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## Evaluation of Caffeine Gels on Physicochemical Characteristics and In Vivo Efficacy in Reducing Puffy Eyes

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### ABSTRACT

The eyelids are the thinnest skin in the body, leading to be easy for the blood vessels to show through the skin caused a swollen and dark appearance called puffy eyes. Placing refrigerated damp tea bags on the eyelids has been believed for a long time that it can reduce the puffy eyes due to the vasoconstriction of caffeine. This study aimed to characterize physicochemical properties and to determine in vivo efficacy in reducing puffy eyes of the prepared caffeine gels. The formulation composed of 3% caffeine, 2% ethanol, 0.3% Uniphen<sup>®</sup> P-23, 7.5% propylene glycol, 0.5% Carbopol<sup>®</sup> Ultrez-21 and water to 100% was selected for eye irritation test and efficacy evaluation since it possessed good characteristics and provided sustained skin permeation. The 34 volunteers (18 women, 16 men) who easily developed puffy eyes after going to bed without sleep and with no irritation to caffeine gel as well as its gel base were treated with the gels in a randomized, double-blind, placebo-controlled trial. The skin permeation profiles showed that all caffeine gels allowed caffeine to permeate through the newborn pig skin. However, the overall efficacy of the selected caffeine gel in reducing puffy eyes was not significantly different from that of its gel base. It could be concluded that the cooling effect of the hydrophilic gels was the main parameter in reduction of eye puffiness rather than the vasoconstriction of caffeine.

**Key words:** Caffeine gel, Skin permeation, Eye irritation, Puffy eyes.

### INTRODUCTION

The skin under the eyes is some of the thinnest in the body. Hence, it is easy for the blood vessels to show through the skin, resulting in a swollen and dark appearance called puffy eyes. Puffy eyes can be caused by several factors such as fluid retention due to high alcohol or salt intake, emotions especially crying, allergies, hormone changes, insufficient sleep and other factors as well (Shelton, 2004). Several methods are proposed to reduce puffiness under the eyes such as placing cold cucumber slices or chilled damp tea bags or refrigerated metal tablespoons on the eyelids, applying a hemorrhoid cream to the puffy area, decreasing alcohol or salt intake, getting enough sleep and etc (Sondra et al., 2011). Putting chilled damp tea bags on the eyelids has been believed for a long time that it can reduce the puffy eyes since caffeine in the tea bags can constrict dilated capillaries, leading to diminishing the swollen and dark appearance. Generally, caffeine is well-known as a mild stimulant to the central nervous system which can be found in coffee, tea and some soft drinks. For cosmetic purpose, caffeine is reported that it can be used as an active compound in an anti-cellulite product (Bertin et al., 2001). Its topical delivery has been investigated by some research groups (Dias et al., 1999; Dreher et al., 2002). However, no clinical trails have evaluated the efficacy of caffeine in reducing puffy eyes. Commercially available topical formulations of caffeine normally contain 3% of caffeine (Dias et al., 1999). A hydrophilic gel is one of the most suitable cosmetic formulations for applying around the eyes since it can provide attractive clear appearance, cooling effect and non-greasy feeling (Boonme et al., 2007;

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Amnuakit et al., 2008). This study aimed to evaluate physico-chemical characteristics and in vivo efficacy in reducing puffy eyes of the prepared caffeine gels.

## MATERIALS AND METHODS

### Materials

Caffeine anhydrous was purchased from Zhejiang Medicines, China. Carbopol® Ultrez-21 (acrylates/C10-30 alkyl acrylate crosspolymer) was purchased from Lubrizol, USA. Uniphen® P-23 (phenoxyethanol (and) methylparaben (and) butylparaben (and) ethylparaben (and) propylparaben) was purchased from Induchem, Switzerland. Propylene glycol (PG), ethanol and triethanolamine were purchased from a local distributor in Thailand. Distilled water was used throughout the experiment. Other chemicals used in analytical process were of reagent grade. All chemicals were used without further modification.

