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Original article

Histological Study on the Possible Ameliorating Consequence of Blocking STAT-3 Pathway on Psoriasis Model in Adult Male Albino rat

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Abstract:

Background: Psoriasis, autoimmune chronic inflammatory skin disease, affects 2-3% of population. It causes poor life-quality due to its disfiguring lesions and co-morbidities. Interleukin (IL)-23/T-helper (Th)-17 &IL-23/Th-22 axes activation, signal transducer and activator of transcription (STAT)-3 pathway stimulation and proinflammatory cytokines (IL-6, IL-17, IL-22 &TNF-α) overproduction are psoriasis main mechanisms. Psoriasis conventional treatment has many adverse effects as immunosuppression and hepatotoxicity with consequent lack of patients' compliance. **Aim of work:** Evaluating the probable curative effect of blocking STAT-3 pathway using ochromycinone [an STA-21 (STAT-3 inhibitor)] on Imiquimod (IMQ)-induced psoriasis model in adult male albino rats. **Materials &Methods:** 1 cm^2 back skin was shaved in twenty-four adult male albino rats. They grouped into; control group [group-I], IMQ group [group-II, received 20mg/cm^2 IMQ-5% topically on the shaved skin for

17 days] & IMQ/STA-21 group [group-III, given daily 3.5mg/kg ochromycinone intraperitoneal injection for 2 weeks]. Psoriasis Area &Severity Index (PASI)-scoring and biochemical, histological, immunohistochemical [for IL-23, STAT-3, Ki-67, connexin (Cx)-26 &vascular endothelial growth factor (VEGF)] and statistical studies were done. **Results:** IMQ produced psoriasis-like skin lesions (inflammation, epidermal proliferation & angiogenesis). However, the use of STA-21 marvelously ameliorated these lesions. **Conclusion:** Topical IMQ provoked psoriasis-like lesions concerning gross, biochemical &histological features. These resulted from IL-23/Th-17 &IL-23/Th-22 axes activation, proinflammatory cytokines production that acted via STAT-3 pathway activation. The use of an STA-21 (STAT-3 inhibitor) as ochromycinone showed amazing improvement of these lesions. Thus, blocking STAT-3 might be a promising treatment for psoriasis instead of the traditional therapy with its marked side-effects.

1. Introduction:

Psoriasis is a chronic noncommunicable inflammatory skin disease affecting nearly 2% of the population with equal incidence in males and females [1]. This disease can affect any age, but its incidence is high in the 20s and 50s [2]. Due to its disfigurement effect, the patients often suffer from poor quality of life, depression and/or anxiety extending to the level of suicidal attempts [3,4]. Sometimes, psoriasis is associated with other serious morbidities (inflammatory bowel diseases namely Crohn's, cancers, and cardiovascular diseases) which, in turn, add to the patients' deteriorating physical and psychological conditions [5,6].

It is an autoimmune disease characterized by disturbances in the immune system as a result of genetic predisposition that alters the immune response of the epidermal and the inflammatory cells disrupting the skin homeostasis [7]. Its mechanism includes 2 phases: initiation and maintenance phases [8].

Early in the initiation phase, the possible autoantigens [antimicrobial peptides (AMPs) such as LL-37 (the only cathelicidin in humans: human cationic antibacterial protein that has 37 amino acids and two leucines at its N-terminal), defensins, S100 and disintegrin] together with interleukin (IL)-6, tumour necrosis factor (TNF)-α and transforming growth factor (TGF)-β1 are produced in excess by the injured keratinocytes and melanocytes in response to physical injury [9,10].

These autoantigens form undegradable complexes with the self-DNA and self-RNA produced by the injured keratinocytes stimulating Toll-like receptors-7 [TLR-7] on dendritic cell (DC) surface [8]. These receptors are normally stimulated by viruses and microbial acids not self-nucleic acids [11]. The activated DCs secrete IL-6, TNF-α & interferon-α [IFN-α] and become mature antigen presenting cells secreting TNF-α and IL-23.

Normally, IL-23 acts a major role in the defence mechanism against bacterial and fungal infection. It is secreted by keratinocytes, DCs and macrophages [8]. T-Naïve CD_4 +ve cells have no receptors for IL-23 [12]. However, the presence of IL-23 in association with IL-6 and TGF-β1 direct the T-naïve cells differentiation towards T helper (Th)-17 cells with subsequent production of IL-17 mainly IL-17A, IL-22 and TNF-α. Moreover, its presence in association with IL-6 and TNF-α promotes the transformation of CD_4 +ve naïve cells into Th-22 cells that secrete IL-22 and TNF-α [8]. Furthermore, IL-23 acts on its receptors (IL-23R) on keratinocytes, macrophages and neutrophils activating the phosphorylation of signal transducer and activator of transcription-3 (STAT-3), increasing their production of chemokines and proinflammatory cytokines [8,13].

The previously mentioned pro-inflammatory cytokines (IL-17A, IL-22 and TNF-α) are responsible for the maintenance phase of psoriasis (chronic inflammatory phase) where they activate the keratinocytes to proliferate and produce more autoantigens, chemokines, and inflammatory mediators like IL-6 and TGF-β1. In addition, they induce neutrophils recruitment creating a viscous circle of inflammation [14].

IL-17A normally exerts its protective action against staphylococcal and candida infections via binding to its receptors (IL-17A-R) on keratinocytes, endothelial cells and fibroblasts and activation of STAT-3 pathway. In cases of psoriasis, the presence of autoantigens and proinflammatory cytokines (IL-23, IL-6, TGF-β1 & TNF-α) recruit more neutrophils and Th17 cells to the skin with more IL-17A production [8,15],

causing exaggerated aberrant inflammatory response [16].

IL-22 is an important anti-inflammatory cytokine secreted by Th-17 & Th-22 under the effect of IL-23 [8]. It interferes with the bacterial, viral, and fungal infections after binding to its receptors (IL-22R) on keratinocytes and activation of STAT-3 pathway [17]. Abnormal production of IL-23 during psoriasis results into the production of abnormally overactive IL-22 with subsequent inhibition of the normal epidermal desquamation process and activation of keratinocytes proliferation, migration & production of AMPs, chemokines and proinflammatory cytokines as well as recruitment of neutrophils [8,17,18].

Psoriasis is used to be treated by either topical therapies such as corticosteroids and vitamin D3 analogues, systemic therapies like methotrexate, cyclosporine or acitretin [19] or even phototherapy [20]. Recently, the use of the biological treatment that interfere with the aberrantly overactivated cytokines is permitted in the management of psoriasis [20]. However, the patient's long-term adherence to these ways of treatment is hindered by their proved harmful effects, attack recurrence, skin cancer development, hyperlipidaemia, and elevated liver enzymes [20,21]. Based on that, the development of a new, safer, and more efficient treatment strategy is crucial.

Aim of work:

This study was directed to investigate the potential therapeutic effect of blocking STAT-3 pathway using ochromycinone [an STA-21 (STAT-3 inhibitor)] on psoriasis-like lesions induced by Imiquimod (IMQ) in adult male albino rats.

2. Materials &Methods:

I) Experimental Design

Twenty-four adult male albino rats (~3 months, ~200 g) were selected for this work. They were housed in cages subjected to the same environmental conditions (24 ± 1°C, in normal light/dark cycle) for 48 hours before the experiment, to acclimatize to the new environmental conditions. All through the experiment they were kept at the same previous environmental conditions and provided with regular chow and water ad libitum. This was done in accordance with Cairo University-Institutional Animal Care and Use Committee (CU-IACUC) guidelines [approval number CU/III/F/33/23] in Laboratory Animal House Unit of Kasr Al-Aini, Faculty of Medicine, Cairo University.

Immediately before the experiment, a square area ~1 cm^2 was shaved on the back of all

rats. Then, the rats were categorized into three main groups:

Group I (control group, 12 rats):

Animals of this group were subdivided equally into three subgroups:

- **Subgroup Ia:** The animals received nothing (negative control).
- **Subgroup Ib:** 19 mg/cm^2 Vaseline [product number 16415, Sigma-Aldrich, St. Louis, MO, USA] was topically applied to the shaved skin area of each rat on a daily basis for 17 days.
- **Subgroup Ic:** Each rat was treated as in subgroup Ib. In addition, it was given 1 ml dimethyl sulfoxide (DMSO) [product number 276855, Sigma-Aldrich, St. Louis, MO, USA] via intraperitoneal (IP) injection once daily for two weeks starting from day 4.

Group II (IMQ group, 6 rats):

The rats were treated by topical application of IMQ cream 5% in Vaseline [Aldara, 3M Health Care Limited - UK, supplied as single use packets, each contains 250 mg cream that contains 12.5 mg IMQ in a ratio of 1 mg IMQ : 19 mg Vaseline] to the shaved skin area on a daily basis for 17 days. Each rat received 20 mg/cm^2 of the cream i.e.,1 mg IMQ & 19 mg Vaseline [22].

Group III (IMQ/STA-21 group, 6 rats):

Each rat was treated as in group II, in addition to IP injection of ochromycinone ((Rac)-STA-21) [STAT-3 inhibitor, S0956-10, AllgenBio, Shanghai] dissolved in 1 ml DMSO once daily for two weeks starting from day 4. STA-21 was used in a dose of 3.5 mg/kg [calculated from the corresponding mouse dose (0.5 mg/kg [23]) according to Paget's equation].

II)Animal studies

1- Psoriasis Area and Severity Index (PASI) scoring system [22,24]:

The degree of psoriasis-like lesions (erythema, scaling, and thickening) in the shaved area in the back skin of rats of all groups was evaluated before the start of the experiment, then during the experiment on the 4th, the 7th, and the 10th day and finally after the end of the experiment, just before sacrifice. Each sign was evaluated based on 0-4 scale (0= no sign, 1= slight, 2= moderate, 3= marked, 4= very marked). Then, a combined score (erythema + scaling + thickening) ranging from 0-12 was done.

2- Animal sacrifice:

All rats were sacrificed after the end of the experiment (day 18), and the shaved area of the skin of each rat was dissected. Each skin specimen was cut into 2 slices [one slice for the

preparation of skin homogenate & quantitative real-time polymerase chain reaction (qRT-PCR), and the second one is to prepare paraffin blocks].

3- Preparation of skin homogenates for enzyme-linked immunosorbent assay (ELISA):

The skin homogenates were prepared [25] at Biochemistry Department, Faculty of Medicine, Cairo University. ELISA was performed based on the manufacturer's instructions to measure the following values using their suitable antibodies:

- IL-6 (pro-inflammatory cytokine) [MBS269892, MyBioSource, USA].
- TGF-β1 (growth factor) [MBS001041, MyBioSource, USA].
- TNF-α (pro-inflammatory cytokine) [MBS175904, MyBioSource, USA].

4- Quantitative real-time polymerase chain reaction (qRT-PCR) [25]:

It was done at Biochemistry Department, Faculty of Medicine, Cairo University, by total RNA extraction followed by complementary DNA synthesis and detection of the relative mRNA expression for IL-17A & IL-22 (pro-inflammatory cytokines) using their primers and the Rotor Gene 6000 series software version 1.7 (Corbett Life Science, USA). The obtained results were stated as a normalized ratio to β-actin (the internal control).

The used primer and internal control PCR sequences were:

- IL-17A: Sense CTACCTCAACCGTTCCACTT Antisense ACTTCTCAGGCTCCCTCTTC.
- IL-22: Sense TTCTCCTCCCAGTTATCAGTTGT Antisense GGTGCGGTTGACGATGTAT.
- β-actin: Sense CTATCGGCAATGAGCGGTTCC Antisense TGTGTTGGCATAGAGGTCTTTACG.

III) Histological Study

Paraffin block preparation:

The skin slices for preparation of paraffin blocks were fixed by 10% formol saline for 24 hours and the process of paraffin blocks preparation was completed. Six μm-thick skin sections were cut and stained with:

1- Hematoxylin and Eosin stain (H&E) [26].

2- Immunohistochemical staining for:

a) IL-23 [rabbit polyclonal antibody, DF13760, Affinity Biosciences, USA]: It appears as a cytoplasmic reaction in the keratinocytes, macrophages and DCs.

b) STAT-3 [mouse monoclonal antibody, ab119352, abcam, USA]: The reaction appears in the cytoplasm of the keratinocytes, macrophages, neutrophils, and endothelial cells.

c) Ki-67 [mouse monoclonal antibody, ab279653, abcam, USA]: Marker for cell proliferation that appears as a nuclear reaction in the proliferating cells.

d) Cx-26 [mouse monoclonal antibody, LS-B6429, LifeSpan BioSciences, USA]: It is a marker for gap junctions in skin that is affected in psoriasis [27]. It appears as a cytoplasmic reaction in the keratinocytes.

e) Vascular endothelial growth factor (VEGF) [mouse monoclonal antibody, ab1316, abcam, USA]: It is a marker for the neo-angiogenesis that appears as a membranous reaction in the endothelial cells.

Immunostaining was done using avidin-biotin technique [26] where all the used materials (citrate buffer, Ultravision One Detection System and Ultravision Mayer's hematoxylin) were purchased from Labvision, ThermoFisher scientific, USA:

- Boiling of the sections in 10 mM citrate buffer (cat no 005000) pH 6 for 10 min for antigen retrieval was managed.
- This was followed by cooling of the sections for 20 min at room temperature.
- The sections were incubated for one hour with the primary antibodies.
- Then, immunostaining was accomplished using Ultravision One Detection System (cat no TL - 060- HLJ) and Lab Vision Mayer's hematoxylin counterstaining (cat no TA- 060- MH).
- The negative control sections were prepared by the same process, but the primary antibodies were excluded.

IV) Morphometric study

In ten non-overlapping fields for each rat in each group (×100), the following was measured:

- The total epidermal thickness in H&E-stained sections.
- The number of inflammatory cells in H&E-stained sections at papillary and reticular dermal layers.
- The area percent of the positive immuno-reaction for IL-23, STAT-3, Ki-67, Cx-26 and VEGF in the corresponding immunostained sections.

Analysis of images was achieved using Leica Qwin-500 LTD-software image analysis computer system (Cambridge, England).

V) Statistical analysis

IBM Statistical Package for the Social Sciences (SPSS) version 21 was used, and all biochemical and morphometric results were stated as mean ± standard deviation (±SD). The accomplishment of their statistical analysis was done using one-way analysis of variance (ANOVA) and "Tukey" post hoc test where the results were counted

statistically significant when P value was < 0.05.

The statistical analysis as well as all histological and morphometric studies were achieved at Histology Department, Faculty of Medicine, Cairo University.

3. Results:

General observations

- No deaths nor abnormal behaviour were observed in all rats of all experimental groups.
- All rats of the control subgroups showed similar biochemical and histological results. So, they were considered as control group (group I).
- **Grossly,** the rats of the control group **[Fig. 1a]** showed complete absence of erythema, scaling, and thickening in the shaved areas on their back skin. These signs started to appear in the rats of IMQ group **[Figs. 1b & 1c]** from day 3 of the experiment and increased progressively till the end of the experiment. However, in the rats of group III (IMQ/STA-21 group) **[Fig. 1d]** these manifestations appeared at day 3 and progressed at a lower rate than in group II till day 7 then regressed gradually throughout the experiment to be limited to few scales at the end of the experiment (day 17). All these changes were shown in the **results of PASI scoring system [Fig. 1e].**

Animal Data

ELISA Results for IL-6, TGF-β1 & TNF-α and qRT-PCR results for IL-17A & IL-22:

In group II (IMQ group), the mean levels of IL-6, TGF-β1 & TNF-α **[Fig. 2a]** in addition to the mean levels of mRNA relative expression of IL-17A & IL-22 **[Fig. 2b]** were significantly increased when compared to group I (control group). However, in group III (IMQ/STA-21), their mean levels revealed significant decrease versus group II and non-significant increase in comparison to group I.

Histological Results

H&E-stained sections:

Group I (control group) [Fig. 3a]: The sections revealed normal histological structure of the thin skin where it was formed of two principal layers (epidermis and dermis). The epidermis, the outermost layer, was formed of keratinized stratified squamous epithelium arranged as 4 layers:

The basal cell layer (stratum basale) was the deepest layer that rested on the basement membrane. It consisted of single row of cuboidal keratinocytes with basophilic cytoplasm and basal nuclei. The next layer was the prickle cell layer (stratum spinosum) that accounted for most of the epidermal

thickness. It was formed of many layers of polyhedral cells with rounded to oval nuclei and prominent nucleoli.

The following layer was the granular cell layer (stratum granulosum) which comprised multiple layers of squamous cells with flattened nuclei and dense basophilic keratohyalin granules. The most superficial layer was the horny layer (stratum corneum) that appeared as acidophilic interlaced keratinized non-cellular layer.

Underlying the epidermal basement membrane there was the thick connective tissue dermis that included the adnexal structures (hair follicles, erector pili muscle and sebaceous glands) and multiple blood vessels.

Group II (IMQ group) [Figs. 3b & 3c]: The sections demonstrated elongated rete ridges (enlarged rete pegs which are the normal epidermal extension projecting into the underlying CT dermis) with thinning of supra-papillary plates. Some keratinocytes appeared with ballooned vacuolated cytoplasm and condensed darkly stained pyknotic nuclei. Other keratinocytes became separated by wide intercellular spaces giving the epidermis a spongy appearance (spongiosis).

Moreover, the horny cell layer showed hyperkeratosis (thickening of the stratum corneum with keratin increase), and parakeratosis (retention of nuclei in the stratum corneum cells). The granular cell layer was decreased. In addition, the prickle cell layer revealed increased thickness (acanthosis), and there was increase in the mitotic activity in both stratum basale and stratum spinosum.

Furthermore, the dermal papillary layer displayed dilated and elongated blood vessels with intraluminal inflammatory cells. The CT showed edema, marked mononuclear inflammatory cell gushing mainly around the blood vessels (perivascular cuffing) and seldom extravasated RBCs. Such inflammatory infiltrates extended to the stratum corneum and stratum spinosum layers forming micro-abscesses.

Group III (IMQ/STA-21 group) [Figs. 3d & 3e]: The histological structure of the skin returned to almost normal features except for the presence of few keratinocytes with vacuolated cytoplasm and pyknotic nuclei, spongiosis, few dermal elongated dilated blood vessels with few intraluminal inflammatory cells, besides few inflammatory cells infiltrate in the dermis.

IL-23 immunostaining:

Sections of **Group I (control group) [Fig. 4a]** demonstrated sparse positive cytoplasmic immunoreaction in the keratinocytes and the mononuclear cells in

the dermis. However, those of **group II (IMQ group) [Fig. 4b]** verified abundant positive immunoreaction in the cytoplasm of the keratinocytes and the dermal inflammatory cell infiltrate. In **group III (IMQ/STA-21 group)** sections **[Fig. 4c]**, the positive immunoreaction was limited to few keratinocytes and few dermal mononuclear inflammatory cells.

STAT-3 immunostaining:

In sections of **group I (control group) [Fig. 5a]**, there was very minimal positive cytoplasmic immunostaining in the keratinocytes, and the dermal inflammatory cells. In sections of **group II (IMQ group) [Fig. 5b]**, this immunopositivity became numerous and extended to the endothelial cells of the dermal blood vessels. But in **group III (IMQ/STA-21 group) [Fig. 5c]**, the sections evidenced barely disseminated positive immunoreaction in the keratinocytes, endothelial and inflammatory cells.

Ki-67 immunostained sections:

In **group I (control group) [Fig. 6a]** denoted proliferating cells mainly in the basal cell and the prickle cell layers of the epidermis, in some of the hair follicle cells and in few inflammatory cells in the dermis. In **group II (IMQ group) [Fig. 6b]**, the proliferating cells were widely spread in basal and prickle cell layers of the epidermis, among the inflammatory cells infiltrating the dermis, and in the endothelial cells of the dermal blood vessels. In **group III (IMQ/STA-21 group) [Fig. 6c]**, the proliferating cells proved marked regression back to its normal distribution in the basale and the prickle cell layers. Additionally, few immune-positive proliferating cells were seen in endothelial cells, and among the inflammatory cells.

Cx-26 immunostaining:

Exhibited very few dispersed positive cytoplasmic reaction in the prickle and granular cell layers of the epidermis of **group I (control group) [Fig. 7a]** sections. This positive reaction increased among the keratinocytes in all epidermal layers of **group II (IMQ group) [Fig. 7b]**. Then it verified marked regression in the epidermal layers of sections of **group III (IMQ/STA-21 group) [Fig. 7c]**.

VEGF immunostained sections:

In the control group (group I) [Fig. 8a] demonstrated slight positive cytoplasmic immunoreaction in the dermal blood vessels. In those of **group II (IMQ group) [Fig. 8b]**, the positive immunostaining was marked in the endothelial cells lining the dilated blood vessels in the papillary dermis. While in the sections of **group III (IMQ/STA-21 group) [Fig. 8c]**, the immunoreaction was scanty.

Morphometric Results:

Statistical analysis of the mean total thickness of the epidermis **[Fig. 3f]**, the mean number of inflammatory cells **[Fig. 3g],** besides the mean area percent of the positive immunoreactions of IL-23 **[Fig. 4d]**, STAT-3 **[Fig. 5d]**, Ki-67 **[Fig. 6d]**, Cx-26 **[Fig. 7d]** & VEGF **[Fig. 8d]** revealed significant increase in IMQ group when compared with the control group. While, in IMQ/STA-21 group they showed significant decrease in comparison with IMQ group and non-significant increase versus the control group.

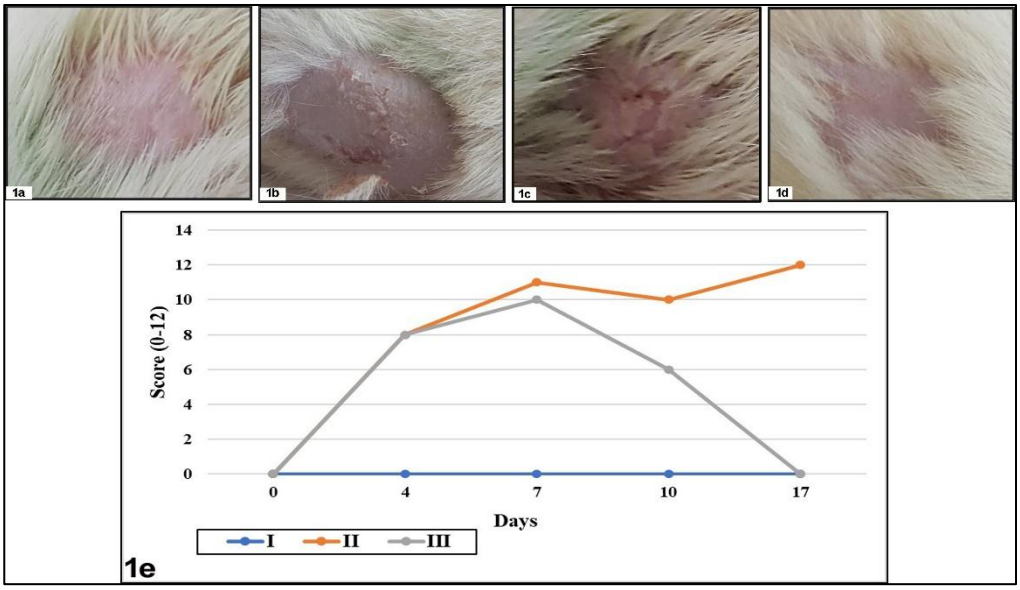


Fig. 1: Showing: 1a: Control group with complete absence of psoriasis signs. **1b & 1c:** IMQ group with erythema, scaling, and thickening in the shaved areas on the rats' back. **1d:** Group III (IMQ/STA-21 group) with regression of psoriasis signs that become limited to few scales at the end of the experiment. **1e:** PASI scoring system.

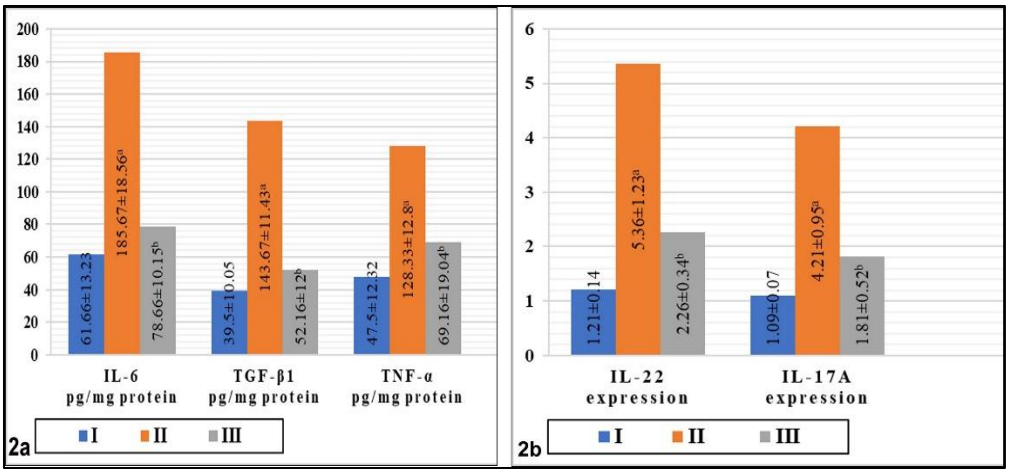


Fig. 2: Showing the mean values of: 2a: IL-6, TGF-β1 & TNF-α. **2b:** IL-22 & IL-17A qRT-PCR expression.

