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Melatonin vitamin C-based nanovesicles for treatment of androgenic alopecia: Design, characterization and clinical appraisal

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ABSTRACT

The present study aimed to develop vitamin C based nanovesicles (aspasomes) loaded with the antioxidant melatonin, as a novel cosmeceutical to be used for clinical treatment of androgenic alopecia (AGA). Aspasomes were assessed regarding their particle size, charge, drug entrapment, anti-oxidant potential, physical stability, *in vitro* release, surface morphology, and *ex-vivo* skin deposition. Clinically, melatonin aspasomes were tested on AGA patients, and assessed by evaluating the degree of improvement through conduction of hair pull test, histometric analysis and dermoscopic evaluation. Results revealed that melatonin aspasomes showed favorable pharmaceutical properties in addition to clinically promising results compared to melatonin solution, manifested by increased hair thickness, density and decreased hair loss, with photographic improvement in most patients. Therefore, melatonin vitamin C-based aspasomes were clinically auspicious in the treatment of AGA, hence, paving the way for their further exploration in other oxidative-dependent dermatological diseases.

1. Introduction

Reactive oxygen species (ROS) are produced upon exposure to exogenous causes such as smoking and UV light. The excessive ROS generation may contribute to a variety of cutaneous diseases, among which is alopecia (Prie et al., 2016). Androgenic alopecia (AGA) is considered a dermatological disease characterized by excessive scalp hair thinning (Torres, 2015). Oxidative stress was assumed to be one of the major causative agents of AGA (Mosley and Gibbs, 1996; Prie et al., 2016), hence it can be postulated that the co-utilization of antioxidant molecules with functional excipients can result in improvement of this disease, especially when formulated in one of the nanodelivery systems which have shown promise in the treatment of dermatological diseases (Bsieso et al., 2015; Bseiso et al., 2015; Abdelgawad et al., 2017; Fadel et al., 2017; Nasr et al., 2017).

Vesicular systems offer many advantages for topical drug delivery such as safety, biocompatibility, controlled drug release and enhanced skin permeation (Nasr et al., 2008a). Bilayer forming agents such as phospholipids and non-ionic surfactants are the most commonly used materials for formulation of vesicles, however they lack antioxidant potential. Therefore, in the current manuscript, we attempted the use of ascorbyl palmitate; a vitamin C derivative as a bilayer forming agent for preparing the vesicles (aspasomes), owing to their previously reported antioxidant potential (Gopinath et al., 2004). Melatonin was chosen as the drug of choice to be loaded in aspasomes owing to its potent anti-oxidant effect (Tan et al., 1993) and its reported clinical influence on hair growth (Fischer et al., 2004; Fischer et al., 2008). The current manuscript represents the first attempt for the clinical use of ascorbyl palmitate vesicles (aspasomes) in treatment of diseases, and the first attempt to treat AGA using nanoparticles.

2. Materials and methods

2.1. Materials

Buffer components were purchased from El-Nasr pharmaceutical company, Egypt. Chloroform, methanol, dicetyl phosphate, ascrobyl palmitate, cholesterol, DPPH (2,2-diphenyl-1-picrylhydrazyl), phosphotungistic acid and spectra/Por dialysis membrane (average flat width 33 mm) were purchased from Sigma Aldrich Chemical Co., USA.

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Table 1
Composition and characterization of melatonin aspasomes.

Formula code	Composition ASP:CHOL:DCP Molar ratio	PS (nm) Mean ± S.D.	Polydispersity index Mean ± S.D.	Zeta potential (mV) Mean ± S.D.	EE% Mean ± S.D.
A1	70:20:10	950.5 ± 16.60	0.501 ± 0.04	−37.3 ± 2.41	91.21% ± 11.50
A2	60:30:10	466.7 ± 12.51	0.360 ± 0.01	−47.6 ± 0.79	75.37% ± 0.22
A3	50:40:10	388.0 ± 15.20	0.340 ± 0.04	−52.7 ± 8.81	72.23% ± 0.22
A4	45:45:10	361.6 ± 11.40	0.314 ± 0.02	−53.8 ± 9.60	63.13% ± 0.66
A5	40:50:10	352.9 ± 12.51	0.311 ± 0.03	−55.2 ± 4.22	61.01% ± 0.02
A6	30:60:10	309.7 ± 7.35	0.251 ± 0.03	−59.1 ± 5.62	59.06% ± 6.17
A7	20:70:10	286.5 ± 5.51	0.255 ± 0.12	−63.3 ± 3.81	52.23% ± 0.64

Melatonin was purchased from Skin actives company, USA.

2.2. Methods

2.2.1. Preparation of aspasomes using thin film hydration

Aspasomes were formulated using the film hydration technique (Gopinath et al., 2004; Nasr et al., 2008a) in which 200 mg of the lipid mixture composed of ascorbyl palmitate (ASP), cholesterol (CHOL) and dicetyl phosphate (DCP) together with 25 mg melatonin were dissolved in a 10 mL organic solvent mixture (chloroform:methanol 9:1). Their composition is described in Table 1. The flask was rotated at 150 rpm under reduced pressure at 50 °C (Rotavapor R114, Buchi, Switzerland) till the formation of thin film, which was then hydrated portion-wise with 10 mL phosphate buffered saline (PBS, pH 7.4), followed by rotation for 45 min. Aspasomes were sonicated for 2 min (Branson Ultrasonic cleaner 3510-DTH, Mexico), and the aspasomal formulations were kept in glass vials in the refrigerator.

