

Topical Management of Acne Vulgaris using Carbohydrate-derived Fulvic Acid (CHD-FA)

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Introduction

In this study of the effect of topically applied Carbohydrate-derived fulvic acid (CHD-FA) we performed 2 clinical trials. In the first pilot study, we attempted to ascertain what formulation of a CHD-FA topical applicator was preferred by patients. 15 Individuals were recruited and were asked to fill in questionnaires regarding the different types of CHD-FA topical applicators. The results from this study would be used to determine the optimal applicator to be used in a larger study where the efficacy of CHD-FA in treating moderate acne would be investigated. In the second clinical trial, a larger pilot study was performed with 33 individuals. The aim of this study was to determine if CHD-FA was effective in the topical management of Acne Vulgaris. The dissertation is split into three parts. The first two parts are the reports for both studies with the smaller pilot study to determine the optimal applicator first and the larger pilot study to determine efficacy of CHD-FA second. The final part is a journal article written according to the guidelines for the South African Medical Journal (SAMJ) summarizing the research done on the topical management of acne using CHD-FA for possible submission to this journal. Each report is shown in its entirety with figures and tables included at the end before the next part begins. An Appendix follows at the end of the dissertation.

A pilot study to optimize the formulation of an applicator for topical carbohydrate-derived fulvic acid (CHD-FA)

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ABSTRACT

Objectives: In this pilot study, our intention was to ascertain what formulation of a carbohydrate-derived fulvic acid (CHD-FA) topical applicator was optimal for patients to use during a larger study where the efficacy of fulvic acid (CHD-FA) in treating moderate acne vulgaris will be investigated.

Methods: 15 individuals with inflammatory acne with an acne grade of III or lower were asked to volunteer for the study. They were split into 3 groups. Each group was asked to use a different formulation (a cream base, gel base and wet applicator formulation) each week. After each week, the individuals returned to fill out a questionnaire evaluating the formulation they used that week. They were also examined by the clinician for any possible side effects, and given the next formulation to use for the following week. The trial was 3 weeks long, and after using all 3 formulations the individuals were asked to fill out a final questionnaire evaluating all 3 formulations. The clinician was also asked to fill out a questionnaire giving his/her opinion on the formulations.

Results: The results did not give conclusive evidence of one particular formulation being favored above all the others. All the formulations performed more or less equally as well. According to the final questionnaire, 6 out of the 15 individuals were most satisfied with the wet applicator, 4 out of 15 preferred the cream base and 5 out of 15 preferred the gel base. 5 out of 15 individuals were least satisfied with the wet applicator, 3 out of 15 with the cream base, and 7 out of 15 were least satisfied with the gel. While there was no conclusive indication for one particular formulation, there were some common complaints or observations by individuals about each formulation. Most individuals said the wet applicator had an initial burning sensation to the skin upon application, but it disappeared a few seconds after application. Many individuals said they felt the cream base was oily and made the skin appear oily after application. The gel base was said to smell the worst, along with the wet applicator, while the cream smelt the least. A few individuals complained that the gel left a residue on the skin.

Conclusion: While the study did not give a clear indication of one particular formulation that was preferred by individuals, it did produce interesting results that can be used to make some of the formulations more favourable. In the main trial, further investigation will be done to optimize the formulation.

Introduction

Acne vulgaris is a disease that affects almost 80% of individuals [1]. It is the most common skin disease [2]. It is commonly known to affect individuals during adolescent years, starting between the ages of 10-14 years, and normally regressing by ages 20-25 years. The severity of acne ranges from mild (few open and closed comedones) to severe inflammation and abscess formation on the face and trunk [3]. Acne is a disease which has a strong psychological effect on persons affected by it. There have been shown to be more than 25 methods of assessing acne and more than 19 methods of counting lesions. There is however no standardized methodology [4].

The pathophysiology of acne vulgaris can be broken up into four different events [4]:

1. Androgen-dependent overproduction of sebum
2. follicular hyperkeratosis (closed and open comedones)
3. increase in microbial flora (*Propionibacteria acnes*)
4. immunological processes and inflammation

These events are not individual events, and are affected by each other [3]. For example, the increase in proliferation of *Propionibacteria acnes* is as a result of increased sebum production, as well as hyperkeratosis. However, the bacteria are responsible in part for producing factors such as bacterial lipases, proteases, hyaluronidases and chemotactic factors that stimulate inflammatory mechanisms [3]. Follicular inflammation can also cause an increase in sebum production [3].

Fulvic acid is one of the components of the humic substance that are formed naturally during the decay of plant and animal material [5]. The humic substances can be divided into humic acid, fulvic acid, and humin. They are separated based on their solubility in water as a function of pH [6]. Fulvic acid is that fraction that is soluble in water at all pHs. Most research done on the therapeutic benefits of fulvic acid has been done using oxifulvic acid. Oxifulvic acid is the name given to fulvic acid that is formed by the wet oxidation of bituminous coal [5, 6, and 7]. Oxifulvic acid has been shown to have antimicrobial activity and anti-inflammatory properties [6, 7]. A pilot study was undertaken to establish the safety and efficacy of topically applied fulvic acid cream (4.5%) compared to hydrocortisone cream (0.1%) in healthy volunteers [5]. Fulvic acid had no significant effect on any of the safety parameters and no sensitisation occurred when applied on the skin.

A new novel method of producing fulvic acid from the controlled wet oxidation of a carbohydrate source has been developed. This method of producing fulvic acid is believed to be better than production by means of wet oxidation of bituminous coal, because there are heavy metals present in fulvic acid produced by oxidation of bituminous coal; whereas carbohydrate-derived fulvic acid (CHD-FA) contains no metals. It is therefore believed to be safer than FA produced from bituminous coal. An *in vitro* antimicrobial study done on CHD-FA showed broad band antimicrobial activity against gram positive cocci, gram negative bacilli, and yeasts [6]. CHD-FA was found to have ten times greater antimicrobial activity than the bituminous coal product.

It is believed that CHD-FA will be effective in topical treatment of acne vulgaris, because of its proven anti-inflammatory and anti-microbial activity. A pilot study done in 2007 by Peacock and Snyman showed *in vitro* efficacy as well as some clinical efficacy demonstrated with Dermawave™ [8]. By eliminating these two pathophysiological causes of acne, it is believed that the severity of the acne will decrease significantly. By decreasing the microbial activity, continual activation of the immune response and subsequent inflammation will decrease. The anti-inflammatory activity of CHD-FA will decrease the inflammation that is already present. Therefore, by treating this inflammation directly, it is believed that the severity of acne will decrease.

In this pilot study, we intended to ascertain what formulation of CHD-FA topical applicator would be optimal for patients to use during a larger study where the intention is to investigate the efficacy of fulvic acid (CHD-FA) in treating moderate acne vulgaris.

Presently, CHD-FA is available as a 4% solution in water. The 3 different formulations used in this study were CHD-FA in a cream base, CHD-FA in a gel base, and CHD-FA as a wet applicator.

Materials and Methods

Individuals with any form of acne Grade III or lower, as defined by Plewig and Kligman (see Table 1), were allowed to take part in the trial [9]. No individuals with nodular acne or cysts

(Grade IV) were allowed to take part in the trial. Comedonal acne was allowed to be present or absent.

15 individuals were recruited to take part in the trial. Participants were recruited by means of word of mouth, and announcement in lectures on the medical campus where they were asked if any volunteers would be interested in taking part in the study. The individuals were randomized to 1 of 3 groups. Each group was given a different formulation to use each week, with each individual being given a diary card (Appendix 1) to fill out each week of the trial. The diary card was intended to record any possible adverse reactions or illnesses due to use of any of the applicator methods. They were also asked to record the skincare regimen they followed as well as any additional medication they may have taken. Each individual was given the opportunity to use all the formulations, but at different times during the study. After each week, the individuals returned to the Department of Pharmacology, University of Pretoria. They returned their diary cards and were asked to fill out a questionnaire (see Appendix 2) to evaluate the formulation they used that week. The clinician examined the individuals for any possible side effects. The individuals were then given the next formulation to use for the following week. At the end of the 3 weeks, after all the individuals had used all formulations, they were asked to fill out a final questionnaire (see Appendix 3) evaluating all 3 formulations. The clinician was asked to fill out a questionnaire (see Appendix 4) giving his/her opinion about the 3 formulations.

Questionnaire

The weekly questionnaire that was filled out by the individuals was based on a likert scale of satisfaction adjusted from a study done by Kellet et. al [10]. Individuals were asked to rate statements on a scale of 1 to 5, with 1 being “strongly disagree” and 5 being “strongly agree”. The statements included the following: “The texture and consistency of the product was acceptable”; “The product was absorbed into the skin easily”; “The product did not leave a residue on the skin”; “The product smelt OK”; “The product made my skin feel uncomfortable”; “Make-up/ facial skincare products were easy to put on after application of the product”; “Overall, I am happy with the product”. In this questionnaire they were also asked to provide any comments on what they liked and disliked about the applicator. At the end of the study, each individual was asked to complete a questionnaire to assess the different methods of application and indicate the method they preferred most. In the

questionnaire, they were asked questions about whether any of the products hurt or irritated their skin, whether any caused an adverse side effect. They were asked to evaluate the 3 products, stating which products they felt were most comfortable on their skin; which was best absorbed into the skin; which form they thought worked the best; which form of application they were most satisfied with, and which form of application they were least satisfied with. The clinician was also to evaluate the different forms of applications, giving their opinion on which ones they felt were most stable, least stable and most acceptable.

Inclusion criteria

Males and females ages 18 to 40.

Individuals with acne Grade III or lower according to guidelines by Kligman and Plewig [9]

Patients who have signed a written informed consent form.

Exclusion criteria

No other skin diseases besides moderate acne vulgaris.

Individuals may not be using any other forms of acne treatment at the period of commencement of the study. This does not include daily cleansing products such as cleansers, toners, moisturizers, etc.

Pregnant or lactating females.

Individuals on antibiotic treatment.

Patients previously found to be unresponsive to fulvic acid may not take part in the study.

Patients with severe nodular or cystic acne vulgaris.

Doses

Individuals will be given a portion of each application that they will be allowed to take home with them and apply intermittently throughout the day, 2 – 5 times a day. Application can be onto the entire face, or only directly to the acne lesion, as preferred by the individual. If applied only to the acne lesion, the individual must be sure to apply it so that it covers the entire lesion fully.

There is no washout period as efficacy will not be tested in this trial. This is also the reason there was no specific form of grading of acne severity.

Results

The results of the questionnaires did not show an outright preference by individuals for one particular type of formulation. The results show the subjective opinion of individuals regarding the different formulations. Although the individuals highlighted the same qualities in each formulation, it was interesting to note the importance of these qualities to the individual when making a final decision as to the formulation they were most satisfied with. The number one complaint about the formulations was the smell. The results of the questionnaire for each week are shown in Table 2, 3 and 4. These show the average results for each statement. The results of the final questionnaire are shown in Table 5, with the number of times each formulation was picked for each statement being recorded.

