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Effect of metformin gel against imiquimod induced psoriasis in mice

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ABSTRACT

Imiquimod-induced psoriasis is an animal model of psoriasis. The antidiabetic metformin had anti-inflammatory, immunomodulation, antiangiogenesis and antiproliferative effect. The objective of this study was to evaluate the possible beneficial effect of metformin gel against imiquimod-induced psoriasis-like form by scoring the erythema and scaling besides measuring of tissue homogenate levels of IL17/IL23. Swiss albino mice were used in this experiment imiquimod 5% cream applied on shaved back of mice for six days, and this is an induction group and compared with a negative control group that involved mice treated with ointment base only for six days. Third group; metformin 10% gel applied on the back of mice with imiquimod for six days. The fourth group included used of metformin 15% gel with imiquimod for six days. This data was analysed for significant level when $p \geq 0.05$ by using either ANOVA test for biochemical parameters levels evaluation. Furthermore, imiquimod 5% cream of induction group caused an elevation of inflammatory parameters; IL17 and IL23 in skin tissue homogenate when being compared to IL17 and IL23 level of the control group. Whereas, metformin 10% gels showed levels of IL17 and IL23 with significant different from induction group. In addition, metformin 15% gels exerted a significantly low level of IL17 and IL23. The possible considerable antipsoriatic activity of topical metformin gel through reducing scaling and erythema and mediated through modest anti-inflammatory effects by suppressing levels of IL17/23.

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INTRODUCTION

Psoriasis is a chronic, non-communicable, painful, disfiguring and disabling disease for which there is no cure and with a great negative impact on patients' Quality of Life (QoL). It can occur at any age and is most common in the age group 50–69 (Organization, 2016). The characteristic of skin lesions mostly consisted of symmetrical, red papules and plaques, covered with white or silver scale and sharply demarcated that present particularly over the extensor surfaces and scalps (Pariser *et al.,* 2016). These lesions cause itching, stinging and pain for individuals with psoriasis (1.3% - 34.7%) who develop chronic, inflammatory arthritis (psoriatic arthritis) that leads to joint deformations and disability (Pariser *et al.,* 2016). The prevalence of psoriasis was estimated to be around 1.3-2.2 % (Springate *et al.,* 2017) in the UK and the psoriasis prevalence was 2.3 % (Al-Samarai, 2009) in Iraq. The pathogenesis of this disease is not completely understood. Multiple theories existed regarding the trigger of the disease process including an infectious episode, traumatic insult, and stressful life

events (Burden and Kirby, 2016). Psoriasis involved hyperproliferation of the keratinocytes, in the epidermis, with an increase in the epidermal cell turnover rate, which causes the loss of control of keratinocyte turnover (Griffiths and Barker, 2007). The disorder primarily of epidermal keratinocytes but is still recognized as one of the commonest immune-mediated disorders where TNF alpha, dendritic cells and T cells all contribute substantially to its pathogenesis (Bejarano and Valdecantos, 2013). The activation of plasmacytoid dendritic cells and other innate immune cells in the skin. Then, the pro-inflammatory cytokines produced by innate immune cells, including interferon-alpha (IFNα) and TNF alpha stimulate the activation of myeloid dendritic cells in the skin (Mckenna *et al.,* 2005). In addition, the myeloid dendritic cells produce cytokines, such as interleukin (IL)-23 and IL-12 that stimulate the attraction, activation, and differentiation of T cells. Finally, the recruited T cells produce cytokines that stimulate keratinocytes to proliferate and produce proinflammatory antimicrobial peptides and cytokines (Damsker *et al.,* 2010). Specifically, the epidermis is infiltrated by a large number of activated T-cells, which appear to be capable of inducing keratinocyte proliferation. Activated T-cells migrate from lymph nodes to the skin, where they stimulate the production of a range of cytokines and factors, which interact to produce changes within the resident epidermal and dermal cells. Two main pathways are known, defined by the T cell class involved: IL-2/Th1/IFNγ and IL-23/Th17/IL-17 (Tan *et al.,* 2017). Metformin is an oral antidiabetic agent, which decreases the hepatic glucose output and acts as an insulin sensitizer by increasing the glucose utilization in muscles and adipocytes (Yang *et al.,* 2016). It is the first line of treatment in overweight and obese type 2 diabetic patients as it has been shown to improve the clinical outcome and the quality of life in those patients as well as decreasing their microvascular and macrovascular complications (Association, 2016). Hence, metformin may play an important role in the reducing pathogenesis of psoriasis by anti-proliferative effect mediated by autophagy and apoptosis mechanisms (Martin *et al.,* 2012). Another interference effect with psoriasis pathogenesis through the anti-angiogenesis effect of metformin (Joe *et al.,* 2015). The mice model used because it is the only models that come close to incorporate the complete genetic, immunologic, and phenotypic changes of the disease. They have shown conclusively that psoriasis is a T-cell mediated disease, and have been used to elucidate novel pathogenic pathways (Gudjonsson *et al.,* 2007). Novel strategies to treat psoriasis are likely to derive from the identification of new targets and the use of mouse models for drug testing (Wagner *et al.,* 2010). Therefore, the present study was aimed to evaluate the potential effect of metformin against imiquimod-induced psoriasis in Swiss albino mice and to evaluate the effect of the topical formula of metformin that may be a beneficial effect through avoiding the common side effects of oral metformin.

