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RESEARCH ARTICLE
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Evaluation of the Anti-Psoriatic Activity of Dapagliflozin and Levofloxacin Ointment on the Tail Mice

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ABSTRACT

The goal of this research study was to prepare topical ointments containing dapagliflozin and levofloxacin and evaluate their anti-psoriatic activity in an animal model. Eighty Swiss albino mice weighing between 24 and 30 g were randomly divided into eight groups of 10 mice each. The first group was the control group, which received a baseline ointment. The second group was treated with clobetasol (5%) cream for six days. The third, fourth, and fifth groups were treated with dapagliflozin ointments at concentrations of 5%, 10%, and 20%, respectively, which were daily applied. The sixth, seventh, and eighth groups were treated with levofloxacin ointments at concentrations of 10%, 20%, and 40%, respectively, which were daily applied. Histopathological changes were observed in the treated skin. The results demonstrated that dapagliflozin and levofloxacin ointments at the specified concentrations could significantly reduce the severity of symptoms such as erythema, scaling, and thickening, as well as inflammation. Therefore, these ointments have strong anti-psoriatic and anti-inflammatory properties.

Keywords: *dapagliflozin, levofloxacin, psoriasis.*

INTRODUCTION

Psoriasis is a chronic, autoimmune, disfiguring, and disabling skin disease and no cure and with reduce the quality of life. It characterized by abnormal enhance in proliferation and less differentiation of the epidermal keratinocytes, with a rapid turnover rate of the epidermal cell. Psoriasis is a common inflammatory condition of human skin characterized by focal to coalescing raised cutaneous plaques with consistent scaling and variable erythema (Lakshmi et al 2020).

Typical histologic features of psoriasis include epidermal hyperplasia (acanthosis) with elongated rete ridges, a less discrete epidermal granular layer, parakeratosis and leucocytic infiltration of the dermis and epidermis. The mice tail model is as natural model for investigating the anti-psoriatic drugs of tested topical drug by measuring the decreasing of parakeratosis and increasing the orthokeratosis (Al-Saedi et al.,2018).

Dapagliflozin (DPG) individuals with type 2 diabetes are treated thru Inhibitors of Na-G co-transporter-2 (NA-G TRANSPORTER), such as DPG, are the first prescription in a new pharmacological class. DPG lowers renal G reabsorption by blocking the DPG in the kidneys, which causes urine G excretion and a drop in blood G levels. DPG effectiveness is dependent on the secretion and action of insulin (Dhillon 2019). DPG could be suppressing inflammatory signaling independently of some of its metabolic effects. DPG acted highly selectively to reduce proinflammatory cytokine secretion from activated macrophages (Abdollahi et al.,2022). DPG has been shown to have anti-proliferative and anti-tumor effects in prior research, and these data suggest that NA-G TRANSPORTER inhibitory treatment induces AMPK-mediated (Zhou et al.,2020). Among the third-generation fluoroquinolone antibiotics is levofloxacin (Castro et al., 2020), used extensively in veterinary and human medicine to slow the development of bacteria by interfering with their ability to replicate their DNA (Khondker et al., 2021). Levofloxacin is an effective third-generation fluoroquinolone antibiotic for the treatment of bacterial infections. In the WHO's list of necessary medications, levofloxacin is a safe and efficient drug. It was granted a patent in 1987 and subsequent approval by the FDA for use in medicine in the US in 1996(Bush et al.,2011). Levofloxacin has been given FDAapproval for the treatment of a number of conditions, such as treating patients with bacterial infections with the help of the medical staff (Podder et al.,2019). The anti-inflammatory and anti-proliferative effects of levofloxacin are mediated by inhibiting inflammatory cytokines (Liu et al.,2019). Levofloxacin hydrochloride (LH) shows universal anti-proliferation activity in all cancer cell lines in our previous study (He et al .,2021).

Aims of study

The present study aimed to evaluate the effects of different concentrations of topical dapagliflozin and levofloxacin on induced psoriasis by measuring their potential anti-psoriatic activity and anti-inflammatory effects besides physical and histopathological evaluations.

MATERIALS AND METHODS

Animal of study and ethical approval

obtain sixty adult male rats, whose weights ranged between (24-33 g) and their ages ranged between (11-15) weeks. The animals were housed in plastic cages at a convenient environment for the aspect of heat, ventilation, and nutrition materials. The animals were allowed to acclimate for two weeks in the College of Pharmacy-University of Karbala. The study protocols were conducted according to the Ethical approval of the Ethics Committee of University of Al-Ameed.