Wet applicator

Most of the individuals complained that the wet applicator left a stinging sensation on their skin, particularly on open cuts or acne lesions. This however was said to be a transient burning sensation and disappeared after a few seconds. 10 out of the 15 individuals felt that the wet applicator was absorbed into the skin the best. 5 out of 15 said the wet applicator was most comfortable on their skin. 7 out of 15 individuals felt the wet applicator worked the best in treating their acne. However, only 6 out of 15 were most satisfied with the wet applicator. 5 out of 15 were least satisfied with the wet applicator. The complaints about the wet applicator were that it dried out the skin slightly. Some individuals felt this helped in treating their acne though, by making the skin less oily. A few individuals were unhappy with the mechanism of application of the wet applicator. They would have preferred if it did not need to be applied with cotton wool. It was inconvenient for them to have to have cotton wool available to apply it. It would have been preferable if it was able to be applied directly to the skin, as a roll-on or as a spray. A few individuals felt the smell was the worst with the wet applicator compared with any of the others. A similar amount of people complained that the gel smelt the worst.

Fulvic acid in a gel base

A number of individuals complained that the gel also left a stinging sensation on their skin, particularly on open cuts or acne lesions. Some complained that the gel burnt the most, while

others said that the wet applicator burnt the most. All individuals did however say that the burning was a transient burning sensation and would disappear after a few seconds. A number of individuals complained that the gel left the skin feeling tight, with some saying that a residue was left on the skin, almost as if the skin were “peeling”. Most individuals said the product was easily applied. 4 out of the 15 individuals felt that the gel was the most comfortable on their skin. Only 3 out of 15 felt the gel was absorbed into the skin the best. This appeared to be the most unattractive feature of the formulation as many complained that the smell lingered the longest with the gel, and left the skin feeling tight and sticky, with a “white-ish film that peels off” left on the skin. 5 out of 15 individuals felt the gel worked the best in improving their acne. 5 out of 15 were most satisfied with the gel base, while 7 out of 15 were least satisfied with the gel base.

Fulvic acid in a cream base

Many individuals complained that the cream left their skin feeling oily. It was interesting that for some this was not a complaint however. They felt that the moisturizing effect of the cream on the skin was pleasant and approved of the product for that. The majority however felt that it made the skin too oily. Some felt that this caused their acne to become worse. The female participants particularly complained that it was difficult to apply make-up after application of the product because of the oiliness the product left on their skin. 6 out of 15 individuals said the cream felt the most comfortable on the skin. 2 out of 15 individuals felt the cream was absorbed the best out of all the formulations. 3 out of 15 individuals felt the cream worked the best in treating their acne. 4 out 15 were most satisfied with the cream formulation while 3 out of 15 were least satisfied with the cream. The consensus appeared to be that the cream caused the least amount of burning to the skin and smelt the least of all the products. This was probably due to the fact that the cream had the least amount of active product compared to the other formulations.

Discussion

While there is not a clear indication that one formulation was preferred above another, there were some interesting results regarding each formulation. The reasons for no clear indication of a preference for one particular formulation could be as a result of the relatively small

sample size. A bigger sample size may have given more clear results. It is apparent however that the use of topical treatments is very subjective amongst different individuals. It appears that the formulations that the participants felt worked the best in improving their acne were the wet applicator, followed closely by the gel base. The wet applicator and the gel caused a transient burning sensation immediately after application, particularly on skin cuts or open acne lesions. This was transient and disappeared soon after application. Some felt the gel burnt more than the wet applicator, while others felt the wet applicator burnt more than the gel. The consensus was that the cream burnt the least. The smell was a complaint in all the formulations with many individuals finding it a very unattractive trait about the product. A recommendation for the wet applicator would be for it to be developed into a roll-on applicator so that application can be directly to the skin without the need for cotton wool. The wet applicator was felt to be absorbed the best, with many individuals complaining about the smell but saying it disappeared quickly. This was a complaint about the gel. The gel appeared to be absorbed more slowly and so the smell was present for much longer. The gel also left the skin feeling sticky with a residue being left on the skin, which peeled off later. The gel and wet applicator both appear to leave the skin dry, while the cream left the skin feeling oily. The cream smelt the least and burnt the least. This was probably due to the fact that the cream had the least amount of active product (cream 2% CHD-FA, gel 3.95% CHD-FA, wet applicator 4% CHD-FA). The least amount of individuals felt the cream worked the best. Many individuals felt the cream was the most comfortable on their skin. According to the final questionnaire, it appears that the wet applicator and gel were the two formulations the individuals were either most satisfied with, or least satisfied with. The cream was a moderate choice with individuals not being particularly unhappy with the product, but also not being especially happy with it. Due to similar results, further investigation into the most optimal formulation for individuals will be done in the main trial. It is suggested that individuals are allowed to use 2 of the formulations in the first week. These will most likely be the wet applicator and the gel formulation. After the first week, the individuals will be allowed to pick one formulation they prefer the most. This formulation will be used by the individuals for the rest of the trial period. It is believed that by doing this, compliance will be increased as each individual will be allowed to use the product they like the most. It is also possible that by increasing the sample size, a more clear indication of preference to one particular formulation will be seen.

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TABLES

Table 1: Plewig and Kligman papulopustular acne severity grading, 1 – 4 [9]

Grade I	Fewer than 10 inflammatory lesions on one side of the face
Grade II	10 - 20 inflammatory lesions on one side of the face
Grade III	20 - 30 inflammatory lesions on on side of the face
Grade IV	more than 30 inflammatory lesions on one side of the face

Table 2: Average results of the likert scale of satisfaction for each statement for each formulation for week 1. Scale is on a 1 to 5, with 1 = “strongly disagree” while 5 = “strongly agree”

	Wet applicator	Gel	Cream
1. Texture and consistency acceptable	4.4	4.6	4.6
2. Absorbed into skin	4	4.2	4
3. Product did not leave a residue	4.6	3	4.8
4. Product smelt OK	1.6	1.8	1.8
5. Product made skin feel uncomfortable	2.2	2	1.8
6. Make-up/facial skincare products easy to apply after	4.2	3.8	4.2
7. Overall happy with product	3.4	4	3.8

Table 3: Average results of the likert scale of satisfaction for each statement for each formulation for week 2. Scale is on a 1 to 5, with 1 = “strongly disagree” while 5 = “strongly agree”

	Wet applicator	Gel	Cream
1. Texture and consistency acceptable	4.2	4.6	4.6
2. Absorbed into skin	4.2	3.6	4.4
3. Product did not leave a residue	4.8	4	4.4
4. Product smelt OK	1.8	2.6	3.4
5. Product made skin feel uncomfortable	2	2.8	2.6
6. Make-up/facial skincare products easy to apply after	3.6	3	3.4
7. Overall happy with product	3.4	3.4	3.6

Table 4: Average results of the likert scale of satisfaction for each statement for each formulation for week 3. Scale is on a 1 to 5, with 1 = “strongly disagree” while 5 = “strongly agree”

	Wet applicator	Gel	Cream
1. Texture and consistency acceptable	4.4	4.4	4.8
2. Absorbed into skin	4.8	3.6	3.4
3. Product did not leave a residue	4.6	3.2	2.6
4. Product smelt OK	2.8	2.6	3.2
5. Product made skin feel uncomfortable	2.2	2.6	1.8
6. Make-up/facial skincare products easy to apply after	3.8	3	3.6
7. Overall happy with product	4.2	3.8	4

Table 5: Results of the final questionnaire. The number of times each formulation is chosen for each statement is recorded in the table.

	Cotton Wool applicator	Cream base	Gel base
Which was most comfortable on your skin?	5	6	4
Which was absorbed into your skin best?	10	2	3
Which did you feel worked the best?	7	3	5
Which of the 3 were you most satisfied with?	6	4	5
Which of the 3 were you least satisfied with?	5	3	7

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ABSTRACT

Objectives: In this study we attempted to determine whether Carbohydrate-derived fulvic acid (CHD-FA) was more effective than placebo as a cosmeceutical in the topical management of mild to moderate inflammatory acne.

Methods: 22 individuals with mild to moderate inflammatory acne (acne grade I-III) were invited to volunteer for the study. They were split into 2 groups, an active group placebo group. The study period was 6 weeks. Individuals filled out questionnaires about the products and VISIA® Complexion Analysis was done to measure any improvement.

Results: No statistically significant difference between group A and B was seen. The questionnaires indicated individuals in both the placebo and active group were happy with the product, but complained extensively about the smell of the product.

Discussion: Although no efficacy could be demonstrated in this study for CHD-FA in the management of inflammatory acne, a larger sample size, together with an improved smell could change the outcome of a second trial.

Introduction

Acne vulgaris is a disease that affects almost 80% of individuals [1]. It is the most common skin disease [2]. It is commonly known to affect individuals during adolescent years, starting between the ages of 10-14 years, and normally regressing by ages 20-25 years. The severity of acne ranges from mild (few open and closed comedones) to severe inflammation and abscess formation on the face and trunk [3]. Acne is a disease which has a strong psychological effect on persons affected by it. There have been shown to be more than 25 methods of assessing acne and more than 19 methods of counting lesions. There is however no standardized methodology [4].

The pathophysiology of acne vulgaris can be broken up into four different events [4]:

5. Androgen-dependent overproduction of sebum
6. follicular hyperkeratosis (closed and open comedones)
7. increase in *Propionibacteria Acnes*
8. immunological processes and inflammation

These events are not individual events, and are affected by each other [3]. For example, the increase in proliferation of *p. acnes* is as a result of increased sebum production and hyperkeratosis. However, the bacteria are responsible in part for producing inflammatory mediators such as bacterial lipases, proteases, hyaluronidases and chemotactic factors [3].

Sebum Production

The sebaceous gland is responsible for the production of sebum [5]. The largest sebaceous glands are found on the face and trunk, incidentally the area's most commonly affected by acne [3]. Sebum is a mixture of relatively non-polar lipids, mostly produced in the sebaceous gland [5]. Acne is sometimes found to occur at birth due to increased androgen production. This soon subsides and very little acne is found during childhood [3, 5]. At about age 8-10 years, androgen production increases with the onset of puberty, and results in enlargement of the sebaceous glands and contributes to acne in teenagers. The primary androgen regulating sebum production in the skin is dehydroepiandrosterone sulphate (DHEA-S) [3]. It is approximately equal in men and women [3]. Increased sebum production can also occur despite normal levels of androgens. This is believed to be due to increased sensitivity to androgens.

Follicular Hyperkeratosis

In normal skin cells, keratinocytes are loosely layered. The dead skin cells are carried to the surface of the skin by sebum flow (desquamated). A balance between newly produced and dead cells exists. During hyperkeratinization however, there is an increased keratinocyte proliferation rate, and the dead cells are not desquamated properly [3]. Retention of these dead cells occur leading to follicular plugging and the formation of a non-visible microcomedo and then a comedo [3, 4]. There are a number of factors that are believed to be responsible for hyperkeratosis. A change in lipid composition of sebum, bacterial metabolites and inflammatory mediators are believed to be among the culprits [3].

