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QR678 & QR678 Neo Hair Growth Formulations: A Cellular Toxicity & Animal Efficacy Study

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Background: Current treatment modalities are limited in their approach and success for hair loss. QR 678 & QR 678 Neo are new formulations, consisting of a combination of growth factors and peptides. This study demonstrates safety analysis of QR 678 & QR 678 Neo formulation, using in vitro cytotoxicity assay and in vivo animal efficacy.

Methods: Factors including vascular endothelial growth factor, basic fibroblast growth factor, insulin-like growth factor-1, keratinocyte growth factor, and copper tripeptide 1 (QR 678) or their biomimetic peptides (QR678 Neo) were suspended in a sterile injectable vehicle. The 3-2,5-diphenyl tetrazolium bromide assay was used to explore the cytotoxic effects of each factor used in the compositions in human keratinocyte cell and human fibroblast cell assays. An in vivo analysis, wherein study animals were given intradermal QR 678 & QR 678 Neo injections, was conducted to assess whether the formulations produce hair growth. Also, hair follicle viability was checked by intradermal injection of the pharmaceutical compositions in secondary alopecia.

Results: In both formulations, a positive response was observed with respect to the number of mice exhibiting hair growth at the injection sites. The injections caused retention of hair in a 0.25-cm radius around the injection site. On cytotoxicity study, all the factors were found to be safe in human keratinocyte cell and human fibroblast cell assay. A positive response was demonstrated in animals on treatment with the chemotherapeutic agent.

Conclusions: Intradermal injections of QR 678 & QR 678 Neo hair growth factor formulations are a safe and efficacious option for alopecia. Results seem encouraging enough to warrant a trial in humans with secondary alopecia, post cancer chemotherapy. *(Plast Reconstr Surg Glob Open 2020;8:e2843; doi: 10.1097/GOX.0000000000002843; Published online 25 August 2020.)*

INTRODUCTION

The most common cause of baldness or hair loss (95%) is androgenetic alopecia.[1] The current surgical, medical, and cosmetic interventions are limited in approach and success.[2] There are several growth factors that have been found to stimulate or inhibit different stages of the hair growth cycle. Various growth factors studied for hair follicle growth are vascular endothelial growth factor (VEGF),[2] epidermal growth factor, insulin-like growth factor-1 (IGF-1), fibroblast growth factor (FGF),[3] wingless-related integration site, noggin, keratinocyte growth factor (KGF), copper tripeptides, and more. These growth factors are safe and cheap nonallergenic tools in the management of alopecia.[4–6]

We have prepared a bioengineered, recombinant formulation, consisting of a combination of growth factors, called the QR 678 hair growth factor formulation. A QR code is a code used in medicine derived from "Quick Response." 678 in the Morse code that signifies "there is no answer." This formulation has been named QR 678 to signify a "Quick Response to a disease which earlier had no answer," that is, to alopecia. The formulation is

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injected in the intradermal layer of the skin of the scalp and may prevent hair loss and stimulate hair growth. The first clinical trial in humans demonstrated a significant reduction in hair loss in 83% of the patients using the hair-pull test. Videomicroscopic image evaluation showed that most patients had a decrease in the number of vellus hairs but an increase in the number of terminal hairs and shaft diameter. Seventy-five percent of the patients believed that the injections reduced or stabilized the hair loss. A positive effect on the hair loss was seen in post-hair–transplant patients. At 1 year, a statistically significant increase in total hair count ($P = 0.002$) was seen. The treatment was well tolerated.[7,8]

This formulation was also compared with the subsequently prepared QR 678 Neo formulation, made up of biomimetic peptides, which demonstrated efficacy equivalent to that of QR678. The results of both the QR 678 and the QR 678 Neo formulations were subsequently also compared with the results of Platelet Rich Plasma (PRP), in a split head human trial and results were almost 80% better with the QR 678 treatment and QR 678 Neo treatments, when compared with PRP therapy.[9]

Considering that the QR 678 and QR 678 Neo have already demonstrated encouraging clinical results in human trials and have been awarded a composition patent by the United States Patent & Trademark Office and Indian Patent & Trademark Office & have also secured Indian FDA approval for commercial use, we thought it pertinent and necessary to publish the preceding animal trial and cytotoxicity data.

MATERIALS AND METHODS

Before conducting the aforementioned pilot human study,[7,8] the QR 678 formulation was tested on cellular assays and animal models to establish the optimum concentration of the multiple growth factors, its safety, and efficacy. The current article documents the same animal study.

Kapoor and Shome[7,8] have also introduced a new variation of the same formulation called QR 678 Neo. It is a plant derivative consisting of biomimetic peptides, including Sh-Polypeptide 9 (biomimicking VEGF), Sh-Polypeptide 1 (biomimicking basic FGF), Sh-Oligopeptide 2 (biomimicking IGF-1), copper tripeptide-1, Sh-Polypeptide 3 (biomimicking KGF-1), Sh-Oligopeptide 4 (biomimicking thymosin β4), vitamins, minerals, and amino acids.[10] Although it has been proved that plant derivatives are biomimetic polypeptides of the growth factors and changing the source does not affect the efficacy of the products,[11,12] we wanted to confirm the same through our study. A similar animal and cytotoxicity trial was carried out using QR 678 Neo, and it was interesting to note that the results obtained were similar in both QR 678 and QR 678 Neo.

Reported therapeutically acceptable ranges of the growth factors used in QR 678 and QR 678 Neo are as follows:

i. VEGF (human oligopeptide-11): 0.01–100 mg/L
ii. Basic FGF (human oligopeptide-3): 0.01–100 mg/L
iii. IGF (human oligopeptide-2): 0.01–100 mg/L
iv. Copper tripeptide-1: 0.1–500 mg/L
v. KGF (human polypeptide-3): 0.01–100 mg/L
vi. Thymosin β4: 0.005–100 mg/L

Determination of the Cytotoxic Effects of the Individual Growth Factors to Determine Safe Levels

Method

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay was used to explore the cytotoxic effects of each of the growth factors used in the composition in human keratinocyte cell and human fibroblast cell.

