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Pharmacological Evaluation of Anti-Psoriatic Activity of *Madhuca longfolia* on Experimental Animals

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Keywords: Anti-proliferation, Anti-psoriatic, *Madhuca longfolia*, UV ray, photo dermatitis,

ABSTRACT

Objective: To carry out pharmacological evaluation of anti-psoriatic activity of *Madhuca longifolia* on experimental animals. **Methods:** In present study we used two models for psoriasis viz., Ultraviolet- C induced photodermatitis model for psoriasis and Perry's Scientific Mice Tail Model, In first model rats were exposed to the UV-C light for 3 days and photodermatitis was produced in the groups treated with standard and *Madhuca longifolia* (Ml) gel (2.5% and 5%). The parameters to be evaluated are epidermal layer thickness, presence of stratum granulosum layer. In case of Perry's mice tail model, the parakeratosis is hallmark for the psoriasis mice tail having the parakeratotic condition; the induction of orthokeratosis in mice tail indicate the drug activity against psoriasis. In this model, orthokeratosis % is calculated by applying ML gel (2.5% and 5%) 5 times in a week for two weeks and the tail samples evaluated with formula for orthokeratosis. **Results**: The UV-C induced photodermatitis model for psoriasis shown significant reduction in epidermal thickness and increased layer of stratum granulosum also in Perry's mice tail model produced significant orthokeratosis with respect to control was observed in groups treated with tazarotene and ML gel 2.5% and 5%. The maximum anti-proliferative activity was observed with 5% ML gel. **Conclusion:** The result obtained in the present study suggests that the gel may prove to be potential therapeutic drug for treating psoriasis. Further detailed experimentation with regards to isolation, purification, mechanism and pharmacological screening of the active principles in *Madhuca longifolia* needs to be done.

INTRODUCTION

Psoriasis is a chronic inflammatory skin disorder characterized by erythematous, sharply demarcated papules and rounded plaques covered by silvery micaceous scale. (Milavec et al. 2011) The increased epidermal proliferation is related to dysregulation of the immune system (Derm et al. 2010). Plaques frequently occur on the skin of the elbows and knees but can affect any area including the scalp and genitals. The cause of psoriasis is not known, but it is believed to have a genetic component (Pandey et al. 2010). As it is known as skin disease, the epidermal transit time is shortened from the normal 28 days to as short as 3-4 days (Derm et al. 2010). The manifestations of psoriasis are not limited to the skin. Co-morbidities may complicate moderate to severe psoriasis. In particular, the relative risks of ischaemic heart disease, stroke, hypertension, dyslipidemia, diabetes and Crohn disease are increased in people with psoriasis. The higher rates of hypertension and diabetes may partly explain the increased risk of heart attacks, stroke, and cardiovascular mortality in people with severe psoriasis reported in large population-based cohort studies. (Rim et al. 2005)

MATERIAL AND METHODS

Collection and Authentication of Plant material:

The fresh leaves of *Madhuca longifolia* were collected from Yavatmal district of India. The shade dried, coarsely powdered leaf material was extracted successively with methanol and aqueous using soxhlet apparatus. Extract was obtained and used for further phytochemical analysis.

Extraction of *Madhuca longifolia:*

About 650 grams of the dry powder was extracted first with Petroleum ether for defatting then powder again extract with 2 liters of Methanol 60-80^{0}C by continuous hot percolation method using soxhlet apparatus. After completion extraction, the methanolic extract was filtered and concentrated to dry mass by vacuum distillation. A dark color residue was obtained.

Preliminary phytochemical screening:

(Trease & Evans et al.) Preliminary phytochemical screening was carried out to find the presence of the active chemical constituents such as alkaloids, flavonoids, tannins, phenolic compounds, steroids, proteins, cardiac glycosides, carbohydrates, fixed oils and fats**.**

Animals:

Rats of Wistar strain weighing 150-200g and mice of swiss albino strain weighing 25-27g were obtained from National Institute of Bioscience, Pune. Animals were housed in group of five under standard laboratory conditions of temperature (25±2^{0}C) and 12hr light, 12 hr dark cycle with free access to standard pellet diet and water *ad libitum.* Laboratory animal handling and experimental procedures were performed in accordance with the guidance of CPCSEA (198/99/CPCSEA) and experiment protocol was approved by Institutional Animal Ethics Committee (DYIPSR/IAEC/15-16/P-01).

Pharmacological Evaluation:

Ultraviolet- C induced photodermatitis model for psoriasis:

The hairs of the skin, on one side of the flank, were depilated by clipping with scissors followed by careful shaving taking precaution to avoid injury to the skin. The animals were then placed on a curved wooden block and their legs tied around it, to avoid contact with the floor. This arrangement prevented the movement of the animal during its subsequent exposure UV radiation. Except for an area of 1.5 × 2.5 cm on the depilated skin, the entire animal was covered with a UV resistant film. The uncovered area of 1.5 × 2.5 cm was then irradiated for 45 min with a UV-C lamp kept at a vertical distance of 20 cm from the skin. Application of the drug was started 12 h after irradiation and was continued for three days. A schedule of two applications per day spaced over 12 h intervals was maintained. On the third day, 2 h after the last treatment, animals were sacrificed using diethyl ether and the exposed area of the skin was removed by surgical incision. The incised skin was then fixed in 10% buffered formalin solution that was followed by gradual dehydration using increasing strength of alcohol (80% to absolute alcohol). The skin was then embedded in paraffin wax and sections of 4 µm thickness were obtained using a microtome. These sections were transferred onto a glass slide and stained with hematoxylin and eosin.

Perry's Scientific Mice Tail Model:

Male albino mice weighing 25–27 g are used. The tails are treated locally with 0.1 ml ointment applied to the proximal part of the tail. For the contact time of 2h, a plastic cylinder is slipped over the tail and fixed with adhesive tape. At the end of contact time, the cylinders are removed and the tails washed. Animals are treated once daily, 5 times a week, for 2 weeks. 5 animals are used per dosage group. Two hours after the last treatment the animals are sacrificed and the tails prepared histologically (fixation in 4% formalin, paraneoplastic embedding). Longitudinal sections of about 5 µm thickness are prepared and stained with hematoxylin-eosin. Number of scale regions with continuous granular layer was counted and expressed as percentages of the total number of scale regions per section. Drug activity is defined by the increasing in percentage of orthokeratotic regions. Ten sequential scales were examined for the presence of the granular layer induced in the previously parakeratotic skin areas. The induction of orthokeratosis in those parts of the adult mouse tail, which have normally parakeratotic differentiation, was quantified measuring the length of granular layer (A) and the length of scale (B).The proportion (A/B)× 100 represents the % orthokeratosis per scale, and the drug activity (DA) was calculated as follows,

DA= (mean OK of treated group – mean OK of control group × 100)/ (100-mean OK of control group)

Where OK means Orthokeratosis

Statistical Analysis Data Analysis:

Arithmetic means of the values of readings were calculated for each experiment. The results obtained were used for statistical analysis using INTA Software. The data obtained from various models of hepatotoxicity in rat experiments were subject to Analysis of Variance (ANOVA) followed by Dunnett's t-test using INTA software. Values of $p < 0.01$ were considered statistically significant.