MATERIALS AND METHODS

The study was conducted on adult Swiss albino mice (22– 32 g; 10-14 weeks of age) procured from Animal House of Medicine College of Al-Nahrain University. The animals were provided with standard pelleted ration and tap water for drinking adlibitum. All animals were maintained under standard management conditions (22 ± 3°C, 50–60% relative humidity, and 12 hours' light-dark cycles). Prior to the start of the experiment, mice were acclimatized in the laboratory conditions for a period of 14 days. All the experimental animals were kept under constant observation during the entire period of study. The experimental protocol was approved by the Ethics Committee of Higher Education of medicine College of Al-Nahrain University. Metformin hydrochloride (purity 99.8%) used in the present study was commercially obtained from India. Imiquimod cream (Aldara)R of MEDA company was purchased from community pharmacies. All other chemicals used in the study for the formulation of metformin gel are purchased from different standard firms. Experimental protocol The metformin gels at the concentration of 10% and 15% were used in this study to determine its effects depending on the unpublished pilot study on tail mice model. Adult Swiss albino mice were randomly allocated into five groups with six mice each. Group, I was treated with vaseline base on shaved back once daily for six days and served as the control. Group II was applied with imiquimod (5%) cream on the shaved back once daily for six days and served as the induction. Group III has treated with both imiquimod (5%) cream and clobetasol ointment for six days. Group IV was treated with both imiquimod (5%) cream and metformin gel (10%) for six days. Group V was treated with both imiquimod (5%) cream and metformin gel (10%) for six days. During the entire period of study that included seven days, all animals were under observations for clinical signs scoring and observing behavioral changes. Collection and processing of samples After the end of six days of daily administrations of treatment, 1 g of the skin of each was collected in 9 ml ice-cold 0.5 M Phosphate buffer saline (pH 7.4) and other part of skin in formal aldehyde (10%) for measuring inflammation parameters and histopathological studies, respectively. Tissue homogenate (10%w/w) was prepared by