2.2.2. Determination of the particle size, polydispersity index and charge of melatonin aspasomes

The particle size, polydispersity index and charge of aspasomes were measured using Zetasizer (ZS3600, Malvern instruments, UK) after appropriate dilution (Mouez et al., 2016; Barakat et al., 2017).

2.2.3. Determination of melatonin entrapment efficiency (EE%) in aspasomes

Entrapment efficiency of melatonin was determined applying the dialysis method (Lingan et al., 2011; Manconi et al., 2012; Bseiso et al., 2016) against 2000 mL stirred distilled water to remove the free drug (magnetic stirrer, Lab Tech LMS, Korea), which was changed twice to provide sink conditions for melatonin. An aliquot of the dialyzed vesicles was disrupted with methanol, and assayed for melatonin content at 278 nm using UV-spectrophotometer (Biochrom Libra S60, UK) using methanol as blank, and the EE% was calculated (Nasr et al., 2008b).

2.2.4. Physical stability of melatonin aspasomes

The stability of melatonin aspasomal formulations was assessed by re-measuring their size, PDI and charge after refrigeration storage for 3 months (Nasr et al., 2008a; Bseiso et al., 2015).

2.2.5. Measurement of the anti-oxidant potential of melatonin aspasomes using DPPH assay

A stock of DPPH in methanol (10 mg DPPH in 400 mL methanol) was prepared, kept in dark for 60 min, then its absorbance was measured at 515 nm (Gonzalez-Paredes et al., 2011). The antioxidant potential of the selected aspasomes was assessed by adding 3.9 mL of the DPPH stock solution to a volume of aspasomal formula containing an equivalent amount of 0.25 mg melatonin, the test tubes were left in the dark for 60 min, and their absorbance values were re-measured at 515 nm using methanol as blank. Melatonin solution at the same concentration was used as control. The extent of lowering of the absorbance of the purple colored DPPH stock solution is indicative of the free radical scavenging ability of the aspasomal formulae (Gonzalez-Paredes et al., 2011). The percentage of DPPH scavenged is calculated using the following equation (Nasr, 2016):

$$\text{DPPH Scavenged (\%)} = \frac{\text{Absorbance of stock solution} - \text{Absorbance of test solution}}{\text{Absorbance of stock solution}} \times 100\%$$

2.2.6. In-vitro release of the selected melatonin aspasomes

Based on the results of EE%, particle size, DPPH assay and physical stability, three formulae were selected for *in-vitro* release evaluation. The study was performed on the freshly dialyzed aspasomal dispersions, in which a specified volume equivalent to 2 mg of melatonin was loaded to a cellulose membrane dialysis bag immersed in 60 mL phosphate buffered saline solution of pH 7.4. Samples were horizontally shaken in a thermostatically controlled shaker (model IKA KS 4000 IC, USA) at 32 °C and 50 strokes per minute. After 0.25, 0.5, 1, 2, 3, 4, 5 and 6 h, 3 mL samples were withdrawn and analyzed spectrophotometrically at 278 nm (Mohammed et al., 2009).

2.2.7. Transmission electron microscopy (TEM) of selected melatonin aspasomes

The shape and surface morphology of selected aspasomes was examined using TEM (JEM – 100S, Joel, Tokyo, Japan) after staining using 1% phosphotungistic acid (Nasr et al., 2008a; Bsieso et al., 2015).

2.2.8. Ex-vivo deposition/permeation of melatonin aspasomes

Defrosted rat skin samples were cut into square pieces and mounted in Franz- type diffusion apparatus (Hanson Research model 60-301-106, California, USA) of cells with diffusion area of 1.77 cm^2 (Nasr and Abdel-Hamid, 2016). The receptor medium was 7.5 mL PBS pH 7.4 at 32 °C and stirred at 100 rpm. Two milliliters of the selected melatonin aspasomes was placed in the donor compartment. Melatonin solution in PBS containing equivalent drug amount was treated equally and used as control. After 24 h, samples were withdrawn from the receptor compartment and the upper part of the skin was washed five times using methanol and water to remove the unpermeated drug/formulation. Tape stripping of the skin was performed twenty times with adhesive tape (Bsieso et al., 2015), and the dermis was detached from the epidermis using scalpel-peeling. The adhesive strips and skin specimens were placed each in 20 mL methanol followed by sonication for 4 h to extract the deposited drug in each skin layer. Furthermore, samples from the receptor compartment after 24 h were analyzed as well. All samples were filtered, and analyzed using UPLC method developed and validated in our laboratory (Agilent 1290 infinity, Germany). The solvent system was water:acetonitrile (25:75), flowing at 0.6 mL/min, and 10 µL was injected into a C18 column and analyzed at 223 nm.