Preparation of dapagliflozin ointment

Dapagliflozin was dissolved in an amount of 5 g, 10 g and 20 g in 2 concentrated ethanol to prepare with the addition of 5 ml of glycerol, then the mixture was supplemented with Vaseline for a final weight of 100 g in a beaker and was mixed at 70oC using water bath to insure melting all components. The mixture was slowly cooled and further stirred for 30 minutes until solidified using a motor at 500 revolutions per minute. The prepared ointment was filtered using filter paper to remove any impurities. After that, the sensory properties and pH of levofloxacin ointment were evaluated, and thermal and centrifugal stability tests were performed. The rheological evaluations using a viscometer and microbiological tests was

done according to procedure given by Jahandideh 2019.

Preparation of Levofloxacin ointment

Levofloxacin was dissolved in an amount of 10 g, 20 g and 40 g in 2 concentrated ethanol to prepare with the addition of 5 ml of glycerol, then the mixture was supplemented with Vaseline for a final weight of 100 g in a beaker and was mixed at 70oC using water bath to insure melting all components. The mixture was slowly cooled and further stirred for 30 minutes until solidified using a motor at 500 revolutions per minute. The prepared ointment was filtered using filter paper to remove any impurities. After that, the sensory properties and pH of levofloxacin ointment were evaluated, and thermal and centrifugal stability tests were performed. The rheological evaluations using a viscometer and microbiological tests was done according to procedure given by Jahandideh 2019.

Animal model and experiment design

In this study, eighty Swiss albino mice were divided into eight groups of ten mice each. The first group received Vaseline daily for six days, while the second group received clobetasol daily for six days. The third, fourth, and fifth groups were treated with dapagliflozin ointment at concentrations of 5%, 10%, and 20%, respectively, and received daily applications for six days. The sixth, seventh, and eighth groups were treated with levofloxacin ointment at concentrations of 10%, 20%, and 40%, respectively, and received daily applications for six days. About 0.1ointment from Vaseline and each concentration of topical dapagliflozin and Two hours of treatment with levofloxacin ointment given to the proximal 2.5 centimeters of the tail. Next, water was used to clean the tails. Throughout the course of two weeks, patients received treatment five times a week, once per day. The animals were euthanized with ether following the last treatment and then sacrificed. The tails were then dissected and the proximal portions were preserved in 10% formalin for histological analysis (Bhatia et al.,2014).

RESULTS

Effects of Different dapagliflozin and levofloxacin ointment Concentrations on Histological Features of the Mice Tail. The histological section of tail skin of mice treated with Vaseline showed a spread of keratin layer and normal appearance of the epidermal layer with a little number of granular cells and normal dermal layers

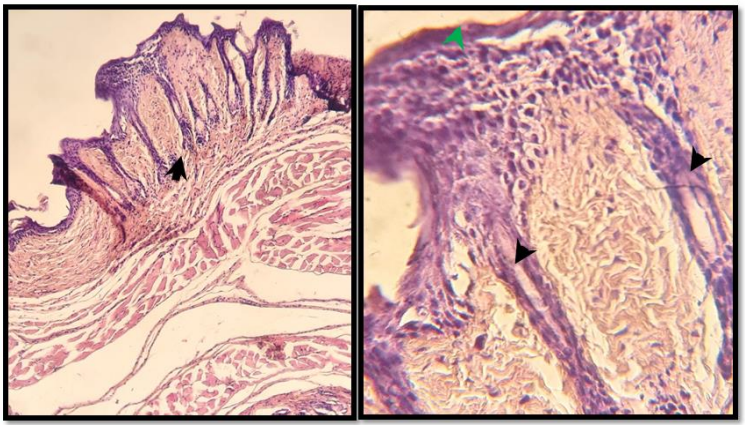

FIGURE 1. skin of Vaseline treated group show normal epithelium in the epidermal layer (black arrow), keratin in the superficial most of the epidermal layer (green arrow) and normal hair follicle in the dermal layer (red arrow) H&EA) 125XB) 500X

