

EFFECT OF NATURAL SUNFLOWER OIL AND ITS COMPONENTS ON THE SKIN PERMEABILITY TO WATER AND SOME DRUGS

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ABSTRACT

Objective: To investigate the effect of natural oils and their components on the water permeability and marker molecules of stratum corneum either when intact or after exposure to mechanical stress.

Methods: Full thickness porcine skin was used. The subcutaneous fat was removed with a scalpel. The remaining tissue was dermatomed. A validated tape-stripping procedure was used to induce skin damage. Tritiated water coupled with a scintillation counter was used for measuring the water permeability of skin, caffeine as hydrophilic molecule and testosterone as hydrophobic one.

Results: Mean flux values for permeation of tritiated water versus time were monitored for intact and stripped skin treated with the oils and for untreated controls shows reduction of water permeability and retain skin barrier function for stripped skin, on the other hand all tested oils increases the reflux of caffeine and testosterone for both intact and stripped skin.

Conclusions: Both sunflower oil and its main components, oleic acid and linoleic acid, significantly decreased the permeability of skin to water. For the damaged skin the oils were effective in reducing the flux to values lower than intact skin. No significant differences between sunflower oil and its components were found to exist, the implication being that either the natural sunflower oil or its components could be employed in skin products.

Keywords: Stratum corneum, Sunflower oil, Oleic acid, Linoleic acid, Tritiated water

INTRODUCTION

Water permeation is considered to be a good indicator of potential changes in the barrier integrity of skin. For this reason, and because tritiated water is one of the most frequently used probes for permeability experiments in general, we considered it to be suitable for evaluating the efficacy of the oils [1,2]. The hypothesis tested being do the oils 'heal' the stratum corneum and restore the water barrier function [3]. To investigate the effect of natural oils and their components on the water and marker molecules permeability of stratum corneum either when intact or after exposure to mechanical stress[4]. Water permeation is considered to be a good indicator of potential changes in the barrier integrity of skin. For this reason, and because tritiated water is one of the most frequently used probes for permeability experiments in general, we considered it to be suitable for evaluating the efficacy of oils [5]. Marker molecules (hydrophilic and lipophilic) also have been tested to measure the permeability of intact and injured skin [6-8]. The hypothesis tested being do the oils 'heal' the stratum corneum and restore the water barrier.

METHODS AND MATERIALS

Materials

- Franz cells, permegear (USA) for diffusion through the skin
- Scintillation counter (Perkin Elmer's) and Tritiated water (Perkin Elmer's) for measuring water permeability of skin tissues.
- Caffeine, testosterone, oleic, conjugated linoleic and monoolein, Sigma Aldrich, UK.
- Zimmer air dermatome (USA)

Methods

Methods have been developed for the excised skin to introduce mechanical stress by the use of tape stripping to view of investigating the effect of the oils on damaged skin tissues.

Skin Tissue preparation

Full thickness porcine skin was obtained from a local slaughterhouse. The skin was cleaned carefully under cold running water. The subcutaneous fat was removed with a scalpel. The remaining tissue was dermatomed to a thickness of ~750 μm using Zimmer dermatome then cut into 2 cm \times 2 cm pieces. Finally, the skin samples were washed under cold running water, dried and sealed in plastic bags at -20°C until used[9].

Application of oils

Skin samples (about 2.7 \times 2.7 cm²) were soaked by sunflower oil, oleic acid and linoleic acid for 1, 8 or 24 hours then wiped with soft tissue prior to run the permeation experiment. A control experiment was also conducted using an untreated skin pieces.

SC tape-stripping

A validated tape-stripping procedure was used to mechanical damage to the skin by removing top layers.