Preparation and Physicochemical Characterization of the Growth Factor Formulation

In a preferred embodiment, the growth factor formulations included the growth factors in the concentrations, as given in Table 2, additionally along with vitamins, minerals, nucleic acids, amino acids, diluents, and/or carriers along with pharmaceutically acceptable diluents and/or carriers.

The formulations as described were formulated for intradermal injection to the treatment area. Suitable vehicles for injection include, but are not limited to, saline and distilled water.

Preparation of the Formulation

Adequate concentrations of VEGF, basic FGF, IGF-1, copper tripeptide-1, KGF, and thymosin β4 additionally with pharmaceutically/cosmetically acceptable and appropriate dose of vitamins, minerals, amino acids, and nucleic acids were added to 1 L of distilled water to yield the concentrations of the solutions as given in Table 2. The formulation was then biologically sterilized and bottled into vials of 5 mL each. The formulation was stable at all concentrations of growth factors and could be stored at room temperature (below 25°C).

Sample Size

The sample size was calculated through the standardized protocol of a priori power analysis using the G* Power Software.[13] The effect size was duly adjusted at 0.5 during computation of power analysis. The power analysis was based on 1-mm hair growth length. The sample size derived was a minimum of 15 for in vivo study arm 1 and 50 for in vivo study arm 2.

In Vivo Study Arm 1: Stimulation of Hair Growth by Representative Growth Factors and Biomimetic Peptides in Mammals

Method. The following study arm evaluated stimulation of hair growth in warm-blooded animals after intradermal injection of representative growth factors and peptides of the formulation.

In this experiment, 15 C3H mice were divided into 5 groups. The backs of the C3H mice (60 days old, telogen hair growth phase) were closely clipped on day 1 with an electric clipper. In mice with an agouti coat, such as C3H/HeJ mice, hair clipping allows easy distinction between anagen and telogen phases, due to the fact that hair follicles switch from light-colored pheomelanin to dark-colored eumelanin production during anagen. A black band

of anagen hairs enables visualization and monitoring of the timing of the anagen-spreading wave. These mice therefore represent an excellent model to study the hair cycle.

When the hair is in telogen, it can grow again only when the hair root enters the anagen phase on its own again after a specific interval of time. So, if a particular substance or molecule needs to demonstrate that it actually stimulates hair growth, it has to be administered in the telogen phase so that it can stimulate the follicle to enter the anagen growth phase, thus shortening telogen and causing premature entry of the resting follicles into the anagen phase. Usually, the dorsal part of the animals is used for testing.[14] As a standard, hair follicles from the dorsal skin from postnatal days 1 to 12 are typically used to represent anagen, from day 17 to represent catagen, and from day 21 to represent telogen.

Most importantly, the 3 stages of the hair cycle are well characterized in the mouse: anagen (growing phase), catagen, and telogen (resting phase). The first 2 cycles of the mouse hair follicle are synchronized; that is, all hair follicles are in one stage of the development. The mouse hair cycle is short, lasting for only 3 weeks, and the hair follicles can be examined at specific time points in the cycle.[15]

A sterile solution of the chosen formulation concentration was then injected intradermally (ie, infiltrated within the skin) at 2 locations within the clipped areas of the mice. Injection at 2 locations provided 2 test locations within the clipped area of each mouse. Each injection (0.1 mL) contained different proportions of IGF 1, VEGF, bFGF, KGF, thymosin β4, and copper tripeptide-1 in distilled water, labeled as solutions 1–4. A group of saline-injected mice (0.1 mL) served as controls. The mice were kept under observation and evaluated at days 1, 3, 7, 14, and 28 (Table 1).

In Vivo Study Arm 2: Hair Follicle Viability by Intradermal Injection of the Pharmaceutical Composition in Secondary Alopecia

Method. The following study arm evaluated maintenance of hair follicle viability by intradermal injection of the pharmaceutical composition in secondary alopecia. The following experiment illustrated the localized maintenance of hair follicle viability (growth) by intradermal (local) injection of the pharmaceutical composition during treatment with chemotherapeutic agent cytosine arabinoside (Ara-C).

In this experiment, Sprague-Dawley rat pups (age: 8 days) were maintained in 5 litters (n = 10–12 rat pups per litter) for the duration of the study. On day 0, the litters received intradermal injection of the pharmaceutical composition in distilled water (solutions 1, 2, 3, or 4 as described in example 3) or a saline control (1 injection per animal, 0.05 mL per injection). Each litter contained 2 normal control animals, which received neither the pharmaceutical composition nor Ara-C; they received saline injection only. On day 1, the designated animals began a series of 7 consecutive daily intraperitoneal injections of Ara-C 25 mg/kg. On day 10, all animals were evaluated for the extent of hair loss at the injection sites by using the below rating:

Grade degree of alopecia

0 normal (no loss of hair),
1 slight thinning,
2 moderate thinning,
3 sparse hair cover, and
4 total loss of hair.

RESULTS

Determination of the Cytotoxic Effects of the Individual Growth Factors to Determine Safe Levels

Reported oral toxicity here is defined as the amount of a material, given all at once, which causes the death of 50% (one-half) of a group of test animals.

i. In vitro, human keratinocyte cell (Fig. 1) and human fibroblast cell (Fig. 2): bFGF is safe (no cellular toxicity) tested up to 10 ppm (μg/mL)—reported oral toxicity, rat: lethal dose, 50% (LD50) > 10,000 mg/kg.
ii. In vitro, human keratinocyte cell (Fig. 3) and human fibroblast cell (Fig. 4): IGF-1 is safe (no cellular toxicity) tested up to 10 ppm (μg/mL)—reported oral toxicity, rat: LD50 > 10,000 mg/kg.
iii. In vitro, human keratinocyte cell (Fig. 5) and human fibroblast cell (Fig. 6): KGF is safe (no cellular toxicity) tested up to 10 ppm (μg/mL)—reported oral toxicity, rat: LD50 > 10,000 mg/kg.
iv. In vitro, human keratinocyte cell (Fig. 7) and human fibroblast cell (Fig. 8): VEGF is safe (no cellular toxicity) tested up to 10 ppm (μg/mL)—reported oral toxicity, rat: LD50 > 10,000 mg/kg.
v. In vitro, human keratinocyte cell (Fig. 9) and human fibroblast cell (Fig. 10): thymosin β4 is safe (no cellular toxicity) tested up to 10 ppm (μg/mL)—reported oral toxicity, rat: LD50 > 10,000 mg/kg.
vi. In vitro, human keratinocyte cell (Fig. 11) and human fibroblast cell (Fig. 12): copper tripeptide-1 is safe (no cellular toxicity) tested up to 10,000 ppm (μg/mL)—reported oral toxicity, rat: LD50 > 10,000 mg/kg.

