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THE ASSOCIATION FINASTERIDE & BLEND-CONTAINING GROWTH FACTORS BY BACTERIAL REVERSE MUTATION ASSAY

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ABSTRACT

The psychologically impactful hair loss caused by a hereditary pattern, known as androgenetic alopecia (AGA), can occur in men as in women. Among medicines, finasteride has been used in association with blend-containing growth factors. The objective of this work was to evaluate the effects of the association between finasteride & blend-containing growth factors using a bacterial reverse mutation assay. Both compounds or in the mixture were subjected to TA97a, TA98, TA100, and TA102 with (+S9) and without (-S9) metabolic activation, using different concentration and combinations. Finasteride (5 mg/plate) showed to be non-toxic to TA100 strain, but not the blend when exposed at concentrations of 2.5 mg/plate. Finasteride + blend association showed to be non-mutagenic in the absence (-S9) of metabolic activation. In the presence (+S9) of metabolic activation, the association was mutagenic in a dose-dependently manner. Under safe concentrations of blend obtained through serial dilution, the association was mutagenic only to TA98 strain (+S9). Finasteride isolated showed to be safe. Safe levels of the blend were found in the absence as in the presence of metabolic activation face to *S.* Typhimurium TA97a, TA98, TA100, and TA102 strains. Even under safe blend concentration, the association induced reverse mutation on TA98 having the finasteride-ω-oic-acid as a suggestive candidate.

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INTRODUCTION

The most common non-cicatricial androgenetic alopecia (AGA) is defined as a genetically predetermined disorder which causes hair loss due to excessive response to androgens affecting up to 50% of males and females (Ho *et al*., 2020). The pathogenesis is excessive activation of the androgen receptor resulting in a decrease of the anagen phase and maintenance of the telogen phase (follicular miniaturization) (Alves, 2017). Two gold-standard pharmacological therapies approved by Food and Drug Administration (FDA) for treating AGA, topical minoxidil and oral finasteride (5-alfa reductase type II inhibitor) (Iamsumang *et al*., 2020) are able in to block the dihydrotestosterone formation, which physiologically stimulates the androgenic receptor. The Brazilian Health Regulatory Agency (Anvisa) designates alopecia as a cosmetic disease (Gensure, 2020) which, in turn, stimulates the off-label use of new emerging treatments aiming to avoid the progression of hair loss and promote hair growth. However, the benefit of controlled trials in certain associations is scarce in the literature, increasing the psychological stress of who receives the treatment (Danyal *et al*., 2018), and causing insecurity about who performs the treatment as well. Among the various emerging treatments arise the growth factors (Aldag *et al*., 2016), which have been off-label used by physicians in association to finasteride for treating AGA, in technical procedures such as Microinfusion of Medications in the Skin (MMS), intradermotherapy, micro-needling using pen or rollers. The expected pharmacokinetics of infused active ingredients, after acting in the tissues, is absorption, biotransformation, and elimination as usually happens with all chemical substances in a living organism (Arbacheand Godoy, 1983). Finasteride is a non-mutagenic medicament under *in vitro* bacterial mutagenicity (*Salmonella*/microsome) and *in vitro* mammalian cell mutagenicity; positive in *vitro* chromosome aberration Chinese Hamster Ovary (CHO) cells, but negative in *vivo* chromosome aberration mouse. The regulatory information was harmful, toxic to reproduction (EU indication of danger); harmful if swallowed, possible risk of harm to the unborn child (EU risk phrases); do not breathe dust, avoid exposure – obtain special instructions before use (EU safety phrases) (Caymanchem, 2020). On the other hand, cytokines or growth factors have been postulated to play a role in hair follicles including Insulin Growth Factor (IGF), which is regulated by

androgens and helps to maintain the anagen stage (Panchaprateep and Asawanonda, 2014); basic Fibroblast Growth Factor (bFGF or FGF-2), which may act as growth-promoting agent by inducing the anagen phase in resting hair follicles (Lin *et al*., 2015); Vascular Endothelial Growth Factor (VEGF), which stimulates the hair follicle dermal papilla cell growth (Nakamura *et al*., 2018), and also could cut 5-alfa-dihydrotestosterone (5α-DHT) against androgen-induced apoptosis (Zhang *et al*., 2020); and tripeptide-cooper complex, which stimulates hair growth through dermal fibroblast stimulation and increased expression of VEGF (Lee *et al*., 2016). In this study, the association (finasteride + blend-containing growth factors such as IGF, bFGF, VEGF, and tripeptide-cooper complex) was evaluated using the bacterial reverse mutation test (*Salmonella*/microsome assay) (OECD, 1997).