Pieces of 2.7 \times 2.7 cm square tapes were prepared using transparent Scotch® No.845 Book Tapes (3M Media, Broken, Germany). To delimit a fixed area for tape stripping, a 5 \times 5 cm square mask was prepared with a cut central aperture of 2 cm in diameter. A strip of adhesive tape was pressed firmly onto the skin surface, and then removed in a single movement. The direction of stripping was changed with each tape to ensure a more uniform removal of the SC with fewer tape-strips. Then, given that the area stripped is kept at known, constant value[9,10].

Water permeability studies using Tritiated water

By using Franz cells 10 μL of tritiated water was applied to the epidermal skin surface of the donor compartment to examine the skin water barrier function and for the next 5 hours. Tritiated water coupled with a scintillation counter were used for measuring the water permeability of skin tissues after 1, 8 and 24 hours exposure to sunflower oil, oleic acid, and linoleic acid by using of Franz cells. Buffer/tritiated water was collected from the acceptor chambers at 1

hour intervals for a total of 5 hours and counted in a liquid scintillation counter. The data collected and analysed using excels sheets.

Permeation studies for oils:

The permeability studies of the skin to caffeine (hydrophilic drug) testosterone (hydrophobic drug) were investigated by means of Franz type diffusion cells (diffusion area of 1.00 cm², donor compartment 2 ml and receptor compartment 8 ml. The dermatomed skin were mounted in the diffusion cells and hydrated with sodium citrate buffer (pH 5.5) for caffeine and PBS (pH 7.4) for testosterone for prior to the experiment. The diffusion cells were placed in a hot plate with the temperature being maintained at 32 °C throughout the experiment.

The donor compartment was filled with 1.5 ml of caffeine solution in citrate buffer (pH 5.5) at a 25 mg/ml or 1.5 ml of testosterone solution in PBS (pH 7.4) at a 2.0 mg/ml concentration. The receptor compartment was completely filled with the respective buffer (8 ml)

and gently stirred using a magnetic stirrer throughout the experiment. The donor compartment was covered with a paraffin film to avoid evaporation of the solution. Sample fractions were collected from the receptor compartment at 1 hour intervals for 5 hours. The total volume of the receptor compartment was maintained by replenishing with fresh buffer. The amount of drug permeated through the model membrane was determined by UV analysis in standard quartz cells after suitable dilution using a Perkin Elmer UV-Vis spectrometer at λ 273 nm for caffeine and λ 250 nm after suitable dilution. A control experiment was also conducted using an untreated skin.

RESULTS AND DISCUSSION

Water permeability studies

Mean flux values for permeation of tritiated water versus time were monitored for intact and stripped skin treated with the oils and for untreated controls (Figures 1-7).

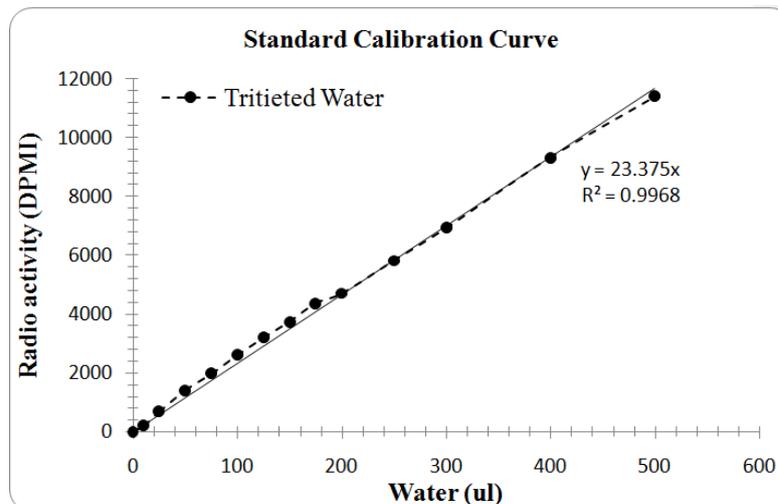


Fig. 1: Standard calibration plot of tritiated water with scintillation counter.