Table 1. Solutions with Varied Concentrations of Growth Factors Injected in Mice in In Vivo Study 1

	VEGF (mg/L)	bFGF (mg/L)	IGF-1 (mg/L)	Cu Tripeptide-1 (mg/L)	KGF (mg/L)	Thymosin β4 (mg/L)
Solution 1	0.01	0.01	0.01	0.1	0.01	0.005
Solution 2	2	1	1	5	0.5	0.001
Solution 3	5	2	2	10	1	0.01
Solution 4	15	5	5	30	2	0.1
Solution 5	50	20	20	100	10	1

1 mg/L means 1 ppm.
Cu, copper.

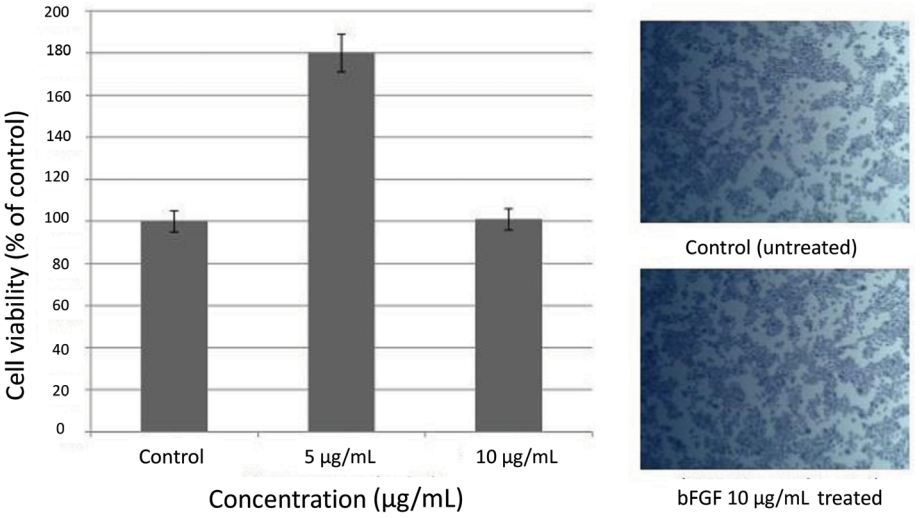

Fig. 1. Cytotoxic effects of bFGF on human keratinocyte cells in vitro.

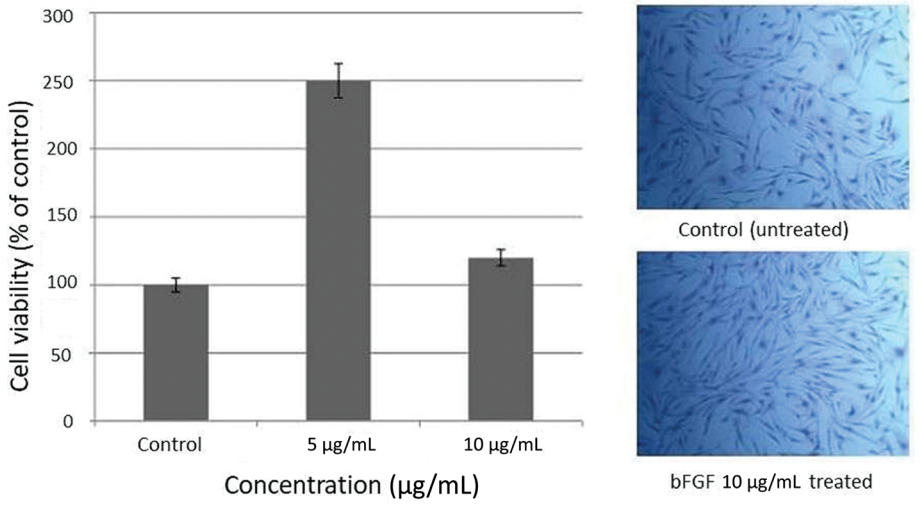

Fig. 2. Cytotoxic effects of bFGF on human fibroblast cells in vitro.

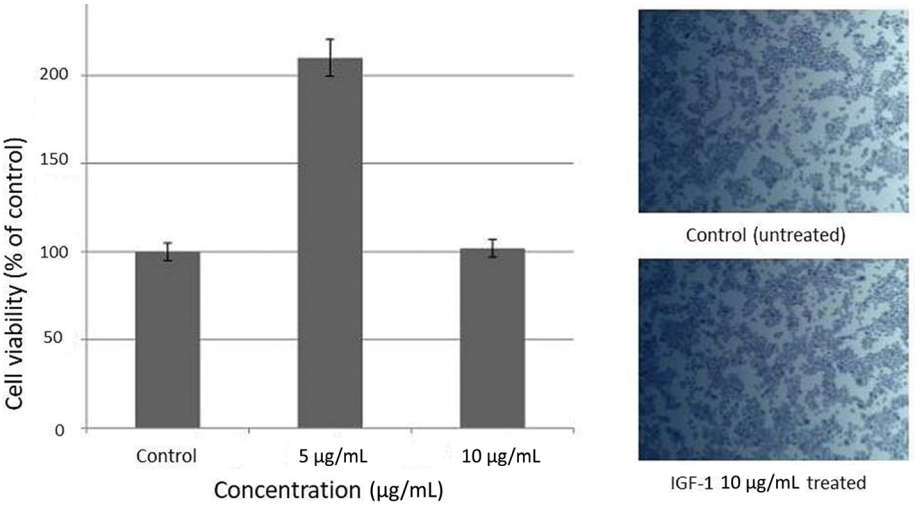

Fig. 3. Cytotoxic effects of IGF-1 on human keratinocyte cells in vitro.