MATERIAL AND METHODS

Finasteride was purchased from Apotheka Pharmacy, Farmácia de Manipulação Enterprise LTDA, Sorocaba, SP, Brazil. The blend-containing growth factors–IGF, bFGF, VEGF, and cooper glycyl-histidyl-lysine complex, GHK: Cu (1:1) – were prescribed and kindly donated for this research by Dr E.D. Machado. The Centro Paulista de Desenvolvimento Farmacotécnico Ltda/Centro Paulista Laboratório, São Paulo, SP, Brazil, was responsible by blend handling.The tested medicine concentrations were the same as those applied in the MMS technique, i.e., finasteride 0.05 mg/mL and the blend 1.2 mg/mL.The breeding of *Salmonella Typhimurium* tester strains (TA97a, TA98, TA100, and TA102) provided by B.N. Ames (Berkeley, CA, USA) was kindly donated by Prof. Dr F.A.R. Nogueira from the University of Araraquara, Uniaras, SP, Brazil.

Salmonella/microsome assessment: Concentration > 5,000 µg/ plate when non-toxic is used as the limit to ensure the safety of a given pure substance or a mixture (Mysore et al., 2019). In this study, the preliminary to xicities of finasteride (0.625; 1.25; 2.5; 3.75; and 5.0 mg/plate), and blend-containing growth factors (0.15; 0.3; 0.6; 0.9; 1.2; 2.5; 5.0; and 6.0 µg/plate) were carried out on TA100 *S.* Typhimurium strain. Further, the decreasing concentration of 5,000 µg/plate was subjected to TA97a, TA98, and TA102, in triplicate, and in absence of metabolic activation (-S9). The assay with metabolic activation (+S9, Moltox, North Carolina, USA) was carried out with finasteride + blend-containing growth factors association, using the same preincubation method and principles as the standard test (OECD, 1997), on 60X15 mm plates where the volume of all components was reduced at 50 %, by the same reason described else where (Brooks, 1995; Burke *et al*., 1996; Diehl *et al*., 2000; Escobar *et al*., 2013; Hamel *et al*., 2016).

Negative and positive controls: All procedure was made using a negative control, dimethylsulfoxide (DMSO – 50 µL/ plate); and positive control (in the absence of S9): 4 -nitro-*o*-phenylenediamine (NOPD – 10.0 µg/ plate– TA98, TA97a); sodium azide (1.25 µg/ plate– TA100); mitomycin (0.5 µg/ plate– TA102); (in presence of S9): 2-anthramine (1.25 µg/ plate– TA 97a, TA98, TA100, and TA102). All controls were purchased from Sigma-Aldrich® (St. Louis, Missouri, USA).

Technical procedure: The assay either in the absence as in the presence of metabolic activation was made as described by Yoshida *et al* (Yoshida *et al*., 2016). In detail, the strains cultures maintained at -80 °C were grown overnight for 12–14 h in Oxoid Nutrient Broth No. 2. The S9 fraction was prepared before each test took 4% of S9 fraction, 1% of 0.4M $MgCl_2$, 1% of 1.65M KCl, 0.5% of 1M D-glucose-6-phosphate disodium and 4% of 0.1M nicotinamide adenine dinucleotide phosphate (NADP), 50% of 0.2M phosphate buffer and 39.5% sterile distilled water. The concentrations of each, pure compound (finasteride), in the mixture (blend-containing growth factors), or in association (finasteride + blend-containing growth factors) were added to 0.5 mL of 0.2M sodium phosphate buffer (pH 7.4), or 0.5 mL de 4% S9 mixture, with 0.1 mL of bacterial culture and then incubated at 37°C for 20 min. After 2 mL of top agar (0.6% agar, histidine and biotin 0.5 mM each, and 0.5% NaCl) was added and the mixture was poured onto a plate containing minimal glucose agar (1.5% Bacto-Difco agar and 2% glucose in Vogel-Bonner medium). The plates were incubated at 37°C for 48 h and the *His*(+) revertant colonies were counted manually. All experiments were carried out in triplicate and compared to the negative or positive control of each strain. A 50 % reduced procedure in all volumes was applied in the test using metabolic activation.