*Increase in *P. acnes**

P. acnes are part of the natural flora of the skin [3, 4, 6]. They prefer anaerobic conditions and colonize regions with high sebum production [3, 4, 6]. For this reason, increased sebum production and follicular hyperkeratinization result in proliferation of *P. acnes*. An increased concentration of *P. acnes* was found in 11-20 year olds with acne compared to those who did not have acne. The role of *P. acnes* in acne is due to release of bacterial metabolites which are proinflammatory [3]. Examples of these metabolites are bacterial lipases that release free fatty acids, proteases, hyaluronidases and chemotactic factors that attract neutrophils [3].

Inflammation

It was believed that inflammation in acne occurred as a result of the three other pathogenic factors. Recently however, it has been found that patients who have acne tend to have a predisposition for follicular inflammation [7]. Inflammatory processes can increase sebum production [3]. This occurs through leukotriene B4 that binds to peroxisome proliferator activated receptor α on sebocytes, which regulates lipid metabolism [3]. Neutrophils that are attracted towards the follicle by bacterial chemotactic factors enter the follicle and phagocytose the bacteria, causing a release of more bacterial metabolites and a subsequent increase in inflammation [3].

Factors that can trigger acne

There are a number of factors that may cause acne. These include (i) hormonal imbalance in females during the menstrual cycle; (ii) drugs including systemic glucocorticoids and oral contraceptives; (iii) chemical contactants and (iv) mechanical irritation [3]. There have also been recent epidemiological observations that have shown that diet may also play a very significant role in the development of acne in Western society [8]. It has been found that in societies where there is a more natural lifestyle, acne does not appear [8]. There are

hypotheses that dairy products in the Western diet are causative effects in the development of acne. The hypothesis is that the hormones and growth factors (IGF-1) found in milk and other dairy products may cause an increase in seborrhea and follicular hyperkeratosis [3, 9].

Pharmacology of Fulvic acid

Fulvic acid is one of the components of the humic substance that are formed naturally during the decay of plant and animal material [10]. The humic substances can be divided into humic acid, fulvic acid, and humin. They are separated based on their solubility in water as a function of pH [11]. Fulvic acid is that fraction that is soluble in water at all pHs. Most research done on the therapeutic benefits of fulvic acid has been done using oxifulvic acid. Oxifulvic acid is the name given to fulvic acid that is formed by the wet oxidation of bituminous coal [10, 11, 12]. Oxifulvic acid has been shown to have antimicrobial activity and anti-inflammatory properties [11, 13]. A pilot study was undertaken to establish the safety and efficacy of topically applied fulvic acid cream (4.5%) compared to hydrocortisone cream (0.1%) in healthy volunteers [10]. Fulvic acid had no significant effect on any of the safety parameters and no sensitization occurred when applied on the skin.

A new novel method of producing fulvic acid from the controlled wet oxidation of a carbohydrate source has been developed. This method of producing fulvic acid is believed to be better than production by means of wet oxidation of bituminous coal, because there is a high amount of heavy metals present in fulvic acid produced by oxidation of bituminous coal; whereas carbohydrate-derived fulvic acid (CHD-FA) contains no metals. It is therefore believed to be safer than FA produced from bituminous coal. An *in vitro* antimicrobial study done with CHD-FA showed broad band antimicrobial activity against gram positive cocci, gram negative bacilli, and yeasts [11]. CHD-FA was found to have ten times greater antimicrobial activity than the bituminous coal product. In a study using the carrageenan-induced rat paw oedema model, CHD-FA was shown to have anti-inflammatory properties (unpublished results).

It is believed that fulvic acid will be effective in topical treatment of Acne Vulgaris, because of its proven anti-inflammatory and anti-microbial activity. A pilot study done in 2007 by Peacock and Snyman showed *in vitro* efficacy against *P. acnes* as well as some clinical efficacy in acne demonstrated with a novel transdermal delivery system called Dermawave™ [14]. By eliminating the inflammatory and antimicrobial causes of acne, it is believed that the

severity of the acne will decrease significantly. By decreasing the microbial activity, continual activation of the immune response and subsequent inflammation will decrease. The anti-inflammatory activity of CHD-FA will decrease the inflammation that is already present. Follicular inflammation can also cause an increase in sebum production [3]. By decreasing acne, we believe that the appearance of the skin will improve dramatically. This will help improve the psychological distress caused by acne.

In this pilot study, we intend to investigate the efficacy of CHD-FA as a **cosmoceutical** in the topical treatment of individuals with mild inflammatory acne vulgaris. Individuals with inflammatory acne, presenting with a minimum of 5 inflammatory lesions (Grade I-III [15]), but no nodular acne or cysts (Grade IV) as defined by Plewig and Kligman [15], will be recruited to take part in this study. Plewig and Kligman grading shown in Table 1. Comedonal acne may or may not be present [16].

Materials and Methods

Recruitment and study design

Individuals between the ages of 13 and 40 with an acne grade of I-III, as defined by Plewig and Kligman, were allowed to take part in the study [15]. No individuals with nodular acne or cysts (Grade IV) were allowed to take part in the study. The study was a Double-blind placebo-controlled randomized study to investigate the efficacy of CHD-FA as a cosmoceutical in the topical management of individuals with mild to moderate acne. 33 individuals were recruited to take part in the trial. 22 completed the study, with 6 individuals in the active group and 5 in the placebo group lost to follow-up. The questionnaires of these individuals suggest that the majority of these individuals discontinued the trial due to the dissatisfaction with the smell of the product. Participants were recruited by word of mouth, announcement in lecture halls on the University of Pretoria's campus and adverts placed on mentioned campus.

In order to be allowed to take part in the trial, individuals had to satisfy a certain set of criteria as listed below.

Inclusion criteria

Males and females between the age of 13 and 40

Individuals with acne Grade I-III according to guidelines by Kligman and Plewig [15]

Patients who had signed a written informed consent form

Exclusion criteria

No other facial skin diseases besides moderate acne vulgaris

Individuals using any other forms of acne treatment less than 30 days prior to taking part in the study (This excluded the use of daily cleansing, toning or moisturizing agents)

Individuals with beards (difficulty with examining severity)

Pregnant or lactating females

Individuals using anabolic steroids

Individuals on antibiotic treatment

Individuals using corticosteroids

Individuals that had changed (or started) oral contraceptive treatment less than 6 months prior to the start of the study

Individuals previously found to be unresponsive to fulvic acid

Participants were randomized to 1 of 2 groups, an active group and a placebo group. The study was done in a double blind fashion. The study period was 6 weeks. Participants were required to return every 2 weeks to have a VISIA® complexion analysis done.

Both groups were given two formulations to use for the first two weeks of the study period. The two formulations were a gel formulation and a water-based roll-on formulation. The active group received the active gel and water-based roll-on formulations, while the placebo group received placebo gel and water-based roll-on formulations. Participants were asked to use both formulations for the first two weeks, after which they returned to the study site. On returning, participants were asked to fill out 2 questionnaires (see Appendix 5, 6) evaluating the formulations they had used. After filling out the questionnaire, participants were asked to choose one formulation which they liked best and would continue using for the remainder of the study period. They were asked to return the formulation they did not like.

The study period was 6 weeks because acne vulgaris is a psychologically debilitating disease where individuals want improvement as soon as possible. If there was no improvement after 6 weeks of treatment, compliance may have decreased as the participants would believe it was not helping.

Questionnaire

Individuals were asked to fill out two questionnaires upon returning for their first two-week follow-up appointment. The first questionnaire asked questions about each individual formulation and participants were asked to give their likes and dislikes about the different formulations. The questionnaire was based on a likert scale of satisfaction adjusted from a study done by Kellet et. al [17]. Individuals were asked to rate statements on a scale of 1 to 5, with 1 being “strongly disagree” and 5 being “strongly agree”. The statements included the following: “The texture and consistency of the product was acceptable”; “The product was absorbed into the skin easily”; “The product did not leave a residue on the skin”; “The product smelt OK”; “The product made my skin feel uncomfortable”; “Make-up/ facial skincare products were easy to put on after application of the product”; “Overall, I am happy with the product”. In this questionnaire they were also asked to provide any comments on what they liked and disliked about the applicator.

In the second questionnaire each individual was asked to compare the two formulations with each other and indicate which formulation they preferred most. They were asked questions about whether any of the products hurt or irritated their skin and whether any caused an adverse side effect. The participants were asked to evaluate the 2 products, stating which products they felt were most comfortable on their skin; which was best absorbed into the skin; which form they thought worked the best; which form of application they were most satisfied with, and which form of application they were least satisfied with. The results of both these questionnaires were assessed to decide the patient-preferred formulations.

Study products

The active gel formulation had a concentration of 3.95% CHD-FA. The water-based roll-on formulation had a concentration of approximately 4% CHD-FA. All the formulations had an acidic pH close to 3.9. All formulations had a mint flavouring added to disguise the smell of the products. The placebo formulations had an approved colouring added to disguise the appearance of the formulations and acetic acid was added in order to lower the pH to an acidic form equal to the active formulations.

Doses

Individuals were required to apply the product 2-5 times daily. Participants were encouraged to apply the product intermittently throughout the day. Application was onto the entire face, or only directly to the acne lesion, as preferred by the individual. If application was only to the acne lesion, the individual was asked to be certain to apply it so that it covered the entire lesion fully.

Assessment

The VISIA® complexion analysis system takes photos of the right cheek and the forehead of the face and is able to measure a number of parameters on and slightly underneath the skin. The parameter we were most interested in was the porphyrin count. Porphyrin is a metabolite of *P. acnes*. Therefore a higher porphyrin count is as a result of a higher presence of *P. acnes*. A higher presence of *P. acnes* is found in individuals suffering from acne [3]. CHD-FA has been shown to have anti-microbial activity against amongst others, *P. acnes*. We therefore expected that the porphyrin count would also decrease with continued use of CHD-FA. A decrease in this parameter would be characteristic of improvement in the appearance of the skin, combined with other factors that may or may not improve as a result. Some measure of compliancy with the treatment was done by issuing each patient with a diary card to fill out, indicating when/how they applied the treatment. Any possible adverse effects that the individual experienced as a result of the treatment were noted in the diary card. The participant was asked to immediately let the clinician or investigator know if they believed they had had a severe adverse effect as a result of the treatment. It should be noted that as is most common when measuring compliancy of an individual taking a product at home, the individual's honesty in filling out the diary card has to be relied on and therefore we cannot be completely certain of the compliancy indicated in the diary cards.

Statistical analysis

The statistical analysis was done by Professor Paul Rheeder at the University of Pretoria. He is a qualified statistician who was given the blinding results after comparing the results of VISIA® complexion analysis. The data was analysed in 3 different ways. Firstly the change between week 0 and week 6 was evaluated with a Mann Whitney U test on the raw data and a two-sample t-test with the normalised values. A repeated measures analysis of variance (anova) on the normalised values was also done. Finally, a poisson regression was performed

in order to give the incident rate ratio (IRR) of having a porphyrin count in each of the two groups. Normalisation was done by log transformation and square root transformation on the data of the forehead and the right cheek respectively.

Ethical considerations

Ethical approval was obtained from the University of Pretoria Research Ethics Committee before commencing the study.