RESULTS

Percentage yield of crude extracts: 650 gm *Madhuca longifolia* powder packed in soxhlet apparatus for extraction of soluble bioactive molecules from leaves by using methanol. The percentage yield was found to be 16.8 w/w.

Ultraviolet- C induced photodermatitis model for psoriasis:

Effect of drug on thickness of epidermal layer of UV-C-induced photodermatitis model for psoriasis:

Animals are exposed with UV-C lamp have increased the thickness of epidermal layer because of hyperproliferation of keratinocytes. Animal treated with standard gel, ML gel 5% and ML gel 2.5% showed significant reduction ($p<0.01$) in epidermal layer thickness as compared to control group.

Effect of drug on thickness of stratum corneum layer of UV-C-induced photodermatitis model for psoriasis:

Animal treated with standard gel, ML gel 5% and ML gel 2.5% showed significant increases ($p<0.01$) in stratum corneum thickness as compared to control group.

Effect of drug on thickness of stratum granulosum layer of UV-C-induced photodermatitis model for psoriasis:

The stratum granulosum layer is absent or reduced when animal exposed with UV-C lamp. Animal treated with standard gel, ML gel 5% and ML 2.5% showed significant increases ($p<0.01$) in stratum granulosum thickness as compared to control group.

Perry's Mice tail model for psoriasis:

Effect of drug on Orthokeratosis % of Perry's Mice tail model for psoriasis

Animal treated with standard gel, ML gel 5% and ML gel 2.5% showed significant orthokeratosis (%) ($p<0.01$) when compared with control group.

% activity of drug on Perry's Mice tail model for psoriasis

The % activity of drug was significant as compared with standard drug.

Table no. 1. Epidermal thickness

Sr.No.	Groups (n=5)	Epidermal Thickness
1	Control	72.932 ± 1.12
2	Standard	38.372 ± 2.15**
3	Gel base	69.102 ± 1.62
4	2.5% ML gel	54.654 ± 2.07**
5	5 % ML gel	45.984 ± 1.87**

Values are expressed as mean ± SEM; statistically analyzed by One Way ANOVA followed by Dunnett's test. *p<0.05, **p<0.01 when all groups compared with Control group. ##p<0.01

Control: Animal exposed with Ultraviolet-C

Standard: Animal exposed with Ultraviolet-C + Standard gel

Gel base: Animal exposed with Ultraviolet-C + Gel base

2.5% ML gel: Animal exposed with Ultraviolet-C + 2.5% *Madhuca longifolia* gel

5% ML gel: Animal exposed with Ultraviolet-C + 5% *Madhuca longifolia* gel

Table no. 2. Thickness of Stratum corneum

Sr. No.	Groups (n=5)	Thickness of Stratum corneum
1	Control	3.74 ± 0.13
2	Standard	10.182 ± 0.58**
3	Gel Base	4.174 ± 0.38
4	2.5% ML gel	7.716 ± 0.14**
5	5 %ML gel	8.71 ± 0.42**

Values are expressed as mean ± SEM; statistically analyzed by One Way ANOVA followed by Dunnett's test. $^{*}p<0.05$, $^{**}p<0.01$ when all groups compared with Control group. $^{\#\#}p<0.01$

Control: Animal exposed with Ultraviolet-C

Standard: Animal exposed with Ultraviolet-C + Standard gel

Gel base: Animal exposed with Ultraviolet-C + Gel base

2.5% ML gel: Animal exposed with Ultraviolet-C + 2.5% *Madhuca longifolia* gel

5% ML gel: Animal exposed with Ultraviolet-C + 5% *Madhuca longifolia* gel

Table no. 3. Thickness of stratum granulosum

Sr. No.	Group (n=5)	Thickness of stratum granulosum
1	Control	0.7 ± 0.1
2	Standard	5.85± 0.27**
3	Gel Base	1.1 ± 013
4	2.5% ML gel	3.67 ± 0.24**
5	5 %ML gel	5.16 ± 0.19**

Values are expressed as mean ± SEM; statistically analyzed by One Way ANOVA followed by Dunnett's test. $^{*}p<0.05$, $^{**}p<0.01$ when all groups compared with Control group. $^{\#\#}p<0.01$

Control: Animal exposed with Ultraviolet-C

Standard: Animal exposed with Ultraviolet-C + Standard gel

Gel base: Animal exposed with Ultraviolet-C + Gel base

2.5% ML gel: Animal exposed with Ultraviolet-C + 2.5% *MadhucaLongifolia* gel

5% ML gel: Animal exposed with Ultraviolet-C + 5% *MadhucaLongifolia* gel

Table no. 4. Orthokeratosis (%)

Sr. No.	Treatments	Orthokeratosis(%)
1	Control	25.2 ± 2.7##
2	Standard	64.7 ± 4.2
3	Gel Base	29.9 ± 1.6
4	2.5%ML gel	37.3 ± 2.5*
5	5% ML gel	51 ± 2.7**

Values are expressed as mean ± SEM; statistically analyzed by One Way ANOVA followed by Dunnett's test. $^{*}p<0.05$, $^{**}p<0.01$ when all groups compared with Control group. $^{\#\#}p<0.01$

Control: Animal has received saline

Standard: Animal has received Standard gel

Gel base: Animal has received Gel base

2.5% ML gel: Animal has received 2.5% *Madhuca longifolia* gel

5% ML gel: Animal has received 5% *Madhuca longifolia* gel

Table no. 5. Drug Activity (%)

Sr. No.	Treatments	Activity (%)
1	Control	-
2	Standard	51**
3	Gel base	-
4	2.5%ML gel	16*
5	5% ML gel	29**

Values are expressed as Mean ± SEM; statistically analyzed by One Way ANOVA followed by Dunnett's test. $^{*}p<0.05$, $^{**}p<0.01$ when all groups compared with Control group. $^{\#\#}p<0.01$

Control: Animal has received saline

Standard: Animal has received Standard gel

Gel base: Animal has received Gel base

2.5% ML gel: Animal has received 2.5% *Madhuca longifolia* gel

5% ML gel: Animal has received 5% *Madhuca longifolia* gel

Model 1: Ultraviolet- C induced photodermatitis model for psoriasis:

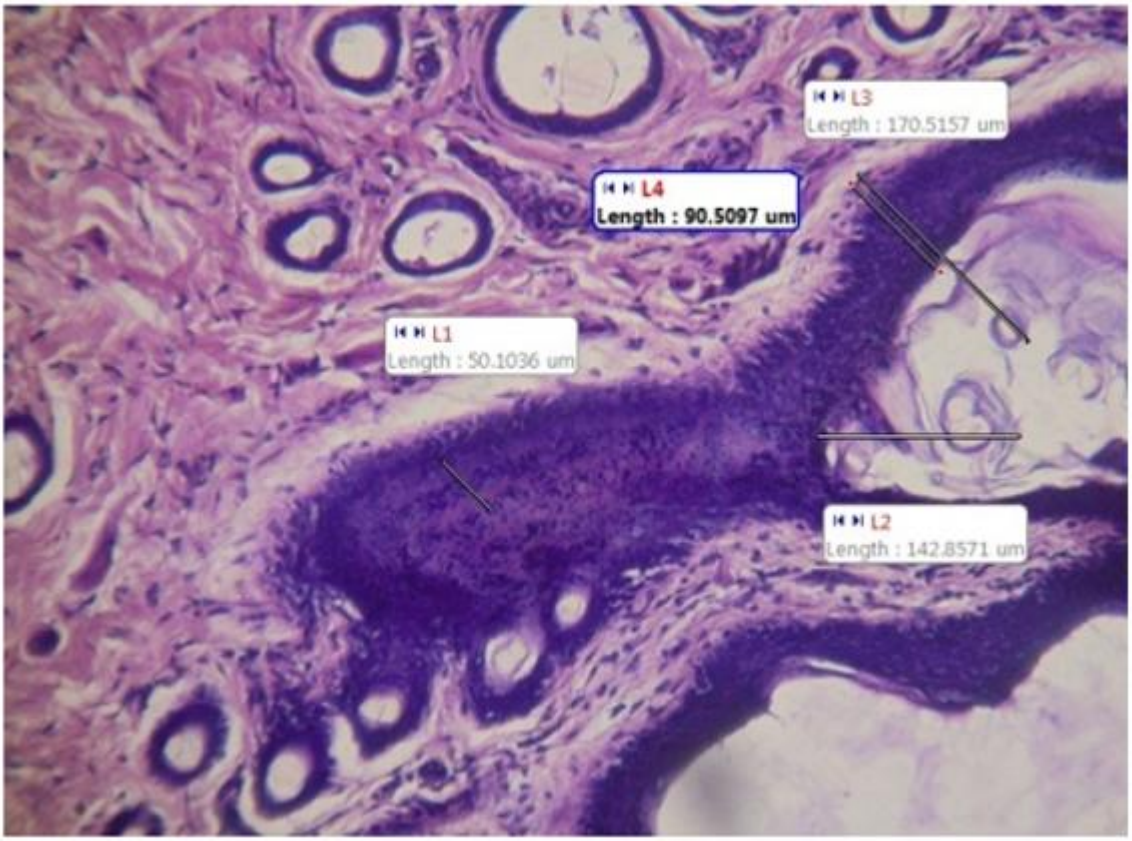


Fig no. 1 Control group

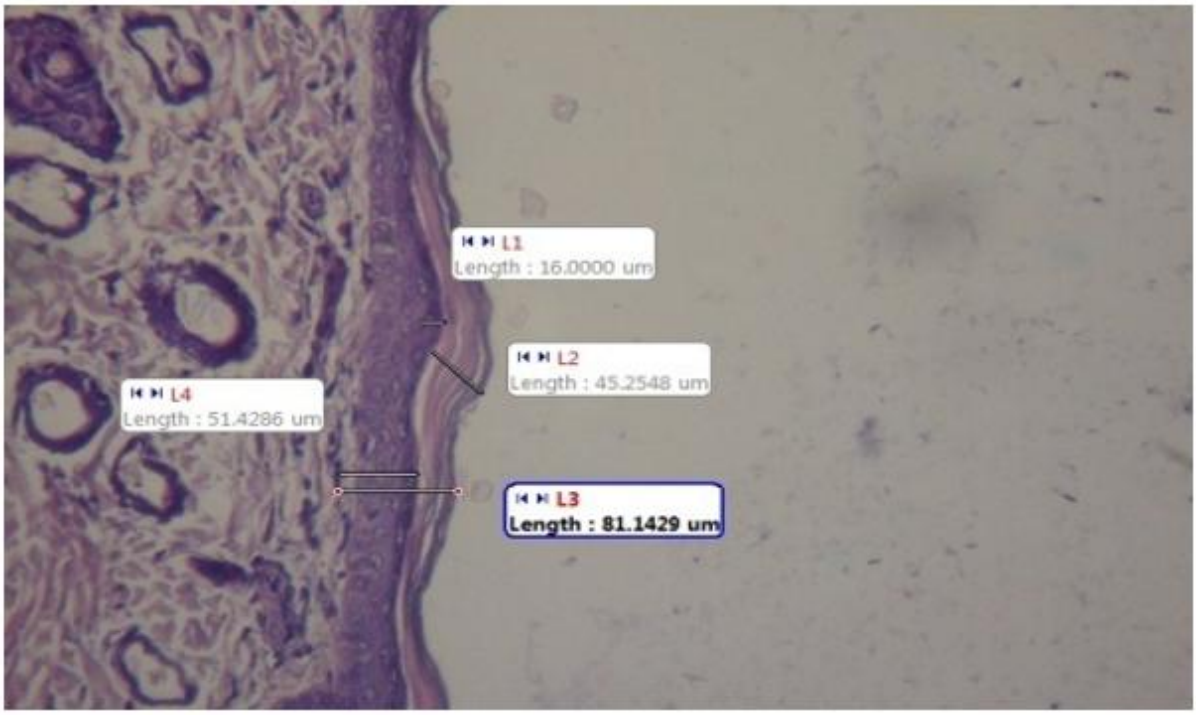


Fig no. 2 Standard group

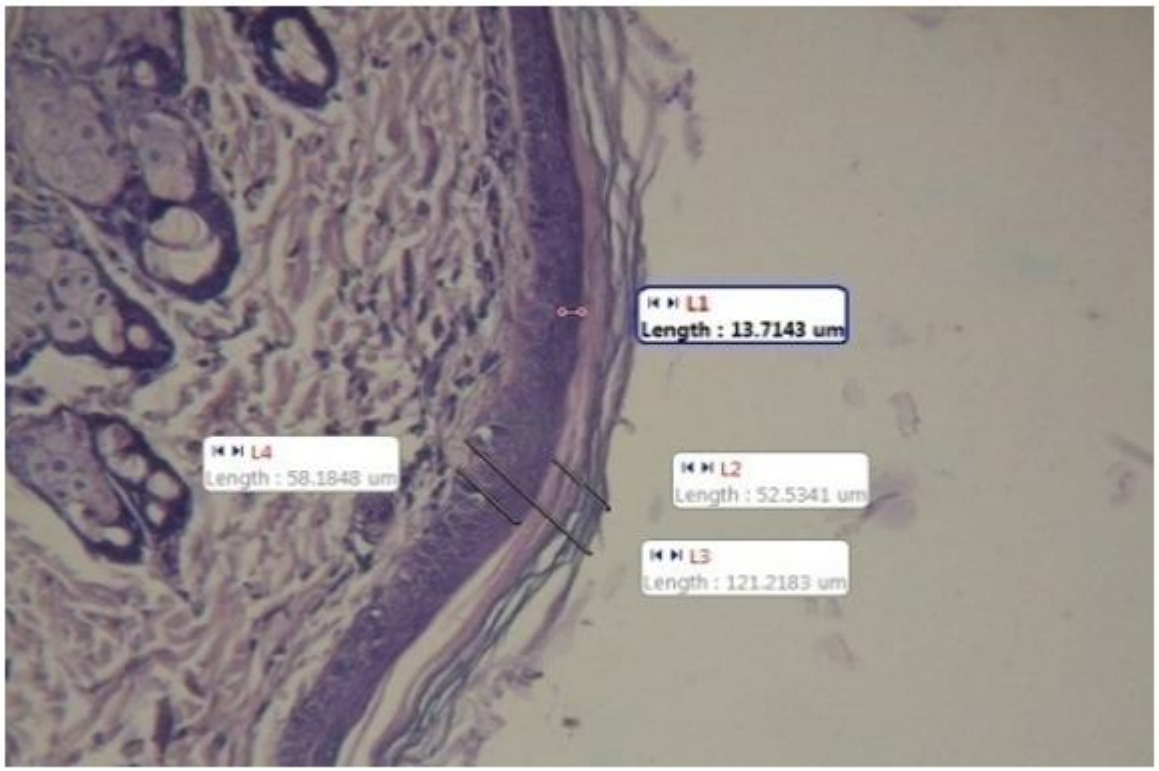


Fig no. 3 Gel base