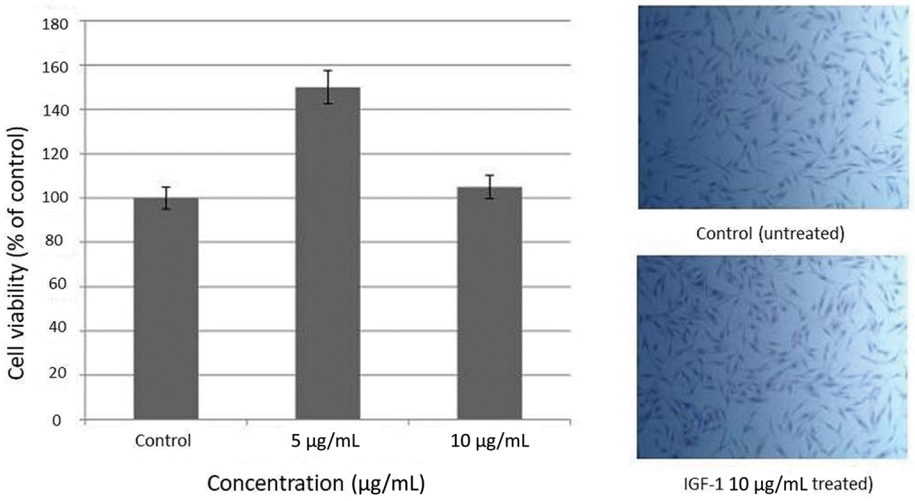

Fig. 4. Cytotoxic effects of IGF-1 on human fibroblast cells in vitro.

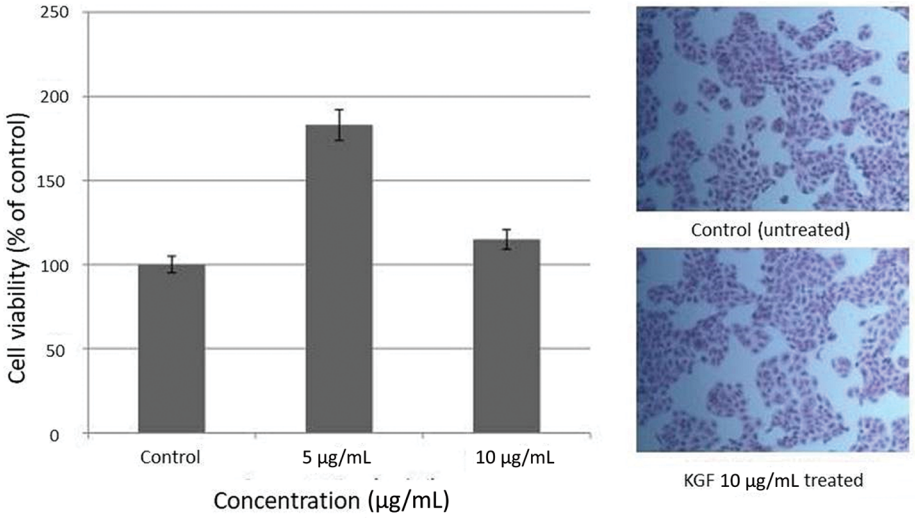

Fig. 5. Cytotoxic effects of KGF on human keratinocyte cells in vitro.

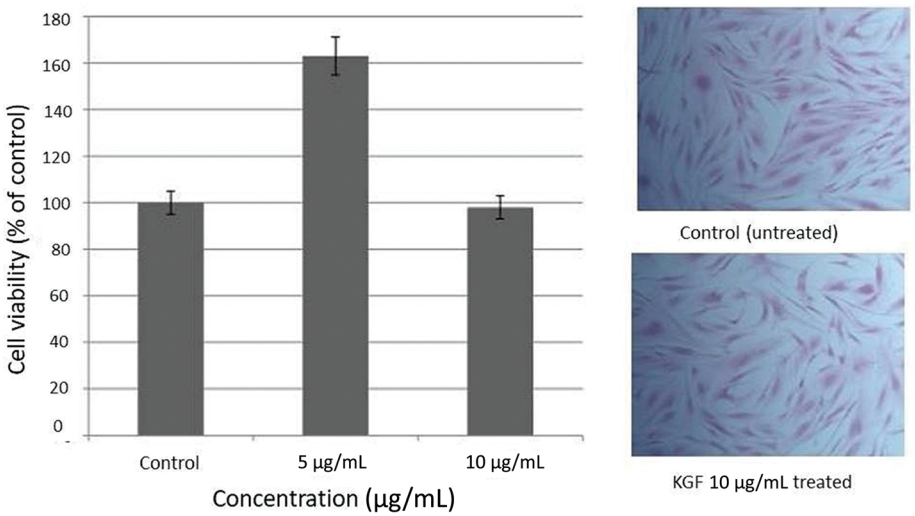

Fig. 6. Cytotoxic effects of KGF on human fibroblast cell in vitro.

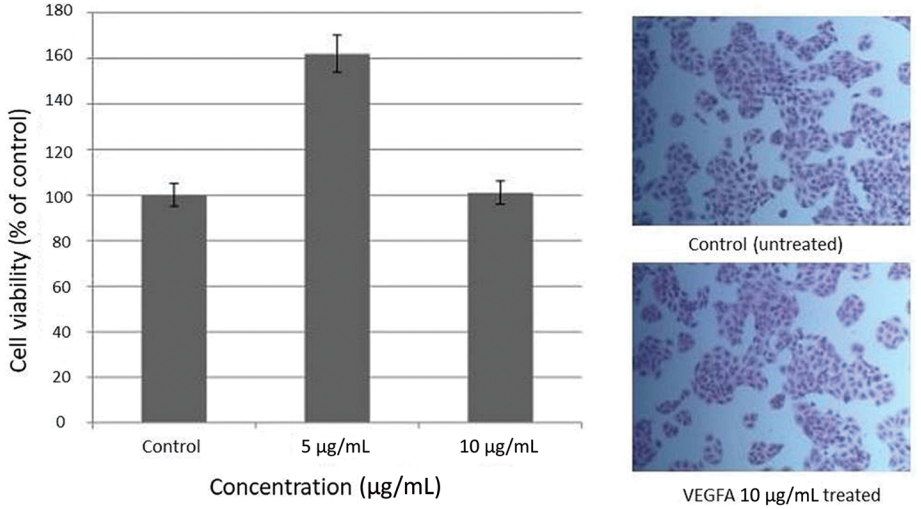

Fig. 7. Cytotoxic effects of VeGF on human keratinocyte cells in vitro.