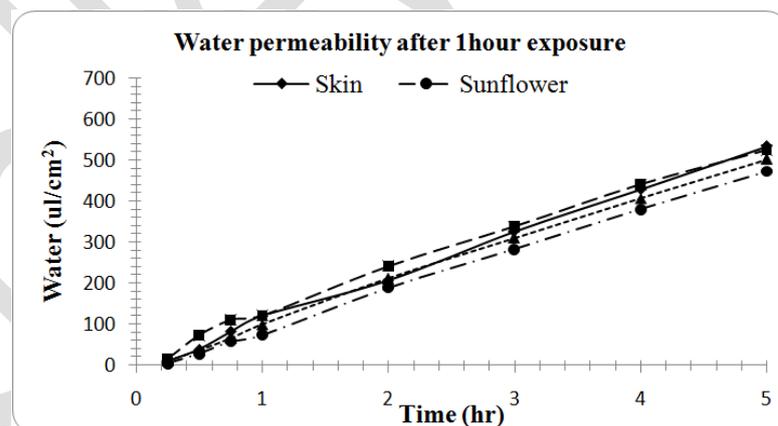


Fig. 2: Effect of oil and oil components on the water permeability through skin after 1hour exposure.

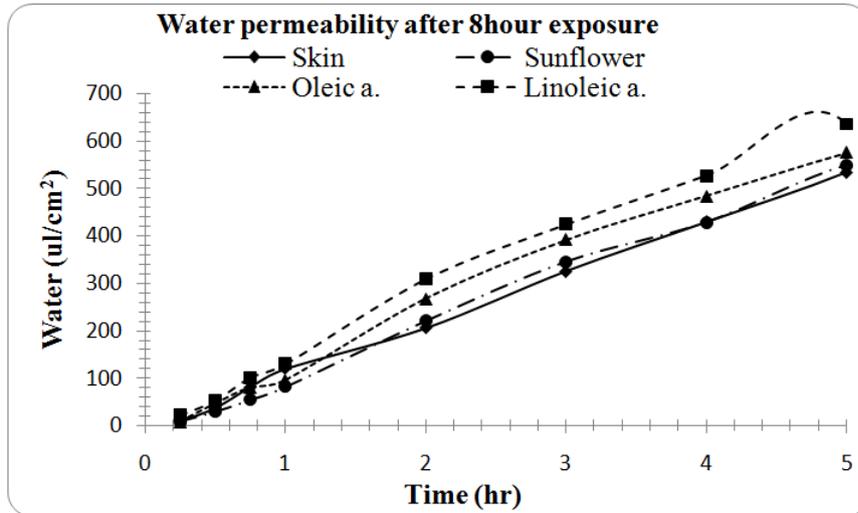


Fig. 3: Effect of oil and oil components on the water permeability through skin after 8hours exposure.

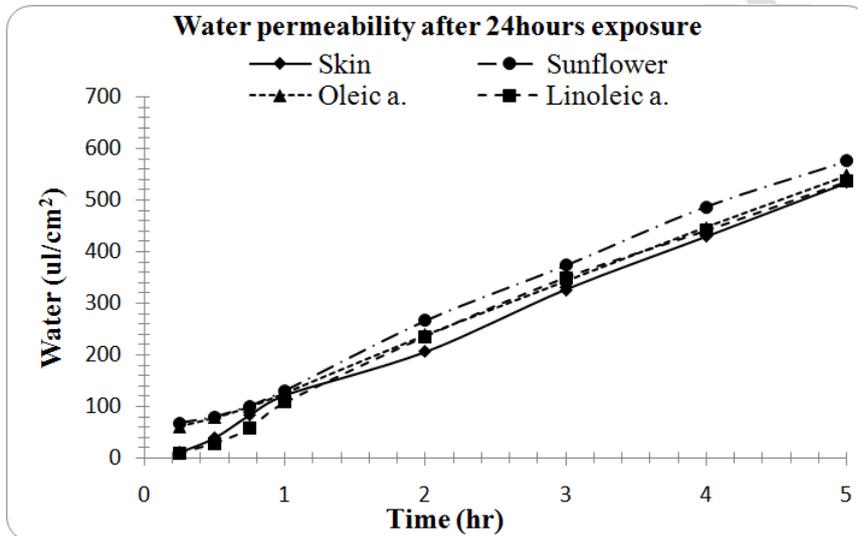


Fig. 4: Effect of oil and oil components on the water permeability through skin after 24hours exposure.