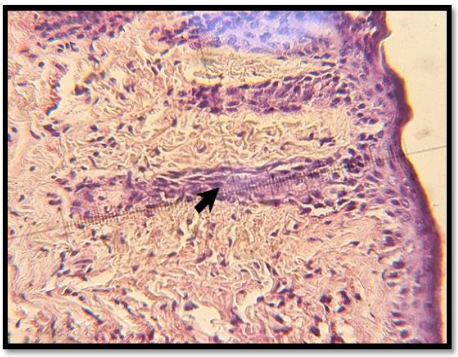

FIGURE 2. Histomorphometry of the skin of Vaseline treated group show an average thickness of the epidermal layer (black arrow) 500X

The effect of clobetasol ointment on the tail skin of mice showed skin of clobetasol treated group show marked thickening of epidermal layer which reveal extension of the epidermis deeply in the dermal layer of the epidermis layer (figure 3). It showed (figure 4) an increment in epidermal thickness in the vertical measurement of in comparison with the control group.

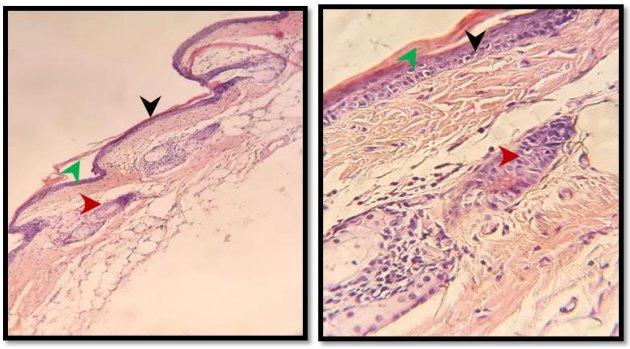

FIGURE 3. skin of clobetasol treated group show marked thickening of epidermal layer which reveal extension of the epidermis deeply in the dermal layer (black arrow), thick keratin layer with parakeratosis (green arrow) H&E A) 125X B) 500X

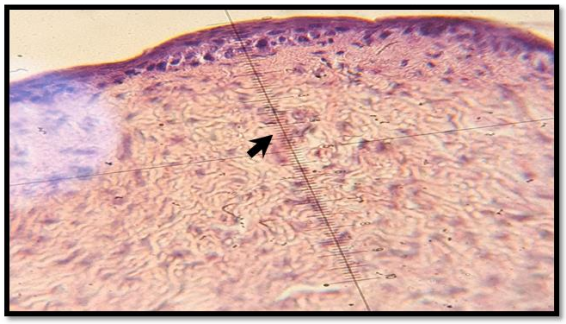

FIGURE 4. Histomorphometry of the skin of clobetasol treated group show an average length of epidermal extensions (black arrow) 500X

The effect of dapagliflozin 5% ointment on histological features showed marked thickening of epidermal layer in comparison with control group (figure 5, and 6).

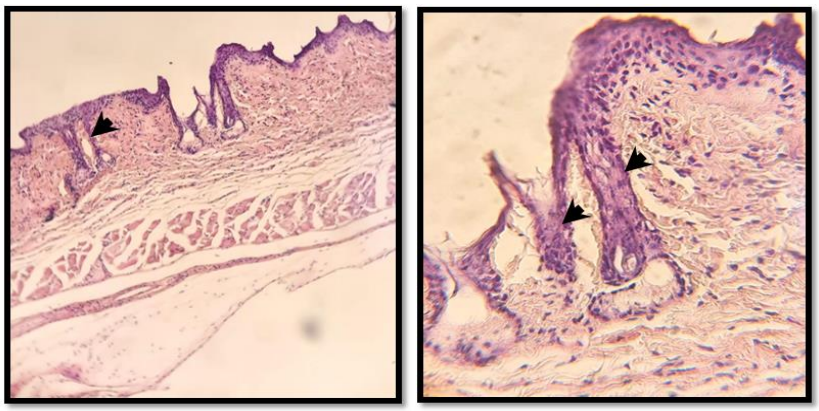

FIGURE 5. skin of dapagliflozin (5%) ointment treated group show marked thickening of epidermal layer which reveal extension of the epidermis deeply in the dermal layer(black arrow). H&E A) 125X B) 500X