homogenizing the skin tissue firstly using mortar and pestle and then homogenizer at 1000 rpm for 5–7 min at 4°C. Before the sample stored in deep freeze (-80), it centrifuged in Eppendorf cool centrifuge at 5000 rpm for 5–7 min at 4°C to obtain a supernatant for each sample. Scoring the clinical symptoms, the objective severity scoring system of inflammation of the back skin was developed based on the Psoriasis Area and Severity Index (PASI), except that for the mouse model, the affected skin area is not taken into account in the overall score. Erythema and scaling were scored independently on a scale from 0 to 4: 0, none; 1, slight; 2, moderate; 3, marked; 4, very marked. The level of erythema was scored using a scoring table with red taints. The cumulative score of erythema plus scaling served as a measure of the severity of inflammation (scale 0–8) (Fits *et al.,* 2009). Determination of interleukin 17 (IL17) in skin tissue homogenate The concentration of tissue IL 17 was measured using the ELISA technique (Shanghai, China). Mouse IL-17 ELISA kit was based on standard sandwich enzyme-linked immune-sorbent assay technology (Liang *et al.,* 2017). Determination of interleukin 23 (IL23) in skin tissue homogenate The concentration of tissue IL 23 was measured using the ELISA technique (Shanghai, China). This assay employs the quantitative sandwich enzyme immunoassay technique (Qiao *et al.,* 2018). Histopathological studies The histopathological studies were carried out according to the standard methods. Formalin-fixed testes of the different group were embedded in paraffin, sectioned and stained with hematoxylin and eosin. It was examined under a light microscope for histopathological studies. Statistical analysis The visual score that considered non-descriptive data was analyzed by using the Mann-Whitney U test that compared between two groups at 0.05 level of significance 0.05. While the inflammatory parameters were analyzed for analysis of variance and post hock Tukey test at 5% level of significance (SPSS 22.0).

RESULTS

In a seven-day experiment, the group II showed a significant increment of erythema scores as compared to group I. At the same time, there was a significant elevation (P≤0.05) of scaling scores of imiquimod effect of group II in comparison with Vaseline effect of group I. The clobetasol treated group III showed significant reduction of erythema scores in comparison with group II besides a significant difference comparing to group I. On the other hand, group III treatment exhibited a considerable reduction of scaling effect of imiquimod when compared to imiquimod treatment only of group II. However, group IV that treated with metformin 10% gel showed significant reduction effects of metformin on erythema as compared to group II that treated with imiquimod only. In a comparison of group IV effect on erythema score with group III effect, there was a significant difference with clobetasol effect on erythema scores. In addition, there was a considerable reduction effect on scaling scores of metformin 10% treated group IV as compared with imiquimod treated group II. Moreover, group IV showed non-significant differences in scaling scores with clobetasol treated group III. Metformin, 15% gel, treated group, group V, exhibited a significant reduction effect on erythema when compared with group II. While the reduction effect found to be significantly different from that shown by clobetasol treated group III. Also, in comparing of group V effect on erythema with group IV, there was a significant reduction effect of metformin 15% gel. On the other hand, the metformin 15% gel treated group V displayed a significant reduction effect on scaling scores compared to the imiquimod scaling effect of group II.

Table 1: Effect of imiquimod, clobetasol, metformin10 %, and metformin 15% on levels of IL17 and IL23 in tissue homogenate

Groups	IL-17 (pg/ml) Mean±SEM	IL23(pg/ml) Mean±SEM
Group I	75.51±11.47	59.15±5.16520
Group II	471.09±41.58[s]	119.16±17.03 [s]
Group III	281.06 ± 33.52*	67.9425±5.777*
Group IV	373.87 ±29.27*	75.4855±11.25*
Group V	347.47±12.19*	83.3950±7.11*

S: p-value ≤ 0.05 significant compared to group I (control). *: p-value ≤ 0.05 significant compared to group II(IMQD)

The effects of metformin found to be significantly different from the effects of both clobetasol and metformin 10% gel on scaling scores. Measurement of skin tissue IL-17 and IL-23 levels in seven days of experiment, as shown in table 1, the level of IL-17 alpha established to be raised significantly (p≤0.05) in group II in comparison with control group I. While, clobetasol treated group III presented a significant fall (p≤0.05) of IL-17 levels when evaluated with imiquimod treated group II. In addition, metformin (10%) gel treated group IV demonstrated a significant (p≤0.05) falling of IL-17 level when being compared to imiquimod treated group II. Furthermore, metformin (15%) gel treated group V exerted significant (p≤0.05) reduction of IL-17 level related to imiquimod group II Measurement of skin tissue IL-23 levels in seven days' experiment, as shown in table (1), the level of IL-23 found to be increased (p≤0.05) in imiquimod treated group II in comparison with control group I. Whereas, clobetasol group presented a significant fall (p≤0.05) of IL-23 levels when being compared to group II. Besides metformin (10%) gel treated group IV demonstrated a significant