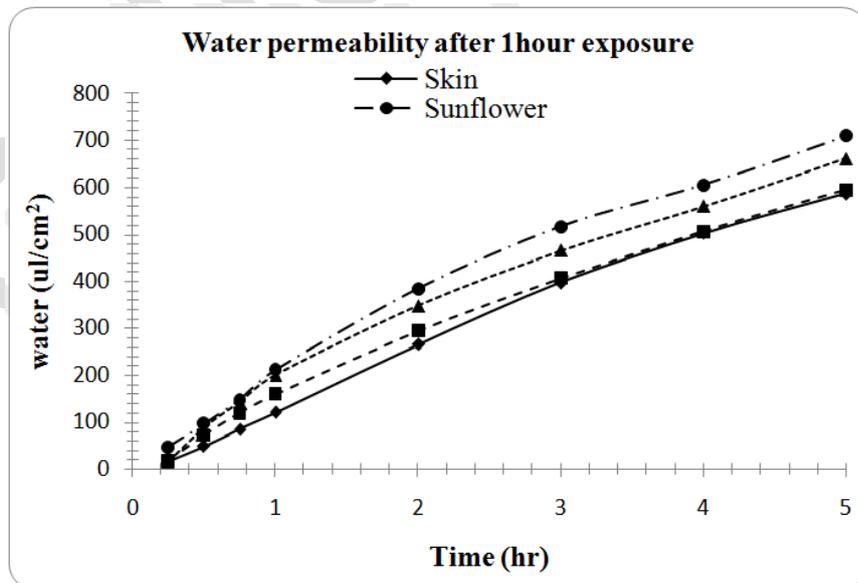


Fig. 5: Effect of oil and oil components on the water permeability through stripped skin after 1hour exposure.

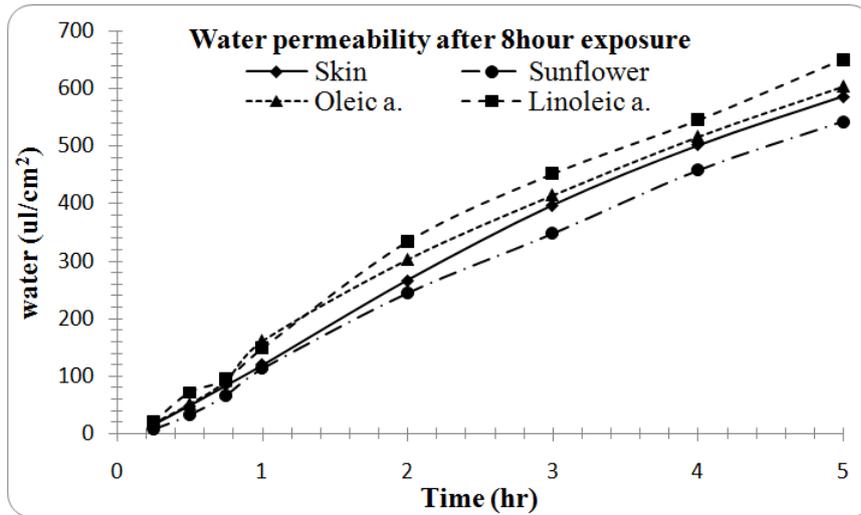


Fig. 6: Effect of oil and oil components on the water permeability through stripped skin after 8hours exposure.