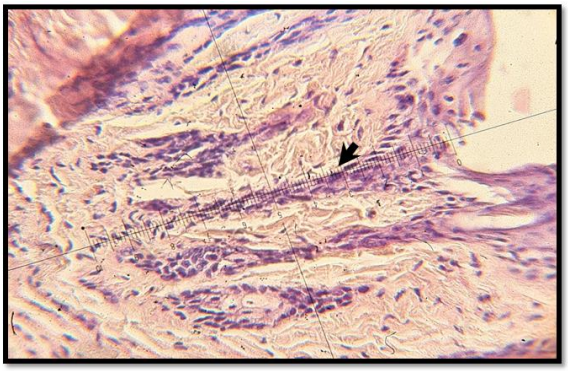

FIGURE 6. Histomorphometry of the skin of dapagliflozin (5%) ointment treated group showed an average length of epidermal extensions (black arrow) 500

The effect of dapagliflozin 10% ointment on the tail skin of mice showed a skin treated group moderate thickening of epidermal layer with deep extensions of the epidermis in to the dermal layer (figure 7 and 8).

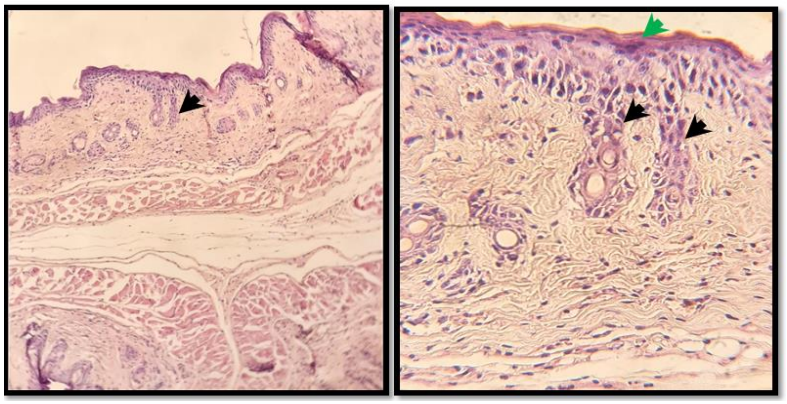

FIGURE 7. skin of dapagliflozin (10%)ointment treated group show moderate thickening of epidermal layer with deep extensions of the epidermis in to the dermal layer (black arrow), minimum keratin in the superficial layer (green arrow) H&E A) 125X B)500X

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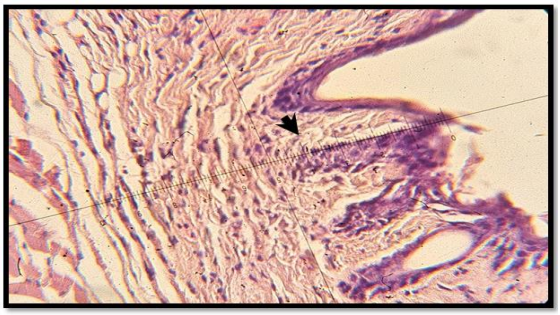

FIGURE 8. Histomorphometry of the skin of dapagliflozin (10%) ointment treated group show an average length of epidermal extensions (black arrow) 500X.

The effect of dapagliflozin 20% ointment on the tail skin of mice showed a skin treated group enhancement thickening of epidermal layer with decrease extensions of the epidermis in to the dermal layer (figure 9 and 10).

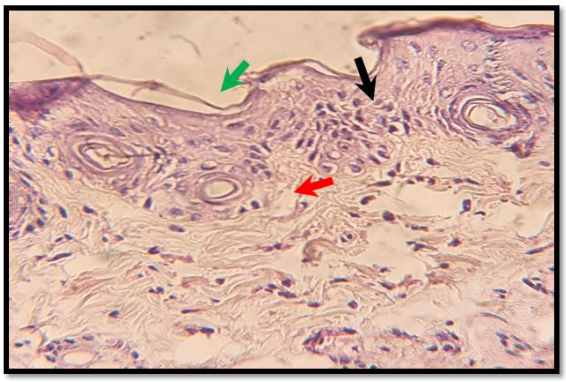

FIGURE 9. skin of dapagliflozin ointment (20%) treated group show normal epithelium in the epidermal layer (black arrow), keratin in the superficial most of the epidermal layer (green arrow) and normal hair follicle in the dermal layer (red arrow) H&E) 500X