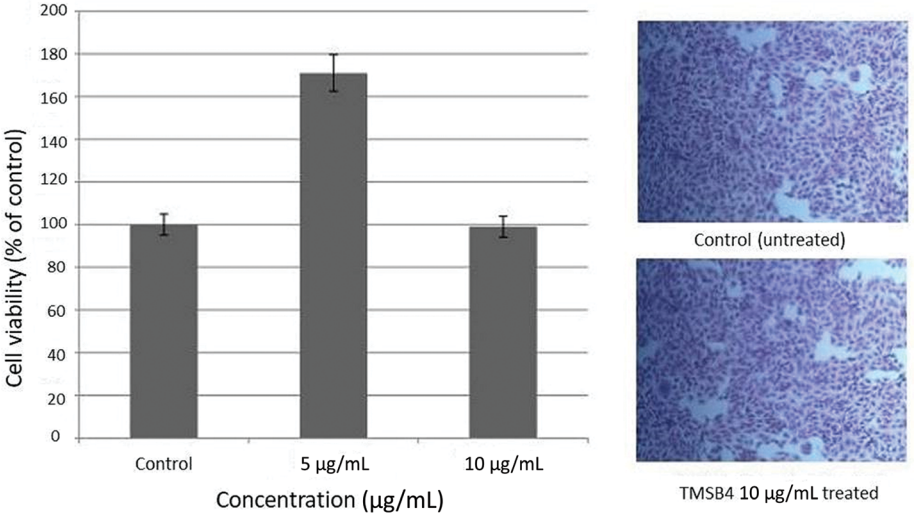

Fig. 8. Cytotoxic effects of VeGF on human fibroblast cell in vitro.

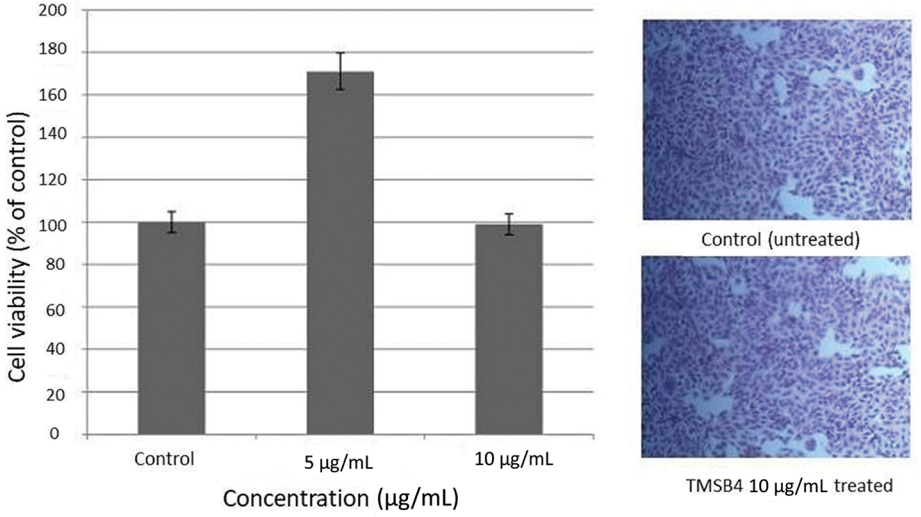

Fig. 9. Cytotoxic effects of thymosin β4 on human keratinocyte cells in vitro.

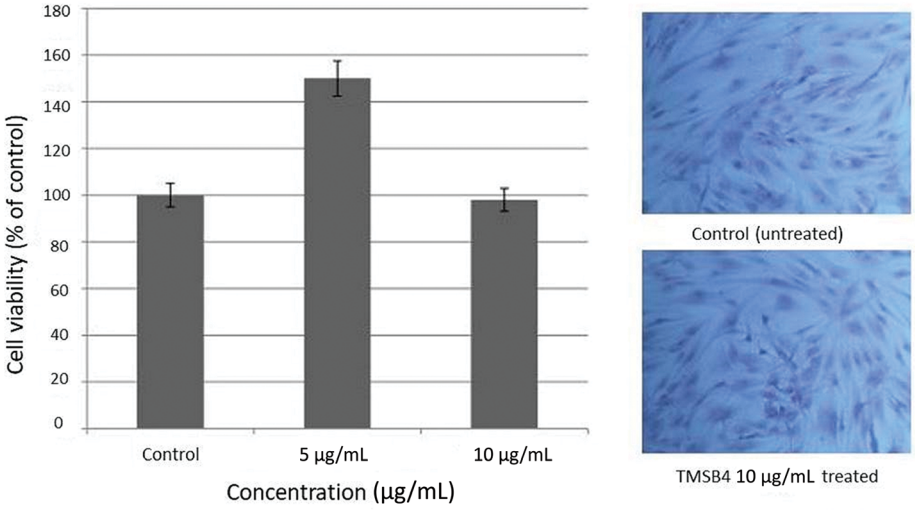

Fig. 10. Cytotoxic effects of thymosin β4 on human fibroblast cells in vitro.