Mutagenicity index calculation (MI): The average number of revertants per plate with the test compound is divided by the average number of revertants per plate with the negative control to obtain the mutagenic index (MI), for each concentration tested. A sample is considered mutagenic when MI ≥ 2 at least in one concentration (Levy, *et al*., 2019).

Statistical Analysis: The results of the mutagenicity tests were analyzed with the Salanal statistical software package (U.S. Environmental Protection Agency, Monitoring Systems Laboratory, Las Vegas, NV, version 1.0, from Research Triangle Institute, RTP, North Carolina, USA), adopting the Bernstein *et al* (Bernstein *et al*. 1982) models. The data (revertants/plate) were assessed by analysis of variance (ANOVA), followed by linear regression.

RESULTS

The preliminary toxicities (Table 1) carried out using TA100 (-S9) exposed to finasteride until a 5 mg/ plate showed absence of toxicity, where as the blend showed absence of toxicity in a range of 0.15 – 1.2 mg/plate, but from this point on was considered positive as showed by 2.5, 5.0, and 6.0 mg/ plate, i.e., these concentrations were indicatives of toxicity to TA100 strain.

Table 1. Preliminary toxicity of Finasteride and Blend (TA100, -S9)

Treatment	(mg/plate)	Revertant colonies	±SD
Finasteride	Control +	2092	± 148
(50 mg/mL)	Control -	153	± 34
	5	134.67 (0.88)	± 3.2
	3.75	159.67 (1.04)	± 8.1
	2.5	173 (1.13)	± 9.5
	1.25	145.3 (0.94)	± 1.52
	0.625	150.3 (0.98)	± 8.4
Blend	Control +	2310	± 126.2
(1.2 mg/mL)	Control -	202	± 60.9
	6	144.7 (0.72)	± 13.87
	5	147.7 (0.73)	± 11.06
	2.5	135 (0.67)	± 15.1
	1.2	236.7 (1.17)	± 24.79
	0.9	258.3 (1.28)	± 11.55
	0.6	230.3 (1.14)	± 17.21
	0.3	289 (1.43)	± 16.46
	0.15	284.7 (1.41)	± 17.62

Control +: sodium azide; Control-: dimethyl sulfoxide (DMSO); ( ): M.I., mutagenic index; SD: standard deviation. Arrow: indicates toxicity to TA100 strain.

Fig. 1shows the results of the reverse bacterial carried out in absence of metabolic activation (-S9). In (A), results from 50 mg/mL finasteride, with the complete absence of mutagenicity, as shown by values of MI in the parenthesis in the legend of the figure; (B) shows 1.2 mg/mL blend, denoting a certain toxicity (#) dose-dependently against TA98 (5 and 1.2 mg/mL) and TA100 (2.5 mg/mL); whereas in (C) the association in the same concentrations used in A and B showed absence of mutagenicity. The asterisk in positive controls represents $p<0.05$ comparatively to all treatments and also negative control. The absence of mutagenicity in the association (finasteride + blend) confirms the safety of finasteride under bacterial reverse mutation assay. The promising result of the association (finasteride + blend), lead us to realize the assay using metabolic activation, as shown in Table 2, showing a high MI > 2.0 (see values in bold in the parenthesis) with all tested concentrations at least to 2 strains concomitantly. The TA98 showed to be the most sensitive strain face to the association.