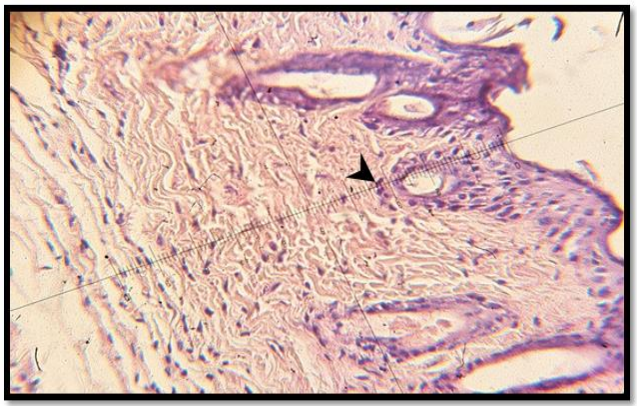

FIGURE 10. Histomorphometry of the skin of dapagliflozin (20%) ointment treated group show an average thickness of epidermal layer was 2.8±0.6 µm (black arrow)B500X

The effect of levofloxacin 10% ointment on the tail skin of mice showed a skin treated group moderate thickening of epidermal layer with decrease extensions of the epidermis in to the dermal layer (figure 11 and 12).

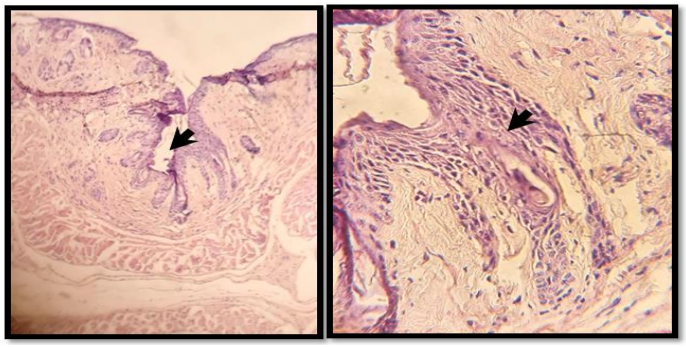

FIGURE 11. skin of levofloxacin (10%) ointment treated group show marked thickening of epidermal layer which reveal extension of the epidermis deeply in the dermal layer (black arrow). (H&EA) 125XB

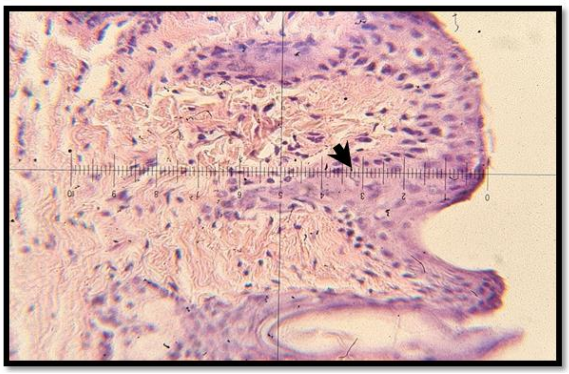

FIGURE 12. Histomorphometry of the skin of levofloxacin(10%) ointment treated group show an average length of epidermal extensions (black arrow) 500X

The tail skin of mice after levofloxacin 20% ointment treatment showed enhancement in epidermis layer (Figure 13). It showed obvious increment in epidermal thickness in vertical measurement in comparison with control group

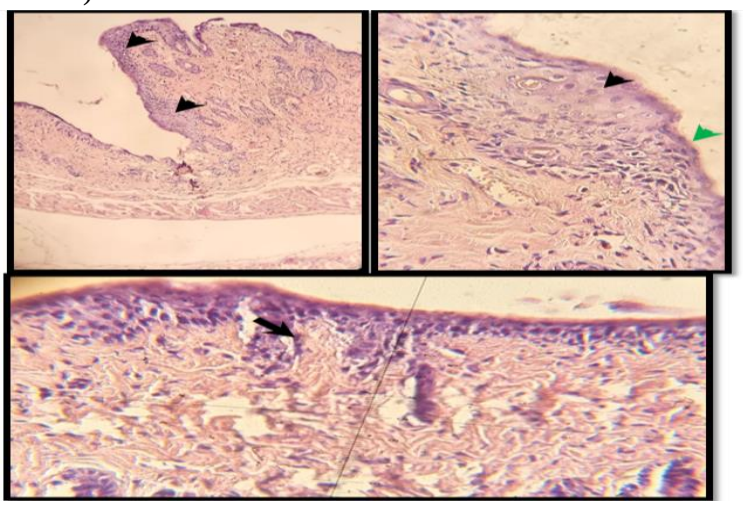

FIGURE 13. Histomorphometry of the skin of levofloxacin (20%) ointment treated group showed an average length of epidermal extensions (black arrow) 500X