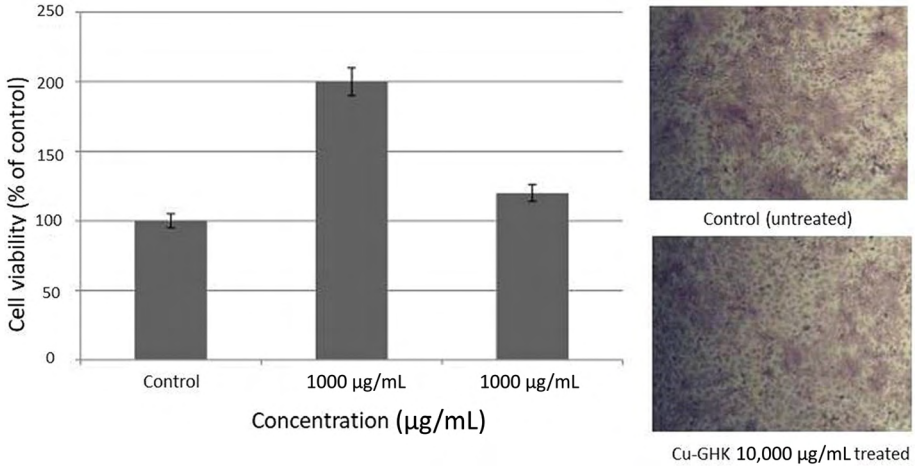

Fig. 11. Cytotoxic effects of Cu GHK (copper peptide GHK-Cu is a naturally occurring copper complex of the tripeptide glycyl-L-histidyl-L-lysine) on human keratinocyte cells in vitro.

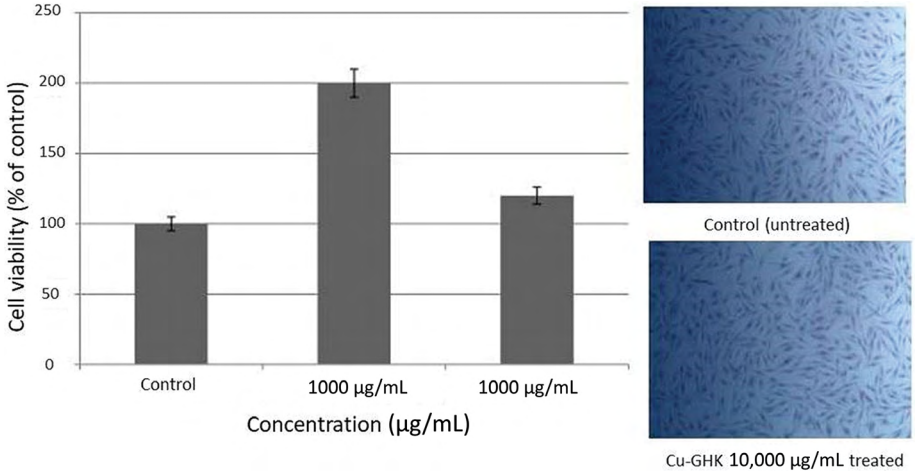

Fig. 12. Cytotoxic effects of Cu GHK on human fibroblast cells in vitro.

Table 2. Toxicity Data of the Growth Factors Used in the Formulations

	Toxicity Data	Reference
IGF-1	Endotoxin level ≤ 10.0 ng/mL using mouse Balb/3T3 cells	1 (IGF-1)
	Endotoxin level: <0.10 EU per 1 μg of the protein by the LAL method	1 & 2 (IGF-1)
bFGF	Rat, oral, LD50: 400 mg/kg	2 (bFGF)
	Mouse, intramuscular, LD50: 108 mg/kg	
	Effect: behavioral: convulsions or effect on seizure threshold.	
	Mouse intraperitoneal, LD50:154 mg/kg	
	Endotoxin level: <0.10 per 1 μg of the protein by LAL method	1 & 2 (bFGF)
KGF	Endotoxin level: <0.10 per μg of the protein by LAL method	3 (KGF)
VEGFA	Endotoxin level: <0.01 per 1 μg of the protein by LAL method	4 (VEGFA)
		5 (TMSB4)
TMSB4	Endotoxin level: <0.1 ng/μg of the protein (<1 EU/μg)	5 (TMSB4)
		5 (TMSB4)
Noggin	Endotoxin level: <0.10 per 1 μg of the protein by LAL method	6 (Noggin)
Copper tripeptide-1	Acute toxicity: LD50 mouse (I.P.) = 160 mg/kg; (I.V.) = 110–120 mg/kg	7 (Noggin)
	Rat (I.V.) ≥ 75 mg/kg; rat (oral) ≥ 150 mg/kg	

BALB 3T3 Cells, cell lines developed from disaggregated BALB/c mouse embryos; bFGF, basic fibroblast growth factor; EU, endotoxin unit; IGF-1, insulin-like growth factor-1; I.P., intaperitoneal; I.V., intravenous; KGF, keratinocyte growth factor; LAL method, limulous amoebocyte lysate test method; LD50, Lethal dose, 50%, or median lethal dose; TMSB4, Thymosin Beta-4; VEGFA, vascular endothelial growth factor A.

vii. Toxicity data of the growth factors used in the composition are seen in Table 2.

In Vivo Study Arm 1: Stimulation of Hair Growth by Representative Growth Factors and Biomimetic Peptides in Mammals

With injection of the above intradermal pharmaceutical formulation, indications of hair growth were seen within 10 days. The first visual signs were darkening of the skin in a circular region surrounding the injection site. The size of this region was generally dose dependent, increasing with an increase in dose of the growth factors, to a certain extent. The 0.1-mL injections used in this experiment produced a circle of hair growth measuring approximately 0.5–5 cm^2 in diameter. Active hair growth occurred between 14 and 20 days of the injection, with a maximum density seen on day 30. Both the number of mice exhibiting hair growth at the injection site and the diameter of the hair growth region were determined on day 21. A positive response was observed with respect to the number of mice exhibiting hair growth at the injection sites compared with the total number of mice injected in the study. The results of this experiment are presented in Table 3 (the day of onset is the day at which hair follicle pigmentation was first observed).

Solution 3 comprising 0.0002 mg/0.1 mL of IGF-1, 0.0002 mg of bFGF, 0.0005 mg of VEGF, 0.0001 mg of KGF, 0.001 mg of copper tripeptide, and 1×10^{-6} mg of thymosin β4 within the distilled water gave the best response. An increase in concentration of the ingredients beyond that in solution 4 did not give any significant benefit in terms of number of mice exhibiting hair growth or the diameter of hair growth region. None of the mice in the group injected with solution 5 died or showed any other signs of clinical or cellular toxicity. This proved the safety of the composition even with very high doses of the growth factors.