2.2.9. Clinical efficacy of aspasomes in treatment of AGA

The clinical study included 40 male patients with AGA from the dermatology outpatient clinic of Minya University hospital. They were separated into two groups of twenty patients each. Group I was treated

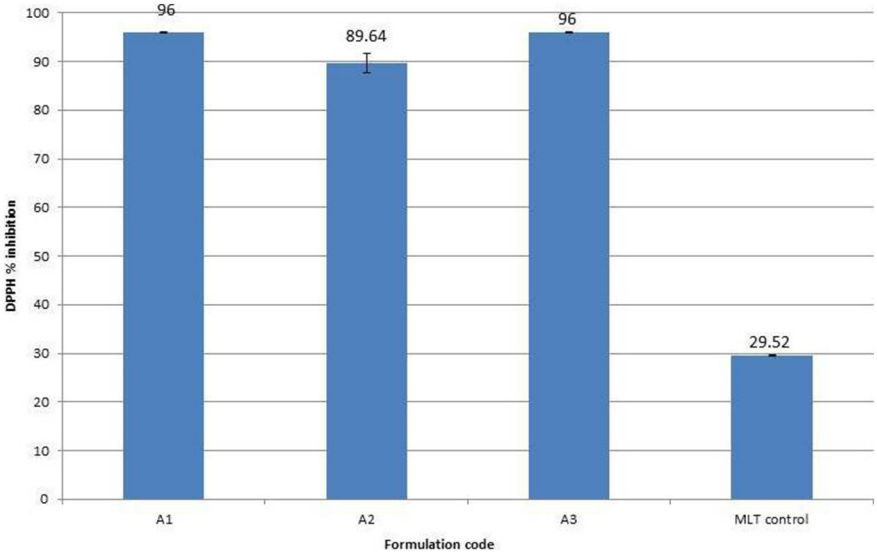

Fig. 1. DPPH% inhibition of selected melatonin aspasomes compared to melatonin control solution (mean ± S.D., n = 3).

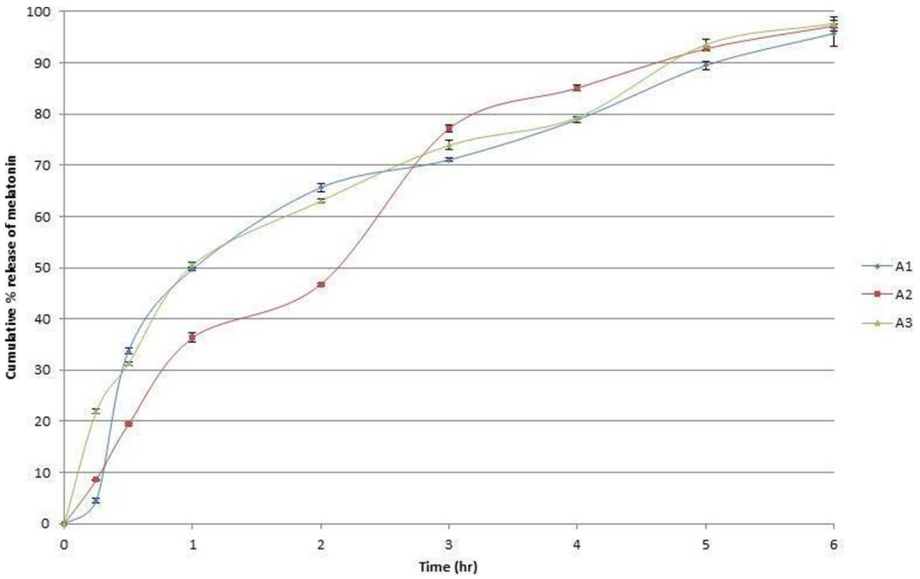

Fig. 2. Melatonin aspasomes release profile over a period of 6 h.

with melatonin PBS pH 7.4 solution and group II was treated with aspasomal formula A3.

Inclusion criteria involved male patients (age 18–50 years) who suffered from mild to moderate AGA (stages I–V Hamilton-Norwood classification) and have not used topical, systemic or intralesional AGA therapy, dyes or hair permanent for six months prior the clinical experimentation. Patients were diagnosed for AGA based on a detailed medical history and clinical examination, in addition to laboratory tests. A written consent was obtained from all patients. The clinical study was consented by both the research committee of the faculty of Medicine, Minya University and the research ethics committee for experimental and clinical studies at the faculty of pharmacy, Ain Shams University (REC ASU-176). The principles outlined in the declaration of Helsinki for human subject experimentation were followed.

Patients were instructed to topically apply the provided aspasomal formula or melatonin solution daily on affected scalp areas for a total period of 3 months. Evaluation of the clinical outcome was performed at the 16th week. Patients were clinically assessed through photography, hair pull test, histometric assessment and dermoscopic examination.

2.2.9.1. Photography. Photographic evaluation was performed through taking digital photos of the scalp before and at the end of treatment. An improvement grade was provided for each patient by comparing the degree of baldness before and after treatment. The grading was expressed as the mean of grades provided by three professional independent blinded observers. Assessment was performed by giving scores of −1 (worse than baseline), 0 (no improvement), 1 (minimum improvement < 20%), 2 (mild improvement 20–39%), 3 (moderate improvement 40–59%), 4 (good improvement 60–79%) and 5 (excellent improvement 80–100%) (Abdallah et al., 2009).

