci, S., & Magnoni, C. (2020). Mesenchymal stem cells for the treatment of psoriasis: a comprehensive review. Clin Exp Dermatol, 45(7), 824-830. doi: 10.1111/ced.14269

35. Wang, Li-Tzu, Liu, Ko-Jiunn, Sytwu, Huey-Kang, Yen, Men-Luh, & Yen, B. Linju. (2021). Advances in mesenchymal stem cell therapy for immune and inflammatory diseases: Use of cell-free products and human pluripotent stem cell-derived mesenchymal stem cells. STEM CELLS Translational Medicine, 10(9), 1288-1303. doi: https://doi.org/10.1002/sctm.21-0021

36. Zhang, B., Lai, R. C., Sim, W. K., Choo, A. B. H., Lane, E. B., & Lim, S. K. (2021). Topical Application of Mesenchymal Stem Cell Exosomes Alleviates the Imiquimod Induced Psoriasis-Like Inflammation. Int J Mol Sci, 22(2). doi: 10.3390/ijms22020720

الملخص العربى

تأثير الخلايا الجذعية الوسيطة المستمدة من نخاع العظم مقابل الإكزوسومات الخاصة بها على الإلتهاب الجلدى الشبيه بالصدفية المستحث بالإيميكويمود في إناث الجرذان البيضاء. دراسة هستولوجية

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المقدمة: تعتبر الصدفية مرض التهابي مزمن يصيب الجلد والأنسجة الأخرى. تُعرف الخلايا الجذعية الوسيطة المستمدة من نخاع العظم وعوامل الباراكرين المشتقة منها بخصائصها المثبطة للمناعة.

الهدف من العمل: التحقيق من تأثير الخلايا الجذعية الوسيطة والإكزوسومات المشتقة منها و التى تم حقنها موضعيا في إناث الجرذان البيضاء المعرضة لالتهاب جلدي يشبه الصدفية و الناجم عن استخدام كريم إيميكويمود (IMQ).

المواد والطرق: تم استخدام خمسين من الجرذان البيضاء (٤٠ إناث و ١٠ ذكور). تم تصنيف إناث الحيوانات بشكل عشوائي إلى أربع مجموعات. المجموعة الأولى (المجموعة الضابطة) ، المجموعة الثانية (مجموعة IMQ) حيث تلقت الفئران كريم IMQ الموضعي على منطقة تم حلاقتها من جلد الظهر مرة واحدة يوميًا لمدة ٥ أيام متتالية ، المجموعة الثالثة (IMQ + BM-MSCs) حيث تلقت الفئران جرعة من ١ مليون BM-MSCs في اليوم الأول فقط بالإضافة إلى IMQ الموضعي كما في المجموعة الثانية ، وأخيرًا المجموعة الرابعة الإكزوسومات المشتقة من الخلايا الجذعية الوسيطة IMQ +) حيث تلقت الفئران تركيزًا مُنقى من الإكزوسومات بالإضافة إلى IMQ الموضعي كما في المجموعة الثانية. استخدم ١٠ ذكور من الجرذان البيضاء كمصدر الخلايا الجذعية الوسيطة المستمدة من نخاع العظم و الإكزوسومات المشتقة منها. في نهاية التجربة (بعد ٥ أيام)، تم التضحية بالحيوانات في جميع المجموعات وأُخذت عينات من المنطقة التى تم حلاقتها ومعالجتها للدراسات المجهرية الخفيفة (H&E و الصبغة المناعية PCNA) وتم تحديد الكروموسوم Y باستخدام تحليل Real-Time PCR . تم عزل وتنقية الإكزوسومات بواسطة طريقة التنبيذ الفائق وتم توصيفها بواسطة دراسات المايكروسكوب الإلكتروني. تم إجراء قياسات مورفومترية وتحليل إحصائي لمتوسط سماكة البشرة، ونسبة المساحة المتوسطة للتفاعل الإيجابي لـ PCNA في الأدمة، ومتوسط عدد الخلايا الكيراتينية الإيجابية لـ PCNA في البشرة.

النتائج: كشفت الملاحظات العامة والفحص المجهري للمقاطع المأخوذة من جرذان المجموعة الثانية عن تفاعلات التهابية شبيهة بالصدفية بما في ذلك الشواك والتهاب الجفن و تخلل شديد لطبقات الجلد المختلفة بخلايا التهابية. كانت هناك زيادة ملحوظة إحصائياً في سماكة البشرة وفي التفاعلات الإيجابية للـ PCNA في المجموعة الثانية مقارنة بالمجموعات الأخرى. أظهرت المجموعتان الثالثة والرابعة تحسنًا ملحوظًا، بينما أظهرت المجموعة الثالثة بنية نسيجية طبيعية تقريبًا.

الاستنتاج: أظهرت الخلايا الجذعية الوسيطة المستمدة من نخاع العظم والإكزوسومات المنقاه المشتقة منها تحسنًا ملحوظًا في التغيرات الالتهابية الشبيهة بالصدفية في جلد النماذج الحيوانية.