### Preparation of caffeine gels

Carbopol® Ultrez-21 0.5 g was dispersed in an aqueous solution containing 3% caffeine, 2% ethanol, 0.3% Uniphen® P-23, various PG concentrations (7.5, 15 and 30%) and water to 100%. Triethanolamine was subsequently added to adjust the pH to 5.5 for neutralizing the polymer to obtain a gel and for stabilizing the ecological balance of the skin. The mixture was mixed with a magnetic stirrer and then sonicated to remove air bubbles.

### Investigation of physicochemical characteristics

All samples were observed for appearance. The pH values were measured by a pH meter (Orion 410A, American Instrument Exchange Inc., USA). Viscosity values were determined by a Brookfield viscometer fitted with RV-7 spindle and 5 rpm spindle speed (Brookfield Engineering Laboratories Inc., USA). All measurements were performed in triplicate at room temperature.

### Determination of the caffeine content in the gels

The caffeine content in the prepared gels was determined by spectrophotometry technique (Amnuakit et al., 2008). A known weight of each gel was dissolved and diluted with phosphate buffer solution pH 7.4 (PBS). The concentration of caffeine was spectrophotometrically measured at 273 nm (Milton Roy Spectronic Genesys 5 Spectrophotometer, USA). The gel base containing the identical amount of ingredients without caffeine was used as a blank. The coefficient of variation for each standard curve was less than 3% and the square of the correlation coefficient was over 0.9995 when the concentrations of standard caffeine ranged from 1 to 16 µg/ml.

### Stability studies

Stability studies were conducted under freeze-thaw conditions. In each cycle, each sample was kept at -10°C for 24 h and at 45°C for 24 h. The sample was then evaluated for its stability after 6 cycles. Changes of physical appearance, pH, viscosity and content of caffeine were determined.

### In vitro skin permeation studies

In vitro skin permeation studies were performed using a modified Franz diffusion cell (Hanson Model 57-6 M, Hanson Research Corporation, USA). Full thickness flank skin of newborn pigs was used as the membrane. The newborn pigs, weighed 1.4 to 1.8 kg and naturally died after birth, were freshly obtained from a local pig farm in Songkhla Province where is regulated by the Department of Livestock Development, Thailand. The epidermal hair at the flank area was clipped with an electric hair clipper as close as possible to the skin without damaging it and excised with a scalpel. The subcutaneous fat and underlying tissues were carefully removed from the dermal surface (Songkro et al., 2003). The obtained skin membrane was mounted on a modified Franz diffusion cell with the stratum corneum facing upwards. The 2.5 g of each gel was placed in a donor compartment. The receptor compartment beneath the skin was filled with 12 ml PBS, thermoregulated with a water bath at 37 °C and magnetically stirred at 400 rpm with an effective skin area of 1.77 cm<sup>2</sup>. The 2 ml of receptor fluid was withdrawn at 0.5, 1, 2, 3, 4, 8, 10 and 12 h. An equal volume of PBS was immediately added to the receptor compartment after each sampling. Each sample was filtered through a 0.45 µm polyamide membrane filter (Sartorius AG, Germany) and then determined for caffeine content by a spectrophotometer as previously described.

### Acute eye irritation test of caffeine gels

The acute eye irritation test was conducted according to the OECD Test Guideline No. 405: Acute Eye Irritation/Corrosion (Anonymous, 1987). This test was held by the Thailand Institute of Scientific and Technological Research with the approval of the Animal Ethics Committee. Three rabbits were employed and acclimatized to the laboratory room for one week. One day prior to the experiment, each rabbit was examined for ocular lesions by applying one drop of 2% w/v fluorescein sodium in 0.9% w/v sterile normal saline solution into both eyes. Approximately 1 minute later, the eyes were gently but thoroughly flushed with 0.9% w/v normal saline solution. The rabbits would be included in the test if no eye lesions were observed. On the experimental day, 0.1 ml of the tested gel was placed in the conjunctival sac of one eye of each rabbit after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the substance. The other eye, which was untreated, served as a control. The eyes were examined at 1, 24, 48 and 72 h after the treatment. The assessments were terminated when the eye had returned to normal. If the eye lesions occurred, the observations would be extended to 21 days in order to assess their reversibility or irreversibility.