2.2.9.2. Hair pull test. The test was performed through gentle pulling of hairs from the vertex scalp area, and the pulled hairs were counted, before and at the end of treatment (Jha et al., 2017).

2.2.9.3. Histometric assessment. The pulled hairs from 10 patients were mounted on a glass slide, and with the aid of a computer program (analysis® Five Olympus Soft Imaging Solutions GmbH, Johann-Krane-Weg 39, D-48149, Munster, Germany), the mean thickness of the hair shaft was assessed before and after treatment (El-Domyati et al., 2017).

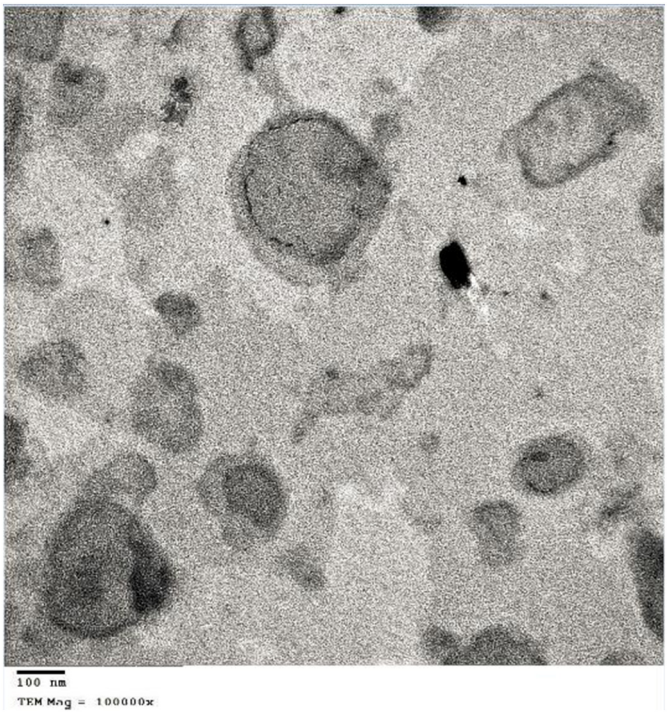

Fig. 3. TEM micrograph of formula A3 at a magnification of 100,000×.

Table 2
Ex vivo skin deposition/permeation data of the selected aspasomal formula A3.

Compartment	A3	Melatonin solution
Stratum corneum	3.69% ± 0.12	2.76% ± 0.04
Epidermis	9.31% ± 0.01	4.46% ± 0.07
Dermis	3.27% ± 0.26	1.21% ± 0.14
Receptor compartment	1.33% ± 0.07	0.58% ± 0.05

2.2.9.4. Dermoscopic examination. Trichoscopy is an assessment tool used for hair visualization based on videodermoscopy. A fixed area of the scalp of patients (10 cm from the glabella) was visualized using Dermlite dermoscope. Baseline and post-treatment photographs were taken in addition to dermoscopic evaluation regarding yellow dots (*i.e.* sebaceous debris), hair shaft thickness and density (Jain et al., 2013; Jha et al., 2017). A score was also given to patients, in which a value of 0 indicated no improvement while a score of 1 indicated improvement of AGA.

2.2.10. Statistical analysis of data

One way ANOVA, Student's paired *t*-test, independent *t*-test, Chi-square test and Tukey Kramer post-test were performed using Graphpad® Instat software. P values less than or equal 0.05 were significant.

3. Results

3.1. Determination of the particle size, polydispersity index and charge of melatonin aspasomes

As shown in Table 1, the size of aspasomes varied from 287 to 950 nm, suggesting that there was an obvious relationship between the vesicles size and the composition. The increase in ascorbyl palmitate concentration (from 20 to 70 mol%) resulted in an accompanying increase in the size of aspasomes ($P < 0.05$).

The polydispersity index values of the aspasomes were in the range of (0.25–0.5), which are low values. Aspasomal formulations were negatively charged and the zeta potential (ZP) values ranged from −37.3 to −63.3 mV, with a significant increase in the negativity of the particles from A1 to A7 ($P < 0.05$).

Table 3
Treatment outcome for group I patients administered melatonin solution for 3 months.

Patient number	Improvement grade[a]	Hair pull test[b]		Dermoscopic score[c]	Histometric hair diameter assessment (μm)	
		Before	After		Before	After
1	0	8	7	0	45.88	54.22
2	1	7	7	0	47.66	53.44
3	1	9	7	0	40.99	50.33
4	1	9	7	0	53.98	56.76
5	0	10	6	0	51.77	51.87
6	1	8	8	0	49.99	50.04
7	2	9	7	1	52.77	53.81
8	0	9	7	0	44.66	45.88
9	1	7	7	0	43.55	44.52
10	0	7	5	0	54.22	54.53
11	0	8	7	0		
12	1	7	6	1		
13	0	9	7	0		
14	0	7	6	0		
15	0	7	7	0		
16	0	7	7	0		
17	0	8	7	0		
18	1	8	7	1		
19	0	7	5	0		
20	0	7	5	0		

[a] 0 indicates no improvement, 1 indicates minimal improvement, 2 indicates mild improvement.
[b] Indicates the number of hairs pulled.
[c] 0 indicates no dermoscopic improvement and 1 indicates dermoscopic improvement.