Table 2. Bacterial reverse mutation assay (+S9) – Association (Finasteride 50 mg/mL + Blend 1.2 mg/mL)

Finateride mg/plate	Blend mg/plate	Number of strands (M ± SD)/plate and (MI)			
		TA 97a	TA98	TA 100	TA 102
0.0[a]		155 ± 49	36 ± 2	136 ± 4	266 ± 27
5	6	639 ± 12**(4.1)	516 ± 72 ** (14.3)	638 ± 40 * (4.6)	823 ± 73* (3.0)
3.75	5	346 ± 60 * (2.2)	558 ± 71 ** (15.5)	558 ± 114* (4.0)	522 ± 57 (1.9)
2.5	2.5	496 ± 15 ** (3.1)	400 ± 99 * (11.1)	348 ± 21 ** (2.5)	530 ± 109 * (1.9)
1.25	1.2	341 ± 60 * (2.1)	364 ± 76 ** (10.1)	330 ± 29 ** (2.4)	571 ± 30 ** (2.1)
0.625	0.9	275 ± 44 (1.7)	167 ± 126 (4.6)	302 ± 11 ** (2.2)	462 ± 76 * (1.7)
Control +		444 ± 41[b]	381 ± 72[b]	848 ± 24[b]	460 ± 37[c]

M ± SD= mean and standard deviation; MI = mutagenicity index; [a]Negative control: polyethylene glycol 400 (PEG 400 - 100 µL/plate); Control + = Positive control: [b]2-aminoanthracene (50 µL/plate- TA97a, TA98, TA100); [c]2-aminofluorine (50 µL/plate- TA102), in the presence of S9 (+S9). *, p<0.05. **, p<0.001.