(p≤0.05) decreasing of IL-23 level related to imiquimod group II. Also, the same effect seemed with metformin (15%) gel treated group which exhibited a significant reduction (p≤0.05) of IL-23 level related to the imiquimod group. Histopathological changes in the control group (Figure 2), the histologically features of the skin had a normal epidermal layer with normal keratin and dermal layer. In imiquimod cream treated group II, there was an increment in epidermal thickness with the penetration of dermo-epidermal extensions (rete ridges) with the infiltration of inflammatory cells. While in clobetasol group III there was a nearly normal appearance of epidermal thickness with the absence of rete ridges. Group IV showed a less epidermal thickness when being compared to group II. Also, group V showed an improvement of histological alterations with the decrement of epidermal thickness and absence of epidermal extensions with the reduction of inflammatory cell infiltration.

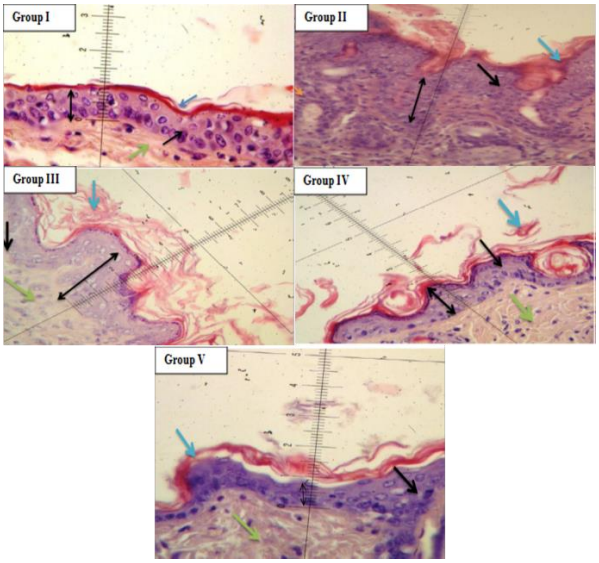

Figure 1: skin section, from animals of study group after 7 days, shows the measuring of epidermal thickness; (black arrow: epidermal layer; blue arrow: keratin layer: double arrow: epidermal thickness; green arrow: dermal layer; orange: rete ridge H&E 500X)

DISCUSSION

Erythema was caused by imiquimod, since it may be acting as a direct mast cell DE granulator through IgE-linked mechanisms. Another possible theory is that imiquimod may also activate mast cells by IgE independent mechanisms (Jacobs *et al.,* 2008; Redegeld *et al.,* 2018). Another clinical sign that was induced by imiquimod was a scaling of back skin of mice. The scaling may be occurred due to imiquimod-induced psoriasis-like skin inflammation in mice via inducing IL-23/IL -17 axis that resembling the pathogenesis of human psoriasis (Girolomoni *et al.,* 2017). Indeed, these phenotypical characteristics appeared by the effect of imiquimod in this study was similar to another study as scoring of erythema and scaling (Arora *et al.,* 2016). While, the reduction of erythema and scaling scores by clobetasol; this result was compatible with Bowman et al. (2002) The reduction of erythema due to potent vasoconstriction blanching effect of clobetasol and the mechanism of vasoconstrictor action is not clearly understood (Uva *et al.,* 2012). It may cause vasoconstriction due to the blocking of action of vasodilators like histamine and bradykinin that lead to reducing the erythema. The vasoconstrictor action may contribute towards the anti-inflammatory effects (Kwatra and Mukhopadhyay, 2018).