### In vivo evaluation of efficacy in reducing puffy eyes

A randomized, double-blind, placebo-controlled trial was performed to evaluate the clinical effects of caffeine gel application on eye puffiness to avoid the bias. This study was approved by the Ethics Committee of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla,

Thailand. The 34 Thai volunteers (18 women and 16 men) in the age of 19-24 years were interviewed to ensure that they had quick response to develop puffy eyes after one sleepless night. Afterwards, they were elucidated for skin irritation by applying the tested caffeine gel and gel base on the lateral arm and left for 24 h. They could participate in the *in vivo* evaluation of efficacy in reducing puffy eyes of the samples if no skin irritation symptoms, e.g., redness and itching were found. The volunteers were divided into 4 groups and the studies were taken for 4 successive days (about 8-9 volunteers/group/day). They were assigned to lie on the bed but did not sleep for one night. No face washing in the next morning was allowed. The length of each puffy eye was immediately measured using a thread starting from the corner of the eye near the nose to the end of the other side of the eye curving via the puffiness and then the thread length was determined using a ruler as illustrated in Figure 1. The swollen area under each eye was marked and then the samples, i.e., a selected caffeine gel and its gel base, were randomly applied on different eyelids. The observations were taken at 10, 15, 30, 90, 150 and 180 min after applying the gel formulations. To minimize variability, this evaluation was performed in a temperature-controlled room ( $24\pm 2^\circ\text{C}$ ) and done by only one investigator.

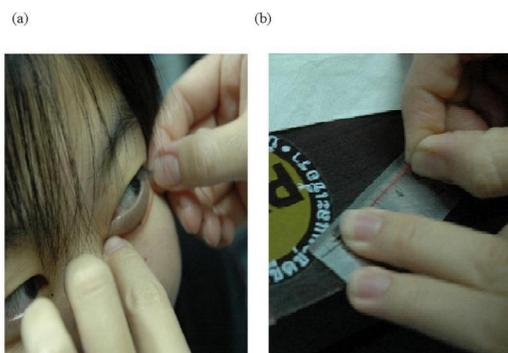


Fig 1. Illustration of the measurement of the swollen area of a puffy eye with two steps: (a) using a thread starting from the corner of the eye near the nose to the end of the other side of the eye curving via the puffiness and (b) determining the thread length with a ruler.

### Statistical analysis

One-way analysis of variance (ANOVA) with Tukey's multiple comparison test and t-test were used to investigate the statistical significance of differences and a *P* value of 0.05 was considered to be significant.

## RESULTS AND DISCUSSION

### Selected formulation of eye gel containing 3% caffeine

All studied formulations were clear gels with the viscosity of 78,000-98,000 cPs and stable after 6 freeze-thaw cycles with caffeine content of 105-111% labeled amount. Their pH values were in the range of 5.51-5.64, not so much different from the skin

pH (about 4.5-6.5). Therefore, it may be assumed that these formulations were applicable for the skin application. The solubility value of caffeine in water is 1 in 60 (Reynolds, 1989). Hence, a co-solvent was required for increasing the solubility in order to prepare 3% caffeine gels. It was found that PG in concentrations of 7.5, 15 and 30% could enhance solubility of caffeine and provide desirable clear gels (Amnuaitkit et al., 2008).

The skin permeation profile as exhibited Figure 2 showed that the concentrations of PG affected the skin permeation of caffeine. Descent ranking of permeation amount of caffeine was from the formulations composed of PG in 15%, 30% and 7.5%, respectively. PG could enhance the skin permeation of several drugs such as hydrocortisone (Polano and Ponec, 1976), metronidazole (Wotton et al., 1985) and ibuprofen (Irwin et al., 1990). It is proposed to provide enhanced activity by increasing the drug solubility in the skin and/or by assisting the partition of the drug into the skin (Higaki et al., 2003). However, the enhancing activity of PG itself is quite controversial since PG has several characteristics (Amnuaitkit et al., 2005). High concentration of PG may contribute to reduced skin permeation of the active compound. The formulation composed of 7.5% PG provided the lowest amount and rate of the skin permeation of caffeine, leading to sustained activity. Therefore, this formulation was selected for further investigation.