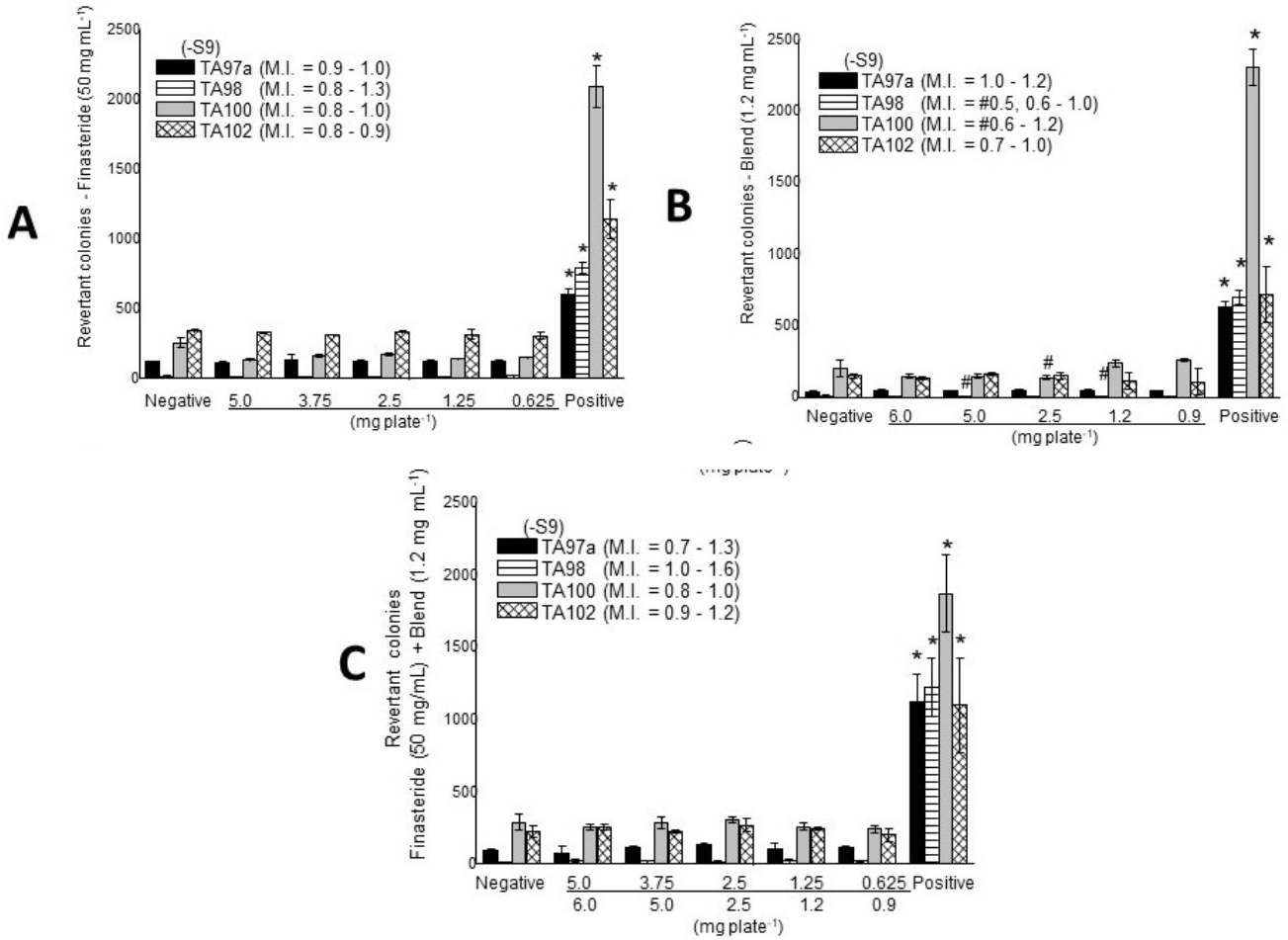

Figure 1. Bacterial reverse mutagenicity assay (-S9). (A) Finasteride 50 mg/mL. (B) Blend 1.2 mg/mL. (C) Association (at the same concentrations used of finasteride and blend). Negative control: dimethyl sulfoxide (DMSO, 100 µL/plate- in A and B), and polyethylene glycol 400 (PEG400, 100 µL/plate in C). Positive controls were: 4-nitro-o-phenylenediamine (NPD, 50 µL/plate- for TA97a and TA98); sodium azide (50 µL/plate for TA100); and mitomycin (10 µL/plate for TA102). *, p<0.05 in comparison to negative control and all tested concentrations. #, concentrations with Mutagenic Index (M.I.) 0.6, which can mean certain toxicity to representative strain

Table 3. Bacterial reverse mutation assay (+S9) – Blend 1.2 mg/mL

Treatment (mg/plate)	Number of strands (M ± SD)/plate and (MI)			
	TA 97a	TA98	TA 100	TA 102
0.0[a]	103 ± 15	29 ± 15	150 ± 25	193± 35
5	UC	UC	UC	UC
2.5	UC	UC	UC	UC
1.2	UC	322 ± 33 ** (11.1)	UC	UC
0.6	UC	311 ± 213 (10.7)	752 ± 316 (5.0)	918 ± 179 ** (4.7)
0.3	409 ± 35 ** (3.9)	UC	265 ± 86 (1.7)	642 ± 102 ** (3.3)
Control +	2054 ± 42[b]	1550 ± 138[b]	2396 ± 292[b]	399 ± 6[b]

M ± SD = mean and standard deviation; MI = mutagenicity index; [a]Negative control: polyethylene glycol 400 (PEG 400 - 100 µL/plate); Control + = Positive control: [b]2-aminoanthracene (50 µL/plate- TA97a, TA98, TA100 and TA102), in the presence of S9 (+S9). UC, uncounted.*, p<0.05. **, p<0.001.