Results

Questionnaire

Investigation into the two formulations gave interesting, yet still inconclusive results. In Table 2 the results of the questionnaire for the active group are shown. In terms of the overall happiness with the product, both formulations scored an average of approximately 4 out of 5 on a likert scale of satisfaction. According to the questionnaires, individuals were happy with the texture and consistency, felt the formulations were absorbed into their skin well and did not leave a residue. All of these statements scored an average of approximately 4 out of 5. In terms of the smell both formulations scored low with the roll-on scoring an average of 2, while the gel slightly higher with an average of 2.5. According to the questionnaires, it is easier to apply make-up and skincare products after using the roll-on product than it is with the gel. There was only a slightly higher average score of 4.5 compared to 3.6.

Table 3 shows the results of the questionnaires for the placebo group. When compared with the results of the questionnaire from the active group, there is very little difference and many of the same comments were made by individuals in both the placebo and active group.

In Table 4, the average scores for each statement in the questionnaire are compared between the active and the placebo group. It is clear that very little difference between the active group and the placebo group was seen with only a slightly lower overall happiness with the product seen in the placebo group.

In Table 5 the results of the final evaluating questionnaire are shown. As can be seen, there is no difference between the overall satisfaction individuals felt towards each formulation. The only slight difference seen between the two formulations was that individuals felt that the gel was absorbed into the skin better than the roll-on. Comments made by the individuals in the questionnaires did say that the gel was easier to spread over the face and this may have given the impression that it is absorbed more easily into the skin. No one formulation appears to be preferred to the other. Of the 13 individuals in the active group who completed the questionnaire, 7 chose to continue with the gel, while 6 continued with the water-based roll-on. One or two of the individuals stated that they liked both formulations and did not have a preference. Their choice was based on only a slight preference for one formulation based on a specific trait such as convenience of application. In the placebo group, there were 6 individuals that continued with the roll-on, and 6 individuals who continued the study with the gel formulations.

At the end of each questionnaire, individuals were asked to comment on what they liked/disliked about the formulations. All the individuals complained about the smell and this was the main problem individuals had with the product. Many complained that the smell was so strong that it made their eyes burn or water after applying the product. This was probably due to the low pH found in both the active and placebo products. Most of the individuals complained that the product burnt or irritated their skin at some point, especially when applied to an open lesion or broken skin. For the majority of individuals, this burning sensation was only a transient burning that went away after a few seconds. One individual did complain that the roll-on product burnt her skin and caused redness and swelling for 2-5 hours after applying the product. This was an isolated event though. A number of individuals felt the products dried out their skin at times. According to the diary card, most individuals applied the product twice a day, with a few individuals applying it more than twice a day on occasion, and a few applying it only once a day. There were comments from a few individuals that the products made their skin feel soft after application. There were a number of individuals that felt the product was working well and commented on this in the questionnaires. This was found frequently in both the active and the placebo group.

Forehead

With the results of the forehead, log transformation was done in order to normalize the data. In Table 6, the descriptive statistics for the active and placebo group are shown. The Geometric mean of the porphyrin count is given since the data was transformed to a logarithmic scale. As can be seen, the mean does not change a significant amount of the 6-week study period. The active group does show a slightly greater change over time. Figure 1 and 2 show the individual changes in the porphyrin count from week 0 to week 6 in the active and placebo group respectively. The graphs indicate that individual porphyrin counts varied a great deal amongst individuals. There is also very little individual change in the porphyrin count over the 6 week study period with most of the data points staying parallel to each other. In Figure 3, the geometric mean porphyrin count change in both groups is shown over the 6 week period. The active group does show a steeper rate of change over time, with the trend in the placebo group staying virtually the same. This is not significant. Table 7 shows the results of the two-sample t-test with differences in the log count between week 6 and week 0 used to determine the mean difference over the 6-week period in each group. The difference in these values between the placebo and active group was approximately half the standard deviation. The study was underpowered as a sample size of 50-70 individuals in each group would be needed to show one half a standard deviation of the mean difference in log counts. The P-value found was 0.4239 and the null hypothesis could not be rejected and no difference between the active and placebo group was found. The P-value for the Mann Whitney U was 0.6444 and thus showed no difference between active and placebo group. Repeated Measures anova showed no significant difference between the two groups. With Poisson Regression the IRR is 1.038 and the 95% confidence interval passes through 1. Therefore no difference between the two groups can be determined from the results of this test either. Table 8 shows the average change in the porphyrin count in each group at each two-week visit. In Table 9, the average results for a number of the other parameters measured with the VISIA® Complexion Analysis system are shown. An increase in the percentage values for those parameters correlates with an improvement in that parameter. As can be seen, these parameters on average did not change dramatically over the 6-week period.

Right Cheek

Log transformation of the porphyrin count for the results of the right cheek was not effective to normalize data. The median and the min and max are used and are shown in Table 10. In

Figure 4, the differences in the porphyrin count between week 6 and week 0 in each group is shown on a box plot. From this figure it is clear that there is no clear difference in the change in porphyrin count between the groups over the 6-week study period. The distribution of the mean difference in the porphyrin count over the 6-week period appears to be larger in the active group with a larger spread of change indicating individuals in the active group experienced a greater variety in the change in porphyrin count over the 6-week study period than did the placebo group. Square root transformation of the porphyrin count was able to normalize data and so this was used to do the two-sample t-test with unequal variances and anova. The square root count difference between week 6 and week 0 in each group was used to compare the active and placebo group. The difference between the two groups was 0.5 and the standard deviation was 7. Once again it should be noted that the study was underpowered. The P-value found was 0.9578 and so the null hypothesis could not be rejected and no difference between the active and placebo group was seen. In the nonparametric Wilcoxon rank sums test, no difference between the two groups was found. The results of the anova test also showed no difference, as did the poisson regression test, with the IRR once again close to 1 (1.043) and the 95% confidence interval passing through 1.

Table 11 shows the average results for the other parameters measured on the right cheek and their change over the 6-week period. Once again, no change was seen over the 6-week period in any of these parameters.

Overall happiness with the Product

Individuals were asked what their overall feelings towards the product were and whether they would consider buying the product in a store. Both the placebo group and the active group were asked this question. Both groups were generally satisfied with the product with only a small number of individuals dissatisfied with the efficacy of the products. All individuals complained about the smell of the product.

When asked after completion if individuals would consider buying the product if it were available in stores, most of the individuals said they would, “if it smelt better”. Individuals in both the placebo group and the active group were asked this question and both groups responded similarly. The general consensus in both groups was that the product worked, but

many said the smell was too much for them. The burning sensation did not appear to be a great problem to them.

Discussion

The primary outcome of this study was the improvement in the appearance of the skin of the face and reduction of inflammatory acne in the individual. This was quantified and assessed using the VISIA® complexion analysis system. Our secondary outcome was investigation into the safety parameters. Any severe adverse effects were noted and individuals who experienced any would be taken off the study immediately. Further information about the two formulations (gel based and water-based) was collected in order to determine which of the two formulations was preferred by participants. The questionnaires also assisted the investigator to gather further information regarding the product and what participants thought of the product.

The study did not produce significant results. There are a number of reasons this could have occurred. Firstly, the sample size was small and decreased the power of the study. Of the 33 individuals that started on the study, only 22 completed the study. Of the 11 individuals that were lost to follow up, 5 were in the placebo group and 6 in the active group. This equates to approximately one third of the individuals falling out. According to the questionnaires and diary cards, this may have been due to the smell of the product and possibly the transient burning sensation it caused.

Those individuals that did complete the study were asked upon completion whether they would consider buying the product if they saw it in a store. The majority of individuals in both the placebo and the active group said that if the smell was better, they would consider buying it. The smell may have affected how frequently individuals applied the product during the day. Had the smell been better, it is likely that individuals would have applied it more frequently and a greater clinical effect may have been seen. There were some individuals who felt the product worked well, especially “when you apply it a number of times during the day”. The majority however felt it helped slightly, but did not cause a dramatic change. This was noted in the placebo group as well. The study period may have been too short to produce clinical results that would be noticed by individuals and clearly seen on the VISIA®

complexion Analysis. Due to the nature of acne, a longer study period would have been impractical. The reason for this is that acne causes severe psychological unhappiness and individuals want immediate results. If the study had been more than 6 weeks long, compliance would probably have been even lower as individuals would have felt the product was not working. It also would have been difficult to motivate individuals to return to the study site for analysis.

Individuals in the placebo group and the active group both felt the product worked and that, had it not been for the smell, they would consider using the product. The pH in both the active and the placebo product was very low and this may have contributed to the small clinical effect seen by the individuals themselves. In a review by Matousek and Campbell [18] it was noted that a low pH on the surface of the skin can decrease acne and eczema as well as a number of other skin conditions. It is possible that the low pH could have contributed to some of the clinical efficacy seen in both groups.

Acne is a disease that is commonly characterised by hormonal changes in the body, specifically increases in the androgen dehydroepiandrosterone-S (DHEA-S) [3]. It is also known that the severity of acne often changes naturally in a cyclical manner so that improvement/deterioration can be seen without any external factors occurring. This is particularly common in females during their menstrual cycle and is the reason that the hormonal contraceptive is also commonly used as an effective treatment for acne in females. It is possible that some of the change seen in the porphyrin count may have been due to these cyclical changes commonly experienced.

As far as any of the other parameters measured by the VISIA® complexion analysis system, there was very little change in any of them. However, the average change did show a slight but insignificant deterioration in these factors.

There were no serious adverse reactions noted during the study. One individual in the active group did note on her questionnaire that the product burnt her skin and caused redness for up to 5 hours after applying the product. This was an isolated event and this individual's participation in the study was discontinued soon after this event. In the active group, seven out of the thirteen individuals who completed the questionnaires noted that the product caused some form of sensitivity or burning when applied, particularly in the beginning. This was

normally only when applied to an open lesion and was in most cases only a transient burning that lasted for a few seconds after application. This burning sensation was seen in the placebo group as well. Almost all individuals in the study complained that the product caused their eyes to burn or water after application. Both the burning on the skin and the slight burning to the eyes is more than likely due to the low pH, as it was found to occur in the placebo group as well as the active group. Two individuals did complain that the product caused their skin to dry and/or peel. This was also seen to occur in the placebo group and could therefore be due to the low pH once again.

Regarding the individual formulations and investigation into the roll-on product versus the gel formulation, in both the active and placebo group it was commented on frequently that the roll-on product was sometimes difficult to distribute over the entire face and did not “come out evenly” from the bottle. While very convenient to carry and apply through the day, many individuals complained that the roll-on ball was too small. One individual in particular said the formulation was “too watery”. The gel in contrast distributed much more evenly over the face and individuals liked this about the product. The smell in the gel however was said to be worse than in the roll-on formulation. The roll-on product allowed individuals to apply the product without using their hands. One individual said they felt this was preferable to applying with the gel because they were not spreading dirt from their fingers onto their face as was the case when applying CHD-FA in the gel. Another individual commented that he thought that if the roll-on applicator was slightly changed so that instead of a ball, a type of “sieve or mesh” was used to distribute the product more evenly across the face, it would be more preferable. This would then need to be combined with a gel formulation as opposed to a water-based formulation so that it would not be as “runny”.