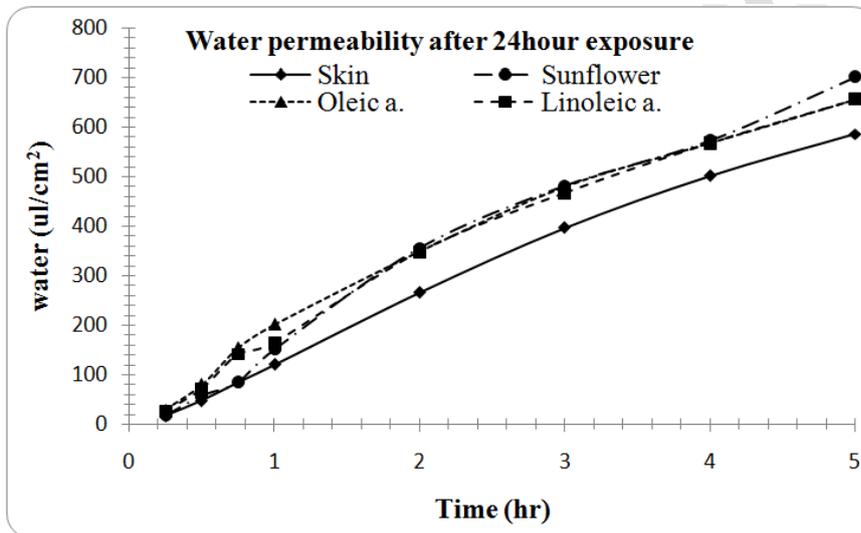


Fig. 7: Effect of oil and oil components on the water permeability through stripped skin after 24hours exposure.

The mean steady state fluxes (1–5 h) for tritiated water (average of three exposure times, 1, 8 and 24 hours) showed that the oils significantly reduced the water flux values from 111.65 $\mu\text{L}/\text{cm}^2\text{hr}$ for the untreated intact skin to 102.99 $\mu\text{L}/\text{cm}^2\text{hr}$ for sunflower oil, 103.38

$\mu\text{L}/\text{cm}^2\text{hr}$ for oleic acid, and 103.54 $\mu\text{L}/\text{cm}^2\text{hr}$ for linoleic acid. For the stripped skin (Table 2) the water flux was 117.82 $\mu\text{L}/\text{cm}^2\text{hr}$, which reduced to 108.70 $\mu\text{L}/\text{cm}^2\text{hr}$ for sunflower oil, 107.36 $\mu\text{L}/\text{cm}^2\text{hr}$ for oleic acid, and 107.16 $\mu\text{L}/\text{cm}^2\text{hr}$ for linoleic acid.

Measure the permeability of the stratum corneum to marker molecules as a function of addition of applied oil and oil components.

Caffeine permeability

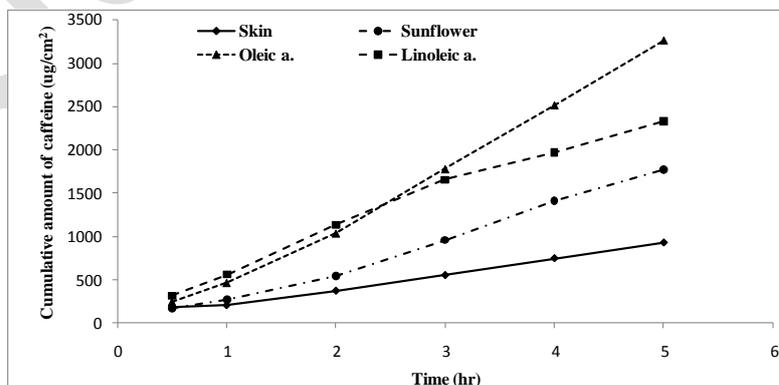


Fig. 8: Effect of oil and oil components on the Permeation of caffeine through the excicated porcine skin after 1 hour exposure.