Table 4
Treatment outcome for group II patients administered melatonin aspasomes for 3 months.

Patient number	Improvement grade[a]	Hair pull test[b]		Dermoscopic score[c]	Histometric hair diameter assessment (μm)	
		Before	After		Before	After
1	3	7	3	1	45.66	70.66
2	3	8	1	1	43.44	67.88
3	4	8	6	1	60.5	65.77
4	3	9	3	1	45.66	66.05
5	4	7	4	1	43.44	71.51
6	3	8	4	0	42.55	60.88
7	3	8	5	0	54.06	62.44
8	2	7	5	0	53.49	68.9
9	4	7	4	1	48.09	62.11
10	3	8	3	1	50.9	68.22
11	3	7	2	1		
12	2	7	1	0		
13	3	8	2	0		
14	3	9	2	0		
15	3	9	4	1		
16	4	8	5	1		
17	3	9	3	1		
18	2	8	4	0		
19	3	7	3	0		
20	3	9	2	1		

[a] 0 indicates no improvement, 1 indicates minimal improvement, 2 indicates mild improvement, 3 indicates moderate improvement, 4 indicates good improvement and 5 indicates excellent improvement.
[b] Indicates the number of hairs pulled.
[c] 0 indicates no dermoscopic improvement and 1 indicates dermoscopic improvement.

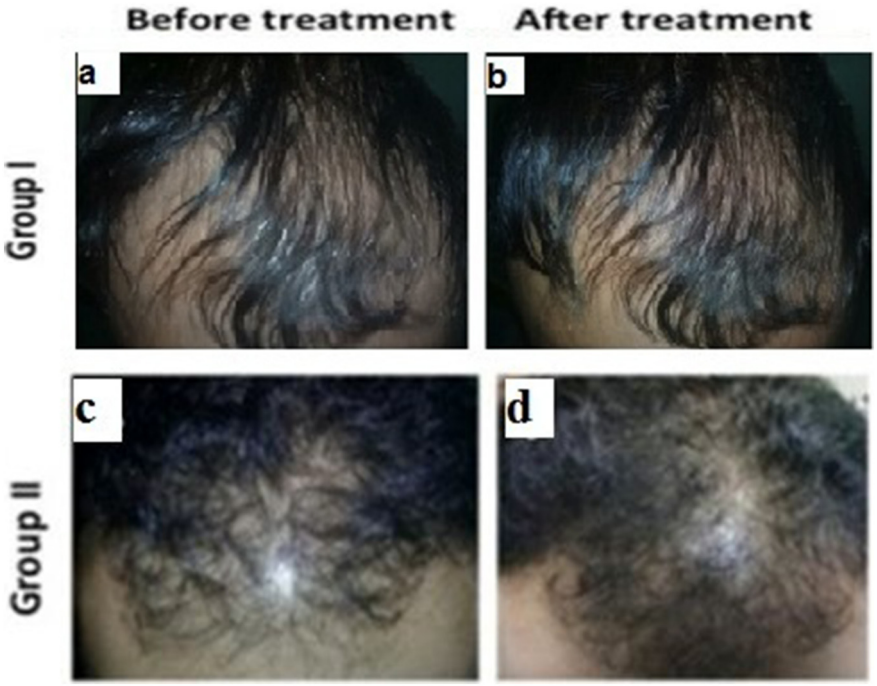

Fig. 4. Two male patients with AGA before treatment (a,c) and after treatment (b,d), showing minimal improvement in group I after topical application of melatonin solution and good improvement in group II after topical application of A3 formula.

3.2. Determination of melatonin aspasomes entrapment efficiency

As demonstrated in Table 1, the entrapment of melatonin ranged from 52.23% to 91.21%. As observed from the results, there was a significant decrease in melatonin EE% by increasing the cholesterol content and decreasing the ascorbyl palmitate content ($P < 0.05$).

3.3. Physical stability of melatonin aspasomes

As shown in Supplementary file 1, storage of aspasomes didn't show any significant variation in particle size ($P > 0.05$) except for formula A7 which was prepared using the lowest amount of ascorbyl palmitate. As for the ZP and polydispersity index, most formulations displayed insignificant changes upon storage ($P > 0.05$).

As can be inferred from the mostly insignificant changes in size, polydispersity index and ZP values after storage, the physical stability couldn't be utilized as an exclusion parameter for aspasomal vesicles. Upon inspection of the combined results, it can be observed that all aspasomal formulations displayed favorable particle size in the nanometer range. Formulations A4, A5, A6 and A7 displayed the lowest EE % values for melatonin, and hence were excluded from the *in vitro* release study. To recapitulate, formulae A1, A2 and A3 were selected for further experimentation (DPPH and *in vitro* release studies).

3.4. Measurement of the anti-oxidant potential of melatonin aspasomes

As observed in Fig. 1, melatonin aspasomes displayed potent anti-oxidant properties, as denoted by their extremely high % DPPH inhibition compared to the control melatonin solution ($P < 0.05$). It can be observed that both formulations A1 and A3 displayed the same DPPH% inhibition, while A2 displayed a significantly lower value ($P < 0.05$). The high DPPH% inhibition encountered with A1 could be attributed to its high ascorbyl palmitate percent, while the high value encountered with A3 could be ascribed to its high content of ascorbyl palmitate provided by taking a large formula volume to compensate for the relatively low EE% of melatonin, to keep the melatonin amount equivalent to A1.

