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A Comparative, Randomized, Double-Blinded, and Vehicle-Controlled Study for the Reduction in Facial Pigmentation after Treatment with both Tranexamic Acid and Tranexamic Acid Ethyl Ester

Alaa A. Ali[1], Zaid Mahdi Jaber Al-Obaidi[2]*, Ayad M.R. Raauf[3], Hasanain Shakir Mahmood[4]
[1]Department of Clinical Pharmacy - College of Pharmacy - Ahlulbait University - Kerbala-Iraq
[2]Department of Chemistry and Biochemistry - College of Medicine – University of Kerbala - Kerbala-Iraq
[3]Department of Pharmaceutical Chemistry - College of Pharmacy - Mustansiriyah University- Iraq
[4]Department of Pharmaceutics - College of Pharmacy – University of Kerbala - Kerbala-Iraq
***Corresponding Author E-mail:** zaid.alobaidi@uokerbala.edu.iq

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ABSTRACT

Background: Tranexamic acid is used to treat or prevent excessive blood loss. Moreover, tranexamic acid is reported to treat the UV light-induced hyperpigmentation. Several tranexamic acid esters were approved to have topical skincare, however, tranexamic acid ethyl ester was not. In this work, a vehicle-controlled, double-blinded, and randomized clinical study was conducted for both tranexamic acid and its synthesized ethyl ester.

Methods: a well-established protocol was adopted to synthesize ethyl-4-(Aminomethyl)Cyclohexanecarboxylate from tranexamic acid and ethanol. Three gel preparations were applied. These contained tranexamic acid, tranexamic acid ethyl ester, and vehicle (gel base). These three prepared gels were applied to thirty-six subjects of three equally-divided groups for thirty days' period.

Results: The synthesis shows 90% yield with purity > 99.7%. While the clinical findings were significant for both tranexamic acid (P = .01) and tranexamic acid ethyl ester (P < .001) whereas it was insignificant for the gel vehicle (P = .176).

Conclusion: The authors concluded that both tranexamic acid and tranexamic acid ethyl ester topical gels are considered favourable choices to counteract facial hyperpigmentation.

Keywords: Tranexamic acid gel, tranexamic acid ethyl ester gel, hyperpigmentation, skin whiteners.

Correspondence:
Zaid Mahdi Jaber Al – Obaidi
Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala
Kerbala, Iraq
E-mail: zaid.alobaidi@uokerbala.edu.iq

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INTRODUCTION

Tranexamic acid is a lysine analogue (figure 1) which is used to treat or prevent excessive blood loss as tranexamic acid possesses an antiplasmin activity. Nevertheless, tranexamic acid has been proven to counteract the hyperpigmentation that is induced by UV light. This is due to that the tranexamic acid interferes with the plasminogen binding to keratinocytes which, in turn, decreases the activity of melanocyte tyrosinase resulting in arachidonic acid and eventually prostaglandins reduction. The latter are considered well-known inflammatory mediators that contribute in melanogenesis especially in the facial area which is considered a major participant in skin aged appearance (1-4). In the same context, melasma was reported to be improved when an intradermal localized dose of tranexamic acid was injected (5). Several tranexamic acid esters were approved to have topical skincare, however, tranexamic acid ethyl ester was not (6).

In this work, the authors aim to seek and compare the effect of the reduction in facial pigmentation after treatment with both tranexamic acid and the synthesized tranexamic acid ethyl ester.

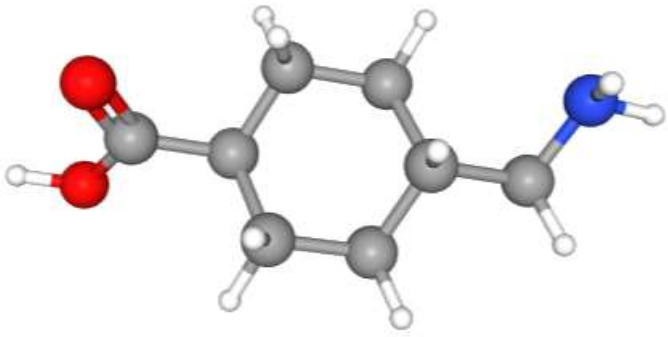


Figure 1: The three dimensional chemical structure of tranexamic acid ($C_8H_{15}NO_2$) (7)