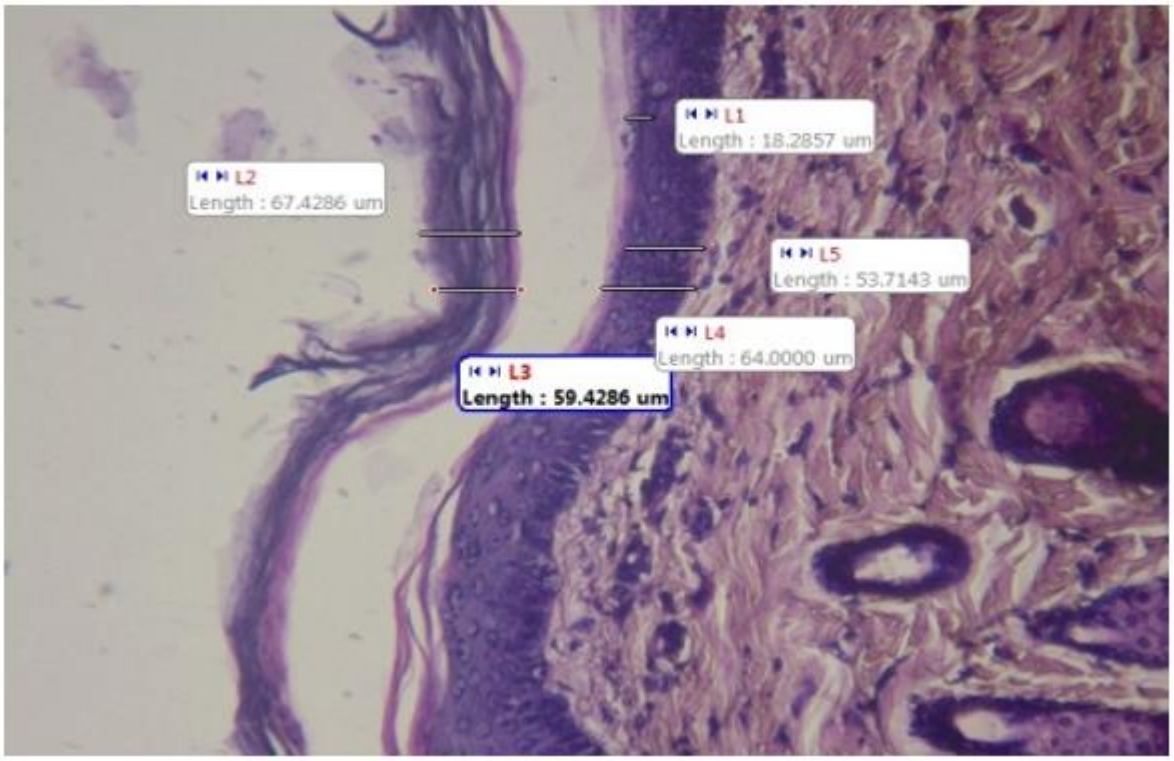


Fig no. 4 ML gel 2.5%

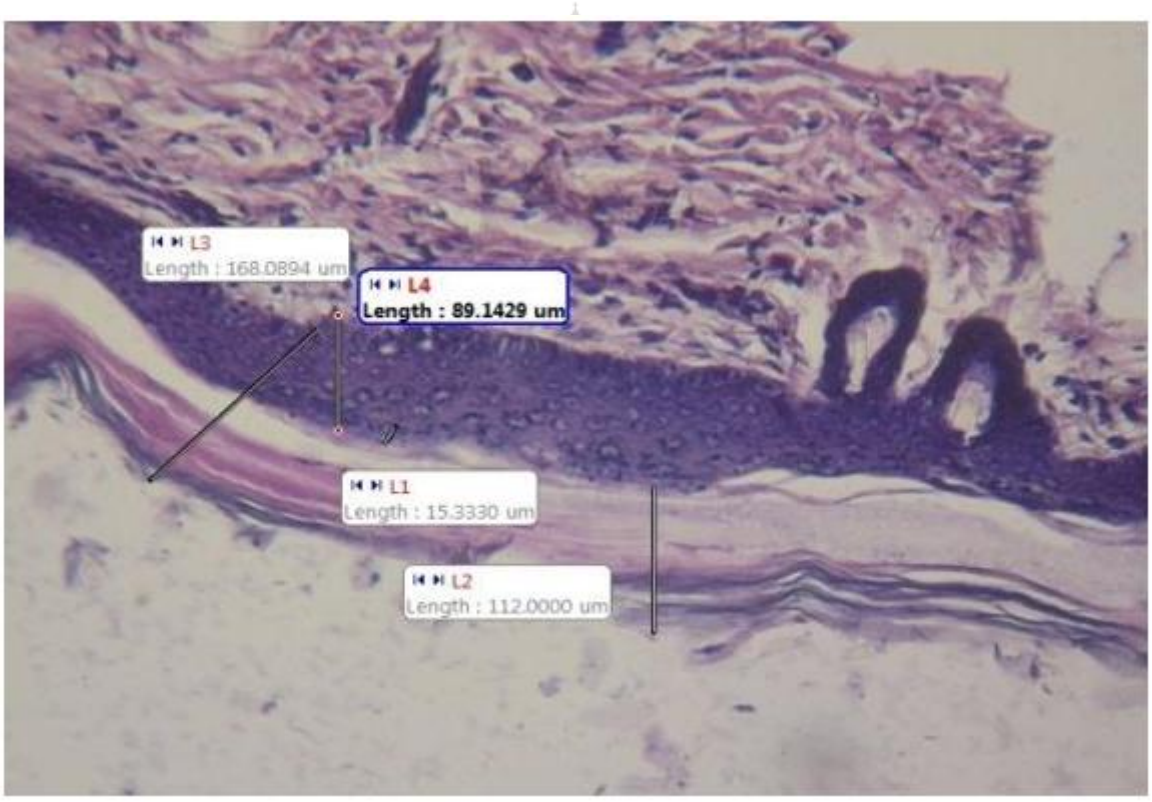
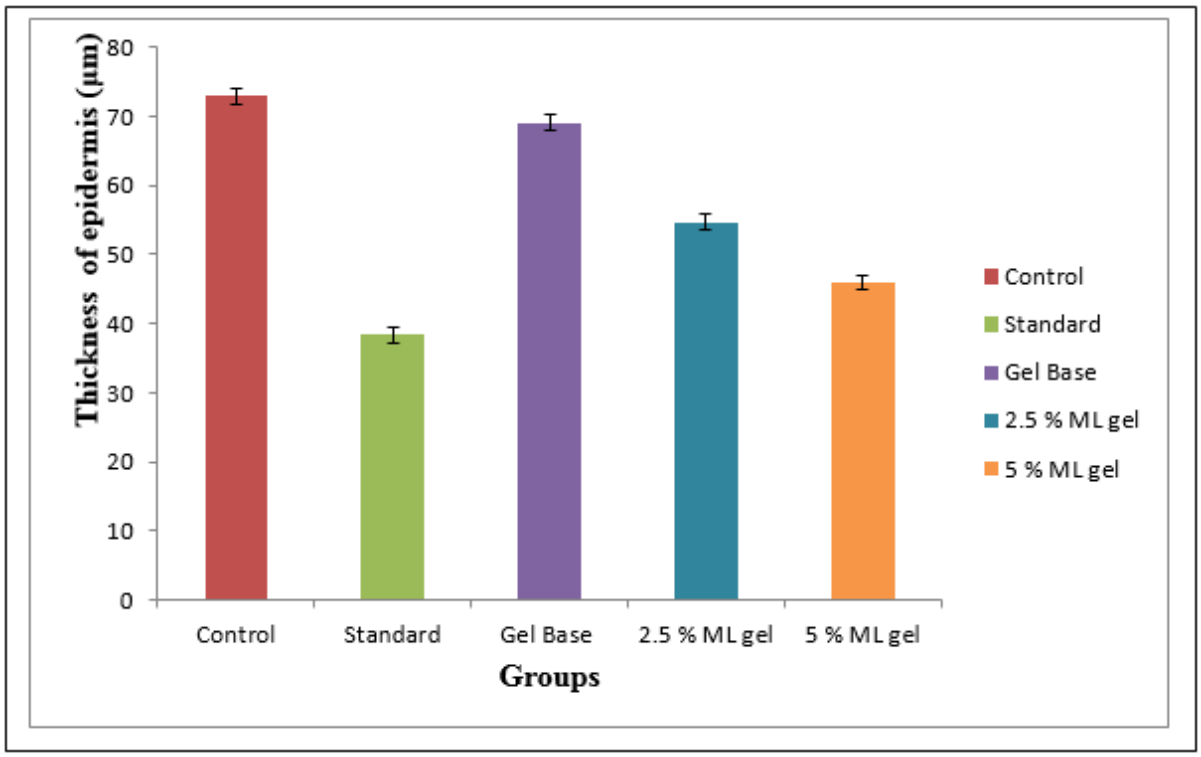
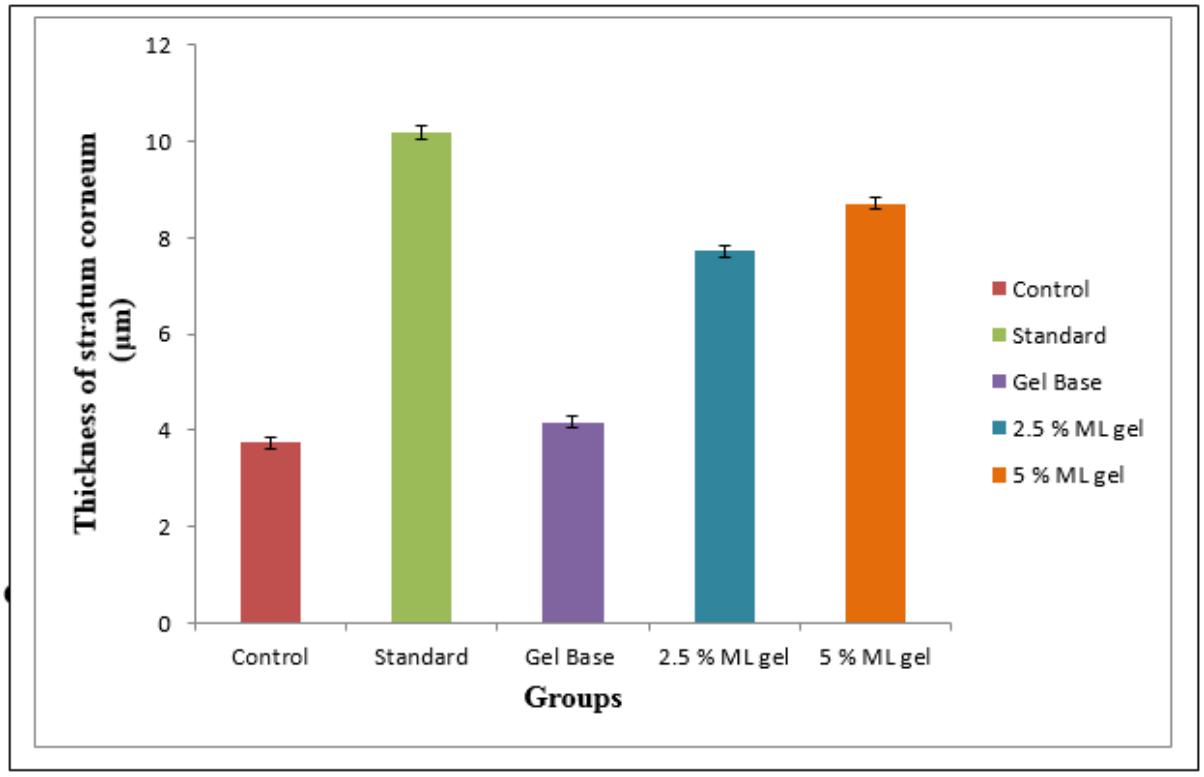


Fig no. 5 ML gel 5%