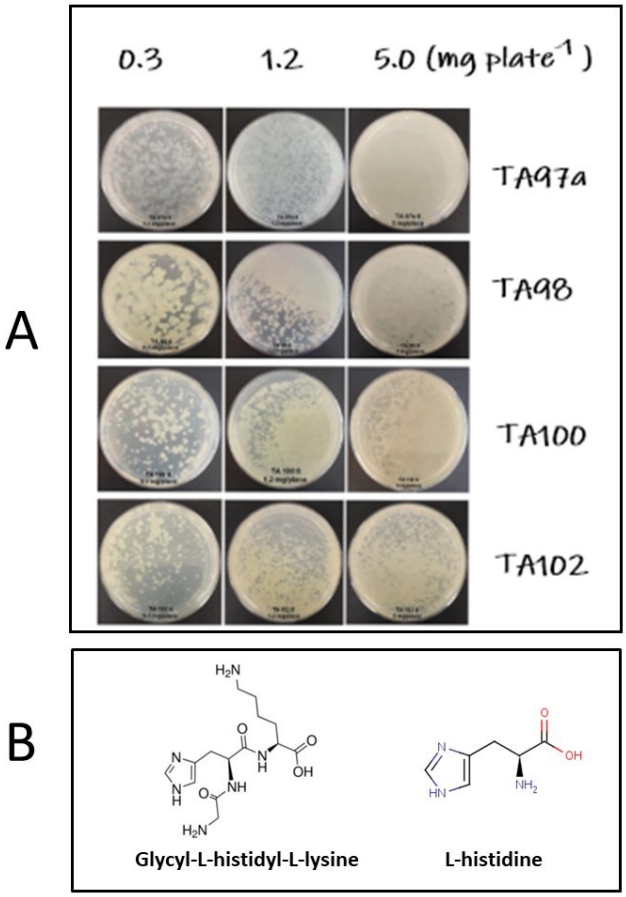

Figure 2. (A) Overgrowth of the background lawn (+S9). Photographs of uncounted plates were submitted to different concentrations of the blend. The blend contains IGF, bFGF, VEGF, and cooper glycyl-histidyl-lysine complex, GHK: Cu (1:1). (B) The cooper complex contains glycyl-histidyl-lysine, which can release histidine into the medium, mediated by microsomal enzymes in S9.

that the minor concentration (0.3 mg/plate) of the blend was mutagenic to TA97 and TA102, whereas TA98 was uncounted, showing that the concentrations were decisive to cause mutation in all tested strains. Fig. 2A shows the uncontrolled growth induced by compounds in the blend (> concentration > growth). The presence of histidine in the cooper complex could explain the obtained results (Fig. 2B), since the uncontrolled growth only occurred in the presence of metabolic activation, i.e., after the biotransformation reaction. In this work, we choose to find a safe range of the commercial blend 1.2% under bacterial reverse mutation parameter, through a serial dilution to obtain (mg plate^{-1}) 0.12, 0.012, 0.0012, 0.00012, and 0.000012 which was resubmitted to all strains (+S9). Note in Table 4, that all tested concentrations (10^{-2} to 10^{-6}) were safe for strains. To confirm the safety of association under the serial dilution of the blend, a new set of experiments was carried out using a single concentration of finasteride (5 mg/plate) (Table 5). Notice that only TA98 was sensitive to association even at lower dilutions of the blend (10^{-5} and 10^{-6}).

DISCUSSION

This bacterial reverse study addressed the off-label use of the growth factors in association with finasteride for treating androgenetic alopecia (AGA). The *Salmonella*/microsome assay is preceded by a preliminary toxicity assay (Table 1) for further to submit the samples to TA tester strains according to Mortelmans and Zeiger (Mortelmans, 2000). Clearly, the assay shows toxicity to 2.5 mg plate^{-1} blend, but further assays were carried out using higher than the recommended concentration of 5 mg plate^{1} 20. In the first assay in absence of metabolic activation (-S9) finasteride, blend, and the finasteride + blend association showed to be non-mutagenic (Fig. 1). Then, the same concentrations of finasteride + blend were submitted to the presence of metabolic activation (+S9), which showed to be mutagenic for all tested strains (Table 2). However, it was unlikely to attribute mutagenicity to finasteride, since it is approved by FDA,[3] and also because of its biotransformation during phase I by the cytochrome P450 enzymes (encoded by the *CYP3A4*, *CYP3A5*, *CYP3A7* and *CYP3A43* genes), renders ω-hydroxyfinasteride with an increased water solubility to increase their rate of urinary excretion by phase II(Huskey *et al*., 1995; Hulin-Curtis *et al*., 2010).

Table 4. Bacterial reverse mutagenicity assay (+S9) – Blend 1.2 mg/mL (serial dilution)

Treatment Mg/plate	Number of strands (M ± SD)/plate[1] and (MI)			
	TA 97a	TA98	TA 100	TA 102
0.0[a]	102 ± 14	14 ± 2	14 ± 2	142 ± 45
0.12	204 ± 36 * (1.9)	9 ± 5 (0.6)	9 ± 5 (0.6)	174 ± 22 (1.2)
0.012	191 ± 40 (1.8)	17 ± 1 (1.2)	17 ± 1 (1.2)	194 ± 26 (1.3)
0.0012	111 ± 7 (1.0)	13 ± 2 (0.9)	13 ± 2 (0.9)	222 ± 20 (1.5)
0.00012	167 ± 12 * (1.6)	17 ± 4 (1.2)	17 ± 4 (1.2)	222 ± 12 (1.5)
0.000012	177 ± 71 (1.7)	15 ± 1 (1.0)	15 ± 1 (1.0)	188 ± 21 (1.3)
Control +	1424 ± 44[b]	1550 ± 138[b]	2396 ± 292[b]	312 ± 11[b]

M ± SD = mean and standard deviation; MI = mutagenicity index; [a]Negative control: polyethylene glycol 400 (PEG 400 - 100 µL/plate); Control + = Positive control: [b]2-aminoanthracene (50 µL/plate- TA97a, TA98, TA100 and TA102), in the presence of S9 (+S9). *, p<0.05.