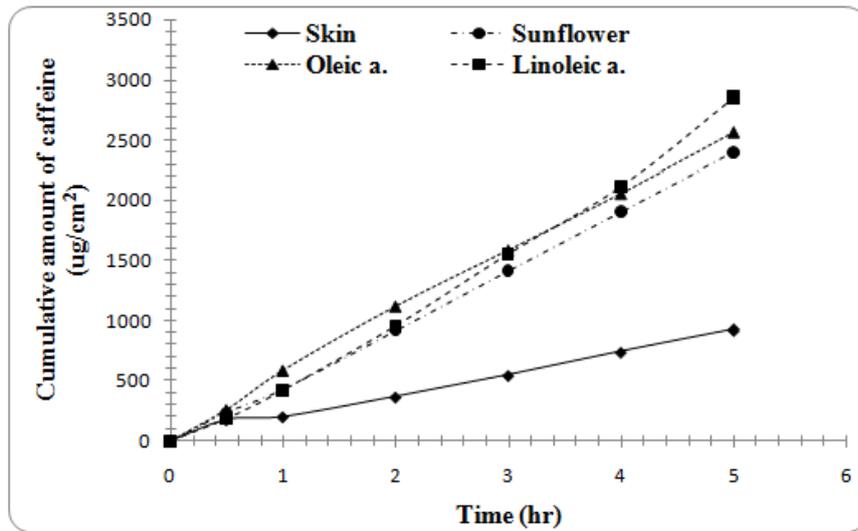


Fig. 9: Effect of oil and oil components on the Permeation of caffeine through the excicated porcine skin after 8 hours exposure.

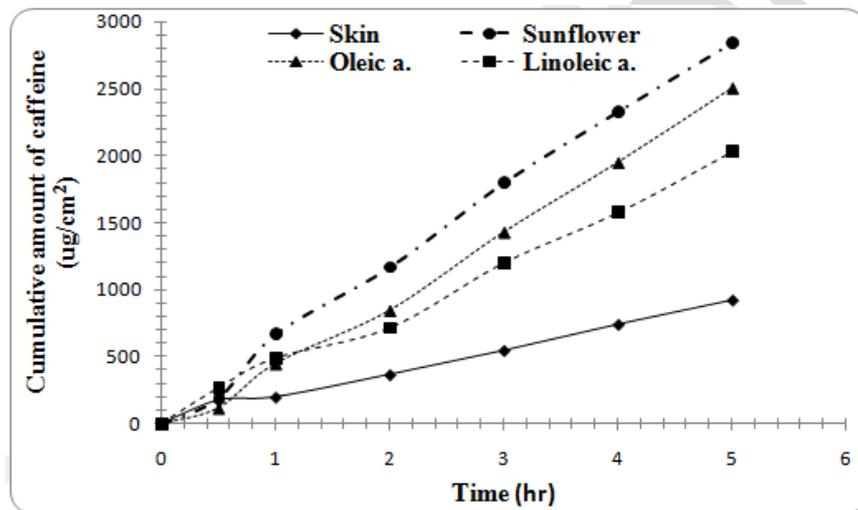


Fig. 10: Effect of oil and oil components on the Permeation of caffeine through the excicated porcine skin after 24 hours exposure.