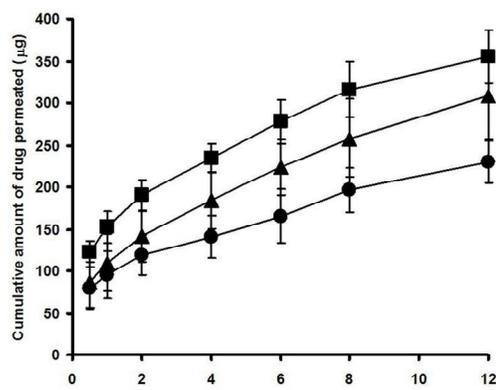


Fig 2. Skin permeation profiles of caffeine from gel formulations composed of PG in the concentrations of: 7.5% (●), 15% (■) and 30% (▲). Each data point was the mean $\pm$ S.D. (n=3).

### Effect of caffeine gel on acute eye irritation of rabbits

To protect against an unwanted effect of the caffeine eye gel when accidentally contacting the eye, an acute eye irritation test was performed. After application of 0.1 ml caffeine gel for 1 h, redness and chemosis of the conjunctiva was observed in three treated rabbits while the cornea and iris were unaffected. These symptoms were completely recovered within 24 h. Moreover, the caffeine gel and its blank counterpart demonstrated no irritation on the lateral arms of the volunteers. These data revealed that the selected caffeine gel was safe for topical application. Like other cosmetic products, the caffeine gel has to be avoided directly contacting to the eyes.

### In vivo effects of caffeine gel on eye puffiness

Both caffeine gel and gel base decreased eye puffiness when the period of application time increased as presented in Figure 3. The reduction of eye puffiness was inversely related to the length of the decrease of swollen area which was calculated from the length of eye puffiness area at the initial minus with that at the investigated time. Insignificant difference between efficacy in reducing puffy eyes of caffeine gel and gel base was found ( $P>0.05$ ). From the results of skin permeation studies, all caffeine gel formulations containing various PG concentrations could facilitate the passage of caffeine through the skin. It meant that the caffeine gel formulation would reduce eye puffiness if caffeine acted as an eye puffiness reducing agent. Thus, the results in Figure 3 implied that the cooling effect of the hydrophilic gels, occurred from water and ethanol evaporation and resulted in heat removal, could reduce eye puffiness rather than the vasoconstriction of caffeine (Sondra et al., 2011).

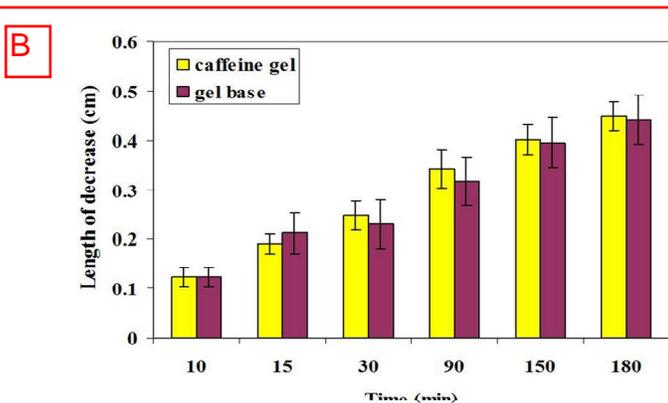


Fig 3. Efficacy of caffeine gel and gel base in reducing puffy eyes after application for various time intervals. Each data point was the mean $\pm$ S.E.M (n=34).

However, it was observed that caffeine gel could significantly higher reduce eye puffiness of 8 from 34 volunteers (23.5%) than gel base ( $P<0.05$ ). It could be explained that different people responded to the vasoconstriction of caffeine after topical application in a different way.

### CONCLUSIONS

Puffy eyes can result from several causes, leading to undesirable looking. Many treatments have been proposed to reduce puffy eyes. Topical application of eye gels is one of interesting ways since people can easily use by themselves. Caffeine is believed that it can reduce puffy eyes due to constriction of dilated capillaries. However, it was found that the cooling effect of the hydrophilic gels provided more effect on reduction of eye puffiness than the vasoconstriction of caffeine. Only 23.5% of the volunteers responded to caffeine activity.

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