In Vivo Study Arm 2: Hair Follicle Viability by Intradermal Injection of the Pharmaceutical Composition in Secondary Alopecia

Ara-C injections caused significant hair loss by day 5–6 in most animals. To evaluate the stimulatory effects of the intradermal QR 678 formulation, the degree of hair loss was evaluated at the injected site daily. Injections generally caused retention of hair in a 0.25-cm radius around the injection site, most notably in the solution 3 group. Table 4 presents the results as evaluated on day 10 using the previously described rating scale, with the degree of alopecia being expressed as the average response seen at the site of injection.

The observation of retained hair within the area of injection was examined histologically. Although normal appearing and functional anagen hair follicles were observed at the site of injection of the hair formulation (Fig. 13), follicles located away from the injection were dystrophic and nonfunctional (disruption of the integrity of inner and outer root sheaths and disrupted hair shafts; Fig. 14). These data confirmed the gross observation of normal hair follicular

Table 3. Hair Growth Response Seen in Mice in In Vivo Study 1

Composition	No. Animals Growing Hair	Day of Onset	Area of Hair Growth around the Injection Site
Solution 1	2/5	10	<1 cm diameter
Solution 2	3/5	10	>1 cm diameter
Solution 3	5/5	10	>1 cm diameter
Solution 4	4/5	10	>1 cm diameter
Solution 5	4/5	10	>1 cm diameter
Solution 6 (saline control)	0	No changes	No changes

Table 4. Localized Maintenance of Hair Follicle Viability (Growth) by Intradermal (Local) Injection of the Invented Composition during Treatment with Chemotherapeutic Ara-C, as Evaluated on Day 10 of Injection

Composition	n	Degree of Alopecia (Mean)
Saline only	8	0.0
Saline + Ara-C	8	4.0
Solution 1 + Ara-C	8	3.25
Solution 2 + Ara-C	8	2.38
Solution 3 + Ara-C	9	1.44
Solution 4 + Ara-C	9	1.91

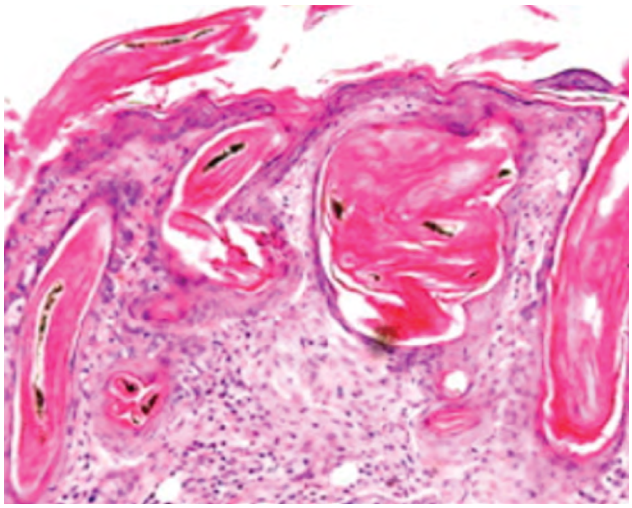

Fig. 13. Histologic section of functional anagen hair follicles, stained with H&E, observed at the site of injection of the hair formulation. Scale bar: 100 µm. H&E indicates hematoxylin and eosin.

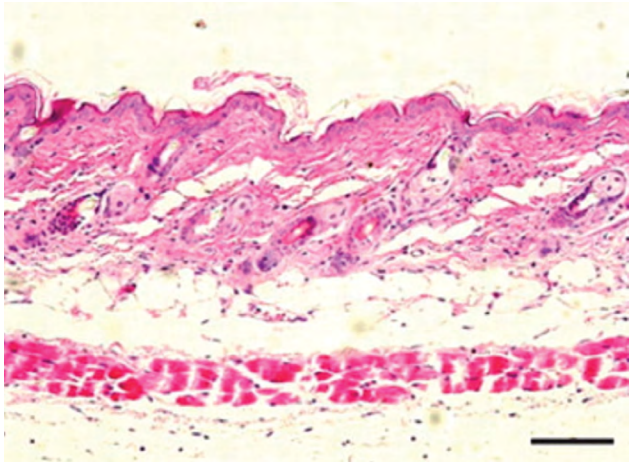

Fig. 14. Histologic section of dystrophic and nonfunctional anagen hair follicles, stained with H&E, observed at the site of injection of the hair formulation. Scale bar: 100 µm. H&E indicates hematoxylin and eosin.

function within the site of QR 678 injections and illustrated the stimulatory effect of the intradermal injections on hair follicles, which maintains the active hair growth cycle during chemotherapy treatment.

DISCUSSION

Hair growth factors, when used in combination, have been shown to have a synergistic impact on human hair growth.[7] Various nonsurgical options for treating hair loss are available. Oral finasteride and topical minoxidil alone or in combination have shown adequate results. However, side effects with their long-term use are not uncommon. Loss of libido with the use of finasteride, and headaches, tachycardia, and increase in body hair with the use of minoxidil have been frequently noted.[16,17]

PRP has also been studied as a method for hair regrowth in male pattern hair loss. Literature review suggests that injections of PRP are a safe and feasible treatment option for androgenetic alopecia, with high overall patient satisfaction.[18–22]

Multiple trials have also been published highlighting the role of PRP on hair growth. However, methodologic inadequacy has been noted in most of the studies.[23] Most noteworthy shortcoming is the lack of standardized device and protocols that define the preferred method for producing PRP. As mentioned by Lynch and Bashir,[24] PRP is usually prepared on a per-patient basis. Approximately 8–60 mL of fresh venous blood is drawn, collected, and centrifuged, leading to the separation of the erythrocytes from lighter plasma with a buffy coat at the interface. The plasma and buffy coat are then aspirated and mixed.[24] Other drawbacks include lack of a reference protocol mentioning the frequency of applications, the amount of PRP to be injected, lack of detailed reports in patients' characteristics heterogeneity in mode of application, lack of controls, small sample size, and used statistical methods.[23] Herein, we publish the results of the preclinical data on file, demonstrating the safety and efficacy of the hair formulation, which we call the QR 678 hair growth factor injections. To evaluate safety, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay[25] was used to explore the cytotoxic effects of each of the growth factors used in the composition in human keratinocyte cell and human fibroblast cell. All the tested individual growth factors were extremely safe, with no cellular toxicity being demonstrated at high concentrations. IGF-1, VEGF, bFGF, KGF, and thymosin β4 are safe, with no cellular toxicity tested up to 10 ppm. Copper tripeptide was safe, with no cellular toxicity tested up to 10,000 ppm.