3.5. In vitro release of melatonin aspasomes

As shown in Fig. 2, aspasomes showed sustained release of melatonin for 6 h with 100% release was achieved with all formulations within this period. Since formula A3 displayed the smallest particle size which would ensure better cell internalization, with comparable release and DPPH% inhibition to A1, formula A3 was chosen for further characterization.

3.6. TEM examination of melatonin aspasomes

As shown in Fig. 3, formula A3 displayed spherical shape with an evident bilayer, as similarly observed by other authors (Gopinath et al., 2004).

3.7. Ex vivo deposition/permeation of melatonin aspasomes

As obvious in Table 2, only a small amount of melatonin (1.33%) could be detected in the receiving compartment, which suggests topical rather than transdermal traits of the aspasomes. The encapsulation of melatonin into aspasomes has significantly augmented the deposition of melatonin in all layers of the skin compared to melatonin solution (1.34 fold more in the stratum corneum, 2.1 fold in epidermis and 2.7 fold in dermis) ($P < 0.05$).

3.8. Clinical efficacy of melatonin aspasomes

Evaluation of the clinical response was carried in terms of follow up photography/scoring, assessment of hair loss and hair diameter. No side effects were reported by patients applying either melatonin solution or aspasomes formulation indicating their safety and patient compliance.

As evident from Tables 3, 4 and Fig. 4, the degree of improvement of hair growth varied depending on the applied treatment. Regarding group I, a total of 8 patients (40%) displayed improvement compared with total significant improvement in all 20 patients (100%) of group II ($P < 0.05$). Upon close inspection of the results, it was evident that the improvement was minimal in 7 patients (35%) and mild in 1 patient