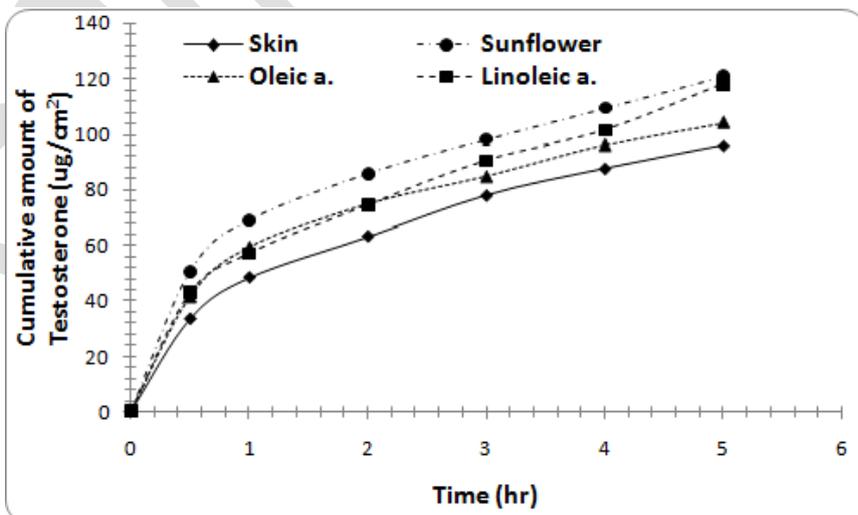


Fig. 11: Effect of oil and oil components on the Permeation of testosterone through the excicated porcine skin after 1 hour exposure.

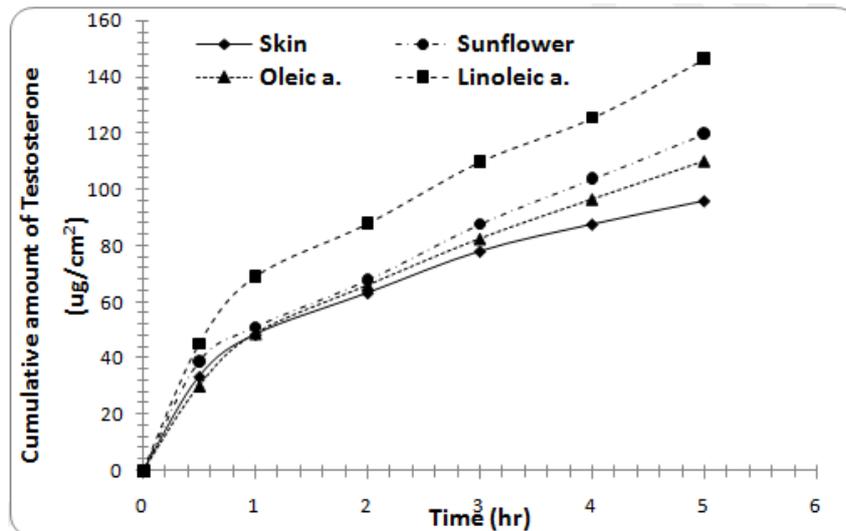


Fig. 12: Effect of oil and oil components on the Permeation of testosterone through the excicated porcine skin after 8 hours exposure.

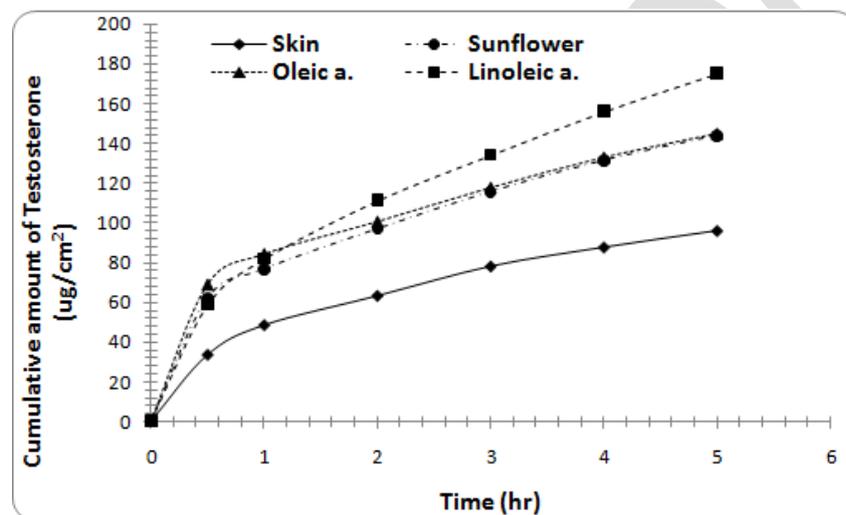


Fig. 13: Effect of oil and oil components on the Permeation of testosterone through the excicated porcine skin after 24 hours exposure.