Table 5. Bacterial reverse mutagenicity assay (+S9) – Association Finasteride 5 mg/plate + Blend (serial dilution)

Treatment mg/plate		Number of strands (M ± SD)/plate and (MI)				
Blend	TA 97a	TA98		TA 100	TA 102	
0.0[a]		120 ± 31	30 ± 4		538 ± 219	212 ± 32
0.12	167 ± 38 (1.3)	45 ± 6 (1.4)		135 ± 13 (0.2)	212 ± 14 (0.9)	
0.012	180 ± 33 (1.4)	67 ± 19 (2.1)		375 ± 52 (0.6)	198 ± 30 (0.9)	
0.0012	127 ± 31 (1.0)	56 ± 16 (1.8)		351 ± 68 (0.6)	238 ± 10 (1.1)	
0.00012	154 ± 21 (1.2)	64 ± 6 ** (2.0)		280 ± 81 (0.5)	233 ± 27 (1.0)	
0.000012	150 ± 22 (1.2)	72 ± 11 * (2.3)		444 ± 87 (0.8)	218 ± 28 (1.0)	
Control +		1296 ± 44[b]	1114 ± 78[b]		1588 ± 78[b]	312 ± 11[b]

M ± SD = mean and standard deviation; IM = mutagenicity index; [a]Negative control: polyethylene glycol 400 (PEG 400 - 100 µL/plate); Control + = Positive control: [b]2-aminoanthracene (50 µL/plate- TA97a, TA98, TA100 and TA102), in the presence of S9 (+S9). *, p<0.05. **, p<0.001.

Thus, the rationale became to test only the blend in the presence of metabolic activation, exempting finasteride from this effect. Table 3 shows the results by which most plates were countless (UC). Note

The investigation was then addressed to the role of the blend on the mutagenic results, which was resubmitted to tester strains, in the S9 presence, which surprisingly induced an unexpected growth (Fig.

2A). An explanation for this growth can arise from Nylund and Einistö (1992), who published about the influence of biological samples and proteins on the Ames/*Salmonella* test, since these molecules have histidine and histidine-related growth factors which, in turn, may produce a false-positive result. We work with a blend which contains growth factors IGF, bFGF, VEGF, and cooper glycyl-histidyl-lysine complex, GHK: Cu (1:1). Gly-His-Lys is a tripeptide composed of glycine, L-histidine and L-lysine residues joined in sequence, which is broken down to histidyl-lysine which is likely further degraded to other metabolites of proteolysis. The tripeptide has a role as a metabolite, a chelator, a vulnerary and a hepatoprotective agent (PubChem, 2020). The presence of histidine in the cooper complex could explain the obtained results (Fig. 2B), since the uncontrolled growth only occurred in the presence of metabolic activation, i.e., after the biotransformation reaction. Besides, Nylund and Einistö (1992) are also alert about the presence of enzyme preparations because of the possible breakdown of protein structures and subsequent release of free histidine and histidine-containing dipeptides into test solutions. Some authors recommend a modified preincubation method with extensive washing before plating to avoid misinterpretations when testing histidine/tryptophan-containing compounds (Aeschbacher *et al*., 1983; Kirkland and Kim, 1995), a method referred to as *treat and wash assay* (Thompson *et al.* 2005); whereas others suggest a turbidimetric bioassay that utilizes a nonreversible *Salmonella Typhimurium* histidine auxotroph, NS 1135 (Busch and Bryan, 1987). Although the Ames test is a sensitive tool in screening for potential genotoxic carcinogens, despite the high correlation, a positive result is difficult to interpret since a mutagen in the Ames test is not necessarily harmful to humans (Hengstler and Oesch, 2001).Then, to offer a safe concentration of blend in the presence of S9, a new set of experiments was done, using a serial dilution of blend achieving amounts of 10^{-2} to 10^{-6} (Table 4), which showed to be non-mutagenic. This result is important concerned to residues from proteins and peptides released into the environment, by which histidine at these concentrations will not interfere with environmental samples (Gratz *et al*., 2011).