Reduction of scaling by clobetasol may be due to anti-inflammatory and anti-proliferative effects — the anti-inflammatory effect through blocking of inflammatory cytokines and suppression of immune cells. Keratinocyte proliferation and keratinocyte growth factors are also decreased. This is accompanied by reduced melanocyte production. The dermis also showed signs of atrophy due to the inhibition of fibroblast proliferation, migration, chemotaxis and protein synthesis (Wei *et al.,* 2017). In the present study, the metformin effect showed a reduction of erythema and scaling scores that was induced by imiquimod. Both topical and oral metformin exerted a preventive and therapeutic effect. The reduction of erythema may be probably due to the possible vasoconstriction effect of metformin (Markos *et al.,* 2014). Metformin reduced the scaling effect of imiquimod that may be attributed to cell cycle arrest and reduce keratinocyte cell proliferation (Ochoa-Gonzalez *et al.,* 2016).

The improving effect of PASI scores of metformin on patients with psoriasis associated metabolic syndrome (Singh and Bhansali, 2017). Additionally, the other studies showed a beneficial effect of oral metformin in obese psoriatic patients (Armstrong *et al.,* 2012; Armstrong *et al.,* 2017).

In the present study, imiquimod exerted an induction of inflammation that may be mediated through the elevation of cytokine levels including IL17 and IL23. These cytokines had a role in the increased proliferation of keratinocytes. Moreover, imiquimod-induced the expression of IL-23/IL-17 in skin tissues of induced psoriasis as similar to the human pathological mechanism of psoriasis. This elevation was found along with another study (Zeichner and Armstrong, 2016). It activated Th17 cells produced IL-17 is a pro-inflammatory cytokine that interacts with IL-17R on the keratinocytes and then mediates the hyperproliferation and differentiation of keratinocytes. It also acts in

synergism with TNF alpha to induce proinflammatory cytokine production by keratinocytes (Zeichner and Armstrong, 2016). However, their roles in the development of psoriasis are still unclear (Monin and Gaffen, 2018). IL-17 and IL-17F have a pro-inflammatory activity inducing the expression of pro-inflammatory cytokines, colony-stimulating factors, and chemokines from dendritic cells, neutrophils, T cells, monocyte/macrophages, and epithelial cells (Armstrong *et al.,* 2011). IL-17 can mobilize, recruit, and activate neutrophils (Weaver *et al.,* 2013). IL-17 is undetectable in normal skin, and biological therapy that inhibits Th17 pathways results in the reduced expression of IL-17 and IL-23, and improved disease outcomes (Michalak-Stoma *et al.,* 2011). Th17 cells and the cytokines produced by these cells are found in increased levels within the skin affected by psoriasis (Kagami *et al.,* 2010). A statistically significant difference in serum IL-17 level had been found in psoriatic patients compared to healthy controls. IL-17 serum levels were correlated with the psoriasis area and severity index (PASI) (Takahashi *et al.,* 2010). The IL-23 was an inflammatory biomarker, and it has a crucial role during the pathogenesis of psoriasis (Boniface *et al.,* 2008). IL-23 is overproduced by dermal dendritic cells (Lee *et al.,* 2004) and keratinocytes in the psoriatic lesion and IL-23 has the influence on sustenance and amplification of the chronic inflammation in psoriasis (Meglio and Nestle, 2010; Wilson *et al.,* 2007). Level of IL-23 decreased after psoriasis therapy (Piskin *et al.,* 2006). The IL-23 was released from dendritic cells and mediated the terminal differentiation and the activation of Th17 cells. Study have demonstrated that the IL-23/Th17 axis, which is critical for the pathogenesis of psoriasis in humans, plays an important role in the mouse skin inflammation induced by IMQ, because the expression of IL-23 and Th17-related cytokines is increased in the lesional skin and deficiency or blockade of IL-23 results in suppression of this inflammation (Cai *et al.,* 2011).