The mean steady state fluxes (1–5 h) for caffeine (figures 8:11) showed that the oils significantly increase the caffeine flux values (average of three exposure times, 1, 8 and 24 hours) from 187.62 $\mu\text{g}/\text{cm}^2\text{hr}$ for the untreated intact skin to 270.29 $\mu\text{g}/\text{cm}^2\text{hr}$ for sunflower oil, 403.22 $\mu\text{g}/\text{cm}^2\text{hr}$ for oleic acid, and 262.73 $\mu\text{g}/\text{cm}^2\text{hr}$ for linoleic acid after 1 hour exposure, and 359.15 $\mu\text{g}/\text{cm}^2\text{hr}$ and 220.53 $\mu\text{g}/\text{cm}^2\text{hr}$ for sunflower oil, 387.67 $\mu\text{g}/\text{cm}^2\text{hr}$ and 398.58 $\mu\text{g}/\text{cm}^2\text{hr}$ for oleic acid, and 299.98 $\mu\text{g}/\text{cm}^2\text{hr}$ and 217.70 $\mu\text{g}/\text{cm}^2\text{hr}$ for linoleic acid after 8 and 24 hours exposure respectively. The oleic acid is the most effective in increasing the caffeine permeability followed by sunflower oil then oleic acid. The exposure of skin to oils for 8 hours is much more effective in increasing the permeability than 1 and 24 hour exposure.

Testosterone permeability

The mean steady state fluxes (1–5 h) for testosterone (figures 11:13) showed that the oils significantly increase the testosterone flux values (average of three exposure times, 1, 8 and 24 hours) from 10.98 $\mu\text{g}/\text{cm}^2\text{hr}$ for the untreated intact skin to 12.67 $\mu\text{g}/\text{cm}^2\text{hr}$ for sunflower oil, 11.06 $\mu\text{g}/\text{cm}^2\text{hr}$ for oleic acid, and 14.80 $\mu\text{g}/\text{cm}^2\text{hr}$ for linoleic acid after 1 hour exposure, which increases to 17.36 $\mu\text{g}/\text{cm}^2\text{hr}$ and 17.24 $\mu\text{g}/\text{cm}^2\text{hr}$ for sunflower oil, 15.29 $\mu\text{g}/\text{cm}^2\text{hr}$ and 16.14 $\mu\text{g}/\text{cm}^2\text{hr}$ for oleic acid, and 19.22 $\mu\text{g}/\text{cm}^2\text{hr}$ and 22.45 $\mu\text{g}/\text{cm}^2\text{hr}$ for linoleic acid after 8 and 24 hours exposure

respectively. The linoleic acid is the most effective in increasing the testosterone permeability followed by sunflower oil then oleic acid. The exposure of skin to oils for 8 hours is much more effective in increasing the permeability than 1 hour exposure, while no big difference between 8 and 24 hours exposure.

CONCLUSIONS

We demonstrated that both sunflower oil and its main components, oleic acid and linoleic acid, significantly decreased the permeability of skin to water. For the damaged skin the oils were effective in reducing the flux to values lower than intact skin. No significant differences between sunflower oil and its components were found to exist, the implication being that either the natural sunflower oil or its components could be employed in skin products. Both sunflower oil and its main components, oleic acid and linoleic acid, significantly increased the permeability of skin to caffeine as an example of hydrophilic drug and testosterone as an example of hydrophobic drug. For caffeine permeability oleic acid was the most effective then sunflower oil and linoleic acid with no significant differences between them were found to exist while for testosterone linoleic acid was the most effective then sunflower oil and oleic acid with no significant differences between them were found to exist. The exposure time for 8 hours is the optimum time for the permeability of both drugs, the implication being that either the natural sunflower

oil or its components could be employed in skin products to restore skin barrier functions.

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Galley Proof