After founding the safe concentration for the blend in the presence of metabolic enzymes (S9), a new association with 5 mg/plate finasteride was carried out (Table 5). Concentrations as small as 0.00012 and 0.000012 mg/plate of blend resulted in mutagenic results on TA98. Some authors state that the sensitivity to compounds is more frequent with TA100 (Jurado, *et al*., 1993), also showed by high response face to mutagen in the positive control (Fig. 1), but in this association study, TA98 showed more sensitivity than TA100. Face to this response is correct to address attention to the metabolites of finasteride. After the work of Huskey *et al*. (1995) who identified 4 metabolites (M1-M4) ω-hydroxyfinasteride (M1), finasteride ω-al (M2), finasteride-ω-oic-acid (M3), and 6α-hydroxyfinasteride (M4), Lundahl *et al*., (2009) showed that M1, M3, and M4 are conjugated with glucuronic acid (phase II); found by the first time in both human, bile and urine. Among them, M3 glucuronide is an acyl glucuronide with toxicological and analytical implications (Shipkova *et al*., 2003), since is an electrophilic conjugate that can cause toxicity by binding to proteins or DNA according to the predictability of the covalent binding of acidic drugs in man (Benet *et al*., 1993). On the other hand, TA98 is a *hisD3052*1 frameshift allele of *Salmonella Typhimurium*, has an addition of the pKM101 plasmid to enhance mutagen sensitivity (DeMarini, 2000), possesses a deletion in *uvrB* ((*uvrB*) including the nitrate reductase and biotin genes (*chl, bio*). The allele *hisD3052 is a* target for reversion, and the mutations could be categorized into five general groups: hotspot, deletions, duplications, insertions, and complex (DeMarini, 1998). Besides, an analysis of the performance of strains TA98 and TA100 alone indicated that these were enough for detecting most bacterial mutagens (93%) (Williams *et al*., 2019), agreeing with the conclusions of the previous analysis (Zeiger *et al*., 1985). These studies attribute reliability to our obtained results using the association between finasteride and a serial dilution of the blend. The results here obtained are unprecedented and the bacterial reverse mutation assay is only the first mutagenicity assay used in a test genotoxic battery together with mammalian cells, and rodents to attend to the regulatory requirements (Cimino *et al*., 2006; Claxton *et al*., 2010), which is not applicable in this off-label use. However, it is known that all *in vitro* assays produce misleading positive results (Walmsley and Billington, 2011), which is commonly described in terms of sensitivity (proportion of genotoxic carcinogens that produce positive results) and specificity (non-genotoxic carcinogens that produce negative results) (Cooper *et al*., 1979). Therefore, the interpretation of these results must be careful, and serve as an alert to professionals from the cosmetic area, which uses the association for treating androgenetic alopecia(AGA), via Microinfusion of Medications in the Skin (MMS), intradermotherapy, and micro-needling. In summary, these results taken together showed an absence of direct mutagenicity (-S9) of the blend, finasteride and association, but in the presence of metabolic activation (+S9) the brake of tripeptide can release histidine inducing false-positive results. A serial dilution of blend achieving 10^{-2} to 10^{-6} concentrations avoided the induction of mutagenicity in all strains (+S9), but not when associated with 5 mg/plate finasteride, which leads us to hypothesize, based on literature and our results, on the involvement at least of finasteride-ω-oic-acid (without to discard other constituents in the blend) to induce mutation on TA98, on the target *hisD3052.*

CONCLUSION

In our experimental conditions, finasteride used isolated is safe. Concerning the commercial blend-containing growth factor, a safe concentration was found either in the absence or in the presence of metabolic activation face to *S. Typhimurium* TA97a, TA98, TA100, and TA102 strains. Even under safe blend concentration, the association induced reverse mutation on TA98 strain, having the finasteride-ω-oic-acid as a suggestive candidate.

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