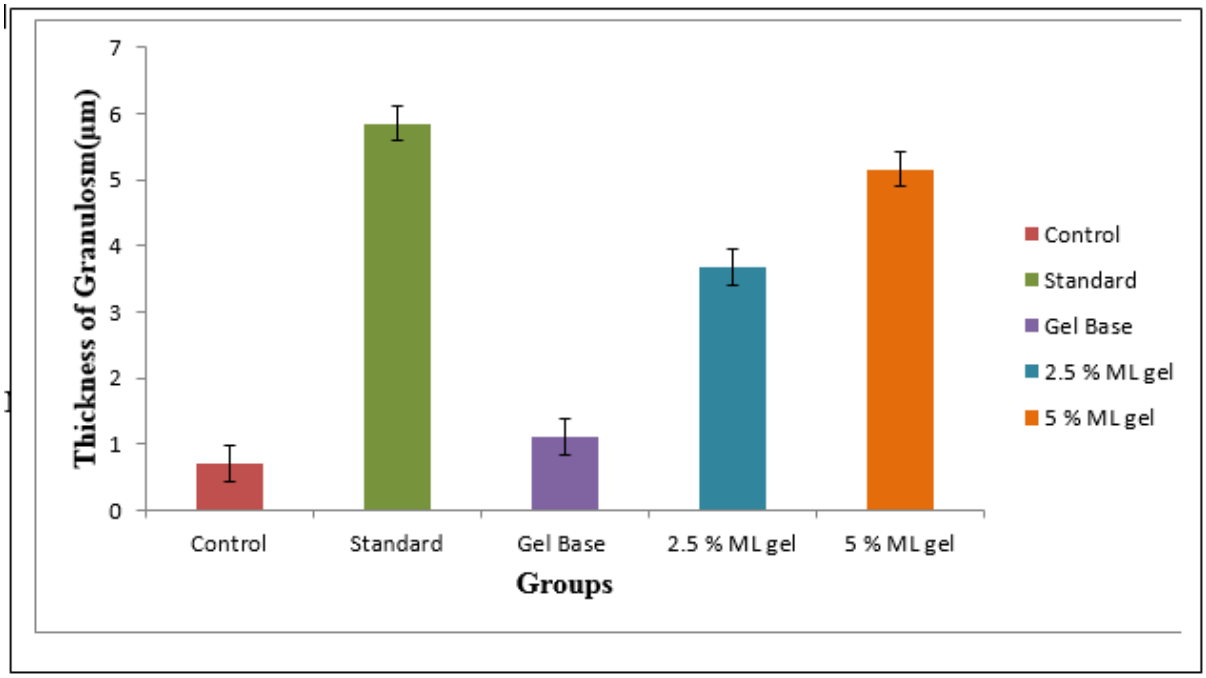
Graph no. 1 Thickness of epidermis



Graph no. 2 Thickness of Stratum corneum



Graph no. 3 Thickness of granulosm



Model 2: Perry's Scientific Mice Tail Model:

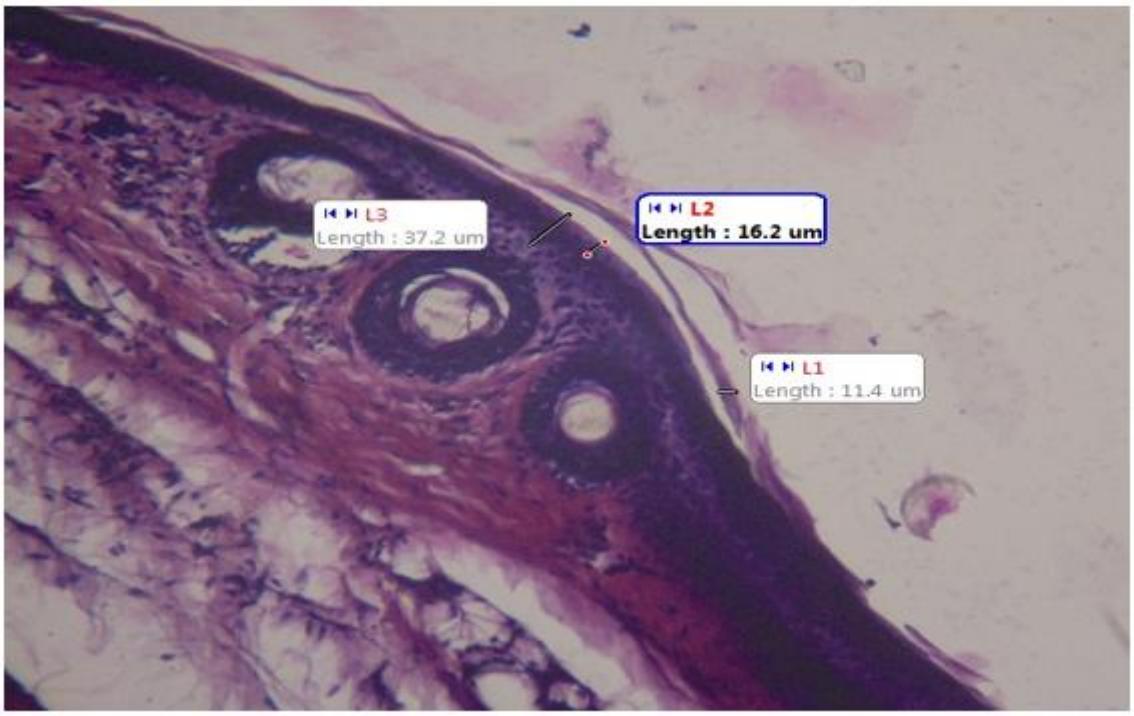


Fig no. 6 Control group

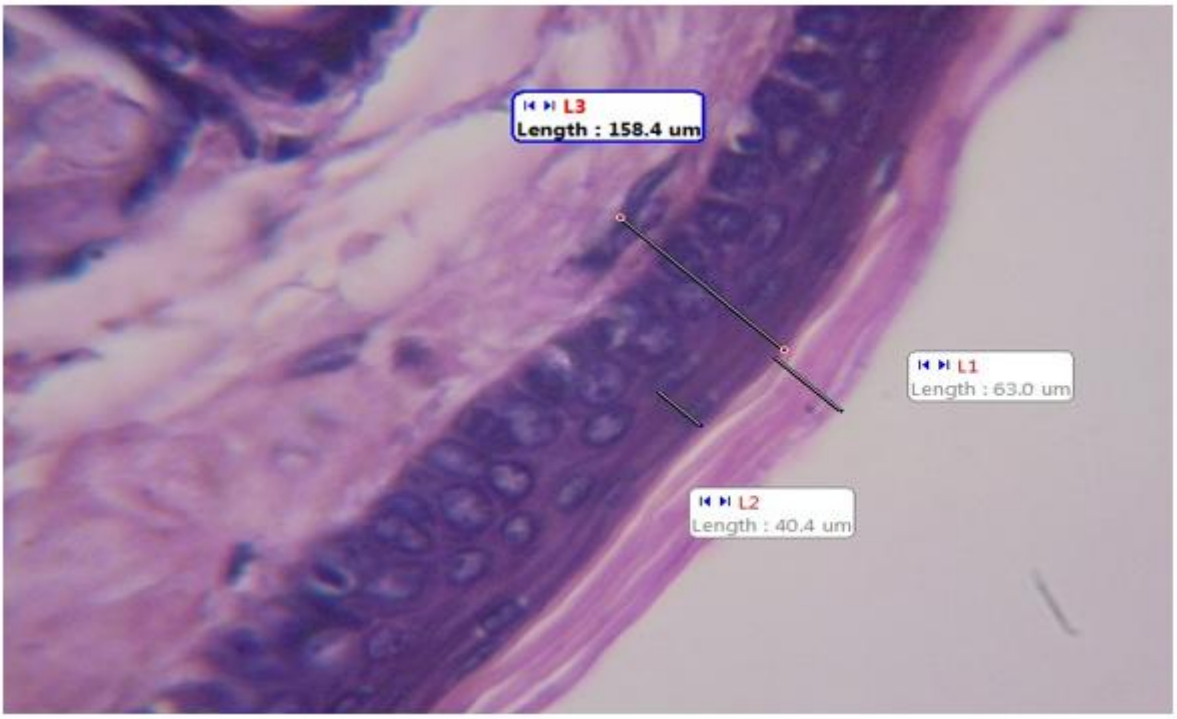


Fig no. 7 Standard group

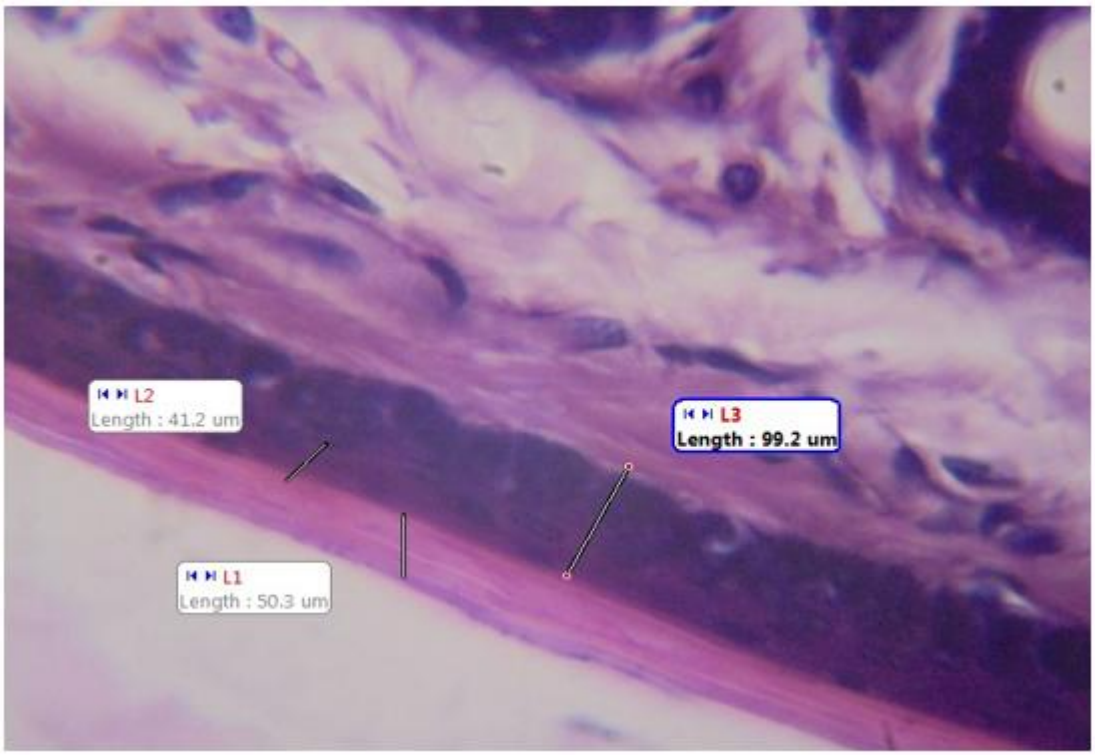


Fig no. 8 Gel Base

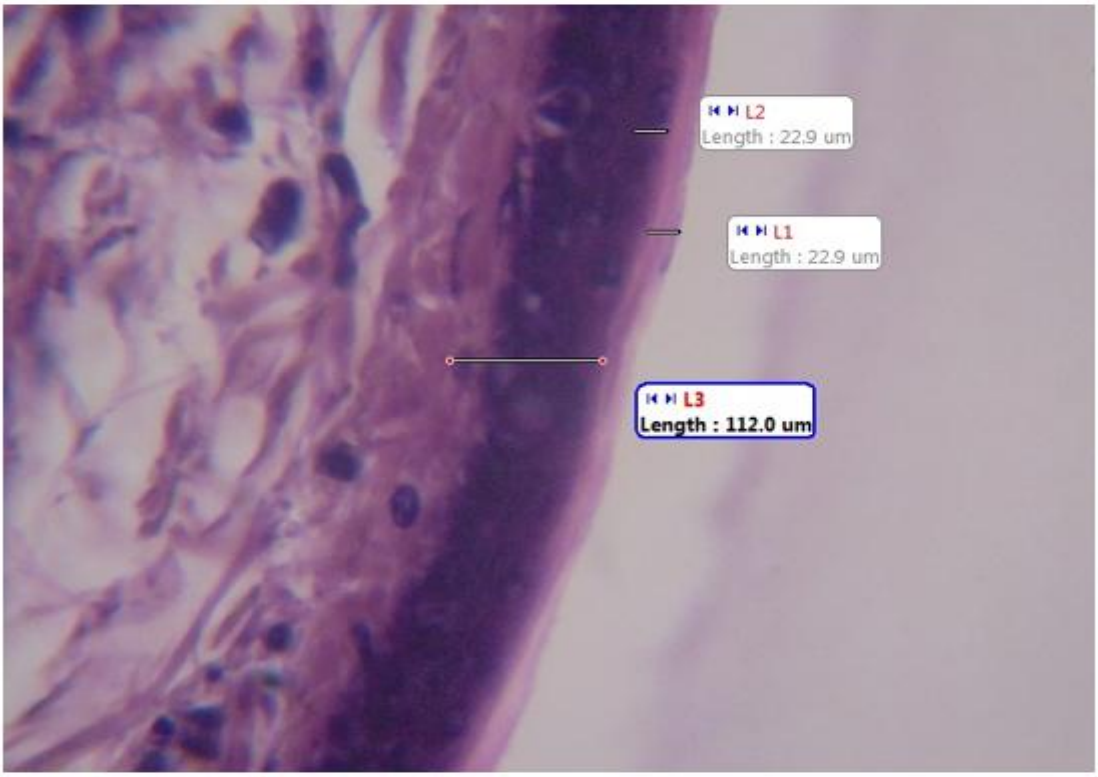


Fig no. 9 ML gel 2.5%

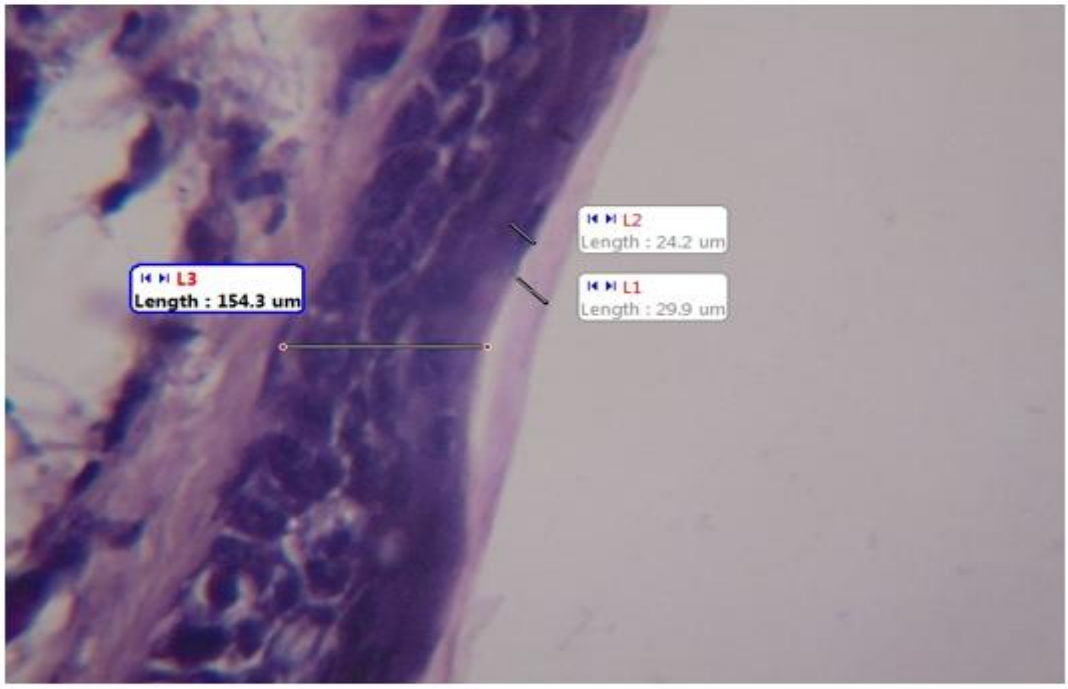
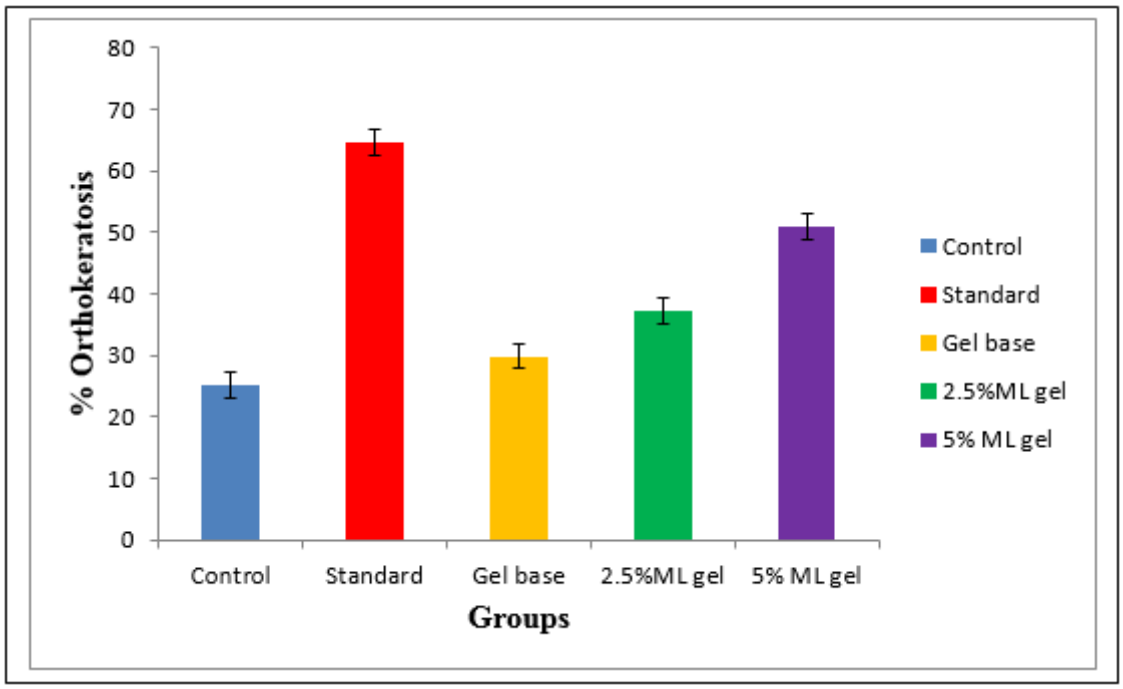
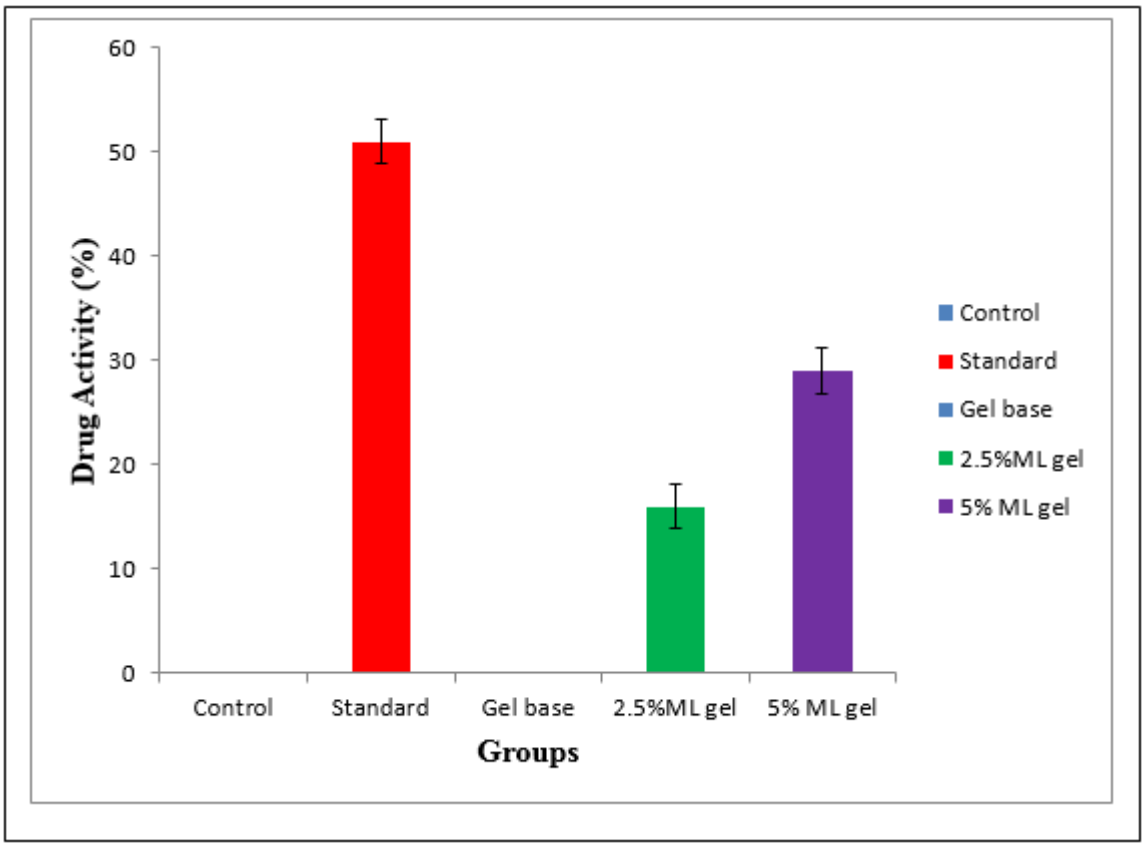


Fig no. 10 ML gel 5%

Graph no. 4 Orthokeratosis %



Graph no.5 Drug activity



DISCUSSION AND CONCLUSION

Quercetin is a flavonol occurs in leaves of *Madhuca longifolia*, it has beneficial effects like anti-bacterial. Antiviral, anti-carcinogenic and anti-inflammatory, traditionally it is used for eczema. The leaves of *Madhuca longifolia* was extracted and used for the evaluation of anti-psoriatic like activity in rat and mice. In the rat ultraviolet photodermatitis model, the extraction of *Madhuca longifolia* (5% ML gel and 2.5% ML gel) produced significant reduction in thickness of epidermal layer as well as presence of stratum granulosum layer as compared to control group. Thickness of the epidermal layer increases in psoriatic condition whereas Stratum granulosum layer is greatly reduced or absent in psoriatic lesions. From the above findings, it might be shown anti-psoriasis activity. In Perry's scientific mice tail model, parakeratotic condition is seen in the adult mouse tail which is hallmarks of psoriasis. Induction of the orthokeratosis in adult mouse tail is the basis behind the mouse tail test. In this model extract of *Madhuca longifolia* (5% ML gel and 2.5% ML gel) showed significant orthokeratosis (%) when compared with control group, it also showed significant changes in epidermal thickness compared with control group. From above data, the formulation of ML gel 5% and 2.5% showed significant reduction in epidermal thickness, presence of stratum granulosum and orthokeratosis %

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