The tail skin of mice after levofloxacin (40%) ointment treatment showed enhancement in orthokeratosis in epidermis layer (Figure 14 and 15). It showed obvious increment in epidermal thickness in vertical measurement in comparison with control group

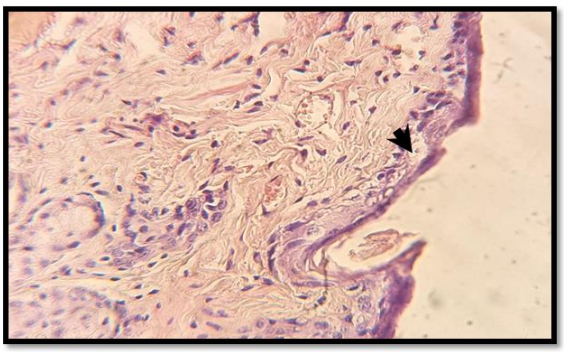

FIGURE 14. skin of levofloxacin (40%) ointment treated group showed normal epidermal layer thickness (black arrow), normal skin appendages (green arrow) H&E A 125X

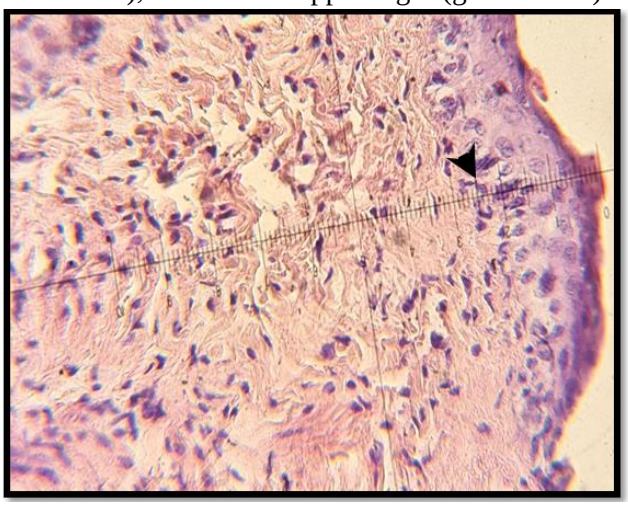

FIGURE 15. Histomorphometry of the skin of levofloxacin (40%) ointment treated group show an average thickness of epidermal layer (black arrow) 500X

DISCUSSION

The investigation on the control group was compatible to based fact that the adult mouse tail normally had parakeratosis which a form of abnormal epidermal keratinizing pattern that is characterized by an absence of the granular layer in the epidermis (Parnami *et al.*,2014). The reasons for parakeratosis could be a substantial surge in the mitosis and decline in the transit time of the differentiating keratinocyte during cornification.

When the dapagliflozin and levofloxacin applied topically on mouse-tail, it showed anti-psoriatic activity by increasing differentiation of parakeratosis into orthokeratosis by increasing numbers of granular cells per scale. This effect similar to the effect of the topical antipsoriatic activity of tamoxifen(Bhatia *et al.*,2014) and tazarotene (Hashim *et al.*,2018).

The antipsoriatic activity results are compatible with other studies for such as aloe vera gel produced a significant differentiation in the epidermis, as seen from its degree of orthokeratosis (Flutter and Nestle,2013). Also, this model used to assess the antipsoriatic activity of tamoxifen (Bhatia *et al.*,2014).

The dapagliflozin ointment affected the differentiation of cell may be through it affected the signaling pathway of insulin-like growth factors that regulate cell proliferation, differentiation, aging, and lifespan(Joshi,2011).

In case of dapagliflozin ointment treated groups, it showed an increment of epidermal thickness with raised of concentration and this elevation in a thickness similar to what was showing by tazarotene, dithranol and tretinoin 0.05% (Sebök *et al.*,2000). This increment of epidermal thickness may not due to hyperproliferation, but possibly caused by abnormal terminal differentiation, which affect stratum corneum formation (Limandjaja et al.,2017).

However, other explanation for the increase of epidermal thickness simply by irritation or a 'keratoplastic' mode of action of the drugs(Sebök *et al.*,2000)

In this study dapagliflozin and levofloxacin ointment in deferent concentration was enhance in histological because dapagliflozin and levofloxacin had strong anti-proliferation and anti-inflammatory (Luo et al.,2021and Zhang et al.,2021).

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Conflicts of interest

Authors declare no conflict of interest

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