Carbohydrate-derived fulvic acid (CHD-FA) has shown antimicrobial and anti-inflammatory properties in previous studies [11, 13]. There is however as yet no substantial evidence that it is effective in the management of acne. It should be noted that due to the small sample size in this study, the power to distinguish a difference between the active and placebo group was small. While some clinical efficacy was noted by individuals themselves in both groups, it is difficult to ascertain whether this is due to CHD-FA, the low pH (in both CHD-FA and placebo) or merely a perceived improvement by the individuals themselves with no true change having occurred. Assessing the severity of acne is extremely difficult due to the subjective nature of the disease. Individuals appeared to be satisfied with the texture and

consistency of the formulations, but were very dissatisfied with the smell of the product. The product did cause a burning sensation in a number of individuals. This did appear, in most cases, to be transient burning and did not result in individuals wanting to discontinue use of the product. The burning sensation occurred in both the placebo and active group and is therefore most likely due to the low pH. A number of individuals felt that the product worked well. There are a number of factors that could have resulted in an actual improvement noted by the individuals. Since clinical efficacy was seen in both groups, it is possible that the low pH in both products could have caused an improvement [18]. The natural course of the disease could have meant that individuals may have improved on their own simply through a natural change in the androgen production of that individual or in the case of female participants; it could be possible that they had noted an improvement due to hormonal changes occurring in their menstrual cycle.

This does not rule out the possibility that CHD-FA is in fact effective in the management of acne, but it is not possible to determine from the results of this study. If further investigation is done, a larger sample size should also be used in order to increase the statistical power of the study. The smell of the product should also be drastically improved which would result in better compliancy. Individuals would use the product more often and this could cause a greater improvement in acne. The overall satisfaction with the product would increase dramatically.

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Legends for figures

Figure 1: Individual changes in the porphyrin count on the forehead from week 0 to week 6 in the active group (group 1) shown for each visit.

Figure 2: Individual changes in the porphyrin count on the forehead from week 0 to week 6 in the placebo group (group 2) shown for each visit.

Figure 3: Geometric mean porphyrin count change over time. The change in the geometric mean porphyrin count of the forehead in each group is shown.

Figure 4: A box plot of the differences in the porphyrin count between week 6 and week 0 in each group

Tables:

Table 1: Plewig and Kligman papulopustular acne severity grading, 1 – 4 [9]

Grade I	Fewer than 10 inflammatory lesions on one side of the face
Grade II	10 - 20 inflammatory lesions on one side of the face
Grade III	20 - 30 inflammatory lesions on on side of the face
Grade IV	more than 30 inflammatory lesions on one side of the face

Table 2: Results of the questionnaire for the active group

ACTIVE									
ROLL-ON	3 fem	10 fem	13 male	16 male	22 fem	30 Male	Ave		
1. Texture and consistency acceptable	4	2	5	3	5	4	3.83		
2. Absorbed into skin	5	3	4	5	4	4	4.17		
3. Product did not leave a residue	5	4	5	1	5	4	4.00		
4. Product smelt OK	3	1	2	1	3	1	1.83		
5. Product made skin feel uncomfortable	2	3	3	1	1	3	2.17		
6. Make-up/facial skincare products easy to apply after	5	4	4		5	3	4.20		
7. Overall happy with product	5	3	4	4	5	3	4.00		
GEL	11 fem	15 male	18 fem	25 fem	26 fem	30 Male	31 Fem	32 fem	Ave
1. Texture and consistency acceptable	4	4	5	4	4	4	4	5	4.3
2. Absorbed into skin	2	4	4	4	5	4	5	5	4.1
3. Product did not leave a residue	2	3	5	4	5	4	5	5	4.1
4. Product smelt OK	4	2	4	3	1	1	3	1	2.4
5. Product made skin feel uncomfortable	2	2	4	2	3	3	2	2	2.5
6. Make-up/facial skincare products easy to apply after	2		5	4	5	3	5	2	3.7
7. Overall happy with product	4	4	5	4	2	3	5	5	4.0

Table 3: Results of the questionnaire for the placebo group

PLACEBO								
ROLL-ON	21 male	23 fem	24 fem	27 fem	29 male	Ave		
1. Texture and consistency acceptable	4	4	3	5	5	4.2		
2. Absorbed into skin	4	3	5	5	3	4		
3. Product did not leave a residue	5	4	5	5	5	4.8		
4. Product smelt OK	3	1	4	5	4	3.4		
5. Product made skin feel uncomfortable	2	3	2	2	2	2.2		
6. Make-up/facial skincare products easy to apply after	2	3	3	5		3.25		
7. Overall happy with product	4	3	4	5	4	4		
GEL	1 fem	8 fem	9 male	12 fem	17 fem	20 male	33 fem	Ave
1. Texture and consistency acceptable	4	5	3	5	4	4	4	4.14
2. Absorbed into skin	4	5	4	4	4	4	4	4.14
3. Product did not leave a residue	5	4	5	4	4	5	5	4.57
4. Product smelt OK	1	1	2	2	2	1	1	1.43
5. Product made skin feel uncomfortable	3	3	2	3	3	3	1	2.57
6. Make-up/facial skincare products easy to apply after	4	3	3	4	4	4	4	3.71
7. Overall happy with product	2	4	4	4	4	5	4	3.86

Table 4: A comparison of the average scores for the placebo and active group for each statement

	Placebo Group Average	Active Group Average
1. Texture and consistency acceptable	4.17	4.09
2. Absorbed into skin	4.08	4.09
3. Product did not leave a residue	4.67	4.00
4. Product smelt OK	2.25	2.27
5. Product made skin feel uncomfortable	2.42	2.27
6. Make-up/facial skincare products easy to apply after	3.55	4.00
7. Overall happy with product	3.92	4.09

Table 5: Results of the final evaluation questionnaire. The number of times each formulation is picked for each statement is recorded in the table. Some individuals did pick both formulations for some statements

<u>Active group</u>	Roll-on	Gel
Which was most comfortable on your skin?	7	7
Which was absorbed into your skin best?	6	9
Which did you feel worked the best?	8	7
Which of the 3 were you most satisfied with?	7	7
Which of the 3 were you least satisfied with?	7	7

Table 6: Descriptive Statistics of the results of the VISIA® Complexion Analysis on the forehead. The porphyrin count was log transformed in order to normalize the data. The Geometric mean is therefore given.

Descriptive Stats					
Group 1 (Group A)					
	Mean age = 20	N (male) = 5	N (fem) = 7		
Variable	Type	Obs	Mean	95% Confidence Interval	
Week 0	Geometric	12	132.3815	42.50523	412.2987
Week 2	Geometric	12	102.9231	29.79567	355.5269
Week 4	Geometric	12	87.73002	31.8899	241.3477
Week 6	Geometric	12	81.04225	21.85964	300.4554
Group 2 (Group B)					
	Mean age = 20	N (males) = 3	N (fem) = 7		
Variable	Type	Obs	Mean	95% Confidence Interval	
Week 0	Geometric	12	120.2766	38.13651	379.3335
Week 2	Geometric	12	102.1145	30.43715	342.5868
Week 4	Geometric	12	107.6487	31.46818	368.2526
Week 6	Geometric	12	116.3565	33.25726	407.0945

Table 7: Results of the two-sample t-test with unequal variances for the log porphyrin count on the forehead.

two-sample t-test with unequal variances							
Group	Obs	mean (log count)	Std error	Standard dev.	P-value	95% confidence interval	
A	12	-0.4907171	0.5220451	1.808417		-1.63973	0.658296
B	10	-0.0331348	0.1891814	0.5982443		-	0.3948234
Combined	22	-0.2827251	0.2954828	1.385937		-	0.3317649
Difference		-0.4575823	0.5552663		0.4239	-1.650229	0.7350642
Difference = mean(A) - mean(B)							
H ₀ : difference = 0							

Table 8: Average change in the porphyrin count at each two-week study visit for both groups.

Average Porphyrin count	Week 0	week 2	week 4	Week 6
Active group (forehead)	312.58	283.09	202.25	249.67
Placebo group (forehead)	338.70	280.80	316.60	345.20
Active group (cheek)	467.92	431.09	408.00	463.42
Placebo Group (cheek)	405.60	339.40	347.90	392.70

Table 9: Average results for each parameter on the forehead for individuals in the active and placebo. The average results of the VISIA® Complexion Analysis for each parameter on the forehead at each two-week period are indicated.

Forehead	Week 0	Week 4	Week 6	Difference (6 wks)	
Porphyrin count	312.58	202.25	249.67	-63	Active
	338.70	316.60	345.20	6.50	Placebo
Red areas	10.42	11.58	11.00	0.58	Active
	8.60	7.80	8.20	-0.40	Placebo
Spots	53%	45%	50%	-2.75%	Active
	56%	50%	49%	-7.10%	Placebo
Texture	54%	38%	40%	-14.00%	Active
	42%	34%	36%	-6.60%	Placebo
Wrinkles	51%	44%	44%	-7.25%	Active
	43%	45%	37%	-6.40%	Placebo
Pores	58%	37%	44%	-13.58%	Active
	45%	37%	38%	-6.60%	Placebo

Table 10: Descriptive statistics of the results of the VISIA® Complexion Analysis on the right cheek. The median and min and max values are given since log transformation of the count does not work.

Descriptive Statistics				
Group 1 (group A)				
Stats	week 0	week 2	week 4	week 6
N	12	11	12	12
Mean	467.92	431.09	408	463.42
Median	337.5	388	330.5	399.5
min	6.00	5.00	7.00	6.00
Max	1622.00	916.00	1298.00	1423.00
Group 2 (group B)				
Stats	week 0	week 2	week 4	week 6
N	10	10	10	10
Mean	405.60	339.40	347.90	392.70
Median	187.50	161.00	215.50	158.50
min	63.00	66.00	26.00	58.00
Max	1310.00	1017.00	916.00	1294.00

Table 11: Average results for each parameter on the right cheek for individuals in the active and placebo. The average results of the VISIA® Complexion Analysis for each parameter on the right cheek at each two-week period are indicated.