MATERIALS AND METHODS

Chemicals and reagents

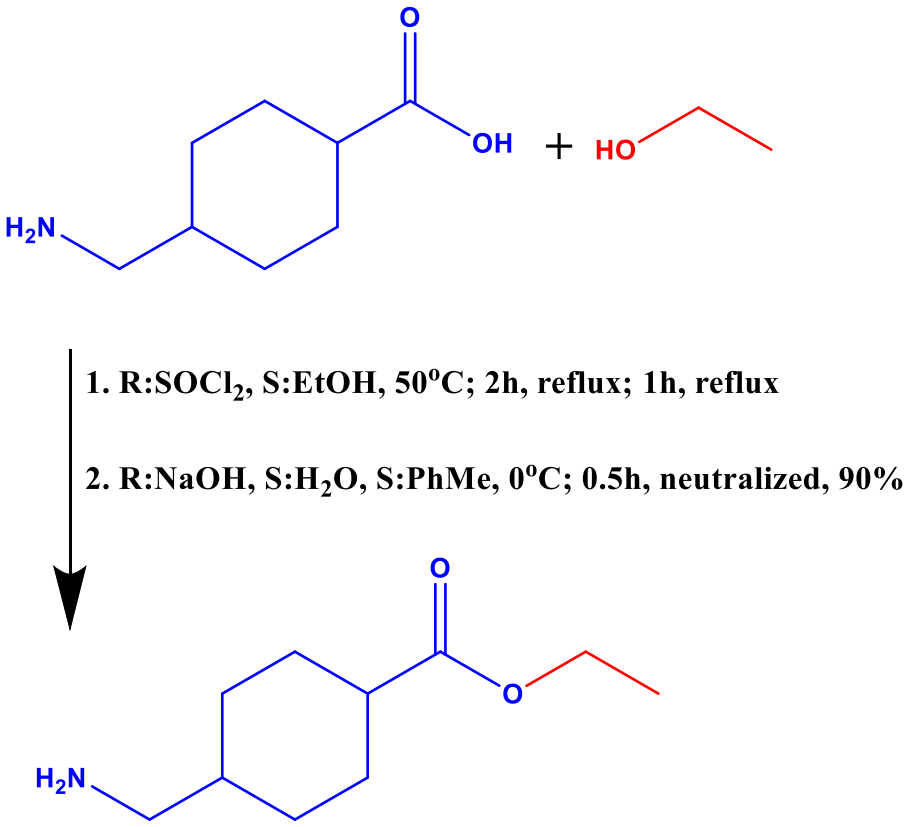
Tranexamic acid was purchased from Haihang Industry (China). Thionyl chloride was obtained from CDH (India). Ethanol (HPLC grade), Diethyl ether (stabilized), Toluene, (A.R.), and Sodium hydroxide (pellets) were purchased from Himedia (India). Dermatologically tested transparent gel base was purchased from local pharmacy.

Instrumentation

The main instrument employed in this work was the portable colorimeter (WR-10QC) was purchased from ShenZhen Wave Optoelectronics Technology Co., Ltd., China.

Chemistry

To a solution of 1 mol (157.2 g) of tranexamic acid in 1L absolute ethanol at 50°C, 2 mol (238 g) of thionyl chloride was wisely dropped. Slight boiling of reaction mixture was noticed during the addition process. Thereafter, the reaction mixture was refluxed for 2 hours. Then, 1.5 mol (178.5 g) of thionyl chloride was carefully added and refluxed at the same temperature (50°C) for an extra 1 hour. The remnant solvent was distilled off and 75 ml of ethanol was added. The ethyl-4-(Aminomethyl) Cyclohexanecarboxylate hydrochloride was precipitated with the addition of 600 ml of anhydrous ether. The precipitant was washed with additional 200 ml of anhydrous ether and was suspended in 500 ml of toluene. The later suspension was vigorously stirred for 30 minutes with 130 ml of 40% of sodium hydroxide solution in water-ice bath at 0°C. The aqueous layer was removed and the toluene was evaporated under vacuum. After drying, white powder of ethyl-4-(Aminomethyl) Cyclohexanecarboxylate was obtained and the yield was recorded as 90% (166.824 g) (8). ^{1}H NMR (500MHz) spectrum ($CDCl_3$), δ, ppm 4.10 q (2H, OCH_2), 3.32 br. (2H, NH_2), 2.61 d (2H, CH_2N), 2.21 t.t (1H, CH), 2.02 m (2H, CH_2), 1.88 d.d (2H, CH_2), 1.42 m (3H, CH_2 + CH), 1.24 t (3H, CH_3), 0.97 m (2H, CH_2).



