IJBPAS, June, 2017, 6(6): 1255-1261

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EVALUATION OF FIVE HERBAL EXTRACTS COMBINATION IN HAIR GROWTH STIMULATION

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Received 17th March 2017; Revised 28th March 2017; Accepted 16th April 2017; Available online 1st June2017

ABSTRACT

Introduction: The use of plant extracts in the cosmetic industry is highly regarded.

Material and methods: In this study, the effects of 5 herbal extracts (rosemary, nettle, sage, peppermint, chamomile) evaluated, in this way water extract prepared from 5 studied plants then the toxicity of solution was determined using MTT assay. In the next step 8 male wistar rats were selected and divided into two groups.

Result: In first group evaluated the effect of compound with 20% concentration on hair growth and were compared with second group that considered as control group results confirmed that number of follicles in the anagen phase increase compared with first group.

INTRODUCTION

There are several factors that can cause hair loss. Such as hormonal imbalance, genetic, mineral deficiency, polycystic ovary syndrome. The use of chemicals for hair loss prevention hasserious side effects like multiple chemical sensitivity. Therefore, herbal extracts are used as new approach in the cosmetic industry. One of the main reasons for the hair loss is reducing the amount of collagen and laminin in the scalp. Free radicals lead to increase Activator protein 1 expression. This factor stimulates metalloproteinase production. Consequently, it has negative impact on the collagen, and degrades it. Also, the oxidative stress of free radicals impairs hair follicle cells. But, antioxidant activity of herb extracts inhibits free radicals, and prevents the degradation of collagen by suppression of Erk/Ap1pathway.

Amino acids

Amino acids are necessary to produce keratin (the main component of hair structure) and extracellular skeleton(1).

Plant	Effective ingredients
Nettle	Quercetin, Alpha tocopherol, Butylatedhydroxytoluene, Butylatedhydroxyanisole
Rosemary	carnosol, carnosic acid, ursolic acid, rosmarinic acid, and caffeic acid.
Salvia officinalis	Luteolin -7-β - glucopyranoside
Peppermint	rosmarinic and caffeic acids, luteolin, eriocitrin, ascorbic acid and carotenoids
Chamomile	apigenin, caffeic acid, chamazulene, chlorogenic acid, chrysoeriol, gentisic acid

Amino acids

Plant	Amino acids
Peppermint	Alanine+ Valine+ Isoleucine+ 4-Aminobutyric acid+ Glutamine+ Tirosine+ Triptophan
Salvia	Arginine+ Alanine+ Threonine+ Glycine+ Serine+ Cystine+ Valine+ Proline+ Methionine+ Lysin
Rosemary	Arginine+ Alanine+ Threonine+ Aspartic acid+ Cystine+ Histidine+ Phenyl alanine+ Tyrosine
Chamomile	Glycine+ Valine+ Proline+ Alanine+ Aspartic acid+ Arginine+ Glutamic acid+ Lysine+ Leucine+ Isoleucine+ Phenilalanine+ Methionine+ Serine+ Theronine+ Histidine+ Glycocoll+ Tyrocine+ Glutamine+ Cystine
Nettle	Aspartic acid + Asparagine+ Threonine+ Serine+ Glutamic acid+ Proline+ Glycine+ Alanine+ Cysteine+ Valine+ Methonine+ Isoleucine+ Leucine+ Tyrosine+ Tryptophan+ Phenylalanine+ Lysine+ Histidine+ Arginine

The enzyme 5-αreductase converts testosterone into Dihydrotestosterone (DHT). Then, DHT acts as a transcriptional factor after binding to androgen receptors, and enters into the nucleus, and has impact on a series of genes involved in apoptosis like TGFβ. It leads to shrink follicle sizes through Wnt/β-catenin pathway suppression.Then,

the follicles enter the catagen phase. Many studies showed that rosemary extract, Salvia, Peppermint, Chamomile, Nettle inhibit the enzyme 5-α reductase (2)(3) extracts of nettle root have been used to treat enlarged prostate glands by inhibiting the body's production of the HORMONE DHT. DHT is also responsible for hair loss, and so nettle root supplements may, by blocking DHT production, also prevent age-related hair loss in men and women. A number of shampoos contain nettle extract. Many also contain other herbs that benefit hair, such as plantain or burdock. Rosemary oil is believed to function similarly to minoxidil, by reinvigorating hair follicles that are thinning or damaged (17).

Chamomile is frequently added to skin cosmetics to serve as an emollient, and for its anti-inflammatory effects. Chamomile is also often used to enhance the color of blonde hair (18).