Cheek	Week 0	Week 4	Week 6	Difference	
Porphyrin count	467.92	408.00	463.42	-4.50	Active
	405.60	347.90	392.70	-12.90	Placebo
Red areas	14.50	21.58	22.08	7.58	Active
	15.90	17.60	18.00	2.10	Placebo
Spots	56%	48%	52%	-4.33%	Active
	65%	63%	65%	0.60%	Placebo
Texture	75%	67%	71%	-4.33%	Active
	66%	61%	61%	-4.50%	Placebo
Wrinkles	61%	52%	56%	-5.08%	Active
	59%	51%	58%	-1.30%	Placebo
Pores	80%	74%	77%	-3.75%	Active
	75%	73%	72%	-2.60%	Placebo

Figures

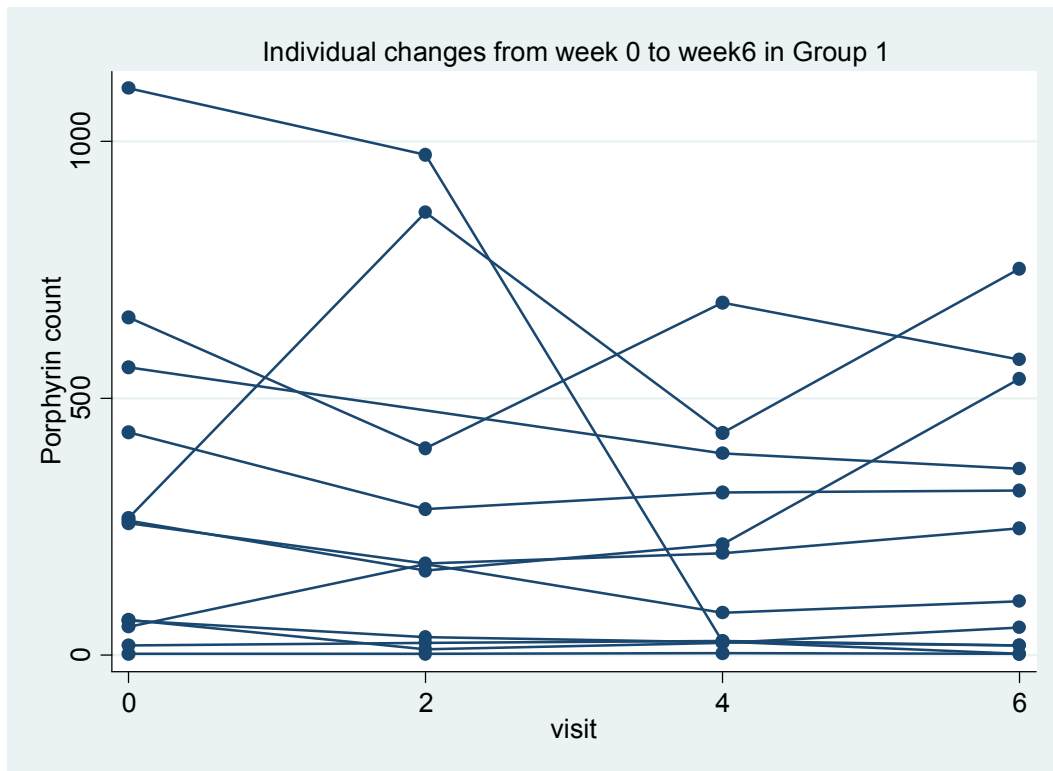


Figure 1

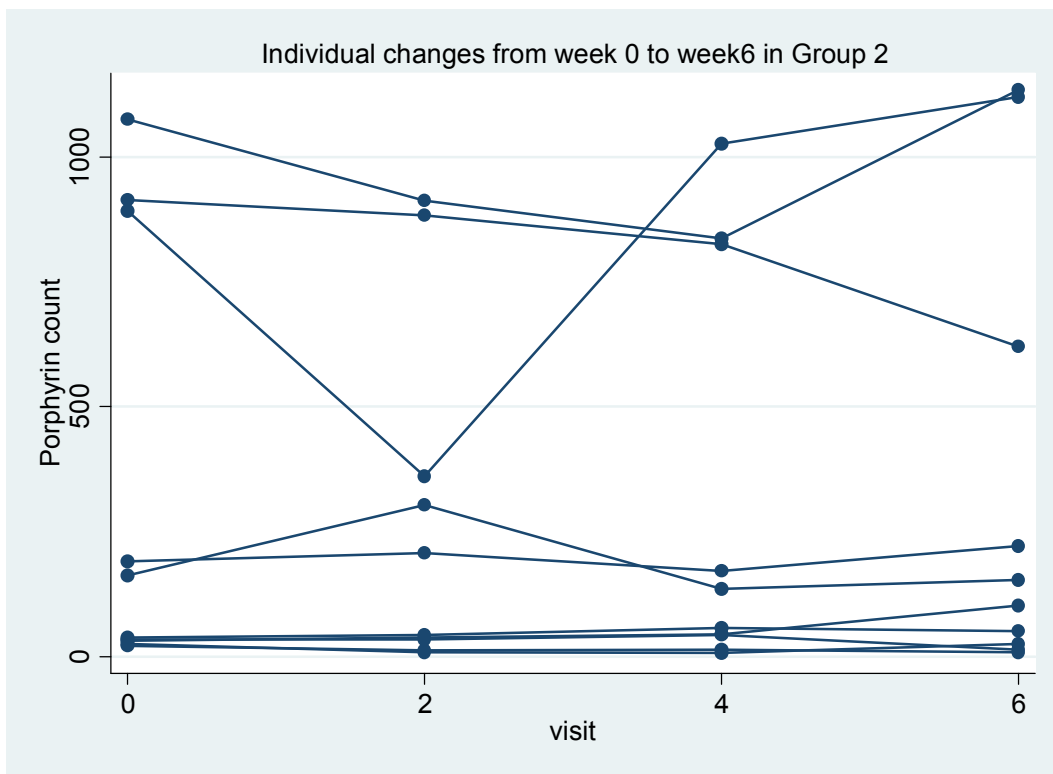


Figure 2

Geometric Mean Porphyrin Count change over time

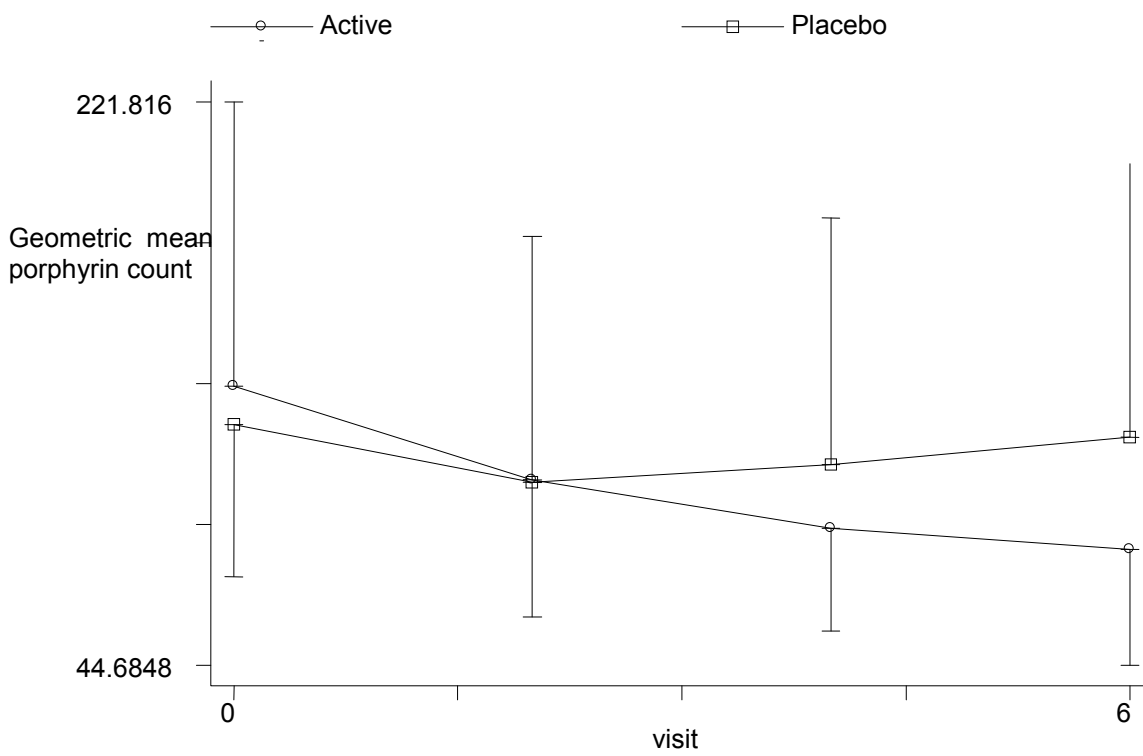


Figure 3

Difference in porphyrin count between week 6 and 0

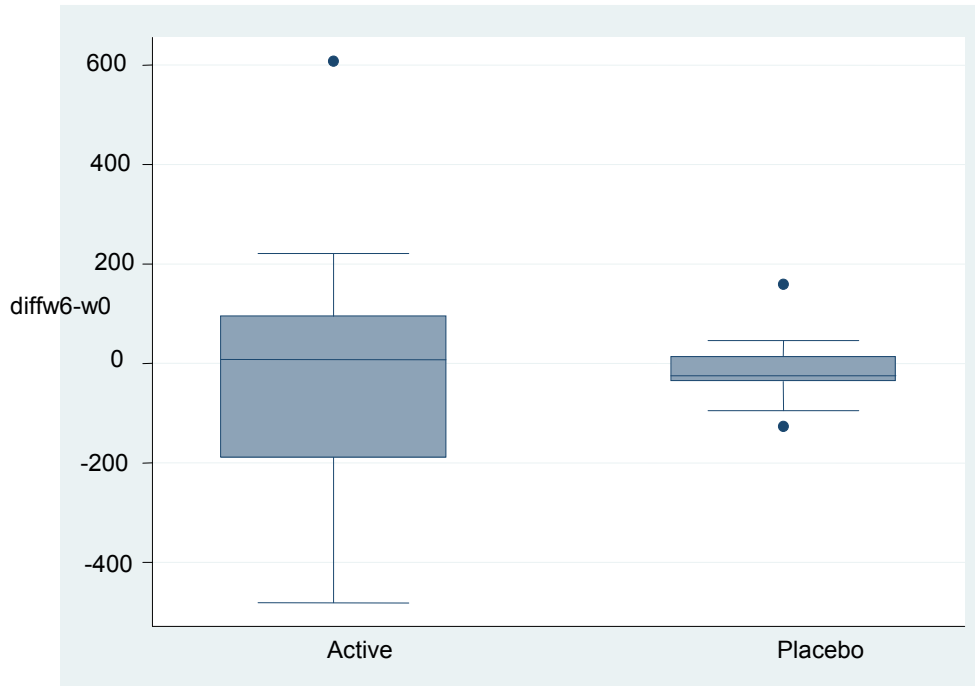


Figure 4

Topical management of acne vulgaris using carbohydrate-derived fulvic acid (CHD-FA)

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Running title: Topical Management of Acne Vulgaris using Carbohydrate-derived Fulvic Acid (CHD-FA)

Key words: fulvic acid, carbohydrate-derived, acne, topical treatment

ABBREVIATION LIST

CHD-FA Carbohydrate-derived fulvic acid

IRR Incident Rate Ratio

ABSTRACT

Objectives: In this study we attempted to determine whether Carbohydrate-derived fulvic acid (CHD-FA) was more effective than placebo as a cosmoceutical in the topical management of mild to moderate inflammatory acne.

Methods: 22 individuals with mild to moderate inflammatory acne (acne grade I-III) were invited to volunteer for the study. They were split into 2 groups, an active group placebo group. The study period was 6 weeks. Individuals filled out questionnaires about the products and VISIA Complexion Analysis® was done to measure any improvement.

Results: No statistically significant difference between group A and B was seen. The questionnaires indicated individuals in both the placebo and active group were happy with the product, but complained extensively about the smell of the product.