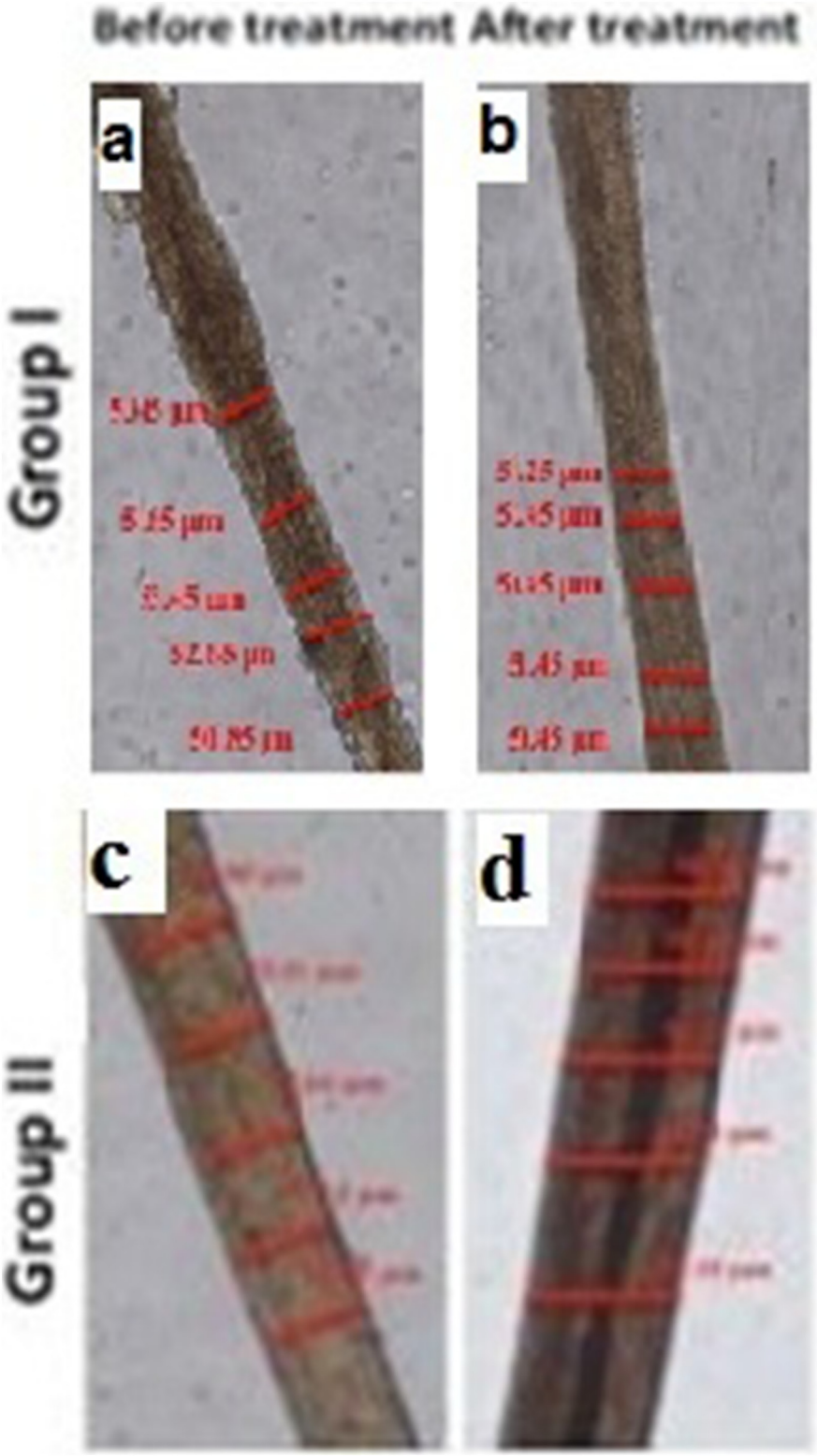

Fig. 5. Microscopic hair pictures from male patients with AGA before (a,c) and after treatment (b,f), showing a significant increase in the hair shaft diameter in group II which were treated with A3 formula (magnification × 400).

(5%) in group I, while in group II, 3 patients showed mild improvement (15%), 13 patients showed moderate improvement (65%), and 4 patients showed good improvement (20%). Therefore, it can be concluded that group II patients displayed significantly better improvement scores for patients ($P < 0.05$).

As also evident in Tables 3 and 4, the average number of pulled hairs before treatment was 7.9 ± 0.96 for group I patients, and 7.9 ± 0.78 for group II patients ($P > 0.05$). At the 16th week, the average number of pulled hairs was significantly reduced ($P < 0.05$) in both groups I (6.6 ± 0.82) and II (3.3 ± 1.38). The extent of decrease of hair loss was significantly higher for group II patients treated with melatonin aspasomal formulation than group I treated with melatonin

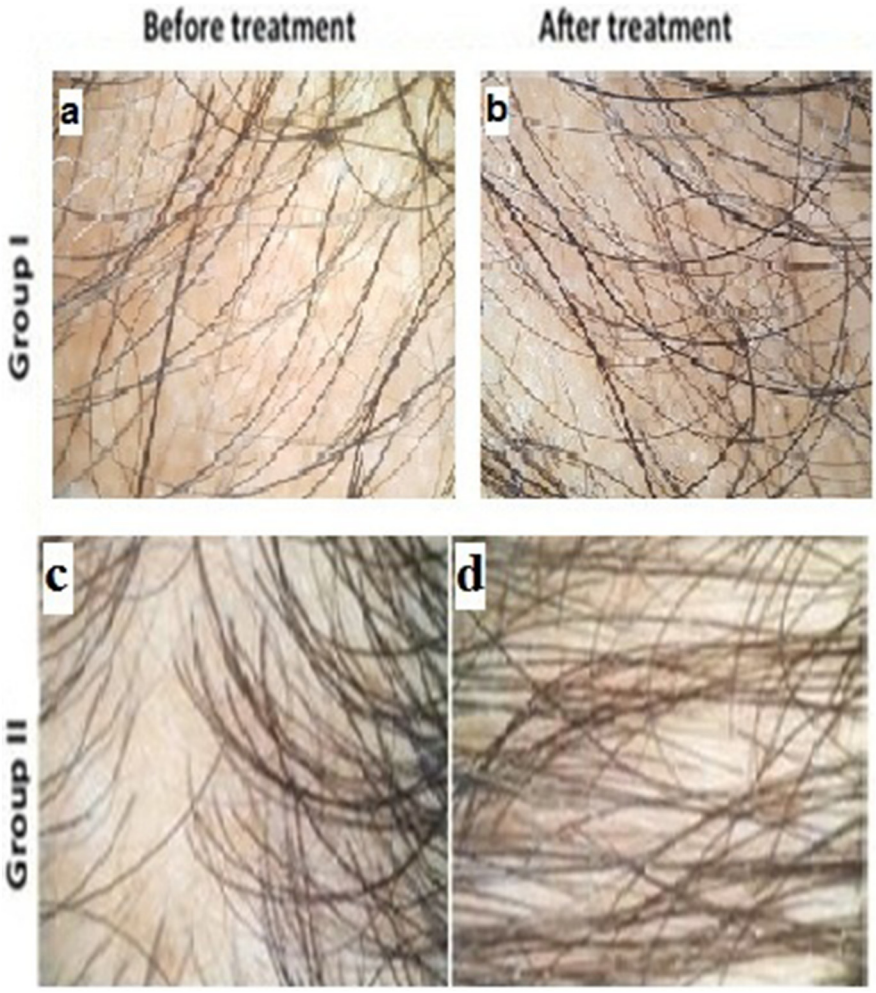

Fig. 6. Dermoscopic features of scalp of male patients with AGA before therapy (a, c) showing yellow dots and variation in the hair shaft thickness. After treatment, there are increase in the hair density, hair shaft thickness and decrease in yellow dots in group II after treatment with formula A3 (d), with no dermoscopic improvement in group I after topical application of melatonin solution (b) (polarized light magnification × 10). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

solution ($P < 0.05$).

As also shown in Tables 3, 4 and Fig. 5, the average hair diameter before treatment was 48.55 ± 4.68 μm for the first group and 48.78 ± 5.85 μm for the second group. After treatment, the average diameter in group I (51.54 ± 3.90, $P < 0.05$) and group II (66.44 ± 3.66, $P < 0.05$) was significantly increased. As similarly encountered with the hair pull test, the extent of hair diameter increase was significantly higher for group II patients treated with aspasomal formulation compared to group I patients treated with melatonin solution ($P < 0.05$).