MATERIAL AND METHODS

Preparation of Plant Extracts

Five plants were selected for the preparation of plant extracts. Namely, rosemary, Salvia, Peppermint, Chamomile, Nettle. After cleaning, the plants were dried in the shade, and made into fine powder by using mill. Next,1g of active ingredient powder was mixed with 9 mL of distilled water. After boiling distilled water, powder was poured into boiled water, and continues to boil for 18 minutes. Then, the liquid was filtered in order to discard 80 percent of solvent (water). Remains were placed into bainmarie at 32°C for 8 hours, and extracts were prepared with high concentration.

Antiproliferation assay

Human dermal fibroblasts passage 3 were prepared from theRoyan institute, and cultured in DMEM mediacontaining10 percent serum, and kept in 5% CO2 at 37°C. Next, cells were subcultured in trypsin and EDTA (cells were passaged every two to three days in order to maintain cells in a logarithmic growth phase).Then, cells were cultured in 96- well culture plates (1 X 10^5 cells in 8 well) in eight different concentrations for 72 hours. Next, 50 µl of 2 mg/ml MTT was added to each well. After 4 hours incubation, the media was removed from the wells, and violet formazan crystals formed, and the MTT-formazan crystals were dissolved in 200 µL DMSO after 20 minutes. The absorbance was measured at 570 nm. Consequently, Cell viability was calculated with the formula:

Viability (%) = [OD (drug-treated sample) –OD (blank)]/[OD (control) –OD (blank)]

Animal test

8 once-month-old male Wistar rats were kept in separated cages. Next, rats were divided into two groups (n=4). The first group was as control group, and second was considered as test group.

First, a small section from the back of the rats was shaved (2cm×4cm). Next, 20 % concentration of each extract was used topically in shaved area of test group, whereas deionized water was used in control group during 30 days on a regular basis. Then, all rats were sacrificed, and samples were taken from shaved area.

Measurement of Histological changes

First, skin samples were fixed in formalin. Next, these were placed in ethanol and xylene. Then, samples were paraffinazed, and were cut at 5 μm thickness, and tissue was stained with hematoxylin and eosin. A microscope equipped with high-definition camera was used to probe the number of hair follicles each mm of skin, the percentage of different phases of hair follicles (anagen and telogen phases) and the variation of epidermal thickness.

Analysis

In this study, analysis of variance (ANOVA) followed by a Tukey honestly significantly different (HSD) tests. Values of $p< 0.05$ were considered significant.

The toxicity of compound was measured by human dermal fibroblasts and determination of EC50.

RESULTS

Determination of chemical compounds: The chemical composition of these extracts was determined by using HPLC. These contain 73% of the total compound. Then, serial dilution of these extracts was made which contains 8 different concentrations of 0/78 % to 100 percent.

The results showed that fibroblast viability in the concentration 84/33 ±%0 of solution is halved after 72 hours treatment. The skin histological appearance of shaved back skin of rats did not alter after treatment with 20% solution in both control and test group. There was a stratified squamous epithelium with normal thickness, and the border between epidermis and dermis were clearly demarcated in both groups.

Numbers of hair follicles multiply after 4 weeks treatment with herbal extracts Skin biopsies showed a significant difference ($p < 0.05$) in the number of hair follicles per mm2 skin of rats in test group in comparison with control group. The number of follicles were 8/9±0/4 per mm2 in test group, and it showed a significant increase of 1.5 -2.5 fold, whereas the number of follicles in control group was 3± 0.1.

The correlation between concentration and fibroblast viability after 72 hours treatment

Concentration	Fibroblast viability
84/33±0%	halved

The comparison of hair follicles numbers in both test and control group

Groups	Test group	Control group
Numbers of hair follicles	8/9±0/4per mm2	3± 0.1per mm2

DISCUSSION

From the histological studies, the increased numbers of hair follicles were clearly seen. Hair follicles entered into anagen phase after 4 weeks in both groups. 70±7% of hairfollicles in control group and 82 ± 6 % hair follicles in test group entered in anagen phase. It showed a significant difference ($p < 0.05$). In vitro toxicology methods were used for the assessment of toxicity of compound. Cytotoxicity test were used for the study of irritant responses of chemicals towards skin. >33% concentration of extract has impact on fibroblast cell. Therefore, low concentration of this extract (20 %) was used for treatment. After 4 weeks, biopsies were taken from both groups, and the thickness of epidermis was compared. The result showed that there was no difference between groups ($p < 0.05$).Cell death was very limited at the concentration below 20 %. In fact, there was no toxicity at concentration 20 % of this blend. Consequently, 20 percent is the best concentration to conserve fibroblast viability. The number of growing hair follicles increased after the treatment with herbal extracts.

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