Discussion: Although no efficacy could be demonstrated in this study for CHD-FA in the management of inflammatory acne, a larger sample size, together with an improved smell could change the outcome of a second trial.

Introduction

Acne vulgaris is a disease that affects almost 80% of individuals [1]. It is commonly known to affect individuals during adolescent years, starting between the ages of 10-14 years, and normally regressing by ages 20-25 years. The severity of acne ranges from mild (few open and closed comedones) to severe inflammation and abscess formation on the face and trunk [2]. It is a disease that has strong psychological effects on persons affected by it. The pathophysiology of acne vulgaris can be broken up into four different events [3]:

1. Androgen-dependent overproduction of sebum
2. follicular hyperkeratosis (closed and open comedones)
3. increase in *Propionibacteria acnes*
4. immunological processes and inflammation

These events are not individual events, but are affected by each other [2]. For example, an increase in proliferation of *P. acnes* is as a result of increased sebum production and hyperkeratosis. However, the bacteria (and their metabolites) are responsible in part for producing proinflammatory mediators such as bacterial lipases, proteases, hyaluronidases and chemotactic factors [2].

Treatment options for acne can be divided into topical and systemic treatment. While systemic treatment remains the most effective means of managing acne, systemic side effects are common. Oral isotretinoin is the mainstay treatment for severe acne [4]. Due to the common and sometimes severe side effects, its use in individuals with mild to moderate acne remains controversial. There are topical treatments available that show some efficacy, however there is still a need for new, safe, effective and affordable topical treatments that can be used in individuals who wish to avoid systemic treatment.

Fulvic acid is one of the components of humic substances that are formed naturally during the decay of plant and animal material [5]. The humic substances can be divided into humic acid, fulvic acid, and humin. They are separated based on their solubility in water as a function of pH [6]. Fulvic acid is the fraction that is soluble in water at all pHs. Most research done on the therapeutic benefits of fulvic acid has been done using oxifulvic acid. Oxifulvic acid is formed by the wet oxidation of bituminous coal [5, 6, 7]. It has been shown to have antimicrobial activity and anti-inflammatory properties [6, 8].

In a pilot study to establish safety and efficacy, fulvic acid had no significant effect on any of the safety parameters and no sensitization occurred when applied on the skin [5].

A novel method of producing fulvic acid from the controlled wet oxidation of a carbohydrate source has been developed; it is known as carbohydrate-derived fulvic acid (CHD-FA). This product contains no heavy metals. CHD-FA showed broad band antimicrobial activity *in vitro* against gram positive cocci, gram negative bacilli, and yeasts [6]. It is believed fulvic acid will be effective in topical management of Acne Vulgaris, because of its proven anti-inflammatory and anti-microbial activity. By eliminating these two pathophysiological causes of acne, it is believed that the severity of the acne will decrease significantly. Follicular inflammation can also cause an increase in sebum production [2]. A pilot study done in 2007 by Peacock and Snyman showed *in vitro* efficacy and some clinical efficacy with a novel transdermal delivery system called Dermawave™ [9]. Decreasing acne will improve the appearance of the skin and the psychological distress caused by acne.

In this pilot study, the efficacy of carbohydrate-derived fulvic acid as a **cosmoceutical** in the topical treatment of individuals with mild inflammatory acne vulgaris was investigated, as well as the patient-preferred formulations of CHD-FA.

Materials and Methods

Assessing formulations

A 3-week long pilot study with 15 individuals with an acne grade less than III (as defined by Plewig and Kligman [10]) was done to establish which of 3 different formulations was preferred by individuals. The formulations were CHD-FA in a cream, in a gel, and in aqueous solution (water-based). Individuals were split into 3 groups and were allowed to use a different formulation every week. At the end of each week, individuals filled out a questionnaire about the formulation they had used that week and at the end of the study period a final questionnaire was filled out comparing the 3 formulations and indicated which one they liked best and which they liked least.

Pilot study to investigate efficacy of CHD-FA

33 individuals between the ages of 13 and 40 with an acne grade of I-III, as defined by Plewig and Kligman, were recruited by word of mouth, announcement in lectures and adverts

placed on the university campus [10]. Only 22 individuals completed the study. 5 individuals in the placebo group and 6 in the active group were lost to follow up. Exclusion criteria included (i) any facial skin diseases other than acne vulgaris (ii) using any other form of acne treatment less than 30 days prior to taking part in the study; (iii) pregnant or lactating females; (iv) using anabolic steroids; (v) individuals on antibiotic treatment; (vi) corticosteroid treatment; (vii) changing oral contraceptive treatment less than 6 months prior to starting the study.

Participants were randomized to 2 group, an active and a placebo group. The study period was 6 weeks. Participants were required to have a VISIA[®] complexion analysis done every two weeks. Participants were given a gel-based and a water-based roll-on formulation to use for the first two weeks. After the first two weeks, participants filled out 2 questionnaires evaluating the formulations they had used and chose the formulation they liked best and would continue using for the remainder of the study period.

Questionnaire

The first questionnaire filled out was based on a likert scale of satisfaction adjusted from a study done by Kellet et. al [12]. Individuals were asked to rate statements on a scale of 1 to 5, with 1 being “strongly disagree” and 5 being “strongly agree”. The statements included questions about the texture, absorption, whether it left a residue, the smell and overall happiness with the product. In the second questionnaire individuals were asked to compare the formulations, indicating which one they were most satisfied with, and which they were least satisfied with.

Study products

The active gel formulation was 3.95% CHD-FA and the water-based roll-on formulation approximately 4% CHD-FA. All the formulations had an acidic pH close to 3.9 and a mint flavouring added to disguise the smell of the products. The placebo formulations had an approved colouring added to disguise the appearance of the formulations and acetic acid was added in order to lower the pH to an acidic form equal to the active formulations.

Doses

Individuals were required to apply the product 2-5 times daily. Application was onto the entire face, or directly to the entire acne lesion/s, as preferred by the individual.

Assessment

Improvement in the appearance of the skin was assessed by the VISIA[®] complexion analysis system (© Canfield Scientific, Inc developed by Einstein Medical). The system takes photos of the forehead and right cheek of the face and measures a number of parameters on and slightly underneath the skin. The parameter used to assess acne severity was the porphyrin count. Porphyrin is a metabolite of *P. acnes* and a higher porphyrin count is due to a higher presence of *P. acnes*. A decrease in the porphyrin count indicated an improvement in acne.

Statistical analysis

The statistical analysis was done by a qualified statistician given blinding results of VISIA[®] complexion analysis. Log transformation and square root transformation of the data from the forehead and the right cheek respectively was done in order to normalize the data. The data was analysed in three different ways (i) looking at the change between week 0 and week 6 with a Mann Whitney U test on the unchanged data and a t-test on the normalized data; (ii) repeated measures analysis (anova) using normalized values, (iii) poisson regression with the result given as an incidence rate ratio (IRR)

Ethical considerations

Ethical approval was obtained from the University of Pretoria Research Ethics Committee before commencing the study.

Results

Questionnaire

In Table 1, the results of the questionnaire for the two active formulations are shown. All the statements scored positively, except for the smell of the product. Individuals complained the smell was so strong it made their eyes burn or water after application. This may be due to

the low pH found in both the active and placebo formulations. Individuals complained the formulations caused transient burning or irritation to their skin especially when applied to an open lesion or broken skin. One individual complained that the roll-on product burnt and caused redness and swelling for 2-5 hours after application. A number of individuals felt the formulations dried out their skin at times, while some felt it made their skin feel soft. Individuals applied the product on average twice a day according to the diary cards. Many individuals felt the formulations were working well, however this was found in both the active and the placebo group. No one formulation appears to be preferred to the other.

Forehead

Figure 1 shows the individual changes in the porphyrin count from week 0 to week 6 in the active group. There is little individual change in the porphyrin count over the 6 week study period and there is a wide spread in porphyrin count amongst the individuals. Table 2 shows the results of the two-sample t-test with differences in the log count between week 6 and week 0 used to determine the mean difference in each group. There were no significant effects. The P-value was 0.4239 and the null hypothesis could not be rejected. The Mann Whitney U test and anova test also showed no significant difference. In the poisson regression analysis, the IRR was 1.038 and the 95% confidence interval passed through 1 indicating no difference between the groups.

Right Cheek

In Figure 2, differences in the porphyrin count between week 6 and week 0 in each group is shown on a box plot. There is no difference between the groups over the 6-week study period, but a wider distribution of mean difference in Group A was seen. A two-sample t-test with the square root transformed data was done but showed no difference between the active and placebo group. The P-value found was 0.9578 and the null hypothesis could not be rejected. The nonparametric Wilcoxon rank sums test and repeated measures anova also showed no significant difference between the two groups. In the poisson regression, the IRR was 1.043 and the 95% confidence interval passed through 1 therefore no difference between placebo and active group can be established.

There were no other side effects reported besides complaints about the smell of the formulations and the transient burning on the skin and the eyes.

11 individuals were lost to follow up. Of these, 6 were in the active group and 5 in the placebo group. The results of the questionnaires suggest the majority of these individuals discontinued the study due to the smell of the formulation and/or the burning sensation it caused.

Discussion

The primary outcome of this study was improvement in the appearance of the skin and reduction of inflammatory acne in the individual. The secondary outcome was investigation into the safety parameters and collecting further information about the two formulations (gel based and water-based) to determine which of the two formulations was preferred by participants. The questionnaires helped gather further information regarding the product and how participants felt about the product.

Carbohydrate-derived fulvic acid (CHD-FA) has shown antimicrobial and anti-inflammatory properties in previous studies [6, 8]. There is however as yet no substantial evidence that it is effective in the management of acne. It should be noted that due to the small sample size in this study, the power to distinguish a difference statistically between the active and placebo group was small. While some clinical efficacy was noted by individuals themselves in both groups, it is difficult to ascertain whether this is due to CHD-FA, the low pH (in both active and placebo) or merely a perceived improvement by the individuals themselves with no true change having occurred. Individuals were very dissatisfied with the smell of the product. The product caused a burning sensation in a number of individuals but did appear, in most cases, to be transient burning and did not result in individuals wanting to discontinue use of the product. This occurred in both the placebo and active group and is therefore most likely due to the low pH. A number of individuals felt that the product worked well. There are a number of factors that could have resulted in an actual improvement noted by the individuals. Since clinical efficacy was seen in both groups, it is possible that the low pH in both the active and placebo group could have caused an improvement [13]. The natural course of the disease could have meant that some individuals

may have improved on their own simply through a natural change in the androgen production of those individuals. In the female participants it is possible they noticed an improvement due to hormonal changes in their menstrual cycle.

This does not rule out the possibility that CHD-FA is in fact effective in the management of acne, but it is not possible to determine from the results of this study. A larger sample size would have increased the statistical power of the study. The smell of the product should be drastically improved. Improvement would result in less people dropping out of the study before completion and individuals would use the product more often which could increase clinical efficacy. The overall satisfaction with the product would increase dramatically.