Scheme 1: The synthesis of ethyl-4-(Aminomethyl) Cyclohexanecarboxylate

Gel preparations

Sufficient quantities of three different types of gels were prepared; the first was blank. The second contains 636 mM (10% w/v) of tranexamic acid. Whereas, the third contains 636 mM (11.8% w/v) of ethyl-4-(Aminomethyl)Cyclohexanecarboxylate.

Subjects

Thirty-six adult volunteers aged 25–63 years with moderate to severe hyperpigmentation were enrolled. These subjects were equally and randomly divided into three groups. The first group received the tranexamic acid, the second group received the ethyl-4-(Aminomethyl) Cyclohexanecarboxylate, and lastly, the third group received the gel base (aka: vehicle control). All subjects were well-informed about the research and the oral consent were confirmed with the employment of Helsinki Declaration.

Study design

This thirty-days randomized study was designed to be prospectively double-blinded vehicle-controlled study. Within the study period, the subjects were allowed to utilize their ordinary cosmetics as these products were not medicated and will not affect the results.

All subjects received a container with 50 grams of the assigned gel. Each subject applied, at evening, 1-mL topical gel applied to the entire face area in daily basis from day-1 to day-30.

Assessment of safety and efficacy

The safety was assessed to all subjects at the base-line, day-15, and day-30 of the study. The subjects were asked for any itching, redness, and/ or pain or any other proposed adverse effect. Whereas, the efficacy assessment was conducted employing a precise colour reader as a portable colorimeter and utilizing the observed *L*-values.

Statistical analysis

The statistical analyses were conducted with the employment of D'Agostino-Pearson omnibus (K2) utilizing two-tailed P value analysed with GraphPad Prism Software version 8.3.1 (549) (GraphPad, LLC, California, USA).

In all circumstances, the observed differences in data sets were statistically deemed significant when $P < 0.05$.

This study was performed in the laboratory of Pharmaceutical Chemistry and Clinical Pharmacy at College of Pharmacy, Ahlulbait University, Karbala, Iraq from 13-Nov-2019 to 15-Mar-2020.

RESULTS

The purity of the synthesized tranexamic acid ethyl ester was calculated relying on the weight deviation in the CHN elemental microanalyses. The calculated and the observed weights for carbon, hydrogen, and nitrogen atoms is revealed in table (1).

Table 1: Reveals the elemental microanalyses for the synthesized tranexamic acid ethyl ester.

Chemical formula	Molecular mass	CHN Elemental microanalyses %		
		Element	Estimated	Found
$C_{10}H_{19}NO_2$	185.27	C	64.83	64.78
		H	10.34	10.27
		N	7.56	7.52
		Sum	82.73	82.57
		% Deviation	0.21%	

The study was conducted with all the 36 subjects. None of the participated subjects withdrawn from the study for inconvenience regarding ineffectiveness or side effects. The facial pigmentation reduction after 30 days' treatment with the specified gels was plotted relying on the mean of the facial skin darkness versus time in days. This was performed with the aid of GraphPad Prism software version 8.3.1 (549) as shown in figure 2.