To evaluate efficacy, we evaluated the stimulation of hair growth in C3H mice, after intradermal injection of various concentrations of the growth factors and peptides of the formulation. The mice were kept under observation and evaluated at days 1, 3, 7, 14, and 28.

This formulation used in solution 3 was called the QR 678 hair growth factor formulation (Table 3), and the same was used in the human trials subsequently.[7] In human trials, our injection protocol was for 8 sessions of treatment, given at 3–4 weekly intervals.[7]

In a study conducted by Shome and Kapoor,[9] comparing the efficacy of QR 678 with PRP in male androgenetic alopecia, a reduction in hair fall (ie, pull test became negative, ie, number of hairs pulled is 3 or less) was noted in all the patients of QR 678 group by the end of the 8th session (6 months), whereas the hair fall was reduced (pull test negative) in just 50% of the PRP group. The results were maintained in QR 678 group at 1-year follow-up although the number of hairs pulled was increased in the PRP group at the end of 1 year.[9] Also, videomicroscopic assessment (evaluating the hair density, terminal hair density, vellus hair density, and shaft diameter) showed significant improvement ($P < 0.005$) in the QR 678 group, whereas the results were not significant in the PRP group. On global photographic assessment and patient self-assessment too, the results of QR 678 were significant and superior to that

of PRP. Side effects like itchy scalp, unsteadiness during injection, and increase in hair fall were also negligible in the QR 678 group as opposed to the PRP group.[9]

In our study, efficacy was further evaluated in another study arm, which evaluated maintenance of hair follicle viability by intradermal injection of the QR 678 formulation in secondary alopecia. Various newer therapies have emerged in the domain, such as dutasteride microinjections, topical finasteride, setipiprant, microneedling, and simvastatin. However, on literature review, no clinical trials were encountered, and the limited number of studies lack enough potential to be termed as efficacious for acceptable treatment outcome for postchemotherapeutic drug-induced alopecia.[26]

This experiment illustrated the localized maintenance of hair follicle viability (growth) by intradermal QR 678 formulation injections, during treatment with chemotherapeutic Ara-C. Injections generally caused retention of hair in a 0.25-cm radius around the injection site, most notably in the solution 3 group. The observation of retained hair within the area of injection was examined histologically. Although normal appearing and functional anagen hair follicles were observed at the site of injection of the QR 678 hair formulation, follicles located away from the injection site were dystrophic and nonfunctional (disruption of the integrity of inner and outer root sheaths and disrupted hair shafts). These data confirmed the gross observation of normal hair follicular function within the site of QR 678 injections and illustrated the stimulatory effect of the intradermal injections on hair follicle, which resulted in the maintenance of the active hair growth cycle during chemotherapy treatment.

VEGF, essential for angiogenesis and vascular permeability, may be responsible for maintaining proper vasculature around the hair follicles, during the anagen growth phase.[27,28] KGF is highly capable of counteracting chemotherapy-induced alopecia, and it is one of the components of our formulation.[29] IGF-1 is critically involved in promoting hair growth by regulating cellular proliferation and migration during the development of hair follicles. IGF-1 has been reported to prevent the follicle from developing catagen-like status.[30–32] Thymosin β4 promotes hair growth in various rat and mice models, including a transgenic thymosin β4 overexpressing mouse, by influencing follicle stem cell growth, migration, differentiation, and protease production.[33] The bFGF has been found to promote hair growth by inducing the anagen phase in resting hair follicles, and has been considered to be a potential hair growth–promoting agent.[34] The effects of L-alanyl-L-histidyl-L-lysine-Cu^{2+} (AHK-Cu) copper tripeptide on human hair growth ex vivo and cultured dermal papilla cells were investigated and shown to promote the growth of human hair follicles.[35]

In summary, we publish the results of the preclinical data on file, demonstrating the safety and efficacy of intradermal QR 678 and QR 678 Neo hair growth formulations. The findings of cellular assays and animal studies suggest that QR 678 and QR 678 Neo formulations are safe and efficacious in treating hair loss in mammals. The second arm, which was introduced in the study to check the effects of QR 678 and QR 678 Neo on cytosine arabinoside-induced hair fall (chemotherapy-induced secondary alopecia), also showed encouraging results. Our evaluation of the QR 678 Neo, which is a plant derivative, consisting of biomimetic peptides, showed similar safety and efficacy to the QR 678. Although it has been proved earlier that plant derivatives are biomimetic polypeptides of the growth factors and changing the source does not affect the efficacy of the product, we wanted to confirm the same through our study.

LIMITATIONS AND FUTURE SCOPE

The limitation of the study was the small sample size. This was a preclinical study, demonstrating the safety and efficacy of the hair formulation, which we call the QR 678 hair growth factor injections. One arm with cytosine arabinoside-induced hair fall was included in the animal trials to check if the QR 678 and QR 678 Neo could also work in chemotherapy-induced secondary alopecia in addition to androgen-induced alopecia. Based on the promising and encouraging results of the current study, a human trial was subsequently conducted and yielded good results.[7]

Also, future studies are warranted showing the effect of QR 678 and QR 678 Neo on disorders affecting hair fall, like inflammatory conditions of the scalp (including, but not limited to, atopic dermatitis and autoimmune hair disorders like alopecia areata). The evaluation of the QR 678 formulation in androgenetic alopecia has already been done in a human trial. A separate trial to evaluate the results of QR 678 and QR 678 Neo hair formulations in human patients with secondary alopecia, post cancer chemotherapy, has been completed and is currently at the publication stage. Another trial showcasing the results of these formulations in female pattern hair loss, has also been completed and is also currently at the publication stage.[36,37]

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