Acknowledgements

Prof. Paul Rheeder for performing statistical tests; Justin Gandy, Dr. Duncan Cromarty and Dr. Kim Outhoff for their valuable advice

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Legends to Figures

Figure 1: Individual changes in the porphyrin count on the forehead from week 0 to week 6 in the active group (group 1)

Figure 2: A box plot of the differences in the porphyrin count between week 6 and week 0 in the active (1) and placebo (2) group.



Tables:

Table 1: Results of the questionnaire for the active group

ACTIVE									
ROLL-ON	3 fem	10 fem	13 male	16 male	22 fem	30 Male	Ave		
1. Texture and consistency acceptable	4	2	5	3	5	4	3.83		
2. Absorbed into skin	5	3	4	5	4	4	4.17		
3. Product did not leave a residue	5	4	5	1	5	4	4.00		
4. Product smelt OK	3	1	2	1	3	1	1.83		
5. Product made skin feel uncomfortable	2	3	3	1	1	3	2.17		
6. Make-up/facial skincare products easy to apply after	5	4	4		5	3	4.20		
7. Overall happy with product	5	3	4	4	5	3	4.00		
GEL									
	11 fem	15 male	18 fem	25 fem	26 fem	30 Male	31 Fem	32 fem	Ave
1. Texture and consistency acceptable	4	4	5	4	4	4	4	5	4.3
2. Absorbed into skin	2	4	4	4	5	4	5	5	4.1
3. Product did not leave a residue	2	3	5	4	5	4	5	5	4.1
4. Product smelt OK	4	2	4	3	1	1	3	1	2.4
5. Product made skin feel uncomfortable	2	2	4	2	3	3	2	2	2.5
6. Make-up/facial skincare products easy to apply after	2		5	4	5	3	5	2	3.7
7. Overall happy with product	4	4	5	4	2	3	5	5	4.0

Table 2: Results of the two-sample t-test with unequal variances for the log porphyrin count on the forehead.

two-sample t-test with unequal variances							
Group	Obs	mean (log count)	Std error	Standard dev.	P-value	95% confidence interval	
A	12	-0.4907171	0.5220451	1.808417		-1.63973	0.658296
B	10	-0.0331348	0.1891814	0.5982443		0.4610929	0.3948234
Combined	22	-0.2827251	0.2954828	1.385937		0.8972152	0.3317649
Difference		-0.4575823	0.5552663		0.4239	-1.650229	0.7350642
Difference = mean(A) - mean(B)							
H ₀ : difference = 0							

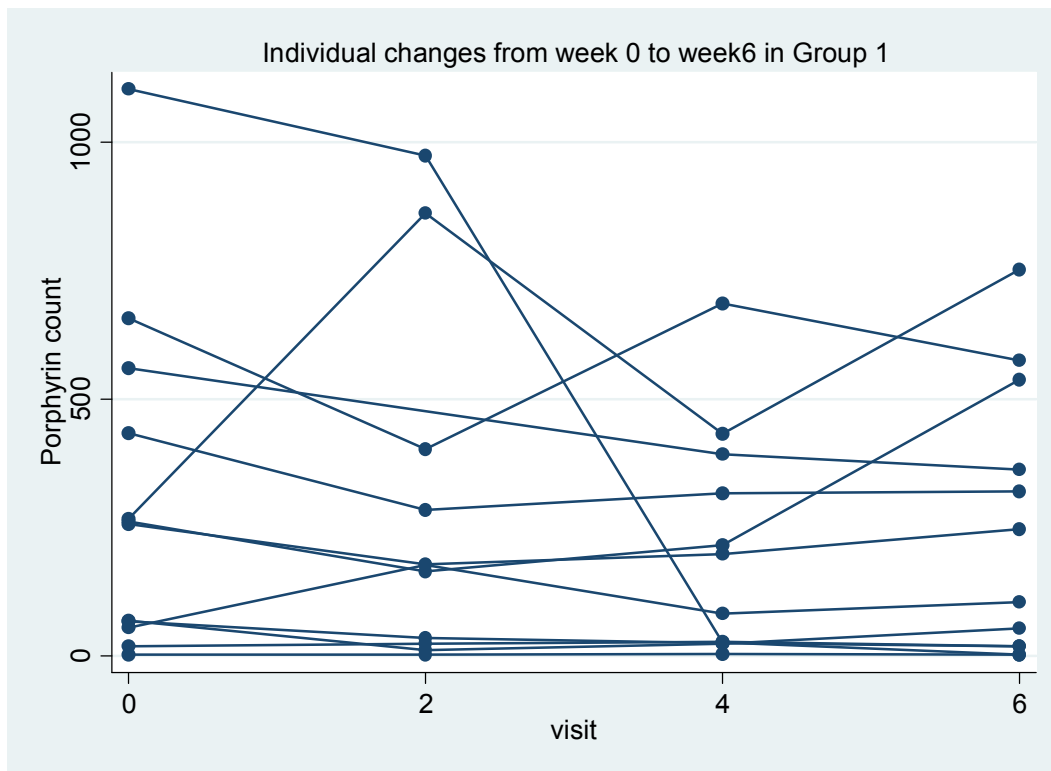


Figure 1

Difference in porphyrin count between week 6 and week 0

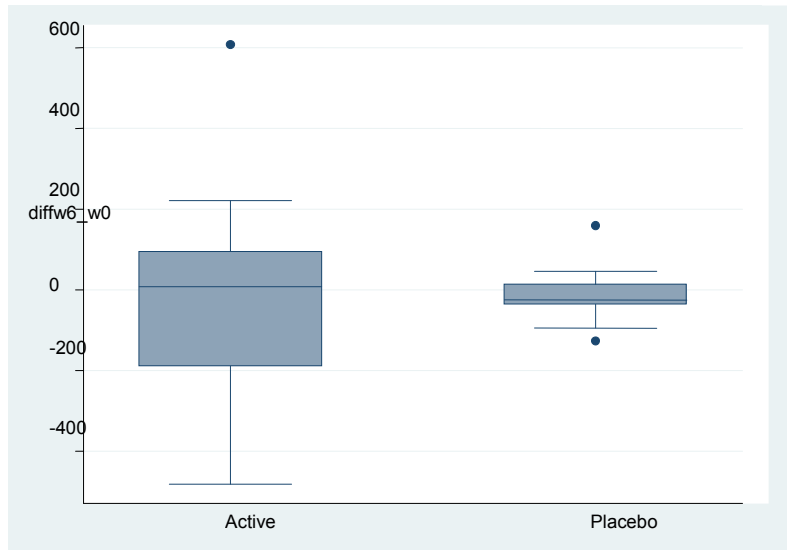


Figure 2



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Appendix 1

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
How many times did you apply the treatment today? At what times did you?							
Did you have any adverse reactions to the treatment, such as sensitivity to the skin, a rash, redness? If so, specify what these were?							
Did you use any other creams or ointments on your face? If so, what were these?							
Did you use any other medications? If so, what were they?							
Comments about the treatment?							

Appendix 2

Questionnaire

Please circle the applicable product you are rating

Fulvic acid cotton wool applicator

Fulvic acid in cream base

Fulvic acid in gel base

Please rate the following statements below on a scale of 1 to 5 depending on how much you agree or disagree with the statement. Circle the number most applicable

1 = Strongly disagree

2 = disagree

3 = neutral

4 = agree

5 = strongly agree

1.	The texture and consistency of the product was acceptable	1	2	3	4	5
2.	The product was absorbed into the skin easily	1	2	3	4	5
3.	The product did not leave a residue on the skin	1	2	3	4	5
4.	The product smelt OK	1	2	3	4	5
5.	The product made my skin feel uncomfortable	1	2	3	4	5
6.	Make-up/facial skincare products were easy to put on after application of the product	1	2	3	4	5
7.	Overall, I am happy with the product	1	2	3	4	5

Please comment on what you liked and disliked about the product:

Appendix 3

Questionnaire: End of trial

1. Did any of the products hurt or irritate your skin?

If so, which one/s?

2. Did any of the products cause an adverse reaction? If so, please explain?

3. Of the three applicators studied, which of the three, in your opinion, was the most comfortable on your skin? Please circle

Fulvic acid cotton wool applicator

Fulvic acid cream base

Fulvic acid gel base

4. Which applicator did you feel was absorbed into your skin the best? Please circle

Fulvic acid cotton wool applicator

Fulvic acid cream base

Fulvic acid gel base

5. Which of the 3 applicators did you feel worked the best? Please circle

Fulvic acid cotton wool applicator

Fulvic acid cream base

Fulvic acid gel base

6. Overall, which of the 3 applicators were you most satisfied with?



Fulvic acid cotton wool applicator

Fulvic acid cream base

Fulvic acid gel base

7. Overall, which of the 3 applicators were you least satisfied with?

Fulvic acid cotton wool applicator

Fulvic acid cream base

Fulvic acid gel base

Appendix 4

Questionnaire: Clinician

1. Did any of the patients complain that any of the products hurt or irritated their skin?

If so, which one/s?

2. Did you notice that any of the products may have caused an adverse reaction? If so, please explain?

3. Of the three applicators studied, which of the three, in your opinion, was the most stable? Cotton wool applicator, cream base, or gel base?

4. Which applicator, in your opinion, was the least stable? Cotton wool applicator, cream base, gel base?

5. In your opinion, which of the 3 applicators was the most acceptable amongst the patients? The cotton wool applicator, cream base, or the gel base? Please explain

Appendix 5

Questionnaire

Age:

Gender:

Education level achieved (highest level achieved thus far):

Please circle the applicable product you are rating

Fulvic acid wet applicator

Fulvic acid in gel base

Please rate the following statements below on a scale of 1 to 5 depending on how much you agree or disagree with the statement. Circle the number most applicable

1 = Strongly disagree

2 = disagree

3 = neutral

4 = agree

5 = strongly agree

1.	The texture and consistency of the product was acceptable	1	2	3	4	5
2.	The product was absorbed into the skin easily	1	2	3	4	5
3.	The product did not leave a residue on the skin	1	2	3	4	5
4.	The product smelt OK	1	2	3	4	5
5.	The product made my skin feel uncomfortable	1	2	3	4	5
6.	Make-up/facial skincare products were easy to put on after application of the product	1	2	3	4	5
7.	Overall, I am happy with the product	1	2	3	4	5

Please comment on what you liked and disliked about the product:

Appendix 6

Questionnaire: Evaluation

1. Did any of the products hurt or irritate your skin?

If so, which one/s?

2. Did any of the products cause an adverse reaction? If so, please explain?

3. Of the two applicators studied, which of them, in your opinion, is the most comfortable on your skin? Please circle

Fulvic acid wet applicator

Fulvic acid gel base

4. Which applicator do you feel is absorbed into your skin the best? Please circle

Fulvic acid wet applicator

Fulvic acid gel base

5. Which of the 2 applicators do you feel works the best? Please circle

Fulvic acid wet applicator

Fulvic acid gel base

6. Overall, which of the 2 applicators are you most satisfied with?

Fulvic acid wet applicator

Fulvic acid gel base



7. Overall, which of the 2 applicators are you least satisfied with?

Fulvic acid wet applicator

Fulvic acid gel base