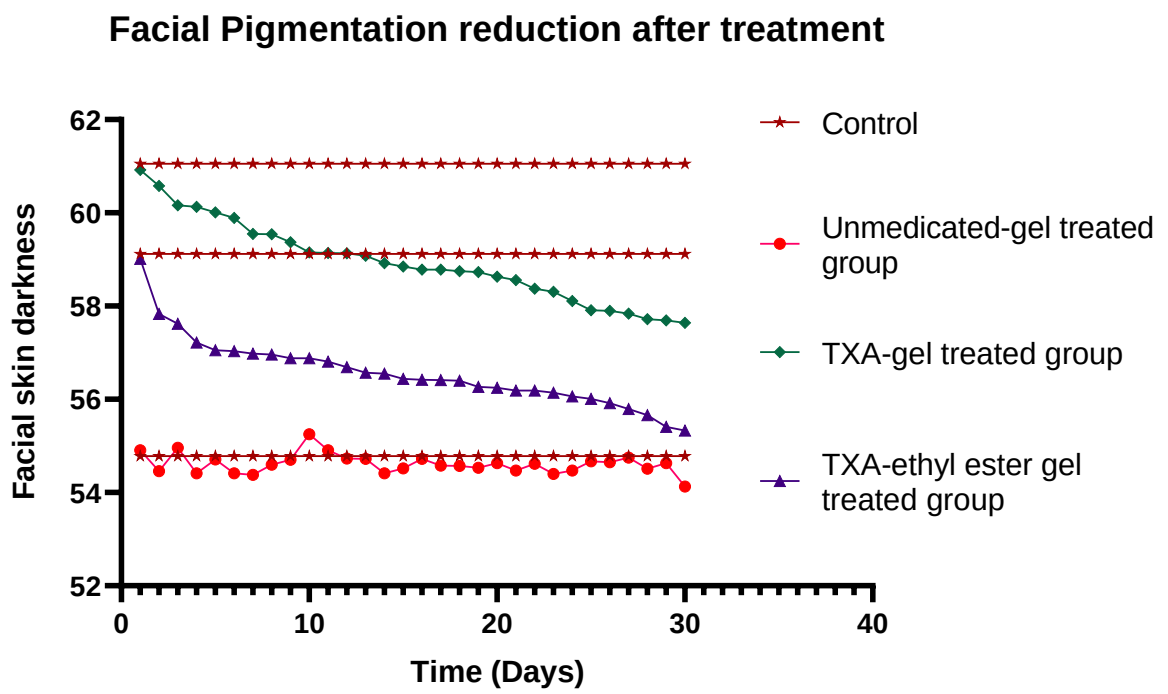


Figure 2: This figure shows the mean of the facial pigmentation reduction after 30-days treatment with tranexamic acid (TRANEXAMIC ACID) gel (), TRANEXAMIC ACID-ethyl ester gel (), unmedicated gel (), in accordance to control ().

In the tranexamic acid gel treated-group, the skin darkness relying on *L*-value were reduced (the skin lightening was increased) from the baseline (*L*-value = 61.05) to the end of day 30 (*L*-value = 57.64) and considered significant (P-value = .01). Moreover, in the tranexamic acid ethyl ester gel treated-group, the skin darkness relying on *L*-value showed greater reduction from the baseline (*L*-value = 59.12) to the end of day-30 (*L*-value = 55.33) and considered significant (P-value < .001). On the other hand, the unmedicated-gel (gel base vehicle only) treated-group, the skin darkness relying on *L*-value was not significantly changed from the baseline (*L*-value = 54.78) to the end of day-30 (*L*-value = 54.13) and considered insignificant (P-value = .176).

The three gel products were well-tolerated by all of the study subjects and none of the subjects encountered any considerable adverse effect.

DISCUSSION

It has been reported that the chemical synthesis of tranexamic acid ethyl ester (ethyl-4-(Aminomethyl) Cyclohexanecarboxylate)) gave a yield of 66% (9). However, in this work the yield is highly enhanced with fewer steps to reach 90% (shown in scheme 1) with typical proton NMR for structural elucidation and high purity (> 99.7%) as calculated from the CHN elemental microanalyses in which the % deviation (revealed in table 1). Basically, it is agreed to have an error to be less than 0.4% for the CHN elemental microanalyses (10). In this study, the synthesized tranexamic acid ethyl ester had a weight deviation of 0.21% which obviously, less than the given limitation of 0.4%. This indicates a high purity or low impurity contents.

In fact, the global demand for safety and effectiveness in skin lightening agents has been increased as the "gold standard" skin lightening, hydroquinone, was banned in 2001 from cosmetic uses due to the reported adverse effects (11, 12). Accordingly, several alternatives have been proposed. For instance, it is commonly agreed that niacinamide, arbutin, azelaic acid, ascorbic acid, kojic acid, resveratrol, licorice, glutathione, soy, and tranexamic acid possess skin lightening effects (12-14). However, topical tranexamic acid showed significant results in a published clinical study (15). Nevertheless, reviewing the parameters affecting percutaneous absorption of drugs (16), tranexamic acid has few limitations to penetrate the skin layer mainly because of its highly polar structure (tPSA = 63.32), ionization (pKa 4.3), and low partition coefficient (logP = 0.49). The previously mentioned physicochemical properties of tranexamic acid can be enhanced via derivatization (16). This prodrug approach has been extensively explained (17-19). Being an advantage, in this study, the synthesis tranexamic acid derivative (i.e. (ethyl-4-(Aminomethyl) Cyclohexanecarboxylate)) showed enhanced the estimated physicochemical properties of tranexamic acid. For example, these physicochemical properties for the ethyl ester were estimated as follows; the topological polar surface area (tPSA = 52.32), the ionization constant (pKa 10.6), and the partition coefficient (logP = 1.09). This enhancement in physicochemical properties are confirmed with the significant enhancement in the clinical findings collected by this study (shown in figure 2).

CONCLUSION

The researchers concluded that both tranexamic acid and tranexamic acid ethyl ester topical gels are considered favourable choices to fight skin hyperpigmentation.

CONFLICT OF INTEREST

"The authors declare that there is no conflict of interest".

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