As shown in Fig. 6, upon dermoscopic examination of patients before receiving treatment, patients displayed an abundance of thin vellus hairs with variability in the diameter of the hair shaft, in addition to the presence of yellow dots. In post-treated patients of group II, there was significant elevation in hair density and hair shaft diameter, and dramatic decrease or disappearance of yellow dots in twelve patients (60%) in group II, and only significant improvement in dermoscopic features for 3 patients in group I (15%) ($P < 0.05$).

4. Discussion

According to previous reports (Capuzzi et al., 1996; Lo Nostro et al., 2000), amphiphilic ascorbyl palmitate was able to form supramolecular self-assembled systems such as liposomes or micelles. Gopinath et al. reported that ascorbyl palmitate was a suitable bilayer forming agent with the aid of cholesterol and dicetyl phosphate to form stable vesicles (Gopinath et al., 2004). Cholesterol stabilizes and improves the rigidity of the aspasomal bilayer, which explains the importance of its inclusion in the aspasomal formulations (Kirby and Gregoriadis, 1980). Cholesterol polar head intercalates in the vesicle-bilayer, and owing to its hydrophobicity, it settles in the lipid bilayers interior portion and fills the gap created due to phospholipid molecules imperfect packing (Kirby and Gregoriadis, 1980). Therefore, it's expected to behave in a similar manner with aspasomes. Dicetyl phosphate was also included as a negative charge inducer in aspasomal formulations, which supports the creation of an electrostatic repulsion between the vesicles, hence prevents aggregates formation and ensures the stability of the dispersion (Nasr et al., 2008a), as evidenced from the physical stability study.

Regarding the particle size of aspasomes, the increase in particle size with ascorbyl palmitate concentration might be attributed to the increased thickness of the bilayer (Barakat et al., 2016, 2017). The low polydispersity index and high zeta potential values of aspasomes suggest their overall homogeneity and stability. The progressive increase in negative charge from A1 to A7 could be ascribed to the accompanying increase of the amount of the negative charge inducer DCP in aspasomal formulations (owing to the decrease of the ascorbyl palmitate ratio which is of higher molecular weight than cholesterol).

The high EE% values for melatonin could be ascribed to its

lipophilic nature (log P = 1.2), which facilitates its incorporation within the hydrophobic ascorbyl palmitate bilayer. Moreover, the thin film hydration technique was reported to be suitable for encapsulation of lipophilic drugs through creation of multilamellar vesicles (Nasr et al., 2008a). It can be inferred that since both melatonin and cholesterol prefer to align themselves in the membrane hydrophobic region, therefore a competition may arise for the space between the ascorbyl palmitate chains, resulting in decreased encapsulation of melatonin with increased cholesterol content (Deniz et al., 2010; Lingan et al., 2011). These results correlate with those of particle size, in which an increase in vesicular particle size was reported to increase EE% of the drugs, by providing additional space for drug entrapment (Tefas et al., 2015).

The preservation of ascorbyl palmitate antioxidant activity in vesicular form might be ascribed to the polar ascorbyl head groups' projection out of the surface of the vesicles. Whereas due to hydrophobic interactions, the chains of ascorbyl palmitate assemble together and hence, retain the antioxidant potential of ascorbyl moiety of ascorbyl palmitate even after its conversion into vesicular systems (aspasomes). The polar head groups of ascorbyl palmitate of aspasomes could then interact effectively with the free radicals and get neutralized (Gopinath et al., 2004).

The significant skin penetration enhancement observed with aspasomes compared to the melatonin solution could be attributed to the amphipathic nature of the aspasomal system, which increases their penetration of the skin hydrophobic domain, where they can function as antioxidants and cause the suppression of lipo-peroxidation. In addition, the nanosize of the aspasomes (388 nm) must have promoted higher skin adhesion, allowing them to localize into deeper skin layers (Gopinath et al., 2004; Gokce et al., 2012).

As can be collectively deduced from the results, melatonin encapsulation in vesicular nanocarriers (aspasomes) maximized its therapeutic potential in AGA, as could be deduced from the clinical assessment criteria. The promising behavior of aspasomes in treatment of AGA could be ascribed to their ability to facilitate the delivery of melatonin to hair follicles, besides interacting with skin lipids and depot formation inside the skin (Kaur et al., 2017). Furthermore, the smaller size of these nanoparticles, their lipophilic nature, their sustained release properties and their enhanced skin deposition compared to the conventional solution form might have improved the drug-skin bioavailability by improving the drug's penetrability compared to the conventional melatonin solution. All the previous advantages were summed with the antioxidant activity of ascorbyl palmitate, being a component of the bilayer forming materials.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejps.2018.06.034.

5. Conclusion

As can be seen from the results, the preparation of melatonin nanovesicles using an antioxidant bilayer forming agent were proven to be effective in the treatment of oxidative based diseases such as AGA, hence, providing a promising cosmeceutical-based platform for treatment of related dermatological diseases.

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