[[a] as compared to group I & [b] as compared to subgroup II (significant difference at *P* < 0.05)]

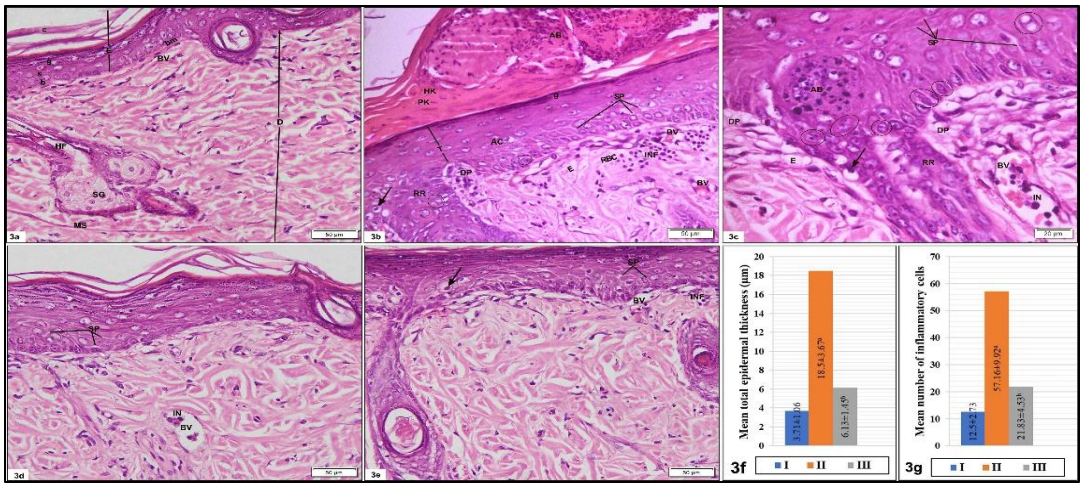


Fig. 3: Photomicrographs of H&E-stained sections in the skin: 3a (the control group, group I): Normal histological structure of thin skin consists of epidermis (E) and dermis (D). The epidermis, the stratified squamous epithelium arranged as 4 layers: stratum basale (b) rested on the basement membrane (bm), stratum spinosum (s), stratum granulosum (g), and stratum corneum (c). The dermis shows multiple blood vessels (BV), hair follicles (HF), sebaceous gland (SG) and erector pili muscle (MS) **[H&E, x200]. 3b & 3c (IMQ group, group II):** There are elongated rete ridges (RR) with thinned supra-papillary plates (T), some ballooned keratinocytes with vacuolated cytoplasm and darkly stained pyknotic nuclei (arrow). Other keratinocytes appear separated by wide intercellular spaces forming epidermal spongiosis (SP). Hyperkeratosis (HK), parakeratosis (PK), decreased granular cell layer (g) and increased prickle cell layer (acanthosis) (AC), and increased mitotic activity (circles) in both stratum basale and stratum spinosum are clearly seen. Dermal papillary layer (DP) shows dilated, and elongated blood vessels (BV) with intraluminal inflammatory cells (IN), beside the noted oedematous CT (E), numerous mononuclear inflammatory cells mainly around the blood vessels [perivascular cuffing] (INF) and seldom extravasated RBCs (RBCs). Such inflammatory infiltrates are seen extended to the stratum corneum and stratum spinosum forming micro-abscesses (AB). **[3b: H&E, x200, 3c: H&E, x400]. 3d & 3e (IMQ/STA-21 group, group III):** Almost normal features of the skin except for the existence of few keratinocytes with pyknotic nuclei and vacuolated cytoplasm (arrow), spongiosis (SP), few dermal dilated elongated blood vessels (BV) with few intraluminal inflammatory cells (IN), in addition to few inflammatory cells infiltrate (INF) in the dermis. **[3d & 3e: H&E, x200]. 3f & 3g:** Showing **the mean values of the total epidermal thickness & the number of inflammatory cells**, respectively. **[^{a} as compared to group I & ^{b} as compared to subgroup II (significant difference at $P < 0.05$)]**

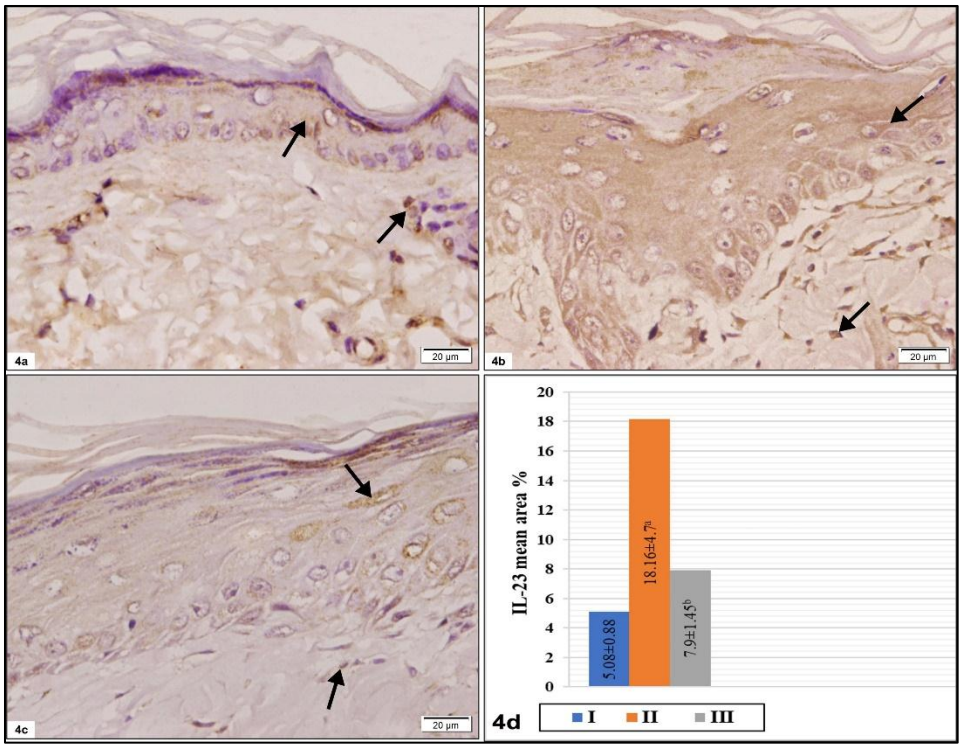


Fig. 4: Photomicrographs of IL-23 immunostained sections in the skin [anti IL-23 immunohistochemical stain, x400]: 4a (the control group, group I): Scarce positive cytoplasmic immunoreaction (arrows) is noted in keratinocytes and dermal mononuclear cells. **4b (IMQ group, group II):** Widely distributed positive cytoplasmic immunoreaction (arrows) in the keratinocytes and the dermal inflammatory cell infiltrate is seen. **4c (IMQ/STA-21 group, group III):** Positive immunoreaction (arrows) can be seen confined to few keratinocytes and few mononuclear inflammatory cells in dermis. **4d:** Showing **the mean area percent of IL-23** positive immunoreaction.

[^{a} as compared to group I & ^{b} as compared to subgroup II (significant difference at $P < 0.05$)]

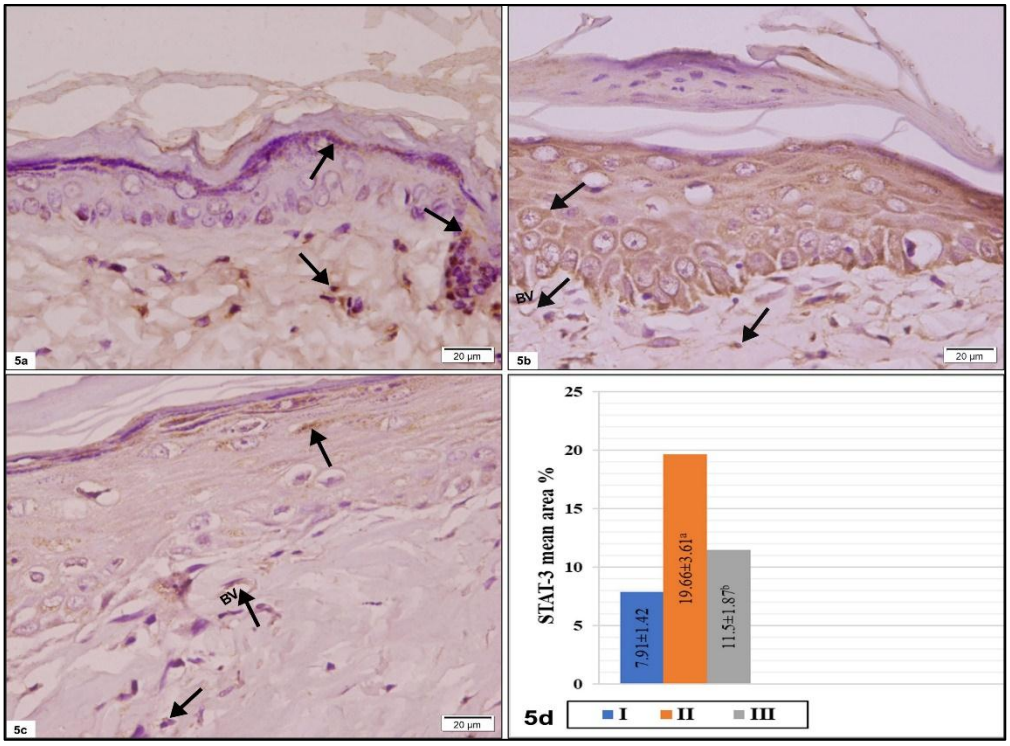
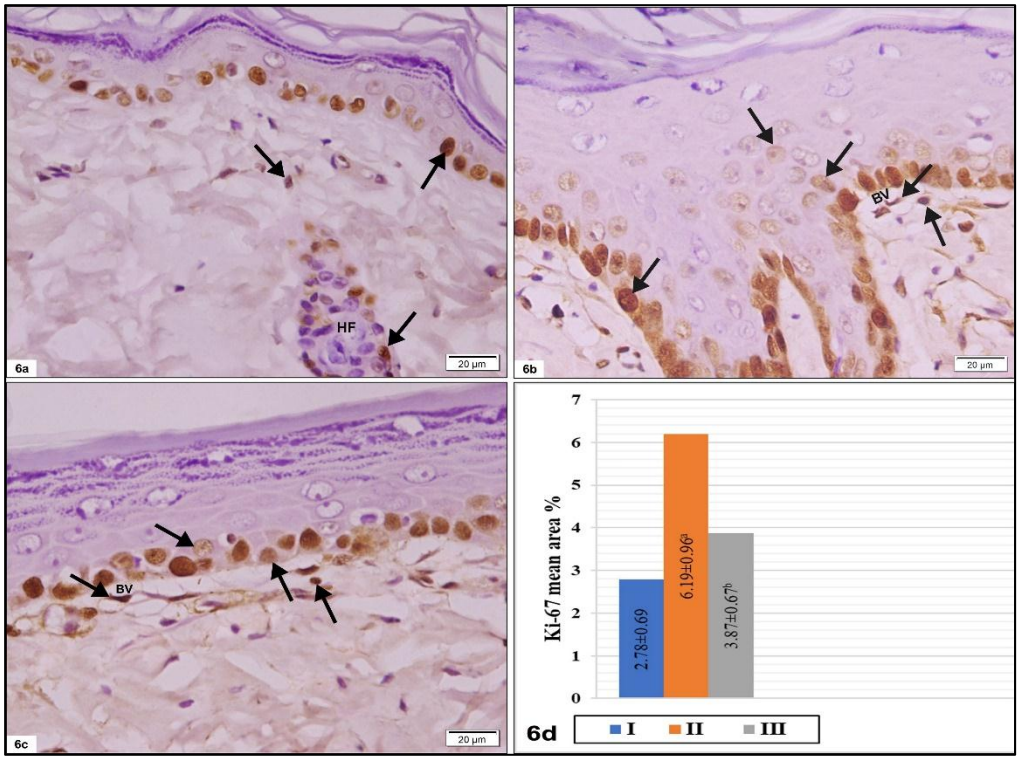


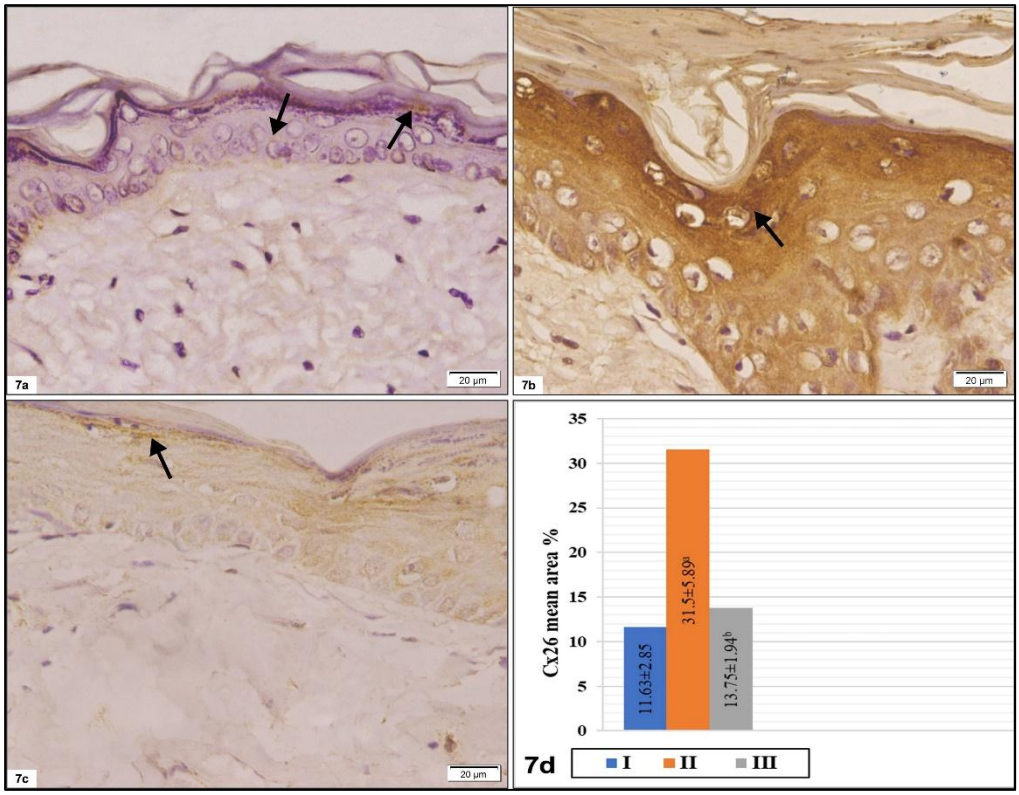
Fig. 5: Photomicrographs of STAT-3 immunostained sections in the skin [anti STAT-3 immunohistochemical stain, x400]: 5a (the control group, group I): There is very few positive cytoplasmic immunostaining (arrows) in keratinocytes, and dermal inflammatory cells. **5b (IMQ group, group II):** Abundant immunopositivity (arrows) in the keratinocytes, in the endothelium of the dermal blood vessels (BV) and in the inflammatory cells is illustrated. **5c (IMQ/STA-21 group, group III):** There is minimal positive immunoreaction (arrows) in the keratinocytes, endothelial cells of the blood vessels (BV) and inflammatory cells. **5d:** Showing **the mean area percent of STAT-3** positive immunoreaction.

[^{a} as compared to group I & ^{b} as compared to subgroup II (significant difference at $P < 0.05$)]



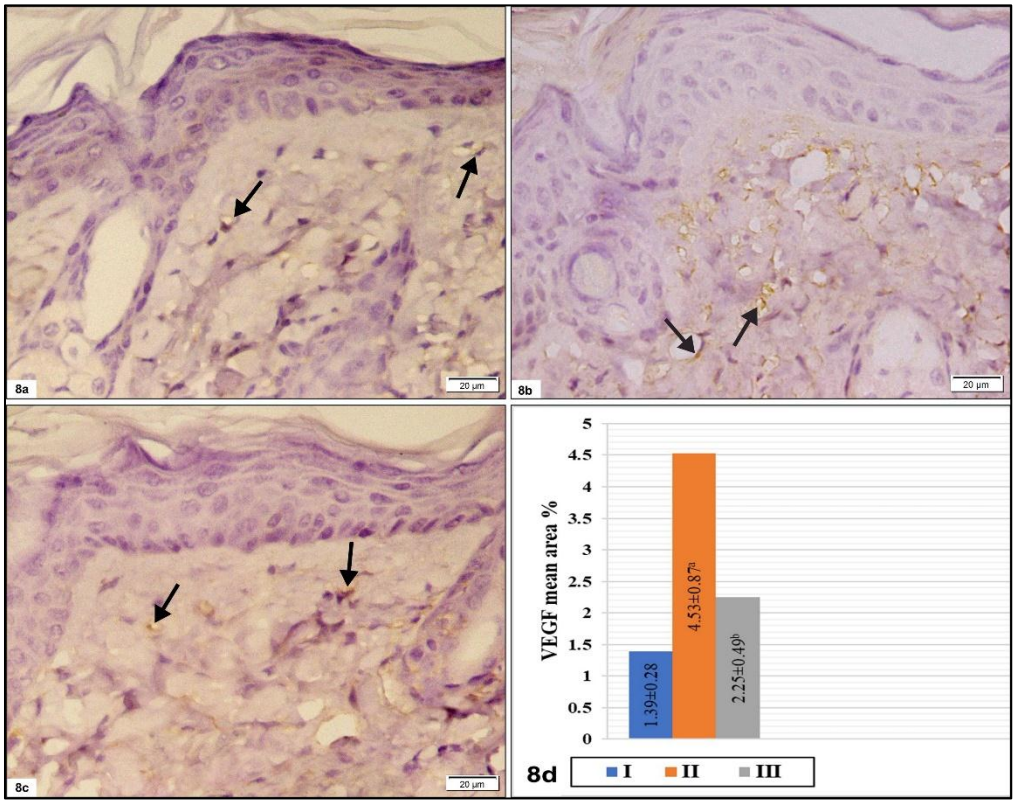
<u>Fig. 6:</u> Photomicrographs of Ki-67 immunostained sections in the skin [anti Ki-67 immunohistochemical stain, x400]: <u>6a (the control group, group I):</u> Nuclear positive immune-reaction in proliferating cells (arrows) are observed in Malpighian layer of epidermis and in some cells of hair follicle (HF) and few dermal inflammatory cells. **<u>6b (IMQ group, group II):</u>** There is numerous positive nuclear immunoreaction (arrows) in the proliferating cells among basal and prickle epidermal layers, in the dermal inflammatory cells infiltrate, and in the endothelial cells of the dermal blood vessels (BV). **<u>6c (IMQ/STA-21 group, group III):</u>** Marked decrease in the positive immunoreaction (arrows) that localised only in the basale and the prickle cell layers. In addition to the presence of positive reaction in few inflammatory cells and endothelial cells of the blood vessels (BV). **<u>6d:</u>** Showing **the mean area percent of Ki-67** positive immunoreaction.

[^{a} as compared to group I & ^{b} as compared to subgroup II (significant difference at *P* < 0.05)]



<u>Fig. 7:</u> Photomicrographs of Cx-26 immunostained sections in the skin [anti CX-26 immunohistochemical stain, x400]: <u>7a (the control group, group I):</u> There is sparsely dispersed positive cytoplasmic immunostaining (arrows) in the prickle and granular cell layers of the epidermis. **<u>7b (IMQ group, group II):</u>** Marked cytoplasmic immunoreaction (arrow) among the keratinocytes in all epidermal layers can be seen. **<u>7c (IMQ/STA-21 group, group III):</u>** There is minimally distributed positive cytoplasmic immunoreactivity (arrows) in epidermal layers. **<u>7d:</u>** Showing **the mean area percent of Cx-26** positive immunoreaction.

[^{a} as compared to group I & ^{b} as compared to subgroup II (significant difference at $P < 0.05$)]



<u>Fig. 8:</u> Photomicrographs of VEGF immunostained sections in the skin [anti VEGF immunohistochemical stain, x400]: <u>8a (the control group, group I):</u> Slight positive cytoplasmic immunostaining (arrows) is detected in the dermal blood vessels. **<u>8b (IMQ group, group II):</u>** Positive immunostaining (arrows) is clearly increased among the endothelial cells lining the dilated blood vessels in the papillary dermis. **<u>8c (IMQ/STA-21 group, group III):</u>** Few positive immunoreaction (arrow) is noticed in the endothelial cells lining the dermal blood vessels. **<u>8d:</u>** Showing **the mean area percent of VEGF** positive immunoreaction.

[^{a} as compared to group I & ^{b} as compared to subgroup II (significant difference at $P < 0.05$)]

4. Discussion:

Psoriasis is a noncommunicable chronic skin inflammatory lesions with 2-3% prevalence among the population and equal incidence in males and females [1]. Its hallmarks are irregular epidermal proliferation, thickening and keratinization together with dermal and epidermal inflammatory infiltration and angiogenesis

[28]. Most probably, it is an autoimmune disease with genetic predisposition that disrupts the immune reaction and the skin homeostasis [29]. It is initially triggered by different environmental factors such as trauma and microbial stresses [9,10].

The interaction between the production of the damaged keratinocytes [autoantigens & cytokines (IL-6, TNF-α & TGF-β1)] and the innate immune cells of the skin (macrophages and DCs) results into their activation to antigen presenting cells (APCs). The activated APCs secretes proinflammatory cytokines as IL-23 which, in turn, activates IL-23/Th-17 and IL-23/Th-22 axes with subsequent production of IL-17A, IL-22 & TNF-α. Such cytokines are the main cause of maintenance phase of psoriasis [28,30,31,32].

Imiquimod (IMQ) is a drug used for treatment of genital warts, keratosis, and basal cell carcinoma. It activates TLR-7 throughout its action, activating DCs & macrophages and consequently activates IL-23/Th-17 & IL-23/Th-22 axes [33]. So, it was used in this study to produce psoriasis-like lesions via these axes. In addition, rats were used in this work as IMQ was proved to induce skin lesions in rats like those of psoriasis in humans regarding the epidermal thickening and scaling in addition to the inflammatory reactions, inflammatory cells infiltration and angiogenesis [33,34].

Such IMQ effects were supported by the findings of this study where the topical use of IMQ for 3 days in group II induced psoriasis-like lesions (epidermal erythema, scales, and thickening) demonstrated in PASI scoring. These lesions were found to be exaggerated till the end of day 7. Such findings were similar to that stated previously [22,24] after topical IMQ use in mice and rats, respectively.

Bearing in mind that IMQ developed limited inflammatory reactions followed by skin adaptation and decline of the lesions after its application for 6 days [35] which was also documented in this study in PASI scoring after 7 days. The use of IMQ, in this work, was continued throughout the whole experimental duration to overcome this drawback and maintain the lesions. This was followed by progression and exaggeration of the gross psoriasis-like skin lesions in PASI scoring to the end of the experiment. Such way of lesions maintenance was in line with what was done in a former study [24].

Moreover, IMQ use in group II demonstrated significant increase in the mean ELISA values of IL-6, TGF-β1 & TNF-α and the mean area percent of IL-23 positive immunoreaction when compared to those of group I. These findings were

assumed to be due to activation of skin macrophages and DCs to APCs. This coincides with the findings of a previous study [24] where topical IMQ use was followed by increase in IL-23 level in skin tissue and serum.

Furthermore, the mean values of IL-17A and IL-22 mRNA expression were found to be statistically significant in group II versus group I. These findings were supposed to be due to conversion of CD4 +ve naïve T-cells into Th-17 with subsequent secretion of IL-17A & IL-22 and their conversion to Th-22 and further production of IL-22 & TNF-α. Such assumption was based on the findings of previous studies [8] where IL-23 stimulated such conversion to Th-17 in the presence of IL-6 and TGF-β1 and to Th-22 in the presence of IL-6 and TNF-α.

Both IL-22 and TNF-α were reported to stimulate keratinocytes to produce more pro-inflammatory cytokines that were also released from the neutrophils recruited to the skin by IL-17A. This was followed by maintenance of the chronic inflammation [8]. Such findings were enforced in group II of the present study by the persistence and exaggeration of the skin inflammatory signs in PASI (thickening and erythema), dermal oedema, presence of micro-abscesses in stratum corneum and spinosum, besides the significant increase in the mean number of the dermal inflammatory cells versus that in group I.

In addition, there were increased mitotic figures in stratum basale and spinosum, significant increase in the epidermal thickness and in the mean area percent of Ki-67 positive immunoreaction and its widespread positive reaction not only in the basal epidermal layers (basal and prickle cell layers) but also in the dermis, when compared to group I. This indicated increased keratinocytes proliferation that was supported previously following IMQ application [22,24]. This could be explained by the marked stimulation of keratinocytes by IL-17A, IL-22 and TNF-α [36].

The stimulated keratinocytes, in addition to increased pro-inflammatory cytokines production, they secrete VEGF causing blood vessels proliferation [37] via acting on its receptors on endothelial cells. This was backed in this study by the presence of dermal dilated elongated blood vessels, VEGF positive immunoreaction in blood vessels' endothelium with significant increase in the mean area percent of such immunoreaction in group II versus group I.

The features of keratinocytes proliferation (appearance of rete ridges, acanthosis, hyperkeratosis, ballooned keratinocytes with pyknotic nuclei) induced by IMQ in this study were like those of

psoriasis. This could be enlightened by the activation of IL-23/Th-17 and IL-23/Th-22 axes with consequent rapid proliferation and imbalance between apoptosis and proliferation [8,17,18,38].

Additionally, such marked proliferation was proved to be followed by improper keratinocytes maturation and disrupted desmosomal junctions [22] that lead to cytoplasmic vacuolations, spongiosis and scales formation provoked by IMQ in group II of this work. Besides, abnormal differentiation [39,40] that results in parakeratosis and decreased stratum granulosum in the same group.

Another explanation for such marked keratinocytes proliferation was suggested in this study by the wide-spread gap junctions between epidermal cells in all layers transporting the proliferation signals and 2nd messengers from one cell to another and from one layer to another. This suggestion was reinforced by the extensive positive immunoreaction for Cx-26 in all epidermal layers together with significant increase in its mean area percent in group II versus group I. Another support came from a former study [41] where mRNA expression and positive immunoreaction for Cx-26 in patients with psoriasis showed significant increase and wide distribution in all layers of the epidermis in contrast to its minimal and normal distribution to the stratum spinosum and stratum granulosum [42].

Based on the poor life quality and anxiety/depression of psoriatic patients due to their severe disfigurement that may lead to suicide [3,4]. In addition to other associated morbidities [5,6] that cause deterioration of the patients' health status. The treatment of psoriasis became a must.

There are different management levels starting from localized treatment, passing through systemic, and phototherapy and ending by the biological therapies blocking the action of the abnormally overactivated cytokines as anti-IL-23 [20]. All these therapeutic ways are of long-term duration which is difficult to be maintained due to their serious side effects related to their prolonged burden on the liver, or their action via inhibition of the systemic immune system with exaggerated infections and cancer development especially that of skin [20,21].

So, this study targeted the evaluation of the effectiveness of a new therapeutic pathway based on blocking the STAT-3 pathway by using STA-21 (a STAT-3 inhibitor). STAT-3 pathway is the main pathway for the action of the overactivated cytokines in psoriasis (IL-23, IL-17A & IL-22) [8,13,15,17] as it is the member of STAT family concerned with cell proliferation,

survival, differentiation, and angiogenesis [43,44]. Moreover, it was proved to be overactive in rapidly proliferating cancers associated with poor prognosis [45].

Binding of the cytokines (IL-23, IL-17A & IL-22) to their receptors stimulates the activation of STAT-3 via its phosphorylation and dimerization. These dimers are translocated to the nucleus where they start the process of transcription and translation of the genes responsible for cellular proliferation, inflammatory infiltration, and angiogenesis [46,47]. This was supported by the significant increase in the mean area percent of STAT-3 positive immunoreaction in group II of this study when compared with group I.

STA-21 blocks STAT-3 dimerization and its DNA binding [48]. So, its use was anticipated to block STAT-3 activation in IMQ-induced psoriasis model as proved previously in case of experimentally induced rheumatoid arthritis [23,47]. This was supported in group III of this work by the significant decrease in the mean area percent of STAT-3 immunoreaction compared to that of group II and its insignificant increase versus group I. This effect was assumed to be followed by improvement of skin proliferation, inflammation, angiogenesis, and psoriasis-like lesions induced by IMQ.

Such assumption was defended in group III of this study by obvious improvement of PASI where it returned to its control score and by the improvement of the histological features of skin inflammation and proliferation.

Besides, the significant decrease in the mean total epidermal thickness, the mean number of dermal inflammatory cells, the mean value of tissue homogenate IL-6, TGF-β1 & TNF-α and the mRNA expression of IL-17A & IL-22 in group III versus group II and their insignificant increase versus group I. Additionally, the mean area percent of the positive immunoreaction of IL-23, VEGF, Cx-26 & Ki-67 were significantly decreased in group III when compared to group II and non-significantly increased compared to group I. Moreover, the immunoreactions of Cx-26 & Ki-67 were regressed to spiny & granular layers and to spiny & basal cell layers, respectively, in group III similar to those in group I.

5. Conclusion:

This study concluded that IMQ topical application to the skin induced skin lesions resembling those of psoriasis regarding their gross, biochemical, and histological characteristics. These characters resulted

from activation of IL-23/Th-17 and IL-23/Th-22 axes and consequently production of IL-17, IL-22, TNF-α and other proinflammatory cytokines. It was also concluded that these cytokines (IL-23, IL-17 & IL-22) exerted their actions via activation of STAT-3 pathway.

The use of ochromycinone [an STA-21 (STAT-3 inhibitor)] in this work showed surprising improvement of IMQ-induced psoriasis-like skin lesions. Thus, blocking of STAT-3 pathway might be a spark of hope in the treatment of psoriasis rather than the traditional therapy with its marked side effects.

Conflict of interests:

There are no conflicts of interest.

6. References:

1- Springate DA, Parisi R, Kontopantelis E, Reeves D, Griffiths CE, Ashcroft DM. Incidence, prevalence and mortality of patients with psoriasis: a U.K. population-based cohort study. Br J Dermatol. 2017; 176:650-8.

2- Mitran MI, Mitran CI, Sârbu MI, Benea V, Tampa M, Georgescu SR. Therapeutic challenges in a case of psoriasis with nail onset. J. Mind Med. Sci. 2017; 4: 186-192.

3- Tampa M, Sarbu MI, Mitran MI, Mitran CI, Matei C, Georgescu SR. The Pathophysiological Mechanisms and the Quest for Biomarkers in Psoriasis, a Stress-Related Skin Disease. Dis Markers. 2018; 2018:5823684.

4- Sarbu MI, Tampa M, Sarbu AE, Georgescu SR. Sexual dysfunctions in psoriatic patients. J. Mind Med. Sci. 2014; 1: 19–27.

5- Oliveira Mde F, Rocha Bde O, Duarte GV. Psoriasis: classical and emerging comorbidities. An Bras Dermatol. 2015; 90:9-20.

6- Woo YR, Park CJ, Kang H, Kim JE. The Risk of Systemic Diseases in Those with Psoriasis and Psoriatic Arthritis: From Mechanisms to Clinic. Int J Mol Sci. 2020; 21:7041.

7- Pithadia DJ, Reynolds KA, Lee EB, Wu JJ. Psoriasis-associated cutaneous pain: etiology, assessment, impact, and management. J Dermatolog Treat. 2019; 30:435-440.

8- Chiricozzi A, Romanelli P, Volpe E, Borsellino G, Romanelli M. Scanning the Immunopathogenesis of Psoriasis. Int J Mol Sci. 2018; 19:179.

9- Lande R, Botti E, Jandus C, Dojcinovic D, Fanelli G, Conrad C, Chamilos G, Feldmeyer L, Marinari B, Chon S, Vence L, Riccieri V, Guillaume P, Navarini AA, Romero P, Costanzo A, Piccolella E, Gilliet M, Frasca L. The antimicrobial

peptide LL37 is a T-cell autoantigen in psoriasis. Nat Commun. 2014; 5:5621.

10- Krueger JG. An autoimmune "attack" on melanocytes triggers psoriasis and cellular hyperplasia. J Exp Med. 2015; 212:2186.

11- Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang YH, Homey B, Cao W, Wang YH, Su B, Nestle FO, Zal T, Mellman I, Schröder JM, Liu YJ, Gilliet M. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature. 2007; 449:564-9.

12- Eberle FC, Brück J, Holstein J, Hirahara K, Ghoreschi K. Recent advances in understanding psoriasis. F1000Res. 2016; 5:F1000 Faculty Rev-770.

13- Fotiadou C, Lazaridou E, Sotiriou E, Ioannides D. Targeting IL-23 in psoriasis: current perspectives. Psoriasis (Auckl). 2018; 8:1-5.

14- Georgescu SR, Sârbu MI, Matei C, Ilie MA, Caruntu C, Constantin C, Neagu M, Tampa M. Capsaicin: Friend or Foe in Skin Cancer and Other Related Malignancies? Nutrients. 2017; 9:1365.

15- Monin L, Gaffen SL. Interleukin 17 Family Cytokines: Signaling Mechanisms, Biological Activities, and Therapeutic Implications. Cold Spring Harb Perspect Biol. 2018;10: a028522.

16- Giunta A, Ventura A, Chimenti MS, Bianchi L, Esposito M. Spotlight on ixekizumab for the treatment of moderate-to-severe plaque psoriasis: design, development, and use in therapy. Drug Des Devel Ther. 2017; 11:1643-51.

17- Yang X, Zheng SG. Interleukin-22: a likely target for treatment of autoimmune diseases. Autoimmun Rev. 2014; 13:615-20.

18- Luan C, Chen X, Hu Y, Hao Z, Osland JM, Chen X, Gerber SD, Chen M, Gu H, Yuan R. Overexpression and potential roles of NRIP1 in psoriasis. Oncotarget. 2016; 7:74236-46.

19- Winterfield L, Cather J, Cather J, Menter A. Changing paradigms in dermatology: nuclear hormone receptors. Clin Dermatol. 2003; 21:447-54.

20- Pouplard C, Brenaut E, Horreau C, Barnetche T, Misery L, Richard MA, Aractingi S, Aubin F, Cribier B, Joly P, Jullien D, Le Maître M, Ortonne JP, Paul C. Risk of cancer in psoriasis: a systematic review and meta-analysis of epidemiological studies. J Eur Acad Dermatol Venereol. 2013; 27:36-46.

21- Dogra S, Yadav S. Acitretin in psoriasis: an evolving scenario. Int J Dermatol. 2014; 53:525-38.

22- Okasha EF, Bayomy NA, Abdelaziz EZ. 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). 2018; 301:166-174.

23- Ahmad SF, Ansari MA, Nadeem A, Zoheir KMA, Bakheet SA, Alsaad AMS, Al-Shabanah OA, Attia SM. STA-21, a STAT-3 inhibitor, attenuates the development and progression of inflammation in collagen antibody-induced arthritis. Immunobiology. 2017; 222:206-17.

24- Na Takuathung M, Wongnoppavich A, Panthong A, Khonsung P, Chiranthanut N, Soonthornchareonnon N, Sireeratawong S. Antipsoriatic Effects of Wannachawee Recipe on Imiquimod-Induced Psoriasis-Like Dermatitis in BALB/c Mice. Evid Based Complement Alternat Med. 2018; 2018:7931031.

25- El Agaty SM. Cardioprotective effect of vitamin D2 on isoproterenol-induced myocardial infarction in diabetic rats. Archives of Physiology and Biochemistry. 2019; 125:210-9

26- Suvarna K, Layton C, Bancroft J. The hematoxylins and eosin, Immunohistochemical techniques & Transmission electron microscopy. In Bancroft's Theory and Practice of Histological Techniques (Eighth Edition), Elsevier,2019, pp: 126-38, 337-94, 434-75

27- Li B, Tsoi LC, Swindell WR, Gudjonsson JE, Tejasvi T, Johnston A, Ding J, Stuart PE, Xing X, Kochkodan JJ, Voorhees JJ, Kang HM, Nair RP, Abecasis GR, Elder JT. Transcriptome analysis of psoriasis in a large case-control sample: RNA-seq provides insights into disease mechanisms. J Invest Dermatol. 2014; 134:1828-38.

28- Kim J, Krueger JG. The immunopathogenesis of psoriasis. Dermatol Clin. 2015; 33:13-23.

29- Lowes MA, Suárez-Fariñas M, Krueger JG. Immunology of psoriasis. Annu Rev Immunol. 2014; 32:227-55.

30- Georgescu SR, Tampa M, Caruntu C, Sarbu MI, Mitran CI, Mitran MI, Matei C, Constantin C, Neagu M. Advances in Understanding the Immunological Pathways in Psoriasis. Int J Mol Sci. 2019; 20:739.

31- Mohd Noor AA, Azlan M, Mohd Redzwan N. Orchestrated Cytokines Mediated by Biologics in Psoriasis and Its Mechanisms of Action. Biomedicines. 2022; 10:498.

32- Baliwag J, Barnes DH, Johnston A. Cytokines in psoriasis. Cytokine. 2015; 73:342-50.

33- Smajlović A, Haverić A, Alić A, Hadžić M, Smajlović A, Mujezinović I, Lojo-Kadrić N, Ramić J, Elez-Burnjaković N, Haverić S, Pojskić L. Molecular and histopathological profiling of imiquimod induced dermatosis in Swiss Wistar rats: contribution to the rat model for novel anti-psoriasis treatments. Mol Biol Rep. 2021; 48:4295-303.

34- Satake K, Amano T, Okamoto T. Low systemic exposure and calcemic effect of calcipotriol/betamethasone ointment in rats with imiquimod-induced psoriasis-like dermatitis. Eur J Pharmacol. 2018; 826:31-38.

35- Sun J, Zhao Y, Hu J. Curcumin inhibits imiquimod-induced psoriasis-like inflammation by inhibiting IL-1beta and IL-6 production in mice. PLoS One. 2013;8:e67078.

36- Na Takuathung M, Wongnoppavich A, Pitchakarn P, Panthong A, Khonsung P, Chiranthanut N, Soonthornchareonnon N, Sireeratawong S. Effects of Wannachawee Recipe with Antipsoriatic Activity on Suppressing Inflammatory Cytokine Production in HaCaT Human Keratinocytes. Evid Based Complement Alternat Med. 2017; 2017:5906539.

37- Shaker OG, Khairallah M, Rasheed HM, Abdel-Halim MR, Abuzeid OM, El Tawdi AM, El Hadidi HH, Ashmaui A. Antiangiogenic effect of methotrexate and PUVA on psoriasis. Cell Biochem Biophys. 2013; 67:735-42.

38- Rückert R, Asadullah K, Seifert M, Budagian VM, Arnold R, Trombotto C, Paus R, Bulfone-Paus S. Inhibition of keratinocyte apoptosis by IL-15: a new parameter in the pathogenesis of psoriasis? J Immunol. 2000; 165:2240-50.

39- Dwarampudi LP, Palaniswamy D, Nithyanantham M, Raghu PS. Antipsoriatic activity and cytotoxicity of ethanolic extract of Nigella sativa seeds. Pharmacogn Mag. 2012; 8:268-72.

40- Buerger C, Shirsath N, Lang V, Berard A, Diehl S, Kaufmann R, Boehncke WH, Wolf P. Inflammation dependent mTORC1 signaling interferes with the switch from keratinocyte proliferation to differentiation. PLoS One. 2017; 12:e0180853.

41- O'Shaughnessy EM, Duffy W, Garcia-Vega L, Hussey K, Burden AD, Zamiri M, Martin PE. Dysregulation of Connexin Expression Plays a Pivotal

Role in Psoriasis. Int J Mol Sci. 2021; 22:6060.

42- Garcia-Vega L, O'Shaughnessy EM, Albuloushi A, Martin PE. Connexins and the Epithelial Tissue Barrier: A Focus on Connexin 26. Biology (Basel). 2021; 10:59.

43- Hanlon MM, Rakovich T, Cunningham CC, Ansboro S, Veale DJ, Fearon U, McGarry T. STAT3 Mediates the Differential Effects of Oncostatin M and TNFα on RA Synovial Fibroblast and Endothelial Cell Function. Front Immunol. 2019; 10:2056.

44- Dong J, Cheng XD, Zhang WD, Qin JJ. Recent Update on Development of Small-Molecule STAT3 Inhibitors for Cancer Therapy: From Phosphorylation Inhibition to Protein Degradation. J Med Chem. 2021; 64:8884-915.

45- Johnson DE, O'Keefe RA, Grandis JR. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. Nat Rev Clin Oncol. 2018; 15:234-48.

46- Sgrignani J, Garofalo M, Matkovic M, Merulla J, Catapano CV, Cavalli A. Structural Biology of STAT3 and Its Implications for Anticancer Therapies Development. Int J Mol Sci. 2018; 19:1591.

47- Park JS, Kwok SK, Lim MA, Kim EK, Ryu JG, Kim SM, Oh HJ, Ju JH, Park SH, Kim HY, Cho ML. STA-21, a promising STAT-3 inhibitor that reciprocally regulates Th17 and Treg cells, inhibits osteoclastogenesis in mice and humans and alleviates autoimmune inflammation in an experimental model of rheumatoid arthritis. Arthritis Rheumatol. 2014; 66:918-29.

48- Song H, Wang R, Wang S, Lin J. A low-molecular-weight compound discovered through virtual database screening inhibits Stat3 function in breast cancer cells. Proc Natl Acad Sci U S A. 2005; 102:4700-5.