Metformin was showing a potential reduction effect on the levels of IL17, and IL23 thus may be mediated by anti-inflammatory effects and immunomodulation which lead to amelioration of psoriatic lesion and reduction of inflammation signs of the skin. The current study data showed that topical and oral administration of metformin improved IMQ-induced skin inflammation in mice. Moreover, metformin acted as an anti-inflammatory agent by activating Adenosine Monophosphate-activated protein kinase (AMPK), and this activation of AMPK might induce inhibition of dendritic cell and T-cell activation as well as cell proliferation, which was considered as the hallmark of psoriasis (Glossmann and Reider, 2013). In the present study, imiquimod showed the high thickness of the epidermis, and epidermal extension and such observed alterations are in agreement with (Rather *et al.,* (2018). While, clobetasol showed improvement of visual and histopathological changes and topical clobetasol, high-potency corticosteroids that have been used in the topical treatment of psoriasis for decades. Their efficacy can be attributed to multiple mechanisms of action, including their anti-inflammatory and immunosuppressive effects (Uva *et al.,* 2012). In another study where clobetasol showed a pronounced effect on imiquimod-induced psoriasis (Sun *et al.,* 2014). In addition, metformin showed a preventive effect on imiquimod. This effect was being obvious on a histopathological study in addition to the biochemical study. The anti-inflammatory effect of metformin exerted irrespective of diabetes mellitus status that was illustrated by the suppression effect on plasma cytokines (Cameron *et al.,* 2016b). In addition, the anti-inflammatory effect introducing it for pleiotropic effects as an alternative drug for neuropathy (Afshari *et al.,* 2018). Metformin has a direct anti-inflammatory action. Studies have suggested that metformin suppresses inflammatory response by the inhibition of nuclear factor κB (NFκB) via AMPK-dependent and independent pathways (Saisho, 2015). Furthermore, in mouse hepatocytes, TNFα-dependent IκB degradation and expression of pro-inflammatory mediators were inhibited by metformin. These effects upon NF-κB signalling could be separated from metabolic effects as or AMPK activation; metformin use was associated with the suppression of several plasma cytokines (Cameron *et al.,* 2016a). The other potential molecular mechanism of anti-inflammatory action of metformin by increasing nitric oxide production and that inhibits the poly [ADP ribose] polymerase 1 (PARP-1) pathway through AMPK activation, leading to the inhibition of inflammatory response. In addition, metformin also suppresses inflammatory response through inhibition of advanced glycation end products formation and its receptor expression (Saisho, 2015). Other metformin effects are against inflammatory, and pro-oxidant stress there found to be having a direct and beneficial effect on arterial and renal endothelial cells function in obese rats, including enhanced NO release and reduced nitroxidative stress, beyond any effects on fasting glucose levels (Sambe *et al.,* 2018). However, there was less or no similar study on the effect of topical or systemic metformin on imiquimod-induced psoriasis, and it is possible to be studied with different concentration and on a human with mild plaque psoriasis.

CONCLUSION

The finding of the present study could be concluded that metformin as topical gel was showing

a potential antipsoriatic activity and anti-inflammatory effects through the reduction of inflammatory parameters, in addition to histopathological improvement.

Declarations, Abbreviations

- IFNα: interferon-alpha
- IL: interleukin
- QoL: quality of life
- TNF alpha: tumour necrosis factor alpha

Ethics approval and consent to participate: Ethics approval form Ethical Committee of Medicine College/Al-Nahrain University

Consent for publication: There is no consent for publication, so it not applicable.

Availability of data and material: The datasets generated and analysed during the current study are available from the University database and also with the corresponding author on reasonable request.

Competing interests: There is no competing author

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Authors' contributions: Haider F. Alsaedi, Pharmacist, M.Sc. pharmacology, and toxicology; assistant lecturer in Pharmacy College/Misan University and PhD. Candidate in the higher education department of pharmacology and toxicology of College of Medicine/Al-Nahrain University act on experimental design and working. Mukhallad A. Al-Rubye, Veterinarian, PhD pathology; lecturer in Medicine College-Misan University; interpretation and done the histopathological pathological part of the study. Professor Adeeb Ahmed Zubaidi; MD, PhD pharmacology and toxicology; Dean of Pharmacy College/Al-Nahrain University/ supervision. All authors read